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CIRCULATING IMMUNE COMPLEXES IN STEROID SENSITIVE NEPHROTIC SYNDROME*

*Ayşın Bakkaloğlu MD**, *Ümit Saatçi MD****

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Steroid sensitive nephrotic syndrome (SSNS), or minimal change nephrotic syndrome (MCNS), is the most frequently encountered childhood nephrosis[1].

Selective proteinuria and minimal histological changes on renal biopsy have been found in this syndrome[2], and various studies have indicated that an immunological mechanism may have an effect on the etiopathogenesis of the disease, the following having been observed: a correlation with atopy and seasonal changes[3,4], good results with corticosteroid and immunosuppressive drug treatment[5,6], a decrease in complement levels and an increase in immunoconglutinins[7] before relapse, and the presence of circulating immune complexes[8].

The type of immunological mechanism responsible for this syndrome has not yet been clearly defined. Glomerular C3 or immunoglobulin deposits on the renal biopsy specimens have not been described in SSNS, in contrast to immune complex-mediated glomerulonephritis.

The aim of the present study is to evaluate the immune complexes in SSNS, both in relapse and remission, using the EA (sensitized erythrocyte) (Fc) rosette inhibition test, in order to discover whether there is any correlation between circulating immune complexes and disease activity and to explore the role of immune complexes in the etiopathogenesis of the disease.

Material and Methods

Twenty-four children (19 males, 5 females) between the ages of 3 and 17 years with MCNS were studied. All of the patients were sensitive to steroid treatment, and none of them had evidence of underlying collagen disease. Fifteen patients were in relapse. Eight of these 15 were studied before steroid therapy and the remaining

* From the Nephrology Unit, Hacettepe University Institute of Child Health, Ankara.
** Associate Professor of Pediatrics, Hacettepe University Institute of Child Health.
*** Professor of Pediatrics and Director, Pediatric Nephrology Unit, Hacettepe University Institute of Child Health.

7 within a week of the commencement of steroid therapy. Clinical and laboratory examinations were carried out on 12 patients in remission. Three of the 12 were also evaluated in relapse (Table I: Cases 10,11,13). All of these cases had been off drugs for four weeks prior to the study.

TABLE I

Immunological Studies of the Patients with SSNS in Relapse

Case number	Age (yrs)	Sex	β_1C mg/dl	IgG mg/dl	IgA mg/dl	IgM mg/dl	IgE IU/ml	% inhibition of EA (Fc) rosette formation
1*	14	F	nd	nd	nd	nd	nd	18.5
2	6	M	91	146	65	125	25	41
3*	6	M	105	360	300	165	2500	3.4
4	13	M	114	800	145	182	54	0
5	17	M	176	215	350	280	93	29
6	7	M	nd	670	201	78	63	0
7	3	M	150	400	155	152	49	39.5
8*	5	M	168	340	146	112	133	7.1
9	6	M	71	167	125	107	508	22
10	16	F	186	360	280	150	74	33.3
11*	16	M	148	168	39	110	1400	17
12*	12	M	93	500	115	230	136	21.4
13	10	M	99	198	299	194	nd	17
14	4	M	183	290	0	106	nd	8.4
15*	11	F	165	510	146	187	885	10.7

* Patients on steroid.
nd: not done.

Circulating immune complexes were detected by the EA (Fc) rosette inhibition test[9]. All sera from these patients were stored at +4°C and tested on the same day. Sera were not subjected to any freeze-thawing or heat-inactivation procedures.

Suspensions of normal human peripheral blood lymphocytes (PBL) were obtained by centrifugation over Ficoll-metrizoate as described by Boyum[10]. Monocytes were removed by adherence to plastic petri dishes leaving virtually pure lymphocytes (97%) of which more than 98% were viable as determined by trypan blue exclusion. Two million lymphocytes were suspended in 0.25 ml neat serum, incubated at 37°C for one hour and following two washes in hepes buffered Earle's medium, rosetted with optimally sensitized chicken erythrocytes (EA) as previously described[9].

Control values were obtained by incubating lymphocytes in medium in place of serum, and the results were expressed as the percentage inhibition of EA (Fc) rosette formation induced by serum in comparison with those control values.

Serum immunoglobulins (IgG, IgA, IgM) and C3 were determined with the radial immunodiffusion method[11]. IgE values were determined by the radioimmunoadsorbent test (RIS)[12] (Phadebas IgE kit, Pharmacia). Student's t test was used for statistical analysis[13].

Results

The results of the immunological studies of the patients with SSNS in relapse are summarized in Table I. The mean (± SE) percentage inhibition of Fc rosette formation by patients' sera in relapse was 17.88 ± 3.45. The mean inhibition value for normal sera was 4.23 ± 1.31. The difference between patient and control group values was significant ($p < 0.001$). The percentage inhibition of Fc rosette formation of the patients with SSNS in remission was 5.94 ± 2.91. A statistically significant difference was found between relapse and remission groups ($p < 0.05$) as seen in Figure 1. IgG levels of SSNS patients in relapse were significantly lower than those of healthy controls (Figure 2). A significant inverse correlation was found between the percentage inhibition of EA (Fc) rosette formation and IgG values ($p < 0.05$). IgA levels were above normal in four out of 14 (Cases 3,5,10,13), and two patients showed decreased levels (Cases 11, 14). IgM values were within normal limits in this group. (Figure 2).

The results for the patients with SSNS in remission are summarized in Table II. Immunoglobulin values are shown in Figure 3. Two out of 8 cases showed decreased levels of IgG (Cases 3,7). IgA levels were above normal in five out of 8 cases (Cases 5,6,8,9,10). IgM levels were within normal limits for all but one patient (Case 7).

IgE levels were above normal in 4 out of 12 cases in relapse and 2 out of 11 cases in remission. No correlation was found between IgE, IgG levels and percentage inhibition of rosette formation.

Discussion

The association of steroid-responsive nephrotic syndrome in childhood with minimal histological changes in the glomeruli ("minimal change nephrotic syndrome, nil disease") is so constant that routine renal biopsy is not necessary in their management. Thus the syndrome is better described by its objective therapeutic response than by its presumed absence of histological change[8].

Immune complexes are thought to play a major role in the pathogenesis of many forms of glomerulonephritis. The main supporting evidence has come from immu-

TABLE II

Immunological Studies of the Patients with SSNS in Remission

Case number	Age (yrs)	Sex	β_1C mg/dl	IgG mg/dl	IgA mg/dl	IgM mg/dl	IgE IU/ml	% inhibition of EA (Fc) rosette formation
1	16	M	110	700	191	116	120	0
2	11	F	nd	nd	nd	nd	52	0
3	6	M	132	430	168	116	170	0
4	11	F	nd	nd	nd	nd	20	0
5*	10	M	129	1050	270	68	2575	8.3
6*	16	F	186	900	380	130	38	0
7*	16	M	148	190	66	25	1025	0
8	17	M	nd	1290	350	130	41	0
9	8	M	nd	1030	363	105	45	31
10	16	M	nd	1505	440	173	55	14.2
11	15	M	nd	nd	nd	nd	43	17.8
12	7	M	nd	nd	nd	nd	nd	0

* Patients evaluated in relapse also (Table I, Cases 10,11,13).
nd: not done.

nofluorescence studies by which granular deposits of immunoglobulin and complement components have been demonstrated in the glomeruli. These deposits are similar in pattern to those found in experimental animals where glomerulonephritis has been induced by the formation of immune complexes in vivo[2,14,15]. Indirect evidence suggests that soluble complexes are present in the circulation of some patients with glomerulonephritis. This evidence includes reduced serum complement levels and the presence of complement split products, cryoglobulins, and rheumatoid factor[16-18].

Recently, circulating immune complexes have been detected in glomerulonephritis by various assays based on complement activation, and in one study by inhibition of uptake of aggregated IgG by guinea-pig peritoneal macrophages[19].

We have demonstrated significantly elevated levels of circulating immune complexes detected by the EA (Fc) rosette inhibition test in patients with SSNS in relapse but not in remission. This assay detects IgG-containing non-complement fixing immune complexes, which have the capacity to react with Fc receptors on lymphocytes in vitro[10]. As stated previously, there are reports in the literature of the detection of complement-activating complexes in glomerulonephritis[19,20]. It is not clear whether two types of complexes (i.e. complement-fixing and non-comple-

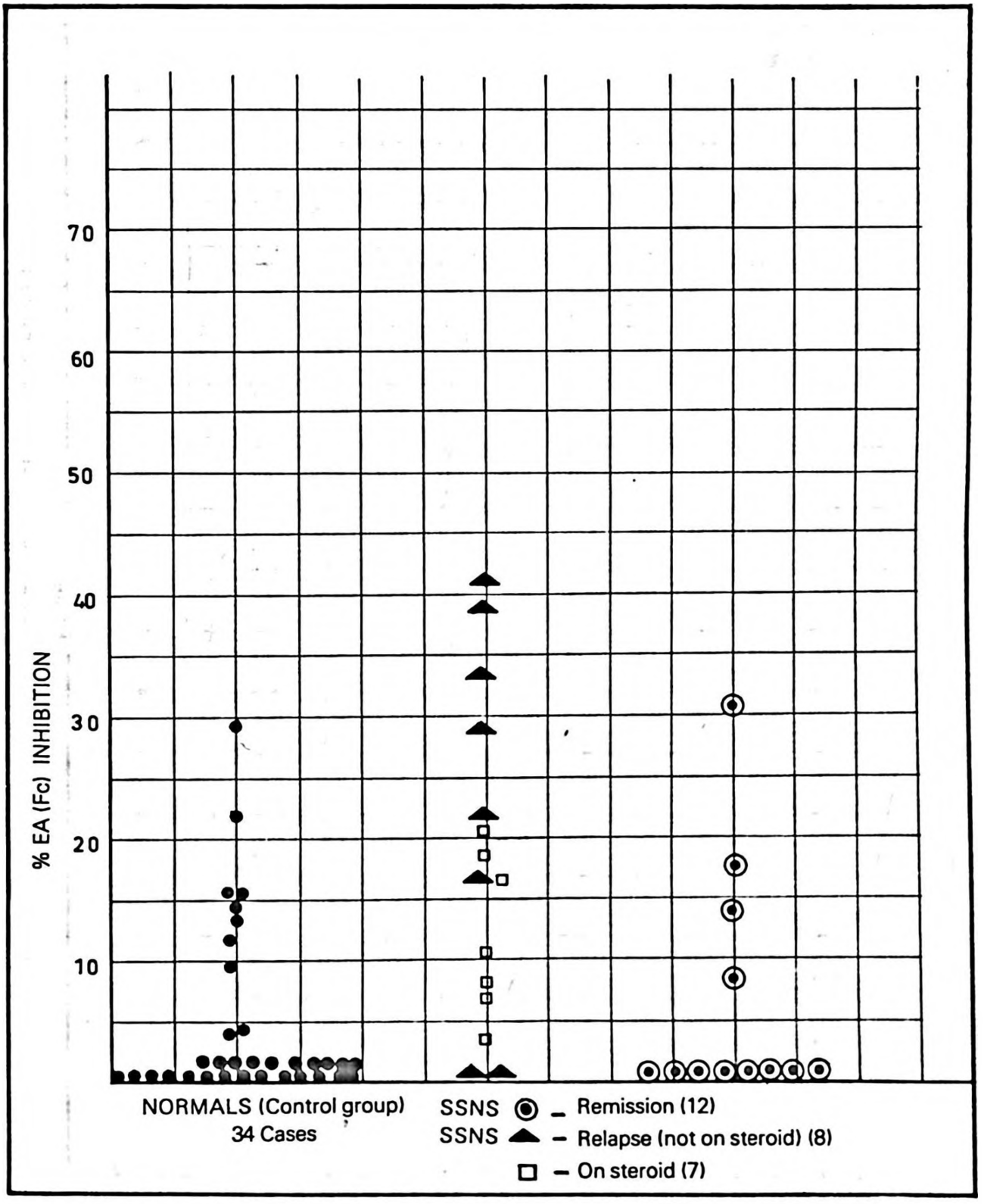

Figure 1

EA (Fc) Rosette inhibition values of SSNS

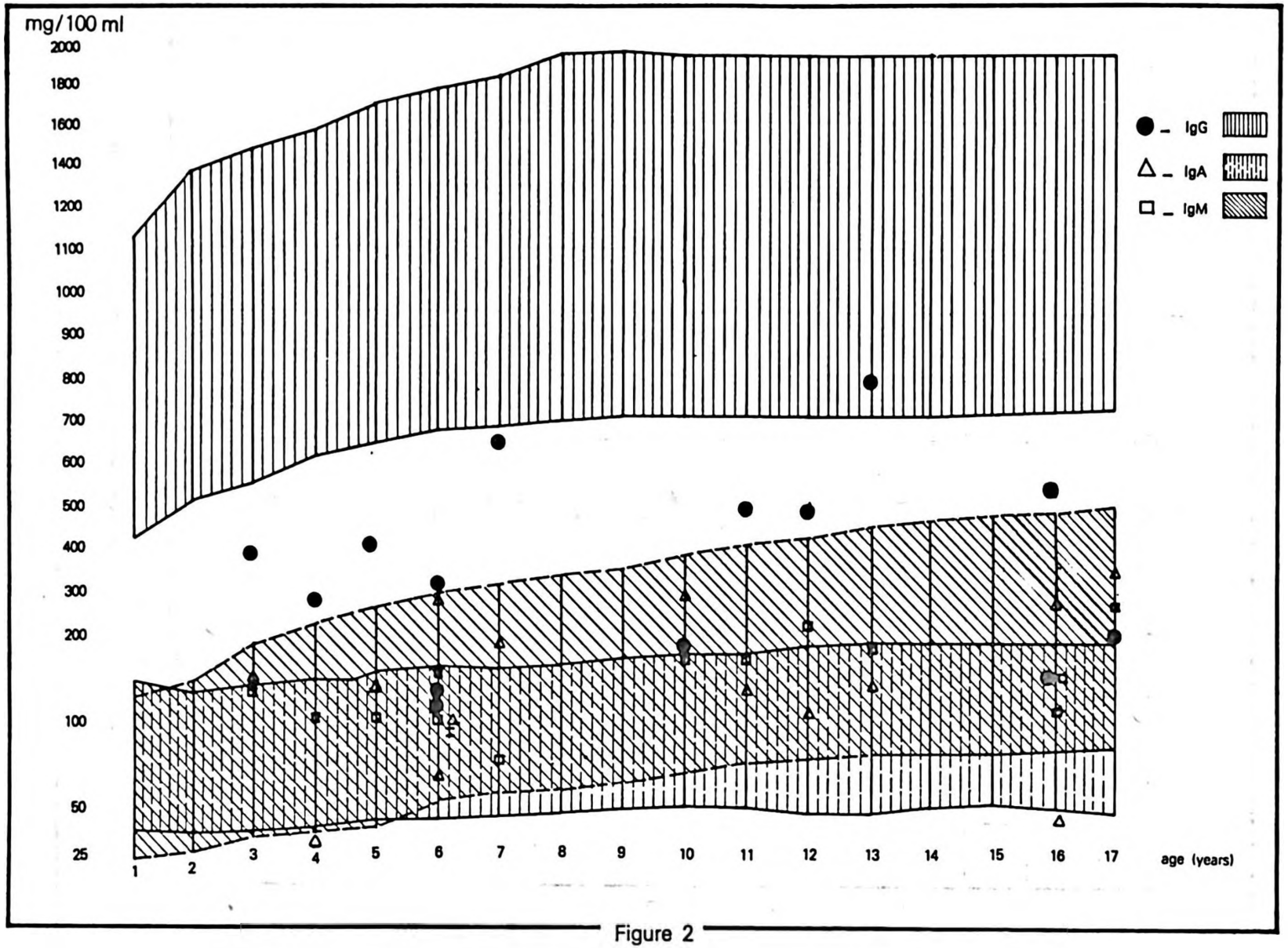

Figure 2

Distribution of immunoglobulins according to normal ranges in SSNS (relapse)

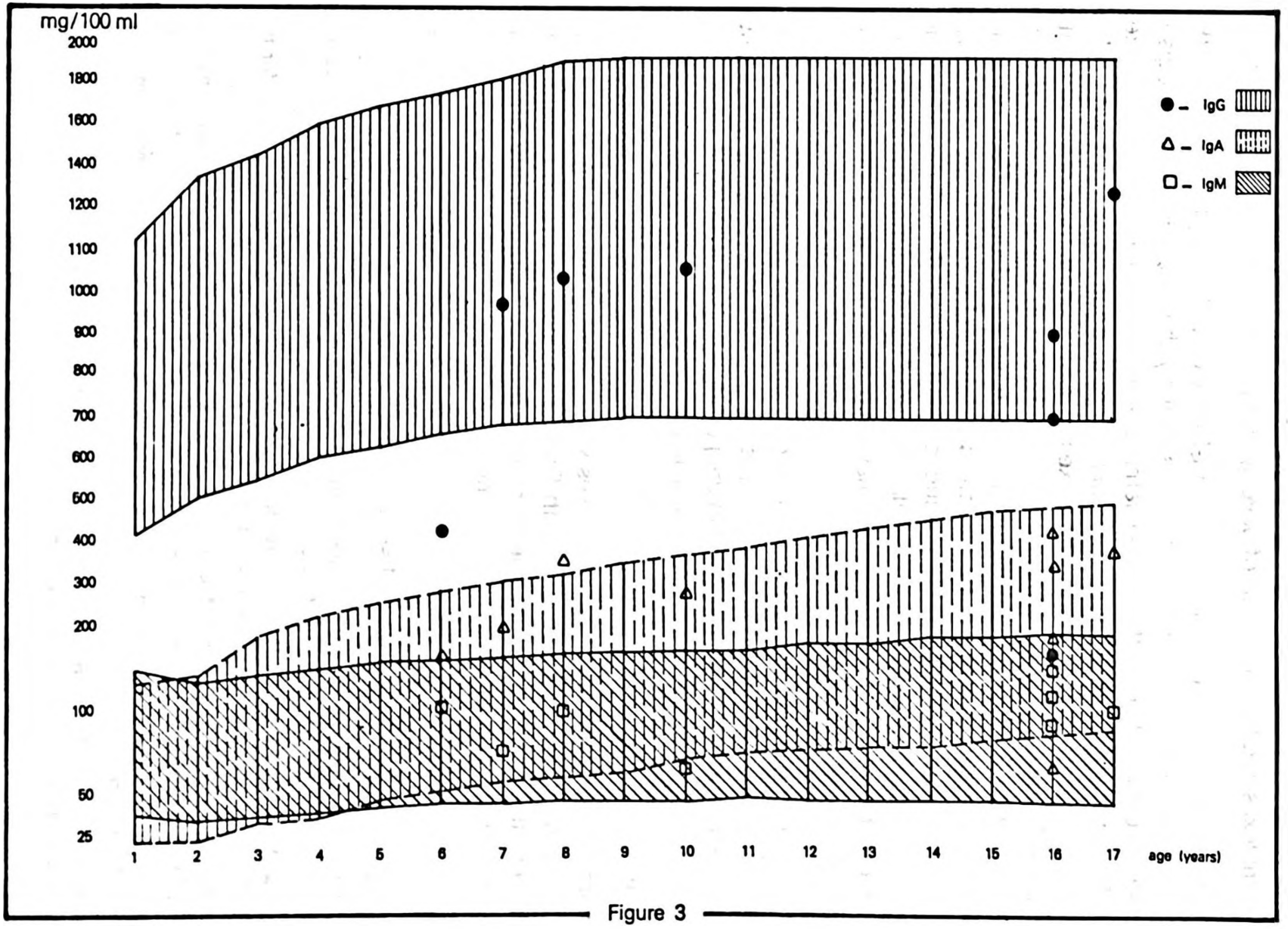

Figure 3

Distribution of immunoglobulins according to normal ranges in SSNS (remission)

ment-fixing) co-exist in glomerulonephritis or whether the various assays detect the same complexes at different stages of development.

Whether these circulating non-complement-fixing complexes play a role in the pathogenesis of SSNS is uncertain. It seems unlikely that they generate phlogistic molecules or contribute directly to renal damage since we have detected complexes in sera from patients with SSNS. Our findings are in good agreement with other reports[7,8,18] of detecting immune complexes in SSNS, and their reactivity with lymphocyte Fc receptors may play a pathogenetic role in this syndrome. If complexes react with Fc receptors in vivo, this may have an adverse effect on many immune functions. For example, Fc-receptor modulation by complexes may inhibit K cell function, influence antibody production and trigger the release of certain lymphokines including vascular permeability factor. Such immune aberrations may be indirectly harmful to the kidney and may help to explain the pathogenesis of SSNS[21-23].

Our immunoglobulin and complement findings were similar to the findings in the literature[24]. Finally, our immune complex results support the idea that the immunological mechanism may be important in the pathogenesis of SSNS[7,18].

Summary

Non-complement-fixing immune complexes were detected by the EA (Fc) rosette inhibition test in the sera of patients with SSNS. The sera of fifteen patients with steroid sensitive nephrotic syndrome in relapse produced significantly greater Fc inhibition than the sera of 12 patients in remission.

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