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Serum immunoglobulin concentrations before and after splenectomy in patients with homozygous β-thalassaemia

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SUMMARY Serum IgM, IgA, and IgG concentrations were measured in 19 patients suffering from blood-dependent homozygous β-thalassaemia. The study was prospective and the same patients served as presplenectomy controls. IgG levels rose rapidly and continuously after splenectomy. IgA levels also rose but not so sharply. IgM fell to low levels within one month after the operation and remained low for about 18 months. A similar pattern was found in the cross-sectional part of the study comprising 39 splenectomised and 51 non-splenectomised patients who were tested once.

Thalassaemia affects patients in many ways. Some of the side effects are related to the abnormal haemoglobin synthesis, others are due to the accumulation of iron in the tissues, and some result from the medical or surgical treatment. One of the serious problems in thalassaemia is the increased incidence of infections both before and after splenectomy. Splenectomy in childhood, irrespective of disease, increases the incidence of infections, especially meningitis and septicaemia (Erickson *et al.*, 1968; Desser and Ultmann, 1972). Splenectomy in thalassaemia increases the incidence and the severity of infections much more than in other diseases (Smith *et al.*, 1962; Eraklis *et al.*, 1967; Erickson *et al.*, 1968).

The indications for splenectomy in homozygous β-thalassaemia are the development of hypersplenism, low leucocyte and platelet counts and continuously increasing blood transfusion requirements, and the discomfort of a large spleen. The need for the operation arises earlier in the more severely affected patients. Serum γ-globulin concentrations may be affected by the removal of the spleen and thus may account, among other factors, for the lower resistance to infections (Caroline *et al.*, 1969; Wasi *et al.*, 1971).

The purpose of our study was to see how splenectomy affected serum immunoglobulin levels both immediately and over a period and what effect these had on the incidence of infections.

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Patients and methods

One hundred and nine patients with transfusion-dependent homozygous β-thalassaemia were separated into two groups.

Group 1 comprised 19 children (8 boys, 11 girls) who were studied prospectively. Their ages at splenectomy ranged from 5 years 8 months to 17 years. Nine of them were followed up for 48 months and the rest for 8 to 36 months. Serum IgG, IgA, and IgM concentrations were measured before splenectomy, one month after splenectomy, and thereafter at regular intervals of two months during the first postoperative year and four to six months thereafter. Clinical follow-up was for up to eight years.

Group 2 comprised 90 patients whose serum immunoglobulin concentrations were measured only once. Thirty-nine of them had had a splenectomy and 51 had not. Their ages at the time of examination ranged between 5 and 22 years. Splenectomy had been performed on the 39 patients one to 15 years previously.

Serum IgG, IgA, and IgM concentrations were measured by the radial immunodiffusion technique. The same commercial plates and standards (Hyland Laboratories) were used throughout the study. All

measurements were duplicated and read by two persons independently. The levels were compared with the normal values in the Greek population (Constantoulakis *et al.*, 1973a). The methodology was similar and most of the estimations were carried out during the same period of time in all groups. The statistical evaluation of the differences between any two means was by Student's *t* test for paired or unpaired observations, as appropriate. The long-term changes in serum immunoglobulin concentrations after splenectomy and the differences in the rates of change among the various subjects were evaluated statistically by regression methods and, more specifically, the analysis of variance technique for evaluating differences between regression slopes. Log transformation was done when necessary (Armitage, 1971; Constantoulakis *et al.*, 1973a).

Results

GROUP 1

Mean serum immunoglobulin levels before splenectomy and at intervals after the operation are shown in Table 1. The mean values were calculated in two ways. The first was from the available data on all 19 patients and the second only from data on the nine patients followed for the entire 48-month period. Since all but one of the patients were older than 8 years (the age by which the adult levels of the three immunoglobulins are reached in the Greek population) the adult mean levels and log-normal ranges of healthy Greeks were used as controls (Table 1). The long-term fluctuations of immunoglobulins after splenectomy are also shown in Fig. 1. The statistical analysis of these data showed the following.

(1) The serum IgG and IgM levels were higher in thalassaemic patients before splenectomy than in the healthy controls of similar age. The differences were statistically significant ($P < 0.01$ and $P < 0.05$, respectively). In contrast, the mean serum IgA level of the same patients did not differ significantly from that of the healthy controls.

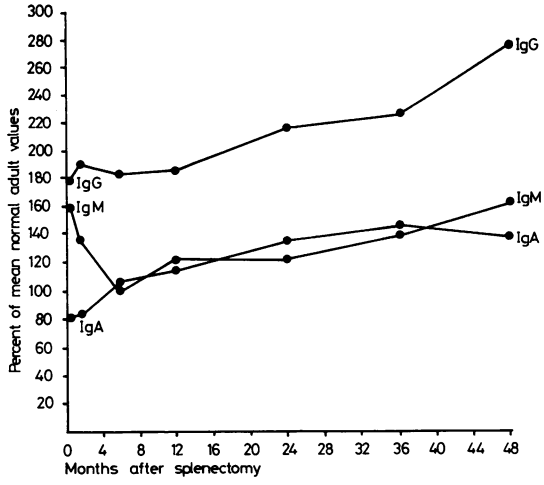

Fig. 1 *Mean serum IgG, IgA, and IgM levels in 19 patients with homozygous β-thalassaemia and their changes with time after splenectomy.*

(2) Splenectomy greatly affected the level of IgG. After the operation serum IgG levels rose in a more or less regular way at an average rate (common slope of the regression lines) of 23 mg/100 ml per month ($P < 0.001$). There was no significant difference in the rate of postsplenectomy rise among the various subjects studied.

(3) Serum IgA levels also rose after splenectomy but tended to level off towards the end of the third postoperative year. The average rate of rise was 3 mg/100 ml per month when only the nine patients with the 48-month follow-up were taken into account but was slightly smaller when all the available data were considered. In the analysis of variance the

Table 1 *Geometric mean serum IgG, IgA, and IgM levels (mg/100 ml) in patients with homozygous β-thalassaemia before and at specified intervals after splenectomy*

	Before splenectomy			*Interval (months) after splenectomy*																	
				1			*6*			*12*			*24*			*36*			*48*		
	IgG	*IgA*	*IgM*	*IgG*	*IgA*	*IgM*	*IgG*	*IgA*	*IgM*	*IgG*	*IgA*	*IgM*	*IgG*	*IgA*	*IgM*	*IgG*	*IgA*	*IgM*	*IgG*	*IgA*	*IgM*
19 patients	2055	261	168	2099	248	122	2241	318	109	2143	339	107	2538	386	109	2623	361	135	3213	348	158
Nine patients followed for 48 months	2065	208	157	2222	213	132	2104	264	99	2147	288	119	2508	340	119	2623	361	135	3213	348	158
96 healthy Greeks	1200	260	100																		
Range (log-) normal	600–2400	120–560	40–230																		

Table 2 *Geometric mean of serum IgG, IgA, and IgM levels (mg/100 ml) in 39 splenectomised and 51 non-splenectomised patients with homozygous β-thalassaemia*

	Age (years) at examination								
	5-9			*10-14*			*>15*		
	IgG	*IgA*	*IgM*	*IgG*	*IgA*	*IgM*	*IgG*	*IgA*	*IgM*
Splenectomised	1652	303	93	2554	393	123	2888	384	134
(No. of patients)		(13)			(14)			(12)	
Non-splenectomised	1462	202	124	1701	261	149	2117	252	149
(No. of patients)		(21)			(18)			(12)	
Healthy Greeks	1200	260	100						
(No. examined)		(96)							

increase in the residual sum of squares due to fitting a common slope rather than separate slopes for every subject was statistically significant ($0{\cdot}01 < P < 0{\cdot}05$), indicating that the postsplenectomy rate of rise in IgA differed among the subjects studied. Notably the most remarkable discrepancy concerned the patient whose age at splenectomy was 5 years 8 months. This patient had a fall rather than a rise in IgA.

(4) The effect of splenectomy on serum IgM levels was quite different. Within one month after the operation the levels were already significantly lower ($P < 0{\cdot}05$). The fall continued for about six months until a low of about 100 mg/100 ml was reached. IgM concentrations remained at this low level for about 18 months and then started to rise, almost reaching the splenectomy levels by the end of the fourth postsplenectomy year.

GROUP 2

Table 2 and Fig. 2 summarise the data on the serum immunoglobulin concentrations in the 90 patients with β-thalassaemia who were not followed up and whose immunoglobulins were measured only once. The data from this large group of patients confirmed several of the observations from the prospective study. Thus the mean serum IgG and IgM were higher in nonsplenectomised patients than in healthy controls, whereas the mean IgA did not differ significantly between the two groups. In addition, the effect of splenectomy on the concentrations of the three immunoglobulins was clearly shown. Since the age at splenectomy varied greatly among the patients, ranging from four to 18 years, the relationship between the time elapsed since the operation and the levels of immunoglobulins could not be studied directly. In the older splenectomised patients the interval between operation and immunoglobulin estimations was on the average longer than in the younger patients and therefore the differences in mean serum IgG and IgA concentrations would be expected to be greater among the older than among the younger patients. This expectation was supported by our data.

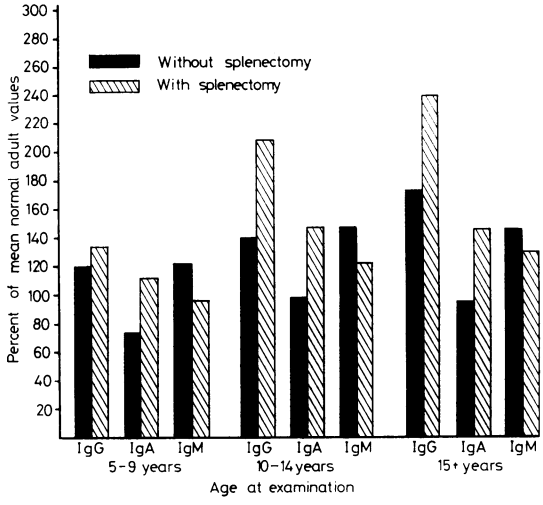

Fig. 2 *Mean serum IgG, IgA, and IgM levels in three age groups of 39 splenectomised and 51 non-splenectomised thalassaemic patients.*

Discussion

The results of the prospective part of this study show that splenectomy induces pronounced changes in the three major serum immunoglobulins. Immediately after the operation and continuously thereafter both IgG and IgA levels rose at a more or less steady rate, especially IgG. IgA did not follow this pattern in only one patient, who had been splenectomised at an early age before this immunoglobulin reaches adult levels. This finding might be significant if it is confirmed in more patients splenectomised under the age of five. IgM levels fell significantly ($P < 0{\cdot}05$) after splenectomy. They remained low for about 18 months and then gradually rose to

reach presplenectomy levels some four years after operation. These results are in general in accord with our preliminary findings (Constantoulakis and Avgoustaki, 1970).

Others have reported various changes in serum immunoglobulin levels after splenectomy in thalassaemic patients (Caroline *et al.*, 1969; Schumacher, 1970; Seitanidis *et al.*, 1971; Wasi *et al.*, 1971; Engelhard *et al.*, 1975). These studies, however, were cross-sectional, usually with small numbers of patients, and both the age of the patients and the interval between splenectomy and immunoglobulin estimations varied greatly. Most of the investigators agree that IgG levels in the splenectomised patients were significantly raised but that the findings for IgA and IgM were conflicting.

We found a similar pattern of immunoglobulin changes in the cross-sectional part of our study. Serum IgA levels in non-splenectomised patients continued to rise until the early teens and IgG levels even beyond that age, whereas among healthy Greeks adult levels were reached by the age of 4 for IgG and 8 for IgA.

It is not known why all thalassaemic patients have high IgG levels. Possibly liver damage and infections play an important role. Also the continuous antigenic stimulus of blood transfusions, with the production of a variety of antibodies, should be considered (Economidou *et al.*, 1971). Neither is it known why after splenectomy IgA and IgG levels rise and IgM levels fall compared with presplenectomy levels and with non-splenectomised patients nor whether this is related to the increased incidence of infections (Constantoulakis *et al.*, 1973b).

The incidence of infections and the causes of death in the 19 patients followed up for up to eight years after splenectomy are shown in Table 3. Six of them had multiple infections, thus the incidence was higher than in the normal population of a similar age. One patient (case 18), and possibly a second (case 13), died of infection 30 and nine months after operation, respectively. This is seldom the case in non-splenectomised patients. The patient who died of septicaemia (case 18) had persistently very low IgM levels after splenectomy and had discontinued the prophylactic penicillin which is given to all splenectomised chidren for many years.

In conclusion, splenectomy affects serum immunoglobulin levels. It is also responsible for an occasional death from a fulminant infection. But splenectomy usually reduces the blood transfusion requirements so greatly that it is advisable when there is hypersplenism.

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Table 3 *Incidence and type of infection and cause of death in the 19 patients of the prospective study*

Case no.	*Age (years months)*	*Sex*	*Age at splenectomy*	*Infections after splenectomy*	*Outcome and cause of death*
1	14	M	8	Three infections in first four years. Tb of liver at operation	Alive
2	13·8	M	5·8	Repeated tonsillitis and bronchitis	Alive
3	15	F	10	None	Alive
4	16·5	M	10	Four infections in six years	Alive
5	16	F	9·5	Repeated tonsillitis, bronchopneumonia thrice	Alive
6	16	F	8	None	Alive
7	18·5	F	11·5	Pericarditis once (1973)	Alive
8	18·5	M	10·5	None	Alive
9	19	F	11	None	Alive
10	20	F	12	None	Alive
11	21	M	15·7	Several infections, recurrent brucellosis, pericarditis	Alive
12	21·5	M	15·5	None	Alive
13	10·8	F	10	Severe gastroenteritis	Died. Bronchopneumonia, cardiac failure
14	13·5	F	9·5	None	Died. Fever unknown aetiology, herpes zoster, cardiac failure
15	14	F	9·2	None	Alive
16	16	M	11	None	Died. Cardiac failure, hepatic failure
17	16	F	10	Cystitis, bronchitis, recurrent brucellosis	Died. Cardiac failure, pulmonary oedema
18	19·5	F	17	None	Died. Septicaemia
19	23·5	M	17	None	Died. Cardiac failure, hepatic failure

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