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Biochemical Findings in Multiple Sclerosis

II. A Detailed Study of the Serum IgA, IgG and IgM Levels of 772 MS Patients Compared with 226 Neurological Controls

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Summary. In the second paper of this series, a detailed study of the three main serum immunoglobulins for a group of 772 MS patients is compared with a group of 226 neurological controls. The results are studied according to sex and different age groups. Except for a slight elevation of the IgM levels in the MS group, especially between 40 and 60 years, no clear cut distinction between the two groups of patients was found.

Key words: Multiple sclerosis — Serum immunoglobulins.

Zusammenfassung. Bei einer Gruppe von 772 Patienten mit multipler Sklerose und einer Kontrollgruppe von 226 Patienten mit anderen neurologischen Krankheiten wurden die drei hauptsächlichen Serumimmunglobuline verglichen. Die Ergebnisse wurden nach Alter und Geschlecht ausgewertet. Außer einer leichten Erhöhung des IgM bei der MS-Gruppe, hauptsächlich im Alter zwischen 40 und 60 Jahren, konnten keine deutlichen Unterschiede zur Kontrollgruppe gefunden werden.

Introduction

As already outlined in the first paper of this series (Delmotte, 1974), it is our aim to present large scale surveys of biochemical data in Multiple Sclerosis.

Over the past few years evidence has been accumulated which indicates that an immunological process plays at least some role in this dreadful disease (Adams, 1974; Rose, 1974; Schuller, 1974). Comparison with experimental allergic encephalomyelitis, the standard model of an autoimmune neurological disorder, as well as findings in Multiple Sclerosis itself, point in this direction. Although most of the immunological process seems to be confined to the central nervous system, the possibility that some disturbance of the immunoglobulin components of the blood might be present, at least at some stage of the evolution of the disease, could not *a priori* be ruled out. In this context, we examined in detail the IgA, IgG and IgM levels of a large group of MS patients and compared the results with a group of neurological controls.

Materials and Methods

Patients

Only patients for wich a definite diagnosis had been established, either MS or another neurological disorder, were retained for the present study. As criteria for the diagnosis of Multiple Sclerosis, the following conditions were taken into account:

age of onset of the disease,
typical evolutionary pattern,
dispersion of neurological signs,
results of C.S.F. analysis.

Patients were taking none or minimal dosage of only symptomatic drugs. As all of the blood samples were taken during the first few days after hospital admission, which was mostly decided because of the appearance of new neurological signs, we can admit that the vast majority of the patients were in an evolutionary state of the disease. All blood samples were taken in the morning after an overnight fast. Serum samples were either analysed immediately or kept frozen at —20°C for not more than 10 days.

Methods

Paper Electrophoresis. Whatman No. 1 paper and veronal buffer pH 8.4 and ionic strength 0.05 were used. After bromophenol staining of the heat denatured proteins, fractions were cut apart and the optimal density of the eluted bromophenol solutions were measured in a filter colorimeter.

Total Protein. The classical biuret method with Lab-trol as standard was used.

Haptoglobin. The enzymatic method, using ethylperoxyde as substrate and gaiacol as a color developer, adapted to the Autoanalyser was used.

Immunoglobulins. All determinations of the immunoglobulins were done with the radial immunodiffusion method of Mancini (1965). Immunoplates of the Behring-Werke, Germany, were used throughout. Serum samples were measured with a 10 µl syringe. Diffusion ring diameters were measured after equilibrium was reached 48 hrs for IgA and IgG and 72 hrs for IgM.

Normal Values

In the course of the last 10 years, a more clear cut definition of the normal levels of the serum IgA and IgG and IgM has followed in the wake of the development of more precise analytical methods. However, a general agreement on the exact limits is still lacking and many parameters seem to influence these limits (Buckley, 1967). Combining figures cited by different authors (LoGrippo, 1967; Störiko, 1968; Kolar, 1972; and others) with figures cited by the makers of the immunoplates (Behring-Werke), we have adopted the following figures as the normal limits for the serum concentrations of the immunoglobulins:

IgA: 110— 400 mg%,
IgG: 800—1800 mg%,
IgM: 80— 280 mg%.

Results

Multiple Sclerosis patients especially, as well as most of those suffering from other chronic neurological ailments, are periodically subjected to a whole series of complications. These secondary ailments, as well as the fact that the patients are practically unable to live a normal life, either in or out of the hospital, have their repercussion on their general state of health. For this reason it is wise to study only very large groups of patients, either MS or neurological controls. Table 2 presents a review of some biochemical data, giving an idea of the general state of health of the patients, and making it possible to judge the matching of the two groups. The serum haptoglobin levels have been included because, in our experience, it is one of the most sensitive parameters to judge the infectious state of the patient. The figures given are the mean and standard deviation.

In Table 3 the general results of the determinations of the IgA, IgG and IgM concentrations in the serum of these patients are presented.

Table 1. Distribution of neurological diseases other than MS

Diagnostic categories other than MS	Number of patients
Inflammatory diseases of the CNS	
Encephalomyelitis-meningitis	2
Poliomyelitis	2
Degenerative diseases of the CNS	
Amyotrophic lateral sclerosis	28
Syringomyelia	17
Myelopathy	53
Heredodegenerative diseases	21
Cerebral degeneration	20
Parkinson's disease	1
Polyneuropathy	7
Tumors	7
Hydrocephalus	2
Occipitocervical malformations	23
Vascular disorders	
Acute accidents	1
Chronic diseases	20
Miscellaneous disorders of the CNS	22
Total number of patients	226

Table 2. Survey of general results

	MS patients $n = 772$		Neurol. controls $n = 226$	
	men $n = 343$	women $n = 429$	men $n = 112$	women $n = 114$
Age (years)	45.5 (± 10)	47.1 (± 10.2)	49.7 (± 13.6)	50.8 (± 13.4)
Tot. prot. (g%)	6.4 (± 0.47)	6.4 (± 0.5)	6.43 (± 0.55)	6.4 (± 0.52)
Albumine (g%)	4.07 (± 0.45)	3.93 (± 0.49)	4.06 (± 0.51)	3.92 (± 0.62)
Gamma glob. (g%)	0.79 (± 0.21)	0.85 (± 0.22)	0.81 (± 0.21)	0.84 (± 0.22)
Alb./Glob. ratio	1.8 (± 0.39)	1.67 (± 0.38)	1.78 (± 0.37)	1.65 (± 0.43)
Haptoglobulin (mg%)	175 (± 90)	153 (± 87)	179 (± 80)	162 (± 95)

Table 3. Results of immunoglobulin determinations

	772 MS patients		226 neurol. controls	
	men $n = 343$	women $n = 429$	men $n = 112$	women $n = 114$
IgA (mg%)	230 (± 97)	218 (± 94)	231 (± 109)	227 (± 90)
IgG (mg%)	997 (± 296)	1030 (± 317)	984 (± 278)	1012 (± 325)
IgM (mg%)	157 (± 82)	202 (± 94)	143 (± 86)	172 (± 81)

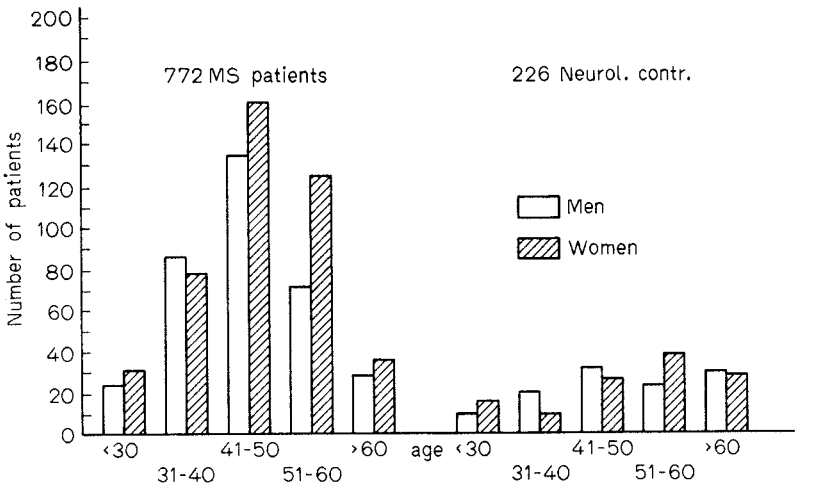

Fig. 1. Age and sex distribution of patients

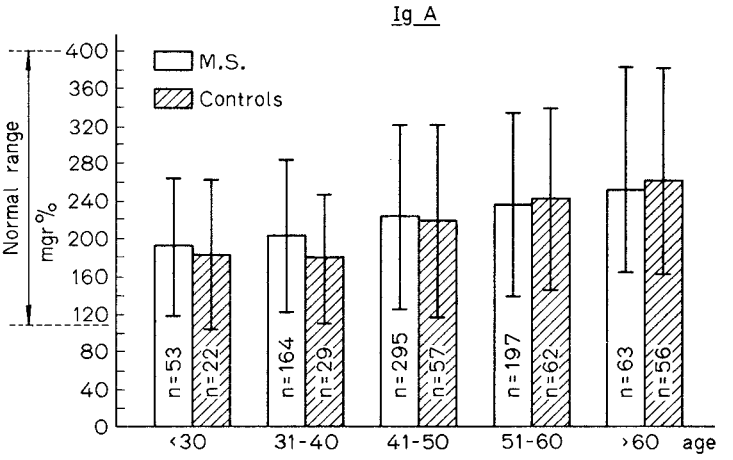

Fig. 2. Serum immunoglobulin A

Statistical significance of difference in mean values between men and women were calculated. Only for the IgM content, both for the MS and the neurological controls, was a significant male/female difference in mean detected ($P = 0.01$). As Multiple Sclerosis is a typically age bound disease, we calculated the age distribution for the three serum immunoglobulin concentrations. The large number of patients studied still permitted statistical calculations in each group. In Figs. 2—4 the results of these calculations are presented. Vertical bars represent the mean for each age group and vertical lines represent the $\pm$ 1 standard deviation. No male/female distinction was made for these calculations. Neither for the IgA nor for the IgG concentrations was there any statistically significant difference in mean between the MS and the neurological controls of the same age group. Concerning the IgM concentration, in all the age groups the MS patients had

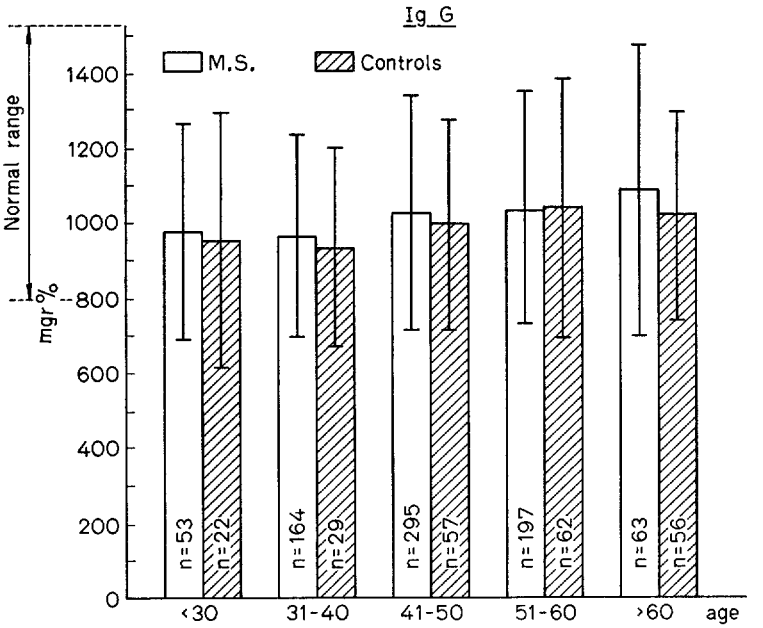

Fig. 3. Serum immunoglobulin G

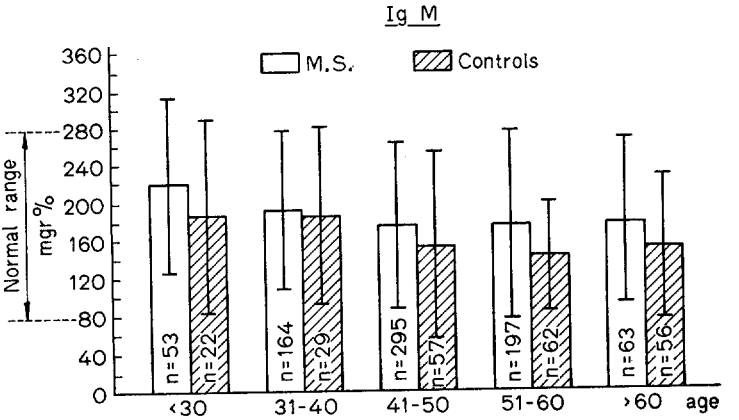

Fig. 4. Serum immunoglobulin M

higher levels of these immunoglobulins than the corresponding group of neurological controls. This difference was most marked for the age groups of 40 to 50 and 50 to 60 years, being significant at the 0.01% level.

A very clear cut correlation could be shown between the age of the patients and the levels of the three serum immunoglobulins. The concentrations rise with age for the IgA and IgG and go down for the IgM. As can be seen from Table 4 giving the correlation coefficients, there is practically no difference between the MS patients and the neurological controls.

Table 4. Correlation of immunoglobulin concentrations with age

	Correlation with age	
	MS patient	neurol. controls
IgA	0.99	0.96
IgG	0.91	0.84
IgM	0.85	0.84

Table 5. Immunoglobulin concentrations before and after treatment

	Before treatment $n = 75$	After treatment $n = 75$
IgA (mg%)	197 (± 91)	211 (± 98)
IgG (mg%)	941 (± 345)	962 (± 269)
IgM (mg%)	177 (± 89)	178 (± 85)

During the course of the present study, 75 subjects, all of them well defined MS patients, underwent treatment with the cytostatic drug cyclophosphamide (Endoxan). Most of them received a total of from 2 to 8 g over a period of 2 to 8 weeks. In every case very striking reduction of the blood lymphocyte count was observed. Serum immunoglobulin levels of these patients were measured immediately before and also a few days after the end of the treatment period. Table 5 presents the results obtained for this group of patients.

Discussion

The serum protein profile in MS has been extensively studied by many workers over the last few decades. The normally used electrophoretic techniques failed to demonstrate any significant difference between MS patients and control subjects (Schüller, 1963; Bergmann, 1964). The discovery of the gamma globulin abnormalities in the CSF of a large percentage of MS patients, when studied by agar electrophoresis (Lowenthal, 1960), attracted new attention to the serum immunoglobulin components. The development of more precise and specific methods to measure the individual classes of immunoglobulin has led to extensive study in this field. Yeda *et al.* (1969) could not detect any significant difference for IgA or IgG between normal controls and a group of MS patients. They found slightly lower levels for serum IgM in the MS group. Kolar *et al.* (1970) reported that about one third of their MS patients did show slight abnormalities in one or more of the serum immunoglobulins. Davis *et al.* (1972) in a study comparing MS patients with and without cytostatic treatment, reported no significant abnormalities. A larger group of MS patients (323) was investigated by Cazzullo *et al.* (1973). They split up their patient material according to several parameters: clinical state, age, duration of disease, disability and CSF immunoglobulin content. Although some abnormalities were reported in their study, no clear cut serum immunoglobulin distribution pattern could be distinguished. No mention of the

Table 6. Percentage of abnormal immunoglobulin levels

	Kolar (1972)	This study	
	87 MS patients	772 MS	226 controls
IgA above 390 mg%	15%	3.75%	5.7%
IgG above 1800 mg%	12.5%	1.42%	1.32%
IgM above 160 mg%	10%	53%	36%

general state of health of the patients was made in this report. As already pointed out in the introduction, there is additional evidence that an immunological mechanism might play some role in the pathogenesis of MS. In this light, we studied extensively the serum levels of the 3 main immunoglobulin classes in a large group of MS patients and compared them to a group of neurological controls. As the result of this detailed study clearly demonstrates, there is no significant disturbance of the serum immunoglobulin content in MS, except for a slight elevation of the IgM concentration. As shown in Figs. 2—4, even the comparison of the different age groups failed to show a clear cut difference between the two groups of patients. Kolar (1972) included a group of 87 confirmed MS patients in his study and calculated the number of patients in whom the immunoglobulin levels reached above an upper limit. In some circumstances, this method can give more information than the calculation of mean concentrations. Table 6 compares our results with those of Kolar, calculated in the same way.

It is evident from these figures that, concerning the IgA and IgG concentrations, a significant smaller percentage of our patients, both MS and controls, have levels above these limits. For the IgM levels on the contrary, accepting the limits used by Kolar, a significantly higher percentage of patients, especially in the MS group, do show results above this limit. It must be pointed out, however, that the upper limit of 160 mg% for the IgM concentration in serum is now considered too low by most workers. Concerning the group of MS patients who were treated with a cytostatic drug (Endoxan), it is evident from the figures in Table 5 that none of the concentrations of the three main immunoglobulin classes was influenced by the treatment described. A study of the clinical observations concerning the group of patients treated will be published in another paper.

In conclusion of this study of the serum immunoglobulins, it can be stated that no significant difference could be detected between a group of MS patients and a group of neurological controls, nor could any effect of short term cytostatic treatment on the serum immunoglobulins content be shown in a group of 75 MS patients.

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