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The Immunoglobulin and Complement Levels in the Active Pulmonary Sarcoidosis

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Background and aim: In sarcoidosis patients the circulating immune complex levels are raised with the activation of the disease but their diagnostic role is not clear. These circulating immune complexes contain complement and immunoglobulins (Ig). Complement and immunoglobulins are mostly accumulated in active sarcoid lesions and in circulating immune complexes. Thus complement and Ig levels in circulation will be reduced in active sarcoidosis patients due to their higher deposition in sarcoid lesions. We tried to make an estimation about the activity of the disease through measuring the IgG, IgA, and IgM and complement (C3-C4) levels in the serum.

Methods: Thirty-two (8 men) sarcoidosis patients (with their symptoms, chest x-ray graphies, bronchioalveolar lavage findings and the other related WASOG criteria) were divided into two groups as active and inactive cases. We measured with the immunodiffusion method the immunoglobulin and complement parameters of these two groups and compared them with each other.

Results: Compared to the active group the IgG and IgA in serum were significantly higher in the inactive group ($p<0.001$, $p=0.07$). Although statistically not significant the IgM and C3, C4 levels were higher too in the inactive group.

Conclusions: The IgG, IgA, IgM and the complement levels in serum are higher in the inactive group. We think that these findings might be useful for the follow up of the disorder's activity in sarcoidosis patients. In order to prove these finding studies with larger volume are needed.

Sarcoidosis is a granulomatous disease of unknown etiology, characterized by multisystem involvement. Although in most cases it remains limited, there are some rare ones with expanded and/or fatal presentations. It is reported that sudden cardiac death was the terminal event in 67 per cent of sarcoid heart disease deaths; the occurrence of sudden cardiac death has been related to the extent of cardiac involvement (1). Thus the determination of activity of the disease and follow up has a very important role in the management of the disease. Chest x-ray, pulmonary function tests, bronchioalveolar lavage findings and clinical symptoms are used to establish the activity of the disease. An increase in the cellular response, especially of the T- helper lymphocytes and macrophages is the first pathological sign of the activation process. This fact shows that T lymphocytes have a major role in the activation but the etiology is still unknown (2,3,4).

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The expression of sarcoidosis activation is not completely defined. According to the 1999 Consensus of the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) it is concluded that the activation should primarily be determined by the following parameters:

1) Clinical findings (symptomatology and examination)
   –Fever –Uveitis –Erythema nodosum –Lupus pernio –Changing scar –Polyarthralgia –Splenomegaly –Lymphadenopathy –Salivary and lacrimal gland enlargement –Myocardial diseases –Neurological symptoms –Progressive dyspnea and Cough
2) Progressive changes in chest x-rays –Ground glass attenuation on HRCT
3) Worsening lung function
4) Positive Gallium –67-uptake
5) High cell count and CD4 /CD8 ratio in bronchio-alveolar lavage (BAL)
6) Serum angiotensin converting enzyme (ACE), abnormal liver function tests and other biochemical parameters (lysosyme, cytokinin, lymphocytes derivates)
7) Abnormal ECG (5).

T cells may be responsible for increased immunoglobulin response through causing B cell growth factor mediation. In active sarcoidosis the humoral immunity is hyperactive and the increase of immunoglobulin is polyclonal. The IgG level is increased much more than the IgM and the IgA and this can be confirmed in the BAL (3,4). With the activation of the disease, circulating immune complexes are elevated but their diagnostic value is not clear. The circulating complexes consist of the complements and the immunoglobulins A, G and M although it is not specific for sarcoidosis, the determination of immunoglobulins and complements in the sarcoid granulomata points the role of the humoral mechanism on pathogenesis and granuloma formation (6,7,8,9). The data from Baumer et al. provide evidence that in pulmonary sarcoidosis, at the site of granuloma formation, an accumulation of Th1 cells as well as of intermediate (between Th1 and Th0) cell types occurs, whereas in the alveolar lumen, high numbers of Th1 and Th2 cells with a simultaneous decrease of Th0 cells can be observed (10).

In active sarcoidosis it is shown that the active T lymphocytes are more than the 10-40 % range and that the helper T to suppressor T lymphocyte ratio (CD4/CD8) has been elevated (2). The alveolar macrophages release substantial mediators, which take part in the pathogenesis of the disease and play an important role in the inflammatory response. Alveolitis in pulmonary sarcoidosis is caused by the progressive accumulation of macrophages and CD4+ lymphocytes. Once the macrophages get activated, they are mixed histocompatibility antigens on the surface release interleukin1, which is a T cell activator (3,11,12,13). Moreover lysozyme, beta-2-microglobulin, neopterin and soluble IL-2 can be used in detecting the activation of the disease. Although these tests show immunological activity of the disease their clinical use is not verified. The BAL findings may be helpful for detecting the activation but their value in clinical follow up is still not proven. Finally no measurement or parameter is found to be good enough to make a clinical decision (3).

Biopsies from active sarcoid lesions have shown some accumulations of immunoglobulins and the complement C3, which make physicians to investigate if sarcoidosis is a result of immunobiological reactions (14,15).

Regarding the accumulation of the complements and the immunoglobulins in active sarcoid lesions and in circulating immune complexes we suppose that one can find out lower blood levels of these components whereas higher in inactive or chronic cases since they accumulate less in lesions of that kind of patients. In order to determine whether the disease is in an acute or chronic phase, we measured IgG, IgA, IgM and C3, C4 levels in serum.

MATERIAL AND METHODS

We studied 32 patients with pulmonary sarcoidosis. All of them underwent a chest x-ray, a thorax CT, a transbronchial biopsy and if necessary a mediastinoscopic biopsy. Cases with lymph nodes got a lymph node biopsy and cases with skin lesions got their lesions biopsied. BAL examination was performed for every patient and his or her lymphocytic subgroups were analyzed with a flowcytometry study through using an immunofluorescence monoclonal antibody test. Additionally all patients got imprint- and BAL-cytological examinations.

We separated 16 cases as active and 16 as inactive (Table 1, Table 2). The distinction between active and inactive cases were accomplished through WASOG criteria. All of the active cases were without any therapy at all and revealed at least one of the activity criteria. Six of them had erythema nodosum, four liver involvement evident with biopsy, two orbital findings, two skin lesions on face and hands and two had lymphadenopathy of neck and axilla. Additionally we found higher CD4/CD8 ratio in nine. Our inactive group comprised routinely checked patients without any activity criteria. Patients with recurrences were excluded. After placing 5 ml of serum on each cavity on plates IgG, A and M and C3 and 4 levels of both groups were quantitatively studied with immunodiffusion plates (callestad). Serum samples were incubated on plates for 72 hours at room temperature. At the end of this period the diameter of precipitation was measured and converted to mg/dl units.

<Normal values of the plates used are as follows:>

IgG: (1009-1388mg/dl), IgA: (150-203 mg/dl), IgM: (102-131 mg/dl),

C3: (115-150 mg/dl), C4: (27.70-35.70 mg/dl)

On the assumption that the IgG has a normal distribution in the active group as 1427.62 ± 311.32 and in the inactive group 2094.06 ± 359.25 the optimum meeting point X @ is found to be approximately as 1730 as a cut–off point. This division is found to be in conformity with the activity criteria (Kappa =0.687 Z=3.89 $p<0.001$ sensitivity =0.81, specificity =0.875). As the average values of both groups were within a standard deviation of ± 1, it was questionable to determine a cut-off value for other parameters.

The chi^2, the student's t and the Mann-Whitney U tests assessed comparisons between groups.

Table I. Active Sarcoidosis Group

Age Sex	X Ray	E.N	OF	Skin Les.	Lam	Liver Invol.	CD4 /CD8	IgG	IgM	IgA	C3	C4
18 M	1		+			+	24.65	1496	310	147	147	21.8
39 F	2	+					4.2	1319	87	212	163	58
31 F	1	+					3.95	1273	288	344	204	44
37 M	3					+	4.2	813	118	186	111	26
53 F	3	+					2.85	1402	134	104	221	42.7
46 M	1			+			4.5	1265	87	430	133	28
55 F	3						10	1592	225	313	244	30.4
42 F	1	+					1.92	1265	288	537	157	30.4
41 F	1			+		+	33	1874	110	465	227	37.6
46 F	1	+						1715	187	275	244	36.3
60 F	3		+				0.58	1820	169	386	210	37.4
60 F	1				+		1.41	1792	80	375	142	26
42 F	1	+					2.26	1310	216	419	244	32.6
55 F	1					+	18.4	909	58	165	76	22
38 F	3			+			1.49	1282	205	320	119	37
34 F	1				+		5.2	1715	178	323	172	366

Table II. Inactive Sarcoidosis Group

Age Sex	X Ray	E.N	OF	Skin Les.	Lam	Liver Invol.	CD4 /CD8	IgG	IgM	IgA	C3	C4
36 M	3	-	-	-	-	-	1.14	2336	196	537	256	42.7
49 F	3	-	-	-	-	-	2.91	2039	803	323	227	35.1
34 F	1	-	-	-	-	-	0.27	2039	256	442	210	31.6
44 F	2	-	-	-	-	-	2.3	2387	235	489	244	35.1
38 F	2	-	-	-	-	-	0.64	2500	246	501	204	19.8
53 F	3	-	-	-	-	-	1.23	2111	299	537	281	33.9
31 M	1	-	-	-	-	-	0.84	2002	256	256	133	20.8
55 F	3	-	-	-	-	-	1.92	2210	235	385	167	24.9
48 F	3	-	-	-	-	-	2.8	1851	129	487	113	46
25 F	3	-	-	-	-	-	1.62	2036	169	342	80	24
47 M	1	-	-	-	-	-	1.49	2363	329	285	90	34
30 M	3	-	-	-	-	-	2.14	2111	368	323	281	45.3
54 F	1	-	-	-	-	-	0.64	2116	143	408	177	41.4
36 F	1	-	-	-	-	-	1.23	1092	134	256	221	42.7
27 M	1	-	-	-	-	-	2.37	1681	220	80	210	42.7
24 F	1	-	-	-	-	-	2.91	2631	430	288	210	35.1

X-RAY: 1: (Bilateral LAM) 2: (Mottling) 3: (1+2)
EN: Erythema nodosum
OF: Orbital findings
LAM: Lymphadenomegalie

RESULTS

We examined 32 sarcoidosis (8 male) patients aged 18 to 60 years (mean age is 43.56 ±11.29 in the first group and 39.43±10.63 in the second group).

Patient characteristics and test results are outlined in the Table III.

The comparison of age, gender and chest x-ray between the active and the inactive group remained statistically not significant. According to the bilateral hilar adenopathy on chest x-ray of both groups there was no difference in distribution ($P>0.05$).

Although the total cell count and their subgroup analysis in the BAL were not significant, the CD4/CD8 lymphocyte ratio of both groups pointed out a statistical significance ($p<0,01$; the Mann-Whitney U test).

IgG and IgA were significantly higher in the inactive group compared to the active group ($p<0.001$, $p=0.07$). Although not significant there were again some difference in the IgM, and C3, C4 levels. The results were shown in the table III.

Table III. Patient's test results

	ACTIVE GROUP n=16	INACTIVE GROUP n=16	p value
Mean age	43.56 ± 11.29	39.43 ± 10.63	p>0.05
Male	3	5	p>0.05
Chest x-ray			
I.BHL	BHL in 10 cases	BHL in 7 cases	p>0.05
II. Mottling	BHL+mottling in 5 Mottling in 1	BHL+mottling in 7 Mottling in 2	
BAL Findings			
Total Cell Count	1704.6 ± 1479.23	654 ± 525.05	p>0.05
% Macrophages	37.76 ± 18.66	36.92 ± 21.67	p>0.05
% Lymphocytes	39.88 ± 12.62	35 ± 14.76	p>0.05
% Granulocytes	22.36 ± 13.74	28.04 ± 22.31	p>0.05
CD4/CD8	7.90 ± 9.69	1.65 ± 0.85	p<0.01*
IgG	1427.62 ± 311.32	2094.06 ± 359.25	p<0.001*
IgA	312.56 ± 123.66	388.81 ± 105.35	p=0.07
IgM	171.25 ± 80.06	215.18 ± 86.40	p>0.05
C3	182.12 ± 57.25	194 ± 62.86	p>0.05
C4	33.60 ± 9.27	34.69 ± 8.61	p>0.05

BHL: bilateral hilar lymphadenopathy

*: Statistically significant

DISCUSSION

Since the etiology of sarcoidosis is still being unknown it has been an attraction for many researchers. Infiltration of lungs, reticuloendotelial system and skin usually dominates the clinical picture. Primary cardiac involvement is not often recognized clinically, although it may be demonstrated at autopsy in 20 to 30 per cent of cases, most of which demonstrate generalized sarcoidosis (16). Negative skin tuberculin test and abnormalities in immune system seen in most of sarcoidosis patients may indicate the effect of immune system on pathogenesis. Determining the Ig G-A-M and the complement fragments in granuloma may prove the presence of immunological reactions, however it is proclaimed that this may not be an evidence for an immunological response (17) because granuloma development occurs in two ways:

First one: etiological agent initiates the formation of granuloma by influencing the immune system directly which has been seen in mycobacteric diseases, mycosis, lepra, and berylliosis.

Second one: the granuloma is developed by nonimmunological inflammatory mechanism but the immune system is affected (17).

The role of serum Ig increase in the pathogenesis of sarcoidosis is not clear. The first possibility is that during the formation of granuloma it is secondary to the presence of active T lymphocytes and the other possibility is that it helps to form some granuloma by joining the formation of immune complexes (18).

In our study in active and inactive cases of sarcoidosis we found higher than normal levels of IgG, IgA, IgM, C3 and C4. Beside that the higher IgG levels compared to the Ig A and Ig M levels were compatible with the literature (3). Some of the patients in the active group had to take steroidal therapy. Since effects of steroidal therapy on Ig levels were out of scope of our study, the changes of Ig levels after therapy were not determined. Furthermore

some of our inactive cases were chronic ones under steroid therapy. In the literature it has been documented that polyclonal hypergammaglobulinemia is a rare finding within patients under therapy (19). Again in the same reference it is said that in patients with pulmonary involvement because of the ability to remain longer in the serum, the IgG-levels might be increased, which is a sign of chronic nature of the disease. They added that the serum IgG-level stays in normal range in active cases with erythema nodosum. Similarly in such cases in our study the IgG-level was lower than our other active ones.

In the early phase of the disease the immune complexes in blood circulation are high. Although not necessary for an explanation of the pathogenesis it may be a sign for identifying the phase of the disease, clinical activity and the presence of some extrapulmonary lesions.

We determined the serum Ig and complement levels in order to make a comment about presence and serum levels of immune complexes since it is not possible to measure out the circulatory immune complexes. Compared to the active patients, in the inactive patients the IgG, IgA were significantly high, IgM and the C3 - C4 were also high though statistically not significant. We comment that the reason for these lower levels of parameters in active patients can be explained that way, that they accumulate in active sarcoid lesions and that they are found as a form of immune complexes in the circulation.

In this study, we found higher than normal laboratory values for immunoglobulins and C3 -C4 in our both case groups. But if we assume 1730 mg/dl for IgG as border values, there is a significant difference between the two groups.

Similar to our results DP Rooney et al. compared the C3-C4 levels in active versus stable patient groups but did not find a significant difference between them (7). In the inactive group of our study the IgM , C3 and C4 levels were higher though statistically not significant.

Saint Remy et al. has noted that the Ig G and A levels were significantly higher in active patients than they are in chronic patients. Patients with BHL and mottling as radiological findings had significantly higher C4 levels than patients without these parameters. In-patients with mottling only C3 levels were significantly higher (8). In our study, there were no difference in C4 levels between patients with BHL and mottling.This may be due to the lower volume of our study group that there is a controversy between our C4 findings and those from Saint Remy et al. Same researchers stated that hypergammaglobulinemia persisted in chronic patients which in fact was the case in our study too.

Although it has been said that in sarcoidosis, IL-12R is expressed both on CD4+ and CD8+ cells (20), it has also been asserted that there is no correlation between circulatory immune complex levels and activation markers like CD4+/ CD8+ ratio in serum and BAL, HLA-DR or Ig levels (6). In this study there is no correlation between CD4 /CD8 and IgG, IgA, IgM, C3, C4. On the other hand IL-18R expression is significantly higher on sarcoid BAL CD4+T cells than on normal and inflammatory control BAL cells (21).

Hol et al. have believed that pulmonary sarcoidosis beginning acutely with erythema nodosum has a limited progress and ends up with spontaneous resolution, however latent beginning would go with fibrosis (13). In six of our study cases we noted acute beginning with erythema nodosum, and their follow-up still goes on.

Giotaki et al. have investigated Ig, complement and autoantibodies in 55 active and inactive cases and IgM, rheumatoid factor and C4 levels have been found high in both sarcoidosis groups (22). In our cases too IgM and C4 were high in both groups but no difference between the two groups.

Humoral immunity in the active period of sarcoidosis is hyperactive. In our study we observed that there is a polyclonal increase in immunoglobulin levels in both active and

inactive groups. On the other hand immunoglobulin G levels in the inactive group were significantly higher compared to the active group. Although statistically not significant the IgM and C3-C4 levels were higher in the inactive group too compared to the active group. It has been thought that the consumption of serum immunoglobulin and immune complexes in the active sarcoidosis lesions might be the reason for this difference.

Conclusively we advise to determine the serum immunoglobulin and complement levels with other parameters in checking the activity and follow up of sarcoidosis. These results suggest that testing in a larger trial with greater power is needed.

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