Anti-Heart Antibodies in Chronic Cor Pulmonale*

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SUMMARY

In patient with chronic cor pulmonale which was caused by pulmonary emphysema, circulating autoantibodies against the heart tissue were investigated by hemagglutination and hemagglutination-inhibition techniques using normal rabbit's myocardium as antigen. Quantitative analysis of serum IgG, IgA and IgM were carried out by use of the radial immunodiffusion method. These results were compared with clinical findings, specifically cardiopulmonary hemodynamics and pathophysiological changes.

Circulating anti-heart antibodies were found in 13 out of 25 (52 per cent) patients with a definite complication of chronic cor pulmonale. The titers of circulating anti-heart antibodies indicated a good correlation with hypoxia and pulmonary hypertension and/or pulmonary circulatory disturbance. Furthermore, a good correlation was noted between anti-heart antibody titers and the serum IgG levels.

These data could be utilized in evaluation and discussion of the pathophysiological findings of the patients with chronic cor pulmonale.

Additional Indexing Words:

Chronic cor pulmonale Anti-heart antibody Pulmonary hypertension Immunoglobulin

SINCE 1956, when Dressler¹⁾ first reported that the post-myocardial infarction syndrome was caused by an antigenic change of necrotic tissue in the myocardium, which leads to the formation of autoantibodies, circulating autoantibodies against cardiac tissue in human and experimental animals have been studied by many investigators.²⁾⁻⁵⁾ Groups of Davies⁶⁾ and Dodson⁷⁾ reported that cardiac damage by rheumatic, traumatic or ischemic lesions lead to antigenic changes which are manifested by autoantibody production. In patients with chronic cor pulmonale, myocardial damage can be caused by the effect of hypoxemia on the myocardial metabolism. We suggest, therefore, that anti-heart antibodies could be seen in patients with chronic cor

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* This paper was presented at the Fifth Asian-Pacific Congress of Cardiology, Singapore, October 8-13, 1972.

Received for publication November 24, 1973.

pulmonale under the same mechanism as in myocardial infarction.

The clinical, radiological, electrocardiographic and hemodynamic studies on cor pulmonale have been done for the purpose of establishing the diagnosis and prognosis of this disorder. However, studies investigating the theory of autoantibodies production in this disease have not been documented other than our preliminary studies.⁸⁾⁻¹²⁾

The aim of this report is designed to explore the diagnostic and prognostic usefulness of determining the occurrence of circulating autoantibodies against cardiac tissue in the serum of patients with chronic cor pulmonale.

MATERIALS AND METHODS

One hundred and fifty-two specimens of serum were collected from normal adults and patients with various diseases. Samples involved 25 sera from patients with chronic cor pulmonale (diagnosed by clinical and laboratory findings and heart catheterization¹³⁾), 10 from emphysema without definite complication, 26 from severe pulmonary tuberculosis, 33 from myocardial infarction and 11 from angina pectoris. As controls, the following subjects were chosen: 12 sera from patients with congenital heart disease, 8 with systemic lupus erythematosus, 2 with cerebral thrombosis and 25 normal subjects aged 43 to 66 who were without any clinical evidences of disease. In this study myocardial infarction was diagnosed on the grounds of ECG findings indicating myocardial necrosis and from the typical history. In addition, blood samples from patients with myocardial infarction were obtained 4 or 5 weeks after the myocardial infarction attack.

Isolation of antigen

The antigen was prepared from freshly killed rabbit's myocardium and from human hearts which were obtained at autopsy within a few hours after death. The heart muscle was stripped of fat and gross fibrous tissue and was divided into small fragments aseptically. These fragments of the heart muscle were washed in cold saline 7 times, and were homogenized in a phosphate buffered normal saline (pH 6.4) for 5 min at 4°C, using a tissue grinder. The homogenates of heart muscle were then centrifuged at 3,000 rpm for 10 min. The supernatant was stored at -20°C until use. The protein concentration of the antigen used was 1-2 mg/ml.

Hemagglutination and hemagglutination-inhibition tests

Hemagglutination and hemagglutination-inhibition techniques were employed for detecting circulating antibodies against cardiac tissue using normal rabbit's myocardium as antigen. That this antibody activity is specific only for heart muscle antigen is shown elsewhere.^{6),14)} Antisera to the heart tissue induced agglutination of tissue extracts only from the myocardium, but not from the other organs. The absorption of these antisera with heart extract abolished hemagglutination activity; whereas, this serologic activity remained after absorption with the extract from the kidney, liver, and lung. Hemagglutination titers were determined using the method outlined by Boyden.¹⁵⁾ The titer of hemagglutinin activity was indicated by the last culture tube in which all erythrocytes are seen to be agglutinated, using the

criteria of Stavitsky¹⁶⁾ in grades of positive, doubtful and negative. The doubtful or non specific group showed partial hemagglutination in 1 out of 8 or less with both heart and kidney. The positive sera showed specific hemagglutination at the dilution of 1/16th or higher. When the test was negative, inhibitors in the specimen were investigated in such a way that the anti-heart antiserum raised in rabbits was added to each tube containing serum negative for the hemagglutination test. Afterwards each tube was incubated for 12 hours at room temperature and then read.

Quantitative analysis of immunoglobulins

Serum levels of immunoglobulin G (IgG), IgA, and IgM were determined by use of the radial immunodiffusion method.*

Hemodynamic studies

The pulmonary artery mean pressure (PAm) and wedge pressure were measured hemodynamically by means of cardiac catheterization.

ECG findings

The ECG findings were collected from 25 cases with chronic cor pulmonale.

RESULTS

Table I shows these results. Circulating anti-heart antibodies were found in 13 of 25 sera (52 per cent) of patients with pulmonary emphysema

Table I. Existence of Anti-heart Hemagglutinin Antibody in Sera from Patients with Various Heart Diseases

Patient groups	Number of patients	Anti-heart hemagglutination test			Occurrence (%)
		Positive	Doubtful/non specific	Negative	
Chronic cor pulmonale	25	13	1	11	52.0
Pulmonary emphysema	10	2	0	8	20.0
Pulmonary tuberculosis	26	3	3	20	11.5
Myocardial infarction	33	13	3	17	39.4
Angina pectoris	11	1	1	9	9.9
Congenital heart disease	12	2	0	10	16.7
Collagen disease	8	0	2	6	0
Cerebral thrombosis	2	0	0	2	0
Normal subjects	25	0	3	22	0
Total	152	34	13	105	

* using commercially prepared immunodiffusion plate and protein standards (Partigen, Behringwerke).

accompanied with chronic cor pulmonale and in 2 of 10 (20 per cent) patients without definite cor pulmonale. The anti-heart antibodies were observed in 13 out of 33 cases (39.4 per cent) of myocardial infarction, whereas only one out of 11 patients with angina pectoris was found to have these anti-heart antibodies. No anti-heart antibodies were detected in 25 normal sera, in 8 cases of SLE and in 2 cases of cerebral thrombosis. The quantitative analysis

Fig. 1. Serum IgG levels in 3 groups divided by the presence of anti-heart antibodies.

Cor pulmonale:

Positive group $2,029.7 \pm 156.5^* \text{ mg/100 ml}$

Negative group $1,279.4 \pm 98.5 \text{ mg/100 ml}$

$t = 3.972, p < 0.01$

Myocardial infarction:

Positive group $1,536.0 \pm 94.5 \text{ mg/100 ml}$

Negative group $1,114.5 \pm 57.8 \text{ mg/100 ml}$

$t = 4.047, p < 0.01$

of IgG, IgA and IgM was carried out in 22 cases of chronic cor pulmonale and in 19 cases of myocardial infarction. In our study, positive sera reacted with both human and animal heart extracts but not with kidney. The animal antigens usually gave titers higher than those given by human heart, probably because the tissues were fresher.

As shown in Fig. 1, specimens were divided into 3 groups, positive ; negative ; and doubtful groups on the appearance of anti-heart antibodies. In patients with chronic cor pulmonale, the mean value of the IgG level in the positive group was significantly higher ($p < 0.01$) than the negative group. The serum IgG value and anti-heart antibody titers are shown in Fig. 2. A good correlation was noted between the IgG value and the anti-heart antibody titer both in chronic cor pulmonale ($p < 0.01$) and myocardial infarction ($p < 0.01$). All of the positive cases of anti-heart antibody activities were more

Fig. 2. Relation between serum IgG levels and the anti-heart antibody titer in chronic cor pulmonale and myocardial infarction.

Chronic cor pulmonale:

$$y = 1,304.1 + 166.5x; n = 22; r = 0.704; t = 4.433; p < 0.01$$

Myocardial infarction:

$$y = 1,119.0 + 95.3x; n = 19; r = 0.771; t = 4.989; p < 0.001$$

than 1,500 mg/100 ml in the IgG concentration while the negative cases were less than 1,500 mg/100 ml. In addition the mean value of IgG level in sera from patients with chronic cor pulmonale seemed to be relatively higher than that in the patients with myocardial infarction. Finally, there were no significant changes in either serum IgA or IgM levels.

The pulmonary artery mean pressure is very important in prognosis of chronic cor pulmonale. Therefore, the pulmonary artery mean pressure (PAm) was measured in 16 cases with chronic cor pulmonale and in 7 cases with congenital heart disease. As shown in Fig. 3, anti-heart antibodies were positive in all 7 cases with more than 22 mmHg of PAm, while all 8 cases with less than

Fig. 3. Relation between pulmonary artery mean pressure (PAm) and the anti-heart antibody titer in chronic cor pulmonale and congenital heart disease.

Cor pulmonale:

$$y = 16.1 + 2.3x; \quad n = 16; \quad r = 0.836; \quad t = 5.695; \quad p < 0.01$$

20 mmHg of PAm showed negative results for anti-heartantibodies. The remaining case with 20 mmHg of PAm showed an anti-heart antibody titer doubtful for the specificity. Anti-heart antibodies were detected in 2 cases of congenital heart disease which showed markedly high PAm (80 and 53 mmHg). A good correlation was noted between the anti-heart antibody titer and PAm values ($p < 0.01$).

In reference to pulmonary wedge pressure, there was some gross correlation between anti-heart antibody titer and wedge pressure levels. And these subjects were measured for arterial oxygen saturation (SaO_2) by means of the Van Slyke-Neill method. As shown in Fig. 4, a correlation was also noted between anti-heart antibody titers and SaO_2 values ($p < 0.01$). Positive cases with higher anti-heart antibody titers showed reduced SaO_2 values.

Results of the investigation on 25 cases with chronic cor pulmonale, as pre-

Fig. 4. Relation between SaO_2 values and the anti-heart antibody titer in chronic cor pulmonale and congenital heart disease.

Cor pulmonale:

$$y = 93.4 - 1.1x; \quad n = 20; \quad r = -0.735; \quad t = 4.597; \quad p < 0.01$$

viously mentioned, are illustrated in the Table II. The materials were divided into 2 groups, pulmonary P and no pulmonary P by the findings of ECG, because pulmonary P wave informs the condition of pulmonary circulatory disturbance. As shown in Table II, positive cases for anti-heart antibodies was larger in the group with pulmonary P than that in the group without pulmonary P.

Table II. Results of the Investigation on 25 Cases with Chronic Cor Pulmonale

	Direct hemagglutination		Inhibition hemagglutination	
	Positive	Negative	Positive	Negative
Pulmonary P	11/17	6/17	0/5	5/5
No pulmonary P	1/8	7/8	1/7	6/7

The materials were divided into 2 groups, pulmonary P and no pulmonary P, by the findings of ECG.

Fig. 5. Relation among serum IgG levels, pulmonary artery mean pressure (PAm) and anti-heart antibodies in chronic cor pulmonale.

A good correlation is noted between serum IgG levels and PAm values;

$$y = 46.4 + 76.9x; n = 13; r = 0.715; t = 3.387; p < 0.01$$

Anti-heart antibodies were positive in all 7 cases which had a high PAm value and more than 1,500 mg/100 ml of IgG concentration, whereas anti-heart antibodies were negative in all 4 cases which showed a low PAm and less than 1,500 mg/100 ml of IgG concentration.

Fig. 5 exhibits the serum IgG level, PAm value and the presence of anti-heart antibodies of 13 patients with chronic cor pulmonale. A good correlation was noted between serum IgG levels and PAm values. Anti-heart antibodies were positive in all 6 cases which had a high PAm value and more than 1,500 mg/100 ml of IgG concentration; whereas, anti-heart antibodies were negative in all 4 cases which showed a low PAm and less than 1,500 mg/100 ml of IgG concentration.

DISCUSSION

The findings of this study indicate that circulating anti-heart antibodies were found in 13 out of 25 sera (52.0 per cent) from patients with pulmonary emphysema accompanied with chronic cor pulmonale. Similar anti-heart antibodies have been demonstrated in various heart diseases by many investigators. Van der Geld¹⁷⁾ demonstrated anti-heart antibodies in 9 of 16 (56 per cent) cases of myocardial infarction; in 13 of 15 cases of post-cardiotomy syndrome; and in 8 of 14 cases of post-myocardial infarction syndrome by use of antiglobulin consumption and immunofluorescence. Ehrenfeld et al²⁾ reported that anti-heart antibodies were found, by the tanned red cell hemagglutination technique in 12 of 53 (23 per cent) patients with a history of acute myocardial infarction and 3 of 33 (9 per cent) patients with chronic coronary heart disease. In our study, sera of 13 out of 33 (39.4 per cent) cases with myocardial infarction gave positive anti-heart hemagglutination test.

As our results indicate, anti-heart antibodies are produced in patients with chronic cor pulmonale. We would consider that in the patients with chronic cor pulmonale, some parts of the heart tissue change to an antigenically different substances upon hypoxia and pulmonary hypertension and/or pulmonary circulatory disturbance. This antigenic substance is probably released into the circulating blood under the same mechanism as in myocardial infarction and acting as antigen that stimulates the formation of autoantibodies in the blood.

The existence of anti-heart antibodies in normal people has been conflicted. Dodson⁷⁾ reported that 3 out of 50 normal human sera had positive hemagglutination reaction, whereas Heine et al¹⁸⁾ demonstrated no anti-heart antibodies in 84 normal sera. The method used by all of these groups were the same. Van der Geld¹⁷⁾ reported that 80 normal people were proven to be negative for anti-heart antibodies by the fluorescence method. In this study, all 25 normal subjects, 2 subjects of cerebral thrombosis and 8 SLE subjects showed negative results. As Davies and Gery¹⁹⁾ point out, only a limited number of individuals may have the capacity to produce circulating anti-

heart antibodies, whereby susceptibility is possibly affected by hereditary factors. Age is also a factor behind the capacity. However, in our experiment no difference in mean age between positive and negative groups.

In the study by Itoh et al,²⁰⁾ the antibody activity in rabbit antiserum against human heart muscle existed mainly in the fraction F_1 (γ -globulin) on DEAE cellulose column chromatography. These cardiac autoantibodies appeared in the second and third weeks after myocardial infarction. Ebringer et al²¹⁾ reported that serum IgG levels fall over a period of 5 to 7 days after myocardial infarction, and subsequently become elevated considerably above control levels. In our study a good correlation was noted between anti-heart antibody titers and serum IgG levels both in chronic cor pulmonale and myocardial infarction. The mean value of IgG level in the positive group for anti-heart antibodies was higher than the negative group in patients with chronic cor pulmonale. A similar tendency was noted in cases with myocardial infarction. These findings suggest a possibility that the antibody activity might be raised in IgG.

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