Immunological results in myocardial diseases

H.-D. BOLTE
M.D.

P. SCHULTHEISS
M.D.

*Medizinische Klinik I, Department of Medicine, University of Munich,
Klinikum Grosshadern, West Germany*

Summary

Immunological studies have shown new diagnostically important changes in alcoholic and viral myocarditis, as well as in congestive cardiomyopathy.

Increased heart size correlated with the degree of congestive heart failure, as well as with negative immunofluorescence and an increased IgA concentration in the serum. These findings may serve as a diagnostic aid in patients with myocardial disease due to alcohol abuse.

Viral heart disease is characterized by a variety of symptoms and nuclear antibodies (IgM) can be of help in the differential diagnosis.

Heart muscle tissue of patients with congestive cardiomyopathy preferentially binds IgG and IgA. In addition to the other changes these findings are of diagnostic importance. It seems likely that results similar to those obtained for humoral antibodies in congestive cardiomyopathy will apply in the correlation of the haemodynamic status of the patients. The pathophysiological implication of these findings is not clear at present, but the evolution of congestive cardiomyopathy appears to be associated with binding of immunoglobulin to the myocardium, as well as with humoral antiheart antibodies.

Introduction

In some myocardial diseases immunological changes are characteristic, for example humoral myocardial antibodies have been demonstrated in the acute phase of rheumatic heart disease, in the postcardiotomy syndrome and in Dressler's syndrome (postmyocardial infarction syndrome) and in disseminated lupus erythematosus (Bolte, 1975; Read, Engle and Zabriskie, 1977).

These antibodies correlate with the clinical symptoms and form a diagnostic index, although their relevance in the pathogenesis of these cardiac diseases is not well understood. Over the years various aspects of cardiomyopathy have been investigated, including the administration of myocardial antibodies (Bolte,

1975, 1976; Bolte *et al.*, 1974; Bolte and Grothey, 1977).

Methods

Immunofluorescent techniques were used to demonstrate antimyocardial humoral antibodies in the serum as well as immunoglobulin at cellular level on biopsy material obtained from patients with myocardial disease. Heart muscle from man, guinea-pig or rat served as antigen. Sections were covered with the patient's serum containing the antibody under investigation. Serum-bound globulins were separated by rinsing the section with phosphate buffer. Conjugates of antiglobulins with a fluorescent agent were used to visualize microscopically the antigen/antibody binding (Coons, Creech and Jones, 1974). The indirect fluorescence test was used to examine sera and the direct test was employed to examine myocardial biopsies with fluorescein-isothiocyanate-conjugated anti-human-globulins from rabbit (anti-IgG, anti-IgA, anti-IgM) (Behring-Werke, Marburg). The sera of the patients were diluted 1 : 5. The conjugates were diluted to 1 : 10, 1 : 30, up to 1 : 40 (Bolte and Grothey, 1977).

This report will concentrate on (1) alcoholic myocardial disease, (2) viral heart disease and (3) congestive cardiomyopathy.

Alcoholic myocardial disease

A total of eighteen patients with an increased cardiothoracic ratio and abnormal electrocardiographic signs were investigated. All patients gave a history of a high daily intake of ethyl alcohol, which was estimated as being 1.4 g/kg body weight/day. The concentrations of serum IgA were quantified and values of $412 (\pm 81) \text{ mg\%}$ were found compared to $198 (\pm 79) \text{ mg\%}$ in fifty-six control patients ($P < 0.001$) corresponding to an increase in alcoholic patients of approximately 110% (Bolte *et al.*, 1974; Bolte, 1976).

By contrast, IgG was only slightly increased and values of up to $1600 (\pm 569) \text{ mg\%}$ (compared to $1178 (\pm 255) \text{ mg\%}$ in control patients) were observed. The difference was, however, significant ($P < 0.001$).

Correspondence: Prof. Dr. H.-D. Bolte, Medizinische Klinik I, der Universität München, Klinikum Grosshadern, Marchioninistrasse 15, D-8000 München 70/West Germany.

Test for indirect immunofluorescence was negative in all alcoholic patients. This finding is of importance, because humoral myocardial antibodies can be demonstrated in various diseases, for example in all the ten patients with lupus erythematosus antibodies were found. In the twenty-four patients with congestive cardiomyopathy, 41% of the patients showed positive indirect immunofluorescence. In 28% of cases with inactive rheumatic heart disease, positive tests were also obtained (Bolte and Grothey, 1977).

None of the patients with alcoholic heart disease showed evidence of cirrhosis of the liver. Transaminase mean values for GPT were 18 and for GOT 22 mEq/ml. Serum alkaline phosphatase was increased to more than 200 mu./ml in only two patients (Bolte *et al.*, 1974).

As can be seen in Table 1, increase in heart size correlated well with congestive failure, negative immunofluorescence tests and increase in IgA concentrations. These parameters can be used in identifying patients with alcoholic cardiac disease.

TABLE 1. Diagnostic profile of alcoholic myocardial disease (n = 23)

Mean ethanol intake daily:	≥ 100–150 g, i.e. ~ 1.4–2.0 g/kg body-weight
Congestive heart failure, NYHA*-class III	
X-ray, cardio-thoracic ratio:	> 0.5
Serum-immunoglobulin A: increased (n = 18)	17/18, i.e. 95%
Antiheart-antibodies (n = 18)	
(Indirect immunofluorescence test)	
Negative 18/18, i.e. 100%	

*New York Heart Association.

Viral heart disease

This may often be preceded by a common cold which a few days later is followed by symptoms of heart disease, which including signs and symptoms of pericarditis, congestive heart failure, disturbances of rhythm such as resting tachycardia, extra-systoles, or atrio-ventricular or sinu-atrial block. Occasionally electrocardiographic ST-T changes may be the only manifestation. Seventeen patients were analysed. Two separate examinations of anti-viral serologic titre of complement binding reaction (Hygiene Institute of the University of Munich) were undertaken.

In 76% of these patients myocardial antibodies were found by the indirect immunofluorescence test: 41% nuclear, 23% sarcolemmar and 12% inter-myofibrillar types. From the nuclear type (41%) antibodies were classified: 100% IgM, 51% IgA, and 14% IgG (Bolte, Schultheiss and Ludwig, personal communication). The results show that viral heart disease can be defined by additional factors, particularly the binding of nuclear antibody

IgM. This may prove to be of value in the differential diagnosis.

Congestive cardiomyopathy

Twenty-four patients, all 45 years of age or under, showing clinical manifestations of cardiomyopathy, were examined. No evidence of factors which could result in heart failure, including those of increased alcoholic intake, was found. All twenty-four patients showed ECG changes, the most common of which was supra-ventricular dysrhythmia (80% of patients); atrial fibrillation, ventricular extrasystole, sinus bradycardia, sinus tachycardia and paroxysmal tachycardia were found with decreasing frequency. In addition 40% of patients also showed ST segment changes as well as atrio-ventricular or intra-ventricular blocks. The cardiothoracic ratio in every patient exceeded 0.5.

The indirect immunofluorescence test was positive in ten of the twenty-four patients (41%): in eight patients sarcolemmar type and in two the inter-myofibrillar type was demonstrated. In only one of thirty control patients (clinically healthy persons) was the immunofluorescence test positive. The results obtained for patients with congestive cardiomyopathy are similar to those found by Das *et al.* (1973); Robinson, Anderson and Griebel (1966) and Sack, Sebening and Wachsmut (1975).

The results correlated well with the clinical symptoms as well as with the length of history. Those patients with myocardial antibodies had a longer history (Bolte and Grothey, 1974; Oevermann, Bolte and Zwehl, 1973).

Endomyocardial biopsies were performed recently, using the King's biptome (Richardson, 1974) in a further sixteen patients with congestive cardiomyopathy who had, in addition, undergone extensive non-invasive and invasive cardiological examinations. Congestive heart failure was present in every patient corresponding to Class III/IV NYHA*. Mean ejection fraction was 30 (±11)% with a range from 10 to 48%. Mean end-diastolic volume was 315 (±102) ml, ranging from 187 to 560 ml. Morphological examination of biopsy material was undertaken in every patient analysed, either by Dr Olsen (London) or Professor Huebner (Munich). Biopsies were obtained at the same time to examine antiglobulin binding by direct immunofluorescence. In addition, indirect immunofluorescence tests for humoral antibodies were undertaken in every patient. The results are summarized in Tables 2 and 3.

It has already been pointed out that humoral myocardial antibodies were demonstrated in 41% of patients with congestive cardiomyopathy. The same

*New York Heart Association.

TABLE 2. Immunological data in sixteen patients with congestive cardiomyopathy: humoral myocardial antibodies (indirect immunofluorescence test) and binding of immunoglobulins in myocardial biopsy tissue

No.	Morphology (biopsy)	AF %	EDV (ml)	Indirect immunofluorescence (heterolog. antigen)			Direct immunofluorescence (biopsy)		
				IgG	IgA	IgM	IgG	IgA	IgM
6	Consistent with COCM*	13	209	0	0	0	I(+)	0	0
10	—	31	245	S+	0	0	S(+)	0	0
15	*	25	288	0	0	0	S+	S+	0
21	Hypertrophy nucl. changes	10	339	0	0	0	0	I+	I+
24	Normal†	32	231	V++	V++	V++	S+	S(+)	0
32	Hypertrophy, dilatation‡	24	318	0	0	0	S+	0	0
33	*	20!	460	S++	0	0	S++	S+	S(+)
36	†	39	422	0	0	0	0	0	0
38	*	21	340	0	0	0	S+	0	0
39	Mild hypertrophy§	31	227	0	0	0	S++	0	0
40	§	37	311	S++	0	0	S+	0	0
41	*	26	380	S+	0	0	0	0	0
7	†	48	187	S(+)	0	0	S(+)	0	0
13	*	43	560	0	0	0	S+I+	S++	0
14	Hypertrophy	44	297	0	0	0	S+	S+	0
26	*	42	218	S++	S+	0	S++	S+	0

S = Sarcolemmal I = Intermyofibrillar N = nuclear V = Vascular AF = Atrial fibrillation
EDV = end diastolic volume

TABLE 3. Diagnostic profile of congestive cardiomyopathies (n = 16)

Unknown aetiology	
Congestive heart failure NYHA*-class III/IV	
Ejection fraction: 31 (s = ± 11)%	
End-diastolic volume: 315 (s = ± 102) ml	
Immunological results	
Anti-heart-antibodies:	
(Indirect immunofluorescence test)	
Positive 7/16 i.e. 43%	
Bound immunoglobulins in heart muscle biopsy tissue:	
IgG	13/16, i.e. 81%
IgA	8/16, i.e. 50%
IgM	2/16, i.e. 8%

*NYHA = New York Heart Association.

percentage result was obtained in the group of sixteen patients in whom endomyocardial tissue obtained by biptome was analysed immunologically: Bound IgG, 81%; IgA, 50%; IgM, 4%. The high percentage of myocardium bound immunoglobulins are in agreement with the findings reported by Das *et al.* (1971). These authors demonstrated bound γ -globulin in the explanted hearts of patients with congestive cardiomyopathy who had undergone cardiac transplantation.

The diagnostic value of the present results was emphasized by analysing a group of nine patients with cardiac disease due to a variety of causes. These patients did not fulfil the criteria of severe congestive myocardial disease. Ejection fractions and end-diastolic volumes were normal. In only two of the nine patients (23%) were irregular types of indirect immunofluorescence demonstrated, and on biopsy

material tissue bound IgG was observed in two of the nine cases (23%), IgA in three (33%) and IgM in one patient (11%).

The findings indicate that heart muscle of patients with congestive cardiomyopathy preferentially binds IgG and IgA and are of importance in those cases where morphological diagnosis does not suggest established congestive cardiomyopathy. It seems likely that these results correlate with the haemodynamic findings, as do humoral antibodies in congestive cardiomyopathy. At present, the pathophysiological implications are not clear, but during the development of congestive cardiomyopathy, there appears to be, in a number of cases, an association with immunoglobulin binding of the myocardium, and the existence of humoral antibodies against the myocardium.

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