

# Role of toxoplasmosis in the aetiology of some cardiac diseases: an immunobiological investigation

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**SUMMARY** The Sabin-Feldman dye test was used to show the presence of toxoplasma antibodies in 183 patients with cardiac disease, as well as in two control groups (A and B), comprising 183 and 2186 healthy subjects, respectively. Toxoplasma specific antibodies were found in 157 (86%) patients with cardiac disease, 39 (21%) of whom had high titres. The prevalence of toxoplasma antibodies was considerably lower than that of control groups: 94 (51%) in group A and 1276 (58%) in group B. High titres were present in 8 (4%) and 83 (4%), respectively. Immunofluorescence antibody testing showed IgM antibodies in five patients, three of whom had the organism itself recovered from the blood or lymph nodes.

Chronic or latent toxoplasma infection as a cause of cardiac disease is not as uncommon as is generally thought.

The organism *Toxoplasma gondii* attacks all tissues of the host, reproducing itself intensively during the acute phase. When immunity has developed in the host *T gondii* withdraws into the brain, muscles, and other organs where it becomes encysted. Acute toxoplasmosis is usually asymptomatic; when it becomes clinically apparent the disease manifestations are protean. *T gondii* infection exists in a chronic asymptomatic form in about 50% of the population of Yugoslavia.<sup>1,2</sup> Siim<sup>3</sup> was the first to describe the clinical features of acute acquired toxoplasmosis. Exanthematous and encephalitic forms of acquired toxoplasmosis cause cardiac diseases in 50% of patients.<sup>4</sup> Cysts in cardiac muscle, probably toxoplasma in origin, were first described by Kean and Grocott in 1945.<sup>5</sup> Subsequently, these parasites were found in myocardial fibres in large aggregates and cysts without a surrounding inflammatory reaction. Experimental studies on rabbits and hamsters<sup>6,7</sup> showed that myocarditis probably occurs after the rupture of affected cells, which liberate the parasites and cause an inflammatory reaction in the surrounding tissue.<sup>8</sup> Later investigations indicated that *T gondii* was found far more often in the heart than in other organs. The most recent investigations show that cardiac transplantation from a seropositive donor to a seronegative recipient can result in severe infection which can be fatal.<sup>9</sup>

As the relation between cardiac disease and toxoplasmosis has hardly been investigated, we carried out a systematic study of this problem, concentrating on the role of acute acquired toxoplasmosis in myocardial disease.

## Material and methods

Sera were obtained from 130 men and 53 women with some form of heart infection or degenerative cardiac disease, aged from 14-54 years. The patients were classified into five age groups: I, 10-19; II, 20-29; III, 30-39; IV, 40-49; and V, > 50. Two control groups were also included in this study: control group A consisted of the same number of healthy subjects matched for age and sex, and control group B consisted of 2186 subjects who had served as healthy controls in a previous investigation, but who were not matched for age and sex: subjects over 40 years of age were classified into one age group.<sup>10</sup>

The persistence of toxoplasma antibodies was shown by the Sabin-Feldman dye test<sup>11</sup> modified by Desmonts into the lysis test.<sup>12</sup> Sera were diluted 1/10, 1/100, 1/1000, or higher if necessary. The titres reported were the final dilutions of the sera in the reacting mixtures.

Specific IgM antibodies were shown by the IgM indirect fluorescence antibody test IgM-IFAT,<sup>13</sup> using "bio-Mérieux" reagents. Sera were diluted 1/50, 1/100, 1/200, or higher if necessary. Attempts were

Table 1 Distribution of toxoplasma antibodies in patients with cardiac disease and in control groups

Age (years)	Patients with cardiac disease titre			Total No examined	Control Group A titre			Total No examined	Control group B titre			Total No examined
	10	100	> 100		10	100	> 100		10	100	> 100	
10-19		5*+++		7	2	3		14	88	106	28	356
20-29	10	10*	6+	31	6	11	3	68	134	190	18	574
30-39	6	21	8**	45	12	20	3	52	90	253	19	646
40-49	10	24	7	46	8	13	1	31	112	220	18	610
Over 50	8	24	18	54	5	6	1	18				
Total	34	84	39	183	33	53	8	183	424	769	83	2186

\*parasite isolated.  
†IgM IFAT positive.

made to isolate *T gondii* by inoculating suspected material (blood samples and peripheral lymph nodes) into Swiss albino mice.

Statistical analysis was carried out using the  $\chi^2$  test, while small groups were analysed by the Fischer test for absolute probability and Student's *t* test for proportions.

**Results**

Table 1 shows the prevalence of infection with *T gondii* and the distribution of the titres obtained in various age groups among the patients with cardiac disease and in the controls. Of the 183 patients with cardiac disease, toxoplasma antibodies were found in 157 (86%). A titre of 1/1000 or higher was found in 39 patients (21%). Two or more serum samples were available from 40 patients; the titre increased in 17 patients and decreased in five.

In contrast, toxoplasma antibodies were found in only 94 (51%) patients in control group A and in 1276 (58%) in control group B, while high titres >1/100 were found in 8 (4%) and 83 (4%), respectively.

It was apparent that toxoplasma antibodies in high titres were far more common among patients with cardiac disease than in the control groups. Statistical analysis showed that there were significant differences between the patients and the control groups both in the prevalence of toxoplasma antibodies  $\chi^2 = 50.324$  and  $\chi^2 = 53.118$ ,  $p < 0.01$  of high titres,  $\chi^2 = 10.303$  and  $\chi^2 = 131.42$ ,  $p < 0.01$ .

The distribution of positive findings by Sabin-Feldman dye test varied among the age groups. Table 2 shows the statistical analysis. In the youngest age

group (10-19 years) no significant difference in the prevalence of toxoplasma antibodies was found between the patients and either control group. In all the other age groups, however, a significant difference was shown, with the exception of patients in the group III age group (30-39 years) compared with the subjects of control group A of the same age.

There were far more men than women in the group with cardiac disease (Table 3) than in control group A (71% and 45%, respectively). This discrepancy can be explained by the fact that we were able to cooperate mostly with cardiologists practising in male departments. On the other hand, the number of women in control group A was slightly greater because the group was derived from hospital administrative employees, many of whom are women.

In spite of the predominance of men in our study statistical comparison of the results obtained from men and women showed no significant difference ( $\chi^2 = 1.363$ ;  $p > 0.05$ ) which implies that heart disorders associated with toxoplasmosis can be expected in both sexes at the same rate.

The patients with cardiac disease were divided into two large groups according to whether the course of the disease was acute or chronic. Some form of heart infection (acute myocarditis, acute myopericarditis, acute pancarditis) was present in 74 (47%); 83 (53%) had degenerative disease (cardiomyopathy) (Table 4), while the remaining 26 patients had cardiac disorders of undefined type. The distribution among the age groups was not even—acute disease was far more common than chronic disease (57%:25%) in the younger patients; among the patients in group II about the same number had both acute and chronic

Table 2 Statistical comparison of positive findings by Sabin-Feldman dye test between patients with cardiac disease and control groups

Age (in years)	Control group A	<i>p</i>	Control group B	<i>p</i>
10-19	$p = 0.119$	$> 0.05$	$T_1 = 0.487$	$> 0.05$
20-29	$\chi^2 = 25.41$	$\leq 0.01$	$\chi^2 = 7.283$	$\leq 0.01$
30-39	$\chi^2 = 1.32$	$> 0.05$	$\chi^2 = 8.133$	$\leq 0.01$
40-49	$\chi^2 = 4.095$	$\leq 0.05$		$\geq 0.01$
Over 50	$\chi^2 = 6.086$	$\leq 0.05$	$\chi^2 = 41.272$	$\leq 0.01$

Table 3 Distribution of toxoplasma antibodies in patients with cardiac disease and in control group according to age and sex

Age group	Patients						Total No examined		Control group						Total No examined	
	Titre 10		100		> 100		M	F	Titre 10		100		> 100		M	F
	M	F	M	F	M	F			M	F	M	F	M	F		
10-19	—	—	3	2	—	—	5	2	1	1	1	2	—	—	7	7
20-29	4	6	6	4	4	2	18	13	3	3	5	6	1	2	30	38
30-39	4	2	17	4	6	2	35	10	5	7	8	12	—	3	20	32
40-49	9	1	17	7	5	2	35	11	4	4	7	6	—	1	16	15
> 50	6	2	18	6	10	8	37	17	2	3	3	3	1	—	10	18
Total	23	11	61	23	25	14	130	53	15	18	24	29	2	6	41	53

Table 4 Distribution of toxoplasma antibodies in acute and chronic cases of cardiac disease according to age and sex

Age group	Acute disease						Total No examined		Chronic disease						Total No examined	
	Titre 10		100		> 100		M	F	Titre 10		100		> 100		M	F
	M	F	M	F	M	F			M	F	M	F	M	F		
10-19	—	—	1	1	—	—	2	1	—	—	1	—	—	—	2	—
20-29	2	5	5	—	2	2	11	8	1	1	2	1	1	1	5	4
30-39	2	1	4	2	5	—	16	3	2	1	11	—	—	1	16	2
40-49	5	1	5	2	1	—	13	4	3	—	13	2	4	1	22	2
> 50	2	1	3	2	1	6	7	9	2	—	11	3	8	3	23	6
Total	11	8	18	7	9	8	49	25	8	2	38	6	13	6	68	15

disease; and among the oldest (groups IV and V) more patients had some form of chronic heart disease (33% : 54% and 33% : 50%, respectively). Among the sixteen patients with acute cardiac diseases in the eldest age group nine were women, six of whom had high titres of toxoplasma antibodies.

The IgM-IFAT was conducted on the first serum sample obtained from each of the patients. Specific IgM antibodies were detected in five of the 183 sera examined, though the titres were low (Table 1). The finding of low titres of specific IgM antibodies indicated that the infection with *T gondii* had been contracted months or possibly a year earlier.

Isolation of the organism was attempted by inoculating suspected blood samples or peripheral lymph nodes into mice in 14 cases. Isolation was successful in three cases (Table 1).

## Discussion

Although there are a great many publications on cardiac toxoplasmosis, these are mostly case reports in which, after thorough clinical and laboratory examinations, toxoplasmosis was diagnosed serologically and confirmed by successful isolation of *T gondii* in some cases.<sup>14-21</sup>

Ludlam and Somers<sup>22</sup> examined the incidence of toxoplasma antibodies in patients with cardiac disease and in healthy blood donors in Uganda: their

results did not allow them to postulate that toxoplasmosis is a potential cause of heart trouble in any age group. In contrast, our study showed a high prevalence of toxoplasma antibodies in patients with cardiac disease (86%) compared with those of the control groups (51%) and (58%), respectively. These findings indicate that *T gondii* might be the aetiological factor in many cases of heart disease. Our study also included the detection of specific IgM antibodies, the presence and especially the rise of which would indicate recent infection with *T gondii*. The results obtained both by the Sabin-Feldman dye test and the IgM-IFAT suggested that heart disorders are seldom associated with acquired toxoplasmosis in the first few months after infection: if this occurs it is mostly in younger age groups. Thus it is more likely that heart disease develop a few years later, after the encystation of the organism and the consecutive reactivation of the illness (serologically shown by high stable titres with the Sabin-Feldman dye test or by slight titre variation in the absence of specific IgM antibodies).

Specific IgM antibodies were found mainly in younger patients. In patients in whom they had not been shown absence did not necessarily mean that the disease had a chronic course; Remington *et al.*<sup>13</sup> Desmonts *et al.*<sup>23</sup> and Couvreur<sup>24</sup> established that in 10% of cases of confirmed acute infection the IgM-IFAT yielded negative results.

The problem of chronic toxoplasmosis is especially relevant nowadays as there are numerous reports of cases of reactivation of latent (chronic) toxoplasmosis in malignant disease, treatment with immunosuppressive drugs, or in elderly people in whom physiological immunosuppression occurs.

Cardiac disease from toxoplasmosis is considered to be extremely rare. Our investigation shows, however, that certain cardiac diseases might be caused by chronic or latent toxoplasma infection. Therefore, in all cases of cardiac disease of unclear aetiology provided symptomatology of acquired toxoplasmosis had occurred (along with a negative Paul-Bunnell test, which excludes infective mononucleosis that may have similar symptomatology), toxoplasmosis should be borne in mind.

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