Toxoplasmosis in heart and heart and lung transplant recipients

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SUMMARY Of the first 250 heart and 35 heart and lung transplant recipients at Papworth Hospital, Cambridge, who survived for more than one month after transplantation, 217 heart and 33 heart and lung patients were investigated serologically for evidence of *Toxoplasma gondii* infection. Six patients acquired primary *T gondii* infection, most probably from the donor organ. Five patients experienced *T gondii* recrudescence, two of whom had recovered from primary infection a few years earlier. Two patients died from primary *T gondii* infection and the severity of symptoms in the other patients with primary infection was related to the amount of immunosuppressive treatment. Prophylaxis with pyrimethamine (25 mg a day for six weeks) was introduced for *T gondii* antibody negative transplant recipients who received a heart from a *T gondii* antibody positive donor after the first four cases of primary toxoplasmosis. Of the seven patients not given pyrimethamine, four (57%) acquired primary *T gondii* infection. This compared with two of the 14 patients (14%) given prophylaxis.

Toxoplasma gondii is a ubiquitous pathogen. In the United Kingdom about 30% of persons aged 40 years have serological evidence of previous infection.¹ *T gondii* infections are usually asymptomatic, but infection may present as a glandular fever-like illness which is usually self-limiting with full recovery. In contrast, primary *T gondii* infections in immunocompromised persons are generally symptomatic, often presenting as necrotising encephalitis, myocarditis, or pneumonitis.² There have been many reports of clinical toxoplasmosis in patients with neoplastic disease,³ with the acquired immune deficiency syndrome (AIDS),⁴ as well as in immunosuppressed recipients of kidney,^{2,5} liver,^{2,6} heart^{7,8} and bone marrow transplants.⁹ Some of these infections seem to have been acquired from the donated organ^{5,7,8} and some follow reactivation of latent *T gondii* cysts,^{7,9} which may lie dormant but potentially active for many years.^{10,11} In some reports, however, the serological findings are insufficient to permit differentiation between primary and reactivated infections.

Having prospectively studied *T gondii* serological responses in 217 heart and 33 heart and lung trans-

plant recipients at Papworth Hospital, Cambridge, we now provide a detailed account of serological and clinical responses to *T gondii* primary and recrudescence infection in these patients.

Material and methods

A total of 250 patients received heart and 35 received heart and lung transplants between January 1979 and January 1988. The first 29 of these patients received conventional immunosuppression with azathioprine and prednisolone as well as a prophylactic course of intravenous equine anti-thymocyte globulin (ATG) for 28 days.¹² The next 60 patients received cyclosporin A and low doses of steroids,¹³ while the following 60 patients received either this regimen or cyclosporin A and azathioprine. Thereafter, patients received triple treatment with cyclosporin A, prednisolone, and azathioprine. The initial maintenance doses of these three agents were: cyclosporin A 8-10 mg/kg/day so as to maintain whole blood "trough" values at 800-1200 ng/kg/day; azathioprine 1-2 mg/kg/day, to obtain a white cell count of $5-6 \times 10^9/l$; heart transplant patients received prednisolone 1 mg/kg/day reducing to a maintenance dose of 0.25 mg/kg/day after two weeks; while heart and lung transplant patients

received prednisolone 375 mg/day for one day. All patients treated with cyclosporin A received a three day course of ATG in the period immediately following surgery.

Serum samples were obtained from patients before transplantation and at weekly intervals during the patient's stay in hospital, and thereafter, when they attended as outpatients or were readmitted to hospital. Serum samples were also received from organ donors. Since June 1984 *T gondii* antibody negative recipients who received an organ from a *T gondii* antibody positive donor—that is, mismatched for *T gondii*—were given a six week prophylactic course of pyrimethamine (25 mg/day) and folinic acid (15 mg three times a day).

T GONDII LATEX AGGLUTINATION TEST

The latex agglutination test (Toxoreagent, Eiken Chemical Company of Japan, supplied by MAST Diagnostics Ltd, Bootle, Merseyside, England) was performed as recommended by the manufacturers on all serum samples. Antibody titres of ≥ 16 were considered to be positive.

T GONDII DYE TEST

The dye test was performed in flat-bottomed, microtitre plates and read directly by means of an inverted microscope, as previously described.¹⁴

HAEMAGGLUTINATION TEST

The indirect haemagglutination test was performed in microtitre plates with V-shaped wells and with sensitised turkey erythrocytes, as previously described.¹⁵

μ -CAPTURE ELISA FOR DETECTING T GONDII-SPECIFIC IGM

The antibody class capture (ACC) enzyme-linked immunosorbent assay (ELISA) for detecting *T gondii*-specific IgM, previously described by Payne *et al*,¹⁶ was used. Results were expressed in enzyme immunoassay units (EIU). A positive control serum was used as a reference standard.

CARDIAC MUSCLE BIOPSY SPECIMENS

Cardiac muscle biopsy specimens were obtained at various times after transplantation, as specified for each patient. They were processed and examined, as described by Pomerance and Stovin.¹⁷ As a result of the small size of the cysts in relation to the standard section thickness of 5–7 μ m it was necessary to examine serial sections to increase the chance of finding cysts. The presence of myocyte necrosis without an accompanying cellular infiltrate or the presence of fibrotic replacement of individual myocytes alerts the histopathologist to the possibility of Toxoplasma myocarditis.¹⁸

TRANSBRONCHIAL BIOPSY SPECIMENS

Transbronchial biopsy specimens were taken as described by Higenbottom *et al*⁶ for investigating new and changed pulmonary symptoms in heart and lung transplant recipients.

Results

Two hundred and seventeen heart transplant and 33 heart and lung transplant patients survived for at least one month after transplantation from whom regular serum samples and the respective donor's serum were available. The numbers of donors and recipients who were *T gondii* antibody positive at the time of transplantation and the numbers of *T gondii* mismatched patients (antibody negative recipient, antibody positive donor) are shown in table 1.

Patients who had no detectable *T gondii* antibody before transplantation and who subsequently developed *T gondii* antibody titres were regarded as having primary infections. Six patients in this series acquired primary *T gondii* infection, probably from the donor's heart. A summary of the antibody responses to infection in these patients is shown in table 2.

Patients who had detectable *T gondii* antibody and subsequently had a rise in *T gondii* antibody titres were regarded as having recrudescence of *T gondii*. Five patients showed evidence of *T gondii* recrudescence at some time after transplantation. Two patients (cases 3 and 4) had previously acquired primary *T gondii* infection. A summary of the antibody responses to *T gondii* recrudescence for the three remaining patients is shown in table 3. A detailed case history for each patient is as follows.

CASE HISTORIES

A 32 year old man (case 1) had received a heart

Table 1 Summary of *T gondii* antibody state of donors and recipients

	Transplant patients (%)		
	Heart (n = 250)	Heart and lung (n = 35)	Total (n = 285)
Total studied	217	33	250 (87.7)
No of <i>T gondii</i> antibody positive donors	49 (22.6)	3 (9.1)	52 (20.8)
No of <i>T gondii</i> antibody positive recipients	64 (29.5)	11 (33.3)	75 (30)
No of patients mismatched for <i>T gondii</i> †	20* (9.2)	1† (3)	21 (8.4)

*The first seven patients were not given prophylaxis with pyrimethamine; the next 13 patients were.

†This patient was given prophylaxis with pyrimethamine.

‡*T gondii* antibody negative recipient, antibody positive organ donor.

Table 2 Antibody titres in heart transplant patients with *T gondii* infections

Case No	Time after transplant	Latex agglutination test titre	Dye test		Haemagglutination test titre	<i>T gondii</i> specific IgM (EIU)
			Titre	IU		
1	Before	< 16	< 8	< 2	< 32	0
	Donor	512	256	62	512	0
	27 d	< 16	< 8	< 2	< 32	0
	33 d	512	64	15	32	101
	44 d	Died—disseminated <i>T gondii</i> found at necropsy				
2	Before	< 16	< 8	< 2	< 32	0
	Donor	256	128	31	1024	0
	29 d	< 16	< 8	< 2	< 32	0
	33 d	< 16	< 8	< 2	< 32	7
	34 d	Died— <i>T gondii</i> seen in heart at necropsy				
3	Before	< 16	< 8	< 2	< 32	0
	Donor	32	64	15	128	0
	22 d	< 16	< 8	< 2	< 32	2
	33 d	128	2048	500	256	85
	1 y	< 16	128	31	32	4
	2 y	256	128	31	32	0
	4.5 y	4096	512	125	512	0
4	Before	< 16	< 8	< 2	< 32	0
	Donor	NT	NT	20	NT	NT
	18 d	< 16	< 8	< 2	< 32	0
	28 d	512	2048	500	128	216
	1 y	16	128	31	< 32	43
5	2 y	1024	4096	1000	64	96
	Before	< 16	< 8	< 2	< 32	0
	Donor	64	64	15	128	4
	6 m	< 16	< 8	< 2	< 32	0
6	14 m	1024	4096	1000	512	86
	Before	< 16	< 8	< 2	< 32	0
	Donor	128	64	15	256	0
	87 d	< 16	< 8	< 2	< 32	0
	115 d	1024	512	125	128	148

transplant. He developed right-sided heart failure and right lower lobe pneumonia. In the second week poorly localised abdominal pain, persistent diarrhoea, and fever were noted but a myocardial biopsy specimen on day 12 proved normal. In the fourth week the patient had two grand mal seizures. Endomyocardial biopsy specimens taken on days 20 and 28 postoperatively showed toxoplasma cysts in the myocardium. Treatment with oral pyrimethamine (25 mg/day), folinic acid, and sulphadiazine (1 g three times a day) was started. His condition deteriorated and he died 44 days after transplantation.

A 49 year old man (case 2) had received a heart transplant. In the third week he received enhanced immunosuppression with high doses of steroids because of a rejection episode. A myocardial biopsy specimen taken on day 24 did not show *T gondii* cysts. From day 32 onwards he became febrile with temperatures of 38.5–39.5°C and rigors. He developed pneumonia and died in the morning of day 34. Disseminated toxoplasmosis was diagnosed histologically at post mortem examination.

A 43 year old man (case 3) had received a heart

transplant. Twenty two days after transplant he developed a persistent fever of 39.5°C. He was treated with oral pyrimethamine, 25 mg twice a day, and sulphatriad, 500 mg twice a day, when a rise in *T gondii* antibody titres was noted. Subsequently, *T gondii* cysts were found in cardiac biopsy specimens taken on days 36 and 58 after transplantation. Fever slowly resolved and he made a good recovery. From day 37 his dose of pyrimethamine was reduced to 25 mg a day. He was discharged 58 days after transplantation. *T gondii* was not found in cardiac biopsy specimens taken from day 78 onwards. A year after transplantation anti-*T gondii* treatment was stopped. He remained generally well, although he complained of occasional headaches and slight blurring of vision. At a routine examination two years after transplantation the patient's *T gondii* latex agglutination test titre had risen from < 16 to 256. A cardiac muscle biopsy specimen taken at this time showed no evidence of *T gondii* cysts. Nevertheless, he had evidently experienced asymptomatic recrudescence of his earlier *T gondii* infection; he remained well five years after transplantation.

A 20 year old man (case 4) had received a heart

Table 3 Patients with *T gondii* recrudescence

Case No	Time after transplant	Latex agglutination test titre	Dye test		Haemagglutination test titre	<i>T gondii</i> specific IgM (EIU)
			Titre	IU		
7	Before	32	16	4	128	0
	Donor	< 16	< 16	< 4	< 32	0
	15 d	32	32	8	128	0
	75 d	256	128	31	512	0
	2.5 y	64	128	31	128	0
8	Before	< 16	16	4	< 32	0
	Donor	16	32	8	128	2
	6 d	16	16	4	32	1
	12 d	64	128	31	64	0
	54 d	1024	8194	2000	2048	3
	2 y	1024	512	125	1024	0
9	Before	256	128	31	256	0
	Donor	16	8	2	32	0
	53 d	256	128	31	256	0
	271 d	16000	16000	4000	16000	160
	406 d	256	256	62	512	0
	473 d	Died—no evidence of Toxoplasmosis at necropsy				

transplant. From day 17 he developed intermittent fever and a dry cough, and culture of bronchial washings grew an *Enterobacter* species. Routine endomyocardial biopsy specimens taken at day 26 showed *T gondii* cysts. Sulphadiazine, 1 g four times a day, and trimethoprim, 250 mg twice a day, were given along with oral pyrimethamine, 25 mg twice a day, and folic acid. Because of deteriorating consciousness, assisted ventilation and subsequently tracheostomy were required. As the patient had not responded to previous treatment, on day 44 spiramycin 1 g, twice a day, and sulphatriad, 500 mg four times a day, were given instead of trimethoprim and sulphadiazine. He made a steady recovery. Sulphatriad treatment was stopped on day 109 and he was discharged. Antitoxoplasma treatment was discontinued at 11 months. A blood sample taken two years after transplantation, however, showed a rise in the *T gondii* latex agglutination, dye test, and haemagglutination antibody titres, although he was asymptomatic.

A 44 year old man (case 5) had received a heart and lung transplant. As he was mismatched for *T gondii* he received prophylaxis with pyrimethamine and folic acid. After five weeks of pyrimethamine he became neutropenic. Treatment with pyrimethamine was therefore stopped. Subsequently, the patient developed CMV infection but symptoms were only mild. He remained well until 14 months after transplantation when a transbronchial biopsy specimen showed evidence of rejection. Immunosuppressive treatment was therefore increased. He subsequently developed a respiratory tract infection with a productive cough and the *T gondii* latex agglutination test antibody titre rose from < 16 to 1024. The patient received a brief course of pyrimethamine (25 mg a day) and made an uneventful recovery. *T gondii* cysts were

not found in myocardial biopsy specimens taken on days 17 and 39 after transplantation and transbronchial biopsy specimens taken on days 23 and 54 after transplantation.

A 47 year old man (case 6) had received a cardiac transplant. As he was mismatched for *T gondii*, he was given prophylaxis with pyrimethamine and folic acid. One hundred and fifteen days after transplantation his *T gondii* latex agglutination test antibody titre rose from < 16 to 1024. He did not have any symptoms. *T gondii* cysts were not found in regular myocardial biopsy specimens taken after transplantation.

A 49 year old man (case 7) had been given a cardiac transplant. Seventy five days after transplantation the latex agglutination test titre had risen from 32 to 256, indicating recrudescence of *T gondii*. The patient was asymptomatic without fever. *T gondii* cysts were not seen in routine myocardial biopsy specimens taken after transplantation.

A 50 year old man (case 8) had received a heart transplant. Twelve days after transplantation he had enlarged cervical nodes, arthralgia of ankles and wrists, as well as an erythematous rash. By day 30, a low grade fever had developed. He subsequently developed general malaise and was treated from day 63 with spiramycin (1 g twice a day), sulphatriad (500 mg four times a day), and pyrimethamine (25 mg twice a day). After six weeks his temperature settled and his general condition gradually improved. Treatment with sulphatriad, pyrimethamine, and spiramycin was stopped on days 77, 100, and 115, respectively. *T gondii* cysts were not seen in numerous cardiac biopsy specimens taken after transplantation.

A 23 year old woman (case 9) had received a heart and lung transplant. At nine months a mild fever and

left lower lobe pneumonia developed, together with a rise in *T gondii* latex agglutination antibody test titre to 16 000. Treatment with intravenous sulphadimidine, 2 g three times a day, was started. Although the fever settled spontaneously, shortness of breath persisted. Two weeks later treatment was changed to oral pyrimethamine, 25 mg twice a day, and folic acid, which was given for one month. She developed *Pneumocystis carinii* infection at 12 months and died with obliterative bronchiolitis 14 months after the operation. *T gondii* cysts were not seen in 11 myocardial biopsy specimens taken at regular intervals from day 7 to day 184, nor in transbronchial biopsy specimens taken on days 142 to 334.

Discussion

In this series we disregarded those patients whose serial serum samples showed rising latex agglutination test antibody titres as a result of *T gondii* antibody passively acquired from blood transfusion, as well as those who had a non-specific rise in latex agglutination test antibody titres concurrently with active CMV infection. These have been reported previously.¹⁹

We have shown that four of seven patients (57%) mismatched for *T gondii* who did not receive prophylaxis with pyrimethamine developed primary donor acquired *T gondii* infection.⁸ In the Stanford series, Luft *et al* found that three of four (75%) patients mismatched for *T gondii* developed life-threatening primary *T gondii* infection.⁷ There is therefore general agreement that when patients are mismatched for *T gondii*, there is a high risk of their developing serious or fatal toxoplasmosis. We have further shown that prophylactic pyrimethamine (25 mg/day for six weeks) prevents or mitigates the effects of *T gondii* infection in mismatched patients. Two such patients (cases 5 and 6) in this study developed primary *T gondii* infection despite prophylaxis with pyrimethamine. Case 5, however, received only a five week course of pyrimethamine because of neutropenia. He developed symptomatic *T gondii* infection 14 months after transplantation, shortly after his immunosuppressive regimen was intensified because of a rejection episode. The enhancement of the immunosuppressive regimen may have been responsible for the activation of the latent *T gondii* in the donor heart, and it may be necessary to give a further prophylactic course of pyrimethamine when rejection episodes necessitate increased immunosuppressive treatment. Case 6 should have received a full course of pyrimethamine, but due to poor compliance, he may not have taken the full course. Primary *T gondii* infection developed around four months after transplantation. His infection was probably asymptomatic because he had not received enhanced

immunosuppressive treatment, and had not experienced episodes of rejection. The primary toxoplasmosis in these two patients had two rather atypical features: infection was noted much later than in the other four patients who did not receive pyrimethamine; and symptoms were mild or absent.

In the first four patients who developed primary *T gondii* infection symptoms were noted within five weeks of transplantation (on days 17, 20, 22 and 32). The first indication of *T gondii* infection in all of them was fever. Three developed respiratory tract symptoms and one had grand mal fits with loss of consciousness. In the series reported by Luft *et al* two patients developed symptoms four to six weeks after transplantation and died.⁷ One was found to have chorioretinitis six months after transplantation. Four months later he became mentally confused and three brain abscesses were found on a computed tomographic scan. Interestingly, the two patients in our series who died from disseminated *T gondii* infection both received an additional course of steroids for the treatment of organ rejection, while the two who survived did not. This increased immunosuppression probably enhanced the severity of disease.

Of the 75 patients in this series who were *T gondii* antibody positive before transplantation, only three (4%) had genuine rises in latex agglutination, dye test, and haemagglutination test antibodies after transplantation, indicating recrudescence of *T gondii*. One (case 7) received azathioprine and prednisolone; case 8 was given cyclosporin A and low dose steroids; and case 9 had triple treatment as the immunosuppressive regimen. This incidence of *T gondii* recrudescence contrasts with that reported by Luft *et al*,⁷ who reported that 10 (53%) of 19 heart transplant recipients in the Stanford series who were antibody positive before transplantation had serological evidence of *T gondii* recrudescence after transplantation. Eight (80%) of their 10 patients who received azathioprine had a $\geq$ four-fold rise in dye test antibody titres compared with only two (25%) of the eight patients receiving cyclosporin A. None of the nine patients in this series, who were *T gondii* antibody positive before transplantation and had been given azathioprine and one (5.9%) of the 17 given cyclosporin A, had a subsequent rise in *T gondii* antibody titre. There does seem to be a discrepancy between the two series which is difficult to explain. It may be that the differences can be attributed to the amounts of immunosuppressive treatment given in the two groups of patients.

None of the patients in the Stanford series developed clinical illness attributable to *T gondii* recrudescence compared with two of the three patients in our series. Interestingly, case 7 did not receive enhanced immunosuppressive treatment as a result of

graft rejection, but cases 8 and 9 did at the time their *T gondii* antibodies increased in titre.

In our series all of the patients with primary *T gondii* infection who produced an antibody response seroconverted in the latex agglutination, dye test, and haemagglutination tests, produced *T gondii*-specific IgM, and had demonstrable *T gondii* visible in biopsy or necropsy specimens. All of the five patients with *T gondii* recrudescence showed an antibody response in the latex agglutination, dye test, and haemagglutination tests, but only two (cases 4 and 9) produced a *T gondii*-specific IgM antibody response. None of these patients had demonstrable *T gondii* in biopsy specimens taken at any time after transplantation. Luft *et al* found *T gondii*-specific IgM in four (40%) of their patients with *T gondii* recrudescence,⁷ which is similar to our findings.

In conclusion, we have found in the Papworth heart transplant series that *T gondii* infection is life-threatening only when acquired from the donor organ and that prophylactic pyrimethamine prevents or mitigates the effect of this infection.

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