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Efficacy of pyrimethamine for the prevention of donor-acquired *Toxoplasma gondii* infection in heart and heart-lung transplant patients

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Abstract. Seven (11%) of the first 65 patients who received heart transplants at Papworth Hospital were mismatched for *Toxoplasma gondii*. Of these, four (57%) experienced *T. gondii* infection and two died. The remaining two had severe symptoms and received anti-*T-gondii* chemotherapy for a year after transplantation. In an attempt to reduce the impact of donor-acquired *T. gondii* in our heart transplant recipients, we decided in April 1984 to give prophylactic pyrimethamine to all *T. gondii*-mismatched patients. In this study, 7 years later, we review the efficacy of this policy. Five of 37 (14%) patients given prophylactic pyrimethamine acquired *T. gondii* infection; only one was symptomatic, and none died. This compares with 100% symptomatic infection in the pre-1984 patients, who did not receive prophylactic pyrimethamine. We believe that our experience has shown that pyrimethamine is effective in reducing the incidence and severity of primary donor-acquired *T. gondii* infection in mismatched heart and heart-lung transplant recipients.

Key words: Pyrimethamine prophylaxis – Heart transplantation, *Toxoplasma gondii* – *Toxoplasma gondii*, in heart transplantation

Toxoplasma gondii infections in organ transplant recipients are usually associated with significant morbidity and mortality, particularly when infection is acquired from the donated organ [2, 11]. Clinical manifestations of primary *T. gondii* infections in transplant recipients are variable [9], but patients usually have a fever and may also develop mental confusion, focal neurological signs, lung consolidation or myocarditis [13].

After primary infection, which in non-immunocompromised persons is often asymptomatic but may be associated with mild glandular fever-like symptoms or lymphadenopathy [8], the organism lies dormant in cysts which have a predilection for muscle [7]. Any organ from a *T. gondii* antibody-positive donor may contain *T. gondii* cysts, which may reactivate when the organ is transplanted into an antibody-negative transplant recipient, producing a primary infection. The risk of seronegative recipients acquiring primary *T. gondii* infection from a seropositive organ donor is greater in heart (57%) than in liver (20%) transplantation, and much lower (< 1%) in kidney recipients [11].

Seven (11%) of the first 65 patients who received heart transplants at Papworth Hospital and survived for at least 1 month after transplantation, were mismatched for *T. gondii* (*T. gondii* antibody-negative recipient, antibody-positive donor). Of these, four (57%) acquired *T. gondii* from the donated organs and two died. The two patients who survived had severe symptoms and received anti-*T. gondii* chemotherapy for a year after transplantation.

In an attempt to reduce the impact of donor-acquired *T. gondii* in our heart transplant recipients, we decided in April 1984 to give a 6-week course of prophylactic pyrimethamine (25 mg a day for 6 weeks, with folinic acid cover) to all *T. gondii*-mismatched patients. In this study, 7 years later, we review the efficacy of this policy.

Materials and methods

Patients

A total of 404 heart, 95 heart-lung and 7 single lung transplants were performed in our centre between January 1979 and October 1990. *T. gondii* infections in the first 250 heart and 35 heart-lung recipients have been described previously [13]. In this study, we describe *T. gondii* infections in 303 heart, 86 heart-lung, and 4 single-lung transplant recipients, who were transplanted between May 1984 and October 1990 and who survived for at least 1 month after transplant, were included in the study. They were aged 6–63 years (mean 42 years).

Immunosuppression

For the period which this study covers, three immunosuppression regimes were used to maintain heart transplant recipients. Patients 74–89 received cyclosporin A and prednisolone [13]. Patients transplanted before April 1986 received cyclosporin A, maintaining a

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Table 1. Number of patients in the three transplant categories mismatched for *T. gondii* who acquired infection

Transplant type	Total no. of patients	Patients mismatched for *T. gondii* *n* (%)	Mismatched patients with primary *T. gondii* infection *n* (%)
Heart	303	29 (9.6)	4 (13.8)
Heart-lung	86	7 (8.1)	1 (14.3)
Single lung	4	1 (25)	0 (0)
Total	393	37 (9.4)	5 (13.5)

Table 2. Length of pyrimethamine treatment and rejection episodes in patients mismatched for *T. gondii* who received pyrimethamine prophylaxis

Patients treated with pyrimethamine	Patients (*n*)	Length of pyrimethamine treatment (weeks)	
		Mean	Range
Patients with a primary infection	5	5	3–7
Patients with no infection	32	7	0–25
Total	37	7	0–25

whole-blood level of 200–400 μg/l (as measured by Cyclo-Trak sp, Instar, Stillwater, USA), and either azathioprine (2 mg/kg per day) or prednisolone (1 mg/kg per day). In addition, a 10-day course of intravenously administered antithymocyte globulin (ATG) was started at day 2.

Heart transplant patients 151–404 were maintained on cyclosporin A, azathioprine and prednisolone as above, with a 3-day course of intravenously administered ATG following transplant. Prednisolone was tapered to 0.2–0.3 mg/kg per day after 2–4 weeks.

For heart-lung and single-lung recipients, routine immunosuppression was with azathioprine to maintain white cell count at $4–6 \times 10^9$ white cells/l blood, and orally administered cyclosporin A, maintaining whole-blood levels at 300–500 μg/l.

A rejection episode was defined as an immunologically mediated dysfunction of the transplanted organ requiring augmented immunosuppression, diagnosed on clinical, physiological and histological findings. Episodes of rejection were treated with orally administered steroids at a dosage of 0.5–1.0 mg/kg per day.

Toxoplasma gondii serological methods

Serum samples from all organ donors and recipients were screened by means of the Toxo latex agglutination test (Eiken Chemical Company of Japan, supplied by Mast Diagnostics, Bootle, UK) [1] at serum dilutions of 1 in 16 to 1 in 256. In order to maximise the detection of toxoplasma-mismatched patients, all samples from donors with a titre of 16 or more and from recipients with a titre of 32 or more were provisionally regarded as positive for *T. gondii* antibody. If a transplant patient was, by these criteria, mismatched for *T. gondii*, then serum samples from both organ donor and recipient were sent to the Toxoplasma Reference Laboratory, where dye test [4], indirect haemagglutination [3] and *T. gondii* IgM enzyme-linked immunosorbent assay [6] tests were performed. In the meantime, patients were started on a course of pyrimethamine prophylaxis, which was discontinued if they were found by the Reference Laboratory not to be mismatched for *T. gondii*. Patients were regarded as mismatched for *T. gondii* if the donor sample had a dye test titre of 8 or more and the recipient sample a titre of less than 8.

Patients were regarded as having primary *T. gondii* infection if they were seronegative before transplant by the dye test and subsequently had a fourfold or greater rise in latex agglutination, dye test and indirect haemagglutination *T. gondii* antibody titres and had *T. gondii*-specific IgM or if *T. gondii* was cultured from the patient.

Pyrimethamine prophylaxis

Heart transplant patients 1–73 did not receive pyrimethamine prophylaxis. The subsequent heart transplant and all heart-lung and single lung transplant patients mismatched for *T. gondii* were given a 6-week course of oral pyrimethamine (25 mg per day, with folinic acid cover).

Statistical analysis

Differences between groups in the number of weeks of prophylaxis was tested using the Mann-Whitney test. The number of rejection episodes in each group was treated as having a Poisson distribution and expressed as number of episodes per 100 patient-days. Differences between groups were tested using the likelihood ratio test, taking into account the length of follow-up of each patient, and adjusting for the type of transplant (i.e., heart or heart-lung/lung).

Results

A total of 331 heart, 95 heart-lung and 7 single-lung transplant recipients have been transplanted since we instituted our policy of giving prophylactic pyrimethamine to patients mismatched for *T. gondii*. However, 40 patients were excluded from evaluation because they survived less than 1 month after transplantation or the *T. gondii* status of the donor or recipient was not known. Of the remaining 393 heart, heart-lung, and single-lung transplant recipients included in this study, 65 (17%) were found to be possibly mismatched for *T. gondii* by means of the Toxo latex agglutination screening assay as we interpret it. However, when organ donor and recipient pre-transplant serum samples were assayed by the dye test, only 37 (57%) of these patients were confirmed as *T. gondii* mismatches. A total of six (9% of the 65) recipient pre-transplant serum samples were found to be false-negative and 27 (42%) donor serum samples false-positive after the dye test had been performed.

The number of patients mismatched for *T. gondii* and the number of patients in the three transplant groups who acquired primary *T. gondii* infection are shown in Table 1. There was no difference between the percentage of mismatched patients in each transplant group, nor in the percentage of patients in the three groups who acquired primary *T. gondii* infection.

The five patients who acquired primary *T. gondii* infection received a similar amount of prophylactic pyrimethamine (median 5, range 3–7 weeks) to the 32 mismatched patients who did not acquire *T. gondii* infection (median 7, range 0–25 weeks). There was no significant difference between these two values (Mann-Whitney $Z = -1.66$, $P = 0.098$; Table 2).

Pyrimethamine was generally well tolerated. In only one patient was treatment stopped because of neutropenia; in the other two patients who did not receive the full

Table 3. Effect of pyrimethamine prophylaxis on the development of *T. gondii* infection and symptoms in *T. gondii*-mismatched patients

Prophylactic pyrimethamine in mismatched patients	Total no. of patients	Patients with primary *T. gondii* infection *n* (%)	Patients with symptoms *n* (%)
Not given[a]	7	4 (57)	4 (100)
Given	37[b]	5 (14)	1 (20)

[a] Previously reported [13]
[b] One patient, scheduled to receive prophylactic pyrimethamine, did not receive any

6 weeks' course the reason was non-compliance. Interestingly, the one patient who developed symptoms received prophylactic pyrimethamine for 7 weeks.

There was no difference in the incidence of rejection or other infections in those five patients who acquired *T. gondii* infection and the 32 who did not. Two (40%) of the five patients who acquired *T. gondii* infection despite pyrimethamine prophylaxis had received steroids to treat rejection episodes in the previous month. However, the one patient who had symptomatic *T. gondii* infection 4 months after the transplantation had only one treated rejection episode, 60 days after transplantation. This is of interest, since the two patients who died of toxoplasmosis in the early part of our transplant programme and who had not received prophylactic pyrimethamine had recently had treated rejection episodes, whereas the two who survived had not [13]. Luft et al. [5] also reported that two of their four patients who acquired primary *T. gondii* infection had recently received steroids for the treatment of rejection episodes.

Discussion

Several heart transplant units have reported *T. gondii* infections in their patients. The percentage of *T. gondii*-mismatched patients who acquired primary infection was 75% in Stanford [5], 50% in Rotterdam [10] and 57% in Papworth [13], with an overall incidence of 58%. Ten (91%) of the 11 patients with *T. gondii* infection in these studies were symptomatic and 45% died. By contrast, in our group of patients who were given prophylactic pyrimethamine, only five of 37 (14%) patients acquired *T. gondii* infection, only one (20%) was symptomatic, and none died.

Since the group of patients receiving prophylactic pyrimethamine in this study underwent transplantation later in the series than those previously reported who did not [13], they cannot be directly compared. Any differences between the groups may be affected by other time-dependent changes to the patient management protocol, such as immunosuppressive treatment. However, mis-

Table 4. Timing of infection and symptoms in the nine mismatched patients who acquired *T. gondii* from their donated organ(s)

Patient no.[a] (Transplant category)	Timing of infection after transplant	Patient received prophylactic pyrimethamine?	Symptoms
1 (Heart)	3–4 Weeks	No	Fever, grand mal seizures. Patient died 42 days after transplantation
2 (Heart)	3–4 Weeks	No	Fever, pneumonia. Patient died 36 days after transplantation
3 (Heart)	3–4 Weeks	No	Persistent fever. Patient still alive 8 years after transplantation
4 (Heart)	3–4 Weeks	No	Fever, cough, loss of consciousness. Patient still alive 7 years after transplantation
5 (Heart)	*T. gondii* seroconversion was noted 3 years after transplantation. No serum samples available for 2.5 years. Infection probably arose in the 1st year after transplantation	Yes (3 weeks)	No known symptoms. Patient died 4 years after transplantation; death not related to *T. gondii* infection
6 (Heart)	*T. gondii* cultured from post-mortem specimens	Yes (5 weeks)	No known symptoms. Patient died 112 days after transplantation from rejection
7 (Heart)	*T. gondii* seroconversion was noted 4 months after transplantation	Yes (7 weeks)	4 Months after transplantation, pyrexia, headache, malaise, flu-like symptoms. Patient still alive 3.5 years after transplantation
8 (Heart-lung)	*T. gondii* seroconversion was noted 14 months after transplantation	Yes (5 weeks)	No known symptoms. Patient still alive 4.5 years after transplantation
9 (Heart)	*T. gondii* seroconversion was noted 8 months after transplantation	Yes (7 weeks)	No known symptoms. Patient still alive 1.5 years after transplantation

[a] Patients 1–4 have been previously reported [13]

matched patients given prophylactic pyrimethamine were less likely to acquire primary *T. gondii* infection and were less likely to have symptoms than those patients in the early part of our transplant programme, previously reported on [13], who were not given pyrimethamine prophylaxis (Table 3). This was not tested statistically because the patients were treated at different times and the cases were subject to other time-dependent changes.

The symptoms associated with primary donor-acquired *T. gondii* infection in our nine patients and the timing of their infection are shown in Table 4. All four patients receiving transplants in the early part of our programme who were not given prophylactic pyrimethamine had severe symptoms; two died. The only patient given prophylactic pyrimethamine who had symptomatic infection experienced pyrexia (38 °C), headache, malaise, and flu-like symptoms which lasted 2 weeks.

Our *T. gondii*-mismatched patients were scheduled to receive a 6-week course of prophylactic pyrimethamine. However, when we reviewed the data, we found that 22 (59%) of the 37 patients had received prophylaxis for more than 6 weeks. The five patients who acquired *T. gondii* infection, however, had received a shorter course of prophylactic pyrimethamine (median 5 weeks) than those who had no evidence of infection (median 7 weeks), but this difference was not statistically significant. The patients who acquired *T. gondii* infection had received prophylactic pyrimethamine for 3, 5, 5, 7 and 7 weeks respectively. One patient failed to receive any prophylactic pyrimethamine. He did not acquire *T. gondii* infection.

Only one organ donor was found to have had recent *T. gondii* infection and the recipient did not acquire *T. gondii* infection, having undergone a 6-week course of pyrimethamine. None of the patients at Papworth who acquired primary *T. gondii* infection have received hearts from donors who had *T. gondii* – specific IgM, a marker of recent infection.

The incidence of primary *T. gondii* infection in heart and heart-lung transplant recipients who received prophylactic pyrimethamine was similar (Table 1). This suggests that (unlike the situation with cytomegalovirus infection [12]) these two groups of patients have a similar risk of acquiring *T. gondii* infection if mismatched. This is most likely because *T. gondii* is principally acquired from dormant cysts in the heart muscle [11].

Sluiters et al. [10] gave their *T. gondii*-mismatched patients prophylactic spiramycin, but this did not prevent infection. By contrast, we believe that our experience has shown that pyrimethamine is effective in reducing the incidence of the primary donor-acquired *T. gondii* infection in mismatched heart and heart-lung transplant recipients.

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