

Surveillance of *Toxoplasma gondii* infection in recipients of thoracic solid organ transplants

Eleonora Sarchi, Francesca Genco, Angela Di Matteo, Barbara Castiglioni,
Lorenzo Minoli, Valeria Meroni

Infectious Diseases Department University of Pavia, Fondazione I.R.C.C.S. Policlinico San Matteo, Pavia Italy

SUMMARY

We evaluated the frequency of seroconversion for toxoplasmosis in seronegative recipients of thoracic solid organ transplants with seronegative or seropositive donors and the efficacy of chemoprophylaxis with pyrimethamine+sulfamethopirazine.

One hundred and sixty one patients seronegative for toxoplasmosis were followed-up at different intervals. Six patients out of 79 R-/D- and twelve out of 82 R-/D+ seroconverted after chemoprophylaxis interruption. There was no difference between matched and mismatched recipients as to the frequency of seroconversion which therefore could not be related to donor seropositivity. Seroconversions were almost asymptomatic. All positive recipients should be tested if symptoms of infection are present.

KEY WORDS: Toxoplasmosis, Thoracic solid organ transplantation, Prophylaxis

In Europe the seroprevalence of *Toxoplasma gondii* is high (Remington and McLeod, *et al.*, 2006). Infection with this protozoon is therefore a possible opportunistic complication in solid organ transplant recipients.

In solid organ transplantation toxoplasma infection may be transmitted via the graft from a seropositive donor to a seronegative recipient. The biological basis of this phenomenon is the propensity of *Toxoplasma gondii* to establish a latent infection in immunocompetent subjects, with "dormant" cystic forms in muscle tissue. Transmission can occur especially with heart transplantation, because of the *Toxoplasma gondii* cysts potentially present in cardiac muscle. There is good evidence that in seronegative

recipients of organs from a seropositive donor, prophylaxis with TMP-SMX immediately after transplant reduces the incidence of primary infection (Montoya and Giraldo, *et al.*, 2001, Munoz and Arencibia, *et al.*, 2003). Particularly in heart transplant the incidence is more than 50% without prophylaxis whereas it is about 5% with prophylaxis (Baden and Katz, 2003).

Moreover, the disease in seropositive patients can be caused by reactivation of a latent infection previously acquired, because of the immunodepression state.

In the period from 1st January 1989 to 31st December 2006 we studied a population of 161 solid organ recipients with negative serology for toxoplasmosis, attending Transplant Infection Surveillance Outpatients of Infectious Diseases Department University of Pavia, Fondazione IRCCS Policlinico San Matteo Pavia.

The purposes of our study were:

- 1) To estimate the frequency of seroconversion after thoracic solid organ transplant in seronegative patients and investigate whether a statistically significant difference exists

Corresponding author

Valeria Meroni
Infectious Diseases Department
University of Pavia
Fondazione I.R.C.C.S. Policlinico San Matteo
Via Taramelli 5, 27100 Pavia, Italy
E-mail: v.meroni@smatteo.pv.it

between the group with a seronegative donor (R-/D-) and the group with a seropositive donor (R-/D+).

- 2) To evaluate the efficacy of prophylaxis with pyrimethamine/sulfametopirazine (Metakelfin® 500 mg quid for 30 days) in the mismatch group and the efficacy of behaviour and diet recommendations in all seronegative patients.
- 3) To observe clinical and serological features of *Toxoplasma gondii* infection in organ transplant recipients, both primary or reactivated.

Serological assays were performed on all patients before and after transplant using ELISA IgG IgM (Diasorin Saluggia Italy) and ELFA IgG (Biomerieux Marcy L'Etoile France). In cases with an uncertain results, or very early when a seroconversion was expected, further tests were used: ISAGA IgM, Avidity IgG (Biomerieux Marcy L'Etoile France), ELISA IgA (Diasorin Saluggia Italy) and Western Blot IgG IgM (LDBIO Lyon France).

When further investigations were needed, a nested PCR with target gene AF 487580 (Clonit Milano Italy) was performed on peripheral blood and other biological sample such as endomyocardial tissue, liver tissue and pleural fluid.

We detected 161 patients seronegative for *Toxoplasma gondii*, followed for a period ranging from 2 months to 17 years after transplant, with clinical and serologic controls every 6 months. The median age at transplant was 43.7 years. Patients were divided in two groups, the first with a seronegative donor (R-/D-), the second with a seropositive donor (R-/D+).

R-/D- group included 79 patients (59 males and 20 females); 53 had heart transplant, 25 lung transplant and 1 heart-liver transplant. Hygienic and alimentary prophylaxis (namely avoiding close contact with cat litters, avoiding raw meat, sausages and eating only thoroughly washed fresh vegetables) was recommended to this group. We observed 6 seroconversions, 5 in heart transplant and 1 in lung transplant.

The R-/D+ included 82 patients (62 males and 20 females), 66 received heart transplant, 15 lung transplant and 1 heart-lung transplant. In this group prophylaxis with pyrimethamine-sulfametopirazine (500 mg orally once a day) was prescribed at transplant and continued for 30 days. In addition hygienic and alimentary prophylaxis as above was recommended. We

observed 12 seroconversions, all of them in heart transplant patients.

The difference in seroconversion rates between group R-/D- and group R-/D+ was not statistically significant ($P= 0.16$ Yates Corrected χ^2). Nevertheless the small size of sample reduced statistical accuracy.

Most of the cases with a seroconversion were asymptomatic or characterized by a mild and aspecific symptomatology, so it was not possible to identify the time of onset. Three patients presented with overt symptomatology. Serologic tests were carried out, and an active toxoplasmosis was diagnosed by a seroconversion. Two out of three patients had a donor seropositive for *Toxoplasma gondii*. In one case the donor's serological state was unknown.

All three patients had been given a 30 days with pyrimethamine-sulfametopirazine prophylaxis. Case 1 was a 26-year-old male who presented four years after heart transplant with mild fever, joint and muscle pain and axillary lymphadenopathy. He was taking cyclosporin -A (CyA), Azathioprine, and steroids; his lymphocytes T CD4+ count was 490/ μ l. He had seroconverted from the last control visit (6 months before), the results of IgG avidity test suggested a recent infection. Transesophageal echocardiography did not show cardiac involvement, histological findings on endomyocardial biopsy were normal, no cysts were detected. Later the patient developed an antibody response overlapping an immunocompetent subject.

Case 2 was a 44-year-old woman on CyA, Everolimus, steroids, with a lymphocyte T CD4+ count of 564/ μ l. She presented with fever, weakness, dyspnoea on mild efforts and inferior limb oedema, 5 years after heart transplant. The transeoesophageal echocardiography showed a depression of heart function and myocardial distress. Histological findings on endomyocardial biopsy were consistent with toxoplasmic myocarditis, with at least one *Toxoplasma* cyst inside a myocyte, surrounded by an intense inflammatory infiltration of lymphocytes and eosinophils.

Serological tests confirmed the diagnosis with a very low *Toxoplasma*-specific IgG Avidity, suggestive of actual, primary infection. PCR on peripheral blood and endomyocardial tissue was negative.

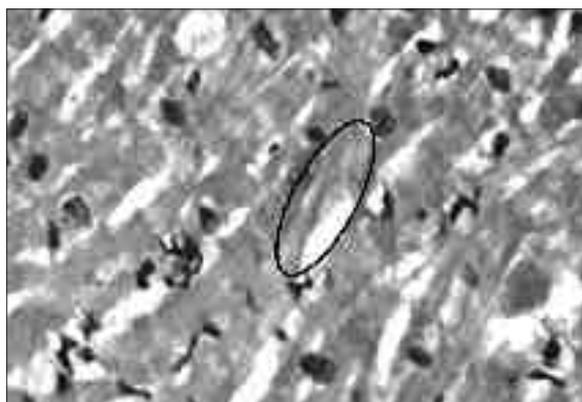


FIGURE 1 - *Toxoplasma* cyst in endomyocardial biopsy.

Case 3 was a 25-year-old male taking CyA + Mycophenolic acid + steroids, with a lymphocytes T CD4+ count of 152/ μ l. 11 months after heart transplant he was hospitalised with persistent high fever, hypotension and severe malaise. A serological test performed at one week from onset was negative for ELISA IgG and IgM, but IgM were detected by using a Western Blot assay. After a week the IgG, IgM and IgA antibody titles became positive also with ELISA, ELFA and ISAGA tests. PCR on peripheral blood was negative such as on liver biopsy and pleural fluid while it was positive on endomyocardial biopsy. The histological study on endomyocardial tissue showed at least one cyst in a myocyte and areas of inflammatory infiltration of lymphocytes and scattered eosinophyle cells more consistent with acute rejection (Figure 1).

Finally we observed two cases of infection reactivation in patients who were seropositive at transplant and received an organ from a seropositive donor. Both occurred after high dose cours-

es of steroids to treat acute rejection. The patients' immunity state was particularly compromised with very low lymphocyte T CD4+ counts (in case 5 was 42/ μ l, in case 6, was 158/ μ l).

Case 6 was a 54-year-old man treated with CyA + Micofenolic acid + steroids and he was asymptomatic. The reactivation occurred 5 months after heart transplant and was diagnosed because of antibody TITRE increase and the neosynthesis of antibodies with different antigenic specificity. The PCR on peripheral blood was negative such as histological study on endomyocardial tissue.

Case 5, who was taking Micofenolic acid + Tacrolimus + steroids, presented with severe heart depression about one year after heart transplant. This evolved into multiorgan failure and death in a few days. The endomyocardial biopsy was consistent with toxoplasmic myocarditis associated with acute cellular rejection. PCR on endomyocardial tissue resulted positive, as well as peripheral blood, suggesting a disseminated infection. The serological tests showed a sharp antibody titre increase and the appearance of new bands at the IgG Western Blot (Figure 2). Probably they represented the antibody response against "new" parasitic antigens. These could be expressed and exposed during reactivation of infection, with the protozoon evolving from the cystic to the trophozoite state (Contini C., Cultrera R. *et al.*, 2002). Other authors regard the antibody neosynthesis as expression of reinfection by a different *Toxoplasma* strain, acquired from the graft (Robert-Gangneux F., Amrein C. *et al.*, 2000).

Our experience prompted us to recommend that all candidates to a solid organ transplant should undergo a serologic screening for *Toxoplasma*

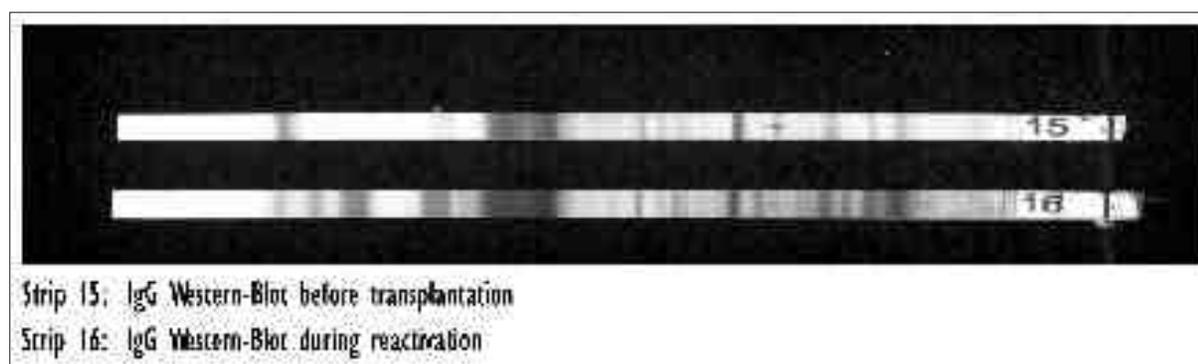


FIGURE 2 - Strip 15: IgG Western-Blot before transplantation. Strip 16: IgG Western-Blot during reactivation.

gondii infection. Seronegative candidates must be re-checked immediately before transplant, in order to avoid unnecessary chemoprophylaxis. All seronegative recipients of organs from a seronegative donor should follow behaviour and dietetic rules to avoid exogenous infection, and should be tested for *Toxoplasma gondii* antibodies every six months.

Seronegative recipients of organs from a seropositive donor are started with pyrimethamine-sulfamethopirazine immediately after transplant; moreover they should follow hygienic and dietetic rules such as those for recipients of organs from a seronegative donor. In these patients serological tests should be carried out at the end of chemoprophylaxis and then every six months.

All patients, including those who were seropositive before transplant, should be tested for *Toxoplasma gondii* serology when present with symptoms suggestive of toxoplasmic disease. Even very mild presentations and aspecific symptoms should raise a suspicion of toxoplasmic disease, particularly after antirejection treatment. The diagnostic work-up ideally includes not only traditional serological assays but also sensitive and rapid techniques like Western Blot and PCR. As far as a target organ is involved, PCR on biopsies (heart, liver, lung) and other biological fluids (pleural effusion, cerebrospinal fluid) is feasible.

REFERENCES

- BADEN, L.R., KATZ, J.T., FRANCK, L., TSANG, S., HALL, M., RUBIN, R.H., JARCHO, J. (2003). Successful toxoplasmosis prophylaxis after orthotopic cardiac transplantation with trimethoprim-sulfamethoxazole. *Transplantation* **75**, 339-343.
- CONTINI, C., CULTRERA, R., SERACENI, S., ROMANI, R., SEGALA, D., FAINARDI, E., CINQUE, P., LAZZARIN, A., DELIA, S. (2002). The role of stage-specific oligonucleotide primers in providing effective laboratory support for the molecular diagnosis of reactivated *T. gondii* encephalitis in patients with AIDS. *J. Med. Microbiol.* **51**, 879-980.
- MONTOYA, J.G., GIRALDO, L.F., EFRON, B. (2001). Infectious complications among 620 consecutive heart transplant patients at Stanford University Medical Center. *Clin. Infect. Dis.* **33**, 629-640.
- MUNOZ, P., ARENCIBIA, J., RODRIGUEZ, C., RIVERA, M., PALOMO, J., JANEZ, J., BOUZA, E. (2003). Trimethoprim-sulfamethoxazole as Toxoplasmosis Prophylaxis for heart transplant recipients. *Clin. Infect. Dis.* **36**, 932-933.
- REMINGTON, J.S., MCLEOD, R., THULLIEZ, P., DESMONT, G. (2006). Toxoplasmosis. In: Remington JS, Klein J, Wilson CB, et al., eds. Infectious diseases of the fetus and newborn infant. 6th ed., Philadelphia: WB Saunders; 947-991.
- ROBERT-GANGNEUX, F., AMREIN, C., LAVARDE, V., BOTTEREL, F., DUPOUY-CAMET, J. (2000). Neosynthesized IgG detected by Western blotting in *Toxoplasma*-seropositive heart or lung transplant recipients. *Transpl. Int.* **13** (6), 448-452.