Rev. Inst. Med. trop. S. Paulo
47(5):303-304, September-October, 2005

LETTER TO THE EDITOR

Amiens, 17/08/2005

SEVERE *Pneumocystis* PNEUMONIA IN A RENAL TRANSPLANT RECIPIENT AFTER LONG TERM MYCOPHENOLATE MOFETIL TREATMENT

Dear Sir,

We read with interest the article by AZEVEDO *et al*.[2] recently published in the REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO (*Journal of the São Paulo Institute of Tropical Medicine*)[2]. The authors suggested that mycophenolate mofetil (MMF) may have a protective role against *Pneumocystis* pneumonia (PCP). Through a retrospective study, they noticed no PCP occurrence in a cohort of renal transplant recipients treated with MMF, despite the absence of trimethoprim-sulfamethoxazole (TMS) prophylaxis. We report herein a case of severe PCP which occurred in a renal transplant recipient after long-term MMF treatment and describe the genotyping of the fungus detected in this patient.

The patient was a 29 year-old man who underwent a renal transplantation because of chronic renal failure related to Berger's disease (IgA nephropathy). The underlying conditions up to 18 months after the renal transplantation were: absence of efficient PCP prophylaxis related to the lack of patient compliance; an immunosuppressive therapy which consisted of tacrolimus (6 mg/d), MMF (1 g/d), and prednisone (15 mg/d). At the end of that period, MMF treatment was replaced with azathioprine (100 mg/d). Six months later, the patient presented an acute alveolo-interstitial syndrome with severe dyspnoea, cough and fever leading to hospitalization in an intensive care unit. The examination of a bronchoalveolar lavage (BAL) specimen by using methanol-Giemsa stain and immunofluorescence assay (Biorad, France) resulted in a diagnosis of PCP in the presence of *P. jirovecii* cystic and trophic forms and in the absence of other microorganisms. The patient was treated with cotrimoxazole at 50 mg/kg/d (dosage adapted to renal failure) and methylprednisolone at 80 mg/d. One week later, because of progressive deterioration, a second BAL was performed in which microscopic *P. jirovecii* detection remained positive. Cotrimoxazole dosage was increased to 75 mg/kg/d and associated with intravenous pentamidine at 100 mg/d. In the absence of improvement, a third BAL was performed in which *P. jirovecii* organisms were still detectable in association with *Streptococcus pneumoniae* and HSV 1. Advancing hypoxemia required connection to mechanical ventilation, increased dosages of cotrimoxazole and pentamidine (up to 100 mg/kg/d and 150 mg/day respectively), and treatment with aciclovir (15 mg/d) and cefotaxime (3 g/d). Despite these treatments, the patient died after a 27 day hospitalization. Sediments of the three subsequent BALs were stored at -80 °C for further genotyping.

A multilocus genotyping was performed. As previously described, it was based on the internal transcribed spacer (ITS) 1 and ITS 2 locus[14], the locus of the dihydropteroate synthase (DHPS)[14], which is the enzymatic target of sulfonamide drugs, and sequence analysis of the mitochondrial large sub-unit (mtLSU) rRNA locus[7,15,16]. In the three BAL specimens, a single and identical ITS 1 and 2 allele combination, B_1a_4, wild sequences of DHPS locus, and identical mtLSUrRNA sequences were observed. Thus, by using a multilocus genotyping at three independent loci, a single *P. jirovecii* genotype was identified, suggesting that the infection was clonal due to only one *P. jirovecii* strain.

In this study, we reported a case of severe PCP in a renal transplant recipient which occurred after long-term MMF treatment. It is noteworthy that the patient did not develop the infection until MMF treatment was interrupted, despite the absence of efficient PCP prophylaxis. PCP appears to be rare in patients with MMF treatment as revealed by HUSAIN & SINGH in a review article: in four controlled trials, none of a total 1068 renal transplant recipients who received MMF developed PCP[6]. These results suggest that the drug is active against *P. jirovecii* (human derived *Pneumocystis*) as it was established in rodent models for *P. carinii* (rat derived *Pneumocystis*)[12]. However, discontinuation of *P. jirovecii* prophylaxis for patients receiving MMF is not yet recommended.

We identified a single *P. jirovecii* genotype in three subsequent BALs performed during this PCP episode. The analysis of the DHPS locus was included in the multi-locus system we chose, since it has previously been suggested that *P. jirovecii* DHPS mutants have a significant impact on the outcome of PCP and patient mortality[3]. For our patient, it was improbable that death was related to such a correlation as no *P. jirovecii* DHPS mutant was detected. We identified a rare allele combination at the ITS locus. The allele combination B_1a_4 [which corresponds to Jf as described by LEE *et al.*[8] (GenBank accession numbers, AF 013815 and AF 013826)] has only been reported in six instances, five concerning PCP cases among patients with HIV[1,4,5,8] and one concerning a primary infection with *P. jirovecii* developed by infants[13]. A relationship between ITS allele combination and virulence has been suggested by MILLER & WAKEFIELD who identified the combination A_2c_1 as a cause of severe pneumonia[10]. However, the ITS allele combination B_1a_4 was not part of the panel investigated by MILLER *et al.* because of its rare occurrence.

In a previous study, by analyzing the ITS locus and sequencing a number of clones from three to five for each specimen, we observed a frequency of mixed infections up to 66%[11], showing that *P. jirovecii* infection is not clonal in most cases. Conversely, in the present study we identified a single and identical ITS allele combination in one patient with PCP, despite examination of three BAL specimens retrieved over a 15 day period, and a high number of clones (a total of 18, data not shown). Furthermore, at each of the mtLSUrRNA and the DHPS loci, identical sequences were also detected. Our genotyping approach, which combined cloning and multi-locus sequence analysis performed on iterative BAL specimens within the same PCP episode reduced the risk of underlooking genotypes and enabled us to assess the presence of a single type in our patient.

However, we should consider the possibility that initially the infection may not have been clonal. The MMF target is the inositol monophosphate dehydrogenase (IMPDH) which appears to have different variants[17] that may conserve activity despite MMF treatment. Thus, the MMF treatment may have killed off susceptible strains leaving resistant strains that corresponded to the single genotype finally detected in our patient. In this case, the patient

may have harbored the fungus, at least over a 6-month period after MMF treatment interruption and before developing the present PCP episode. This is consistent with previous studies which established that immunosuppressed patients can frequently be colonized by the fungus[11]. In fact, it cannot be ruled out that the *P. jirovecii* genotype corresponds to virulent organisms proliferating initially in a clonal context or secondarily after selection by MMF treatment. The severity of PCP in our patient may be partly related to his past history of long-term MMF treatment. The present case-report pleads in favor of *P. jirovecii* prophylaxis maintenance in transplant recipients even if the immunosuppressive therapy is based on MMF.

Anne TOTET[1]
Pierre François WESTEEL[2]
Gilles NEVEZ[1]
1. Service de Parasitologie et Mycologie médicales
2. Service de Néphrologie, Centre Hospitalier Universitaire, 80054 Amiens, France

Correspondence to: Gilles Nevez, MD, PhD, Service de Parasitologie et Mycologie médicales, Groupe Hospitalier Sud, Centre Hospitalier Universitaire d'Amiens, 80054 Amiens, France, Tel: 33 3 22 45 59 75, Fax: 33 3 22 45 56 53. Email: nevez.gilles@chu-amiens.fr

REFERENCES

1. ATZORI, C.; VALERIO, A.; FANTONI, G. *et al*. - *Pneumocystis carinii* ITS typing: doubtful evidence of genotype-related virulence. **J. euk. Microbiol., 48 (suppl.)**: 147S, 2001.

2. AZEVEDO, L.S.; CASTRO, M.C.R.; PAULA, F.J.; IANHEZ, L.E. & DAVID-NETO, E. - Mycophenolate mofetil may protect against *Pneumocystis carinii* pneumonia in renal transplanted patients. **Rev. Inst. Med. trop. S. Paulo, 47**: 143-145, 2005.

3. HELWEG-LARSEN, J.; BENFIELD,T.L.; EUGEN-OLSEN, J.; LUNDGREEN, J.D. & LUNDGREEN, B. - Effects of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of AIDS-associated *P. carinii* pneumonia. **Lancet, 354**: 1347-1351, 1999.

4. HELWEG-LARSEN, J.; LEE, C.H.; JIN, S. *et al*. - Clinical correlation of variations in the internal transcribed spacer regions of rRNA genes in *Pneumocystis carinii* f. sp. *hominis*. **AIDS**, **15**: 451-459, 2001.

5. HOSOYA, N.; TAKAHASHI, T.; WADA, M. *et al*. - Genotyping of *Pneumocystis carinii* f. sp. *hominis* isolates in Japan based on nucleotide sequence variations in internal transcribed spacer regions of rRNA genes. **Microbiol. Immunol.**, **44**: 591-596, 2000.

6. HUSAIN, S. & SINGH, N. - The impact of novel immunosuppressive agents on infections in organ transplant recipients and the interactions of these agents with antimicrobials. **Clin. infect. Dis., 35**: 53-61, 2002.

7. LATOUCHE, S.; ORTONA, E.; MAZARS, E. *et al*. - Biodiversity of *Pneumocystis carinii hominis*: typing with different DNA regions. **J. clin. Microbiol., 35**: 383-387, 1997.

8. LEE, C.H.; HELWEG-LARSEN, J.; TANG, X. *et al*. - Update on *Pneumocystis carinii* f. sp. *hominis* typing based on nucleotide sequence variations in internal transcribed spacer regions of rRNA genes. **J. clin. Microbiol., 36**: 734-741, 1998.

9. MILLER, R.F.; AMBROSE, H.E. & WAKEFIELD, A.E. - *Pneumocystis carinii* f. sp. *hominis* DNA in immunocompetent health care workers in contact with patients with *P. carinii* pneumonia. **J. clin. Microbiol., 39**: 3877-3882, 2001.

10. MILLER, R.F. & WAKEFIELD, A.E. - *Pneumocystis carinii* genotypes and severity of pneumonia. **Lancet, 353**: 2039-2040, 1999.

11. NEVEZ, G.; TOTET, A.; JOUNIEAUX, V. *et al*. - *Pneumocystis jiroveci* internal transcribed spacer types in patients colonized by the fungus and in patients with pneumocystosis from the same French geographic region. **J. clin. Microbiol., 41**: 181-186, 2003.

12. OZ, H.S. & HUGHES, W.T. - Novel anti-*Pneumocystis carinii* effects of the immunosuppressant mycophenolate mofetil in contrats to provocative effects of tacrolimus, sirolimus, and dexamethasone. **J. infect. Dis., 175**: 901-904, 1997.

13. TOTET, A.; PAUTARD, J.C.; RACCURT, C.; ROUX, P. & NEVEZ, G. - Genotypes at the internal transcribed spacers of the nuclear rRNA operon of *Pneumocystis jiroveci* in nonimmunosuppressed infants without severe pneumonia. **J. clin. Microbiol**., **41**: 1173-1180, 2003.

14. TOTET, A.; DUWAT, H.; MAGOIS, E. *et al*. - Similar genotypes of *Pneumocystis jirovecii* in different forms of *Pneumocystis* infection. **Microbiology, 150**: 1173-1178, 2004.

15. TSOLAKI, A.G.; BECKERS, P.; WAKEFIELD, A.E. - Pre-AIDS era isolates of *Pneumocystis carinii* f. sp. *hominis*: high genotype similarity with contemporary isolates. **J. clin. Microbiol., 36**: 90-93, 1998.

16. WAKEFIELD, A.E. - DNA sequences identical to *Pneumocystis carinii* f. sp. *carinii* and *Pneumocystis carinii* f. sp. *hominis* in samples of air spora. **J. clin. Microbiol., 34**: 1754-1759, 1996.

17. YE, D.; LEE, C.H. & QUEENER, S.F. - Differential splicing of *Pneumocystis carinii* f. sp. *carinii* inosine 5'-monophosphate dehydrogenase pre-mRNA. **Gene, 263***:* 151-158, 2001.