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Lifelong Prophylaxis With Trimethoprim-Sulfamethoxazole for Prevention of Outbreak of *Pneumocystis jirovecii* Pneumonia in Kidney Transplant Recipients

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Background. Outbreaks of *Pneumocystis jirovecii* pneumonia (PCP) in kidney transplant recipients are frequently reported worldwide. However, the general guidelines propose only short-term prophylaxis with trimethoprim-sulfamethoxazole after kidney transplantation. We experienced 3 PCP outbreaks in the last 10 years despite providing the recommended prophylaxis. The purpose of this study was to find a prophylaxis regimen that could successfully prevent future PCP outbreaks in immunosuppressed kidney transplant recipients. **Methods.** Occurrence of PCP at our hospital since 2004 was reviewed. A total of 48 cases were diagnosed from July 2004 through December 2014. Genotypes of *P. jirovecii* were determined in these cases. **Results.** Three PCP outbreaks by 3 different genotypes of *P. jirovecii* in each outbreak occurred with 2-year intervals in last 10 years. Molecular analysis showed that each intraoutbreak was caused by identical *P. jirovecii*, whereas interoutbreaks were caused by different genotypes. Although short-term prophylaxis was provided to all kidney recipients after each outbreak after identification of a single PCP case, additional outbreaks were not prevented because the universal prophylaxis had already been completed when new case of PCP emerged. **Conclusions.** The contagious nature of *P. jirovecii* allows easy development of outbreaks of PCP in immunosuppressed kidney transplant recipients. Although the universal short-term prophylaxis is effective in controlling ongoing outbreak, lifelong prophylaxis of kidney transplant recipients should be considered to prevent new outbreaks.

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N.G., K.F., M.O., T.Y., M.T., T.H., S.N., and Y.W. collected the samples and data. A.T.-N. conducted the molecular experiment and analysis with help from K.T., H.G., and S.O. K.T. and H.G. contributed to the study design and analysis. N.G., A.T.-N., and S.O. wrote the article. All authors participated in revising the article for important intellectual content, and approved the final version for publication.

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Outbreaks of *Pneumocystis jirovecii* pneumonia (PCP) in kidney transplant recipients have been documented worldwide in the last 2 decades.[1-7] One of the possible reasons for the outbreaks is the use of aggressive immunosuppressive therapy. In this regard, kidney transplant recipients account for the largest proportion of organ transplant recipients, and accordingly, have a greater chance of sharing time and space in the outpatient clinics. Therefore, the occurrence of a single case of PCP under such circumstances could easily result in a PCP outbreak.[8]

Reactivation of endogenous organisms is considered a potential mechanism of PCP in an immunocompromised host; however, recent studies indicate that de novo infection is the main reason rather than reactivation.[9] There are 3 sources of PCP: transmission from patients with active PCP,[10] environmental exposure,[11-13] and transmission from asymptomatic carriers.[14] *P. jirovecii* can exist ubiqitously in the hospital environment and personnels could be frequently exposed to *P. jirovecii*, especially during PCP outbreak. Advances in genetic testing of Pneumocystis-specific DNA have provided evidence for nosocomial infection of PCP.[7,15]

Prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX) against PCP is highly effective and is recommended

for HIV patients especially those with CD4 count of less than 200/μL.[16] However, long-term prophylaxis with TMP-SMX is discouraged for all kidney transplant recipients based on its renal toxicity. In fact, the majority of treatment guidelines recommend such prophylaxis to be used only just after the transplantation and for a short period of time.[17-19] However, in the case of an outbreak, some guidelines advise universal prophylaxis until the outbreak has abated, or for a minimum of 6 to 12 months.[4,7]

We experienced previously a PCP outbreak involving 27 cases, which occurred mainly in the outpatient clinic during a single year and 6 additional cases in the following 3 years. All 33 cases were caused by the same genotype of *P. jirovecii*. After the identification of the first 27 cases, TMP-SMX was given to only those recipients who had received kidney transplantation in the previous 12-month period, but not to all recipients, resulting in 6 additional cases in the following 3 years. The outbreak was finally controlled by adding a 3-month prophylaxis to all recipients (universal short-term prophylaxis). After that event, we recommended a case-guided 6-month prophylaxis for all kidney transplant recipients in the unit because the longest incubation period was 6 months.[4]

It is true that universal short-term prophylaxis is effective in controlling a single outbreak. However, we identified 2 other outbreaks thereafter with 2-year intervals between the 3 outbreaks. In this study, we analyzed the genotypes of *P. jirovecii* that caused the latter 2 outbreaks and, based on our experience, explored possible protocols that could prevent future PCP outbreaks in kidney transplant recipients under current immunosuppression.

MATERIALS AND METHODS

Study Design and Diagnosis of PCP

PCP outbreaks at our hospital since 2004 was reviewed. A total of 48 cases of PCP diagnosed between July 2004 and December 2014 underwent genotyping for *P. jirovecii*. PCP was suspected based on clinical features, such as fever, nonproductive cough, and/or dyspnea, and imaging findings (X-ray and high-resolution computed tomography scans), and high serum levels of [1–3]-β-D-glucan (Fungitec G test MK).[20] The diagnosis was confirmed by detection of *P. jirovecii* with specific staining and/or detection of *P. jirovecii* DNA by polymerase chain reaction (PCR) in bronchoalveolar lavage fluid (BALF). All BALF samples were stored at −80°C until genotyping.

Maintenance Immunosuppression in ABO Compatible Kidney Transplantation

Since May 2002, all patients who underwent transplantation were treated with intravenous basiliximab (Simulect). Furthermore, a triple-therapy regimen is used for standard maintenance immunosuppression, consisting of a steroid, a calcineurin inhibitor (cyclosporine microemulsion [CsA-ME] or tacrolimus [TAC]), and an anti-metabolite (mycophenolate mofetil [MMF] or mizoribine). Prolonged-release TAC (Gracepter) and everolimus (Certican) became available at our institution in July 2008 and January 2007, respectively. An initial dose of 500 mg methylprednisolone was administered intravenously, followed by oral dose of 60 mg, which was gradually tapered to 5 mg/d from month 3 onward. The target levels for calcineurin inhibitors were as follows: for CsA-ME (Neoral), an area under the curve in 0 to 4 hours (AUC [area under the blood concentration time curve], 0-4) of 3500 ng/h per mL for the first 3 months then tapered to a maintenance dose of 2000 ng/h per mL; and for TAC (Prograf), an AUC of 0 to 4 of 80 ng/h per mL for the first 3 months and then tapered to a maintenance dose of 50 ng/h per mL. Treatment with MMF (Cellcept) commenced at 3.0 g/d for 2 weeks then tapered to a maintenance dose of 2.0 g/d in recipients treated with CsA-ME as calcineurin. For recipients treated with TAC, MMF was administered at 2.5 g/d then tapered to 1.5 g/d due to enterohepatic circulation. Instead, a fixed-dose, concentration-controlled administration of MMF was initiated in 2012. A target exposure of 40 to 80 ng/h per mL for AUC of 0 to 12 determined using the enzyme multiplied immunoassay technique method was used.

Maintenance Immunosuppression in ABO Incompatible Kidney Transplantation

Splenectomy was performed in such patients. The immunosuppression induction regimen consisted of basiliximab, a steroid, a calcineurin inhibitor, and cyclophosphamide, which was later switched to mizoribine at 1 month. Low-dose steroid and cyclophosphamide were administered 2 weeks before transplantation surgery, and 4 rounds of double-filtration plasmapheresis were performed to remove anti-ABO antibodies at 6, 4, 2, and 1 day before transplantation. Rituximab (Rituxan) became available at our institution from 2006 to avoid splenectomy, and MMF was used instead of cyclophosphamide thereafter. The standard maintenance immunosuppression regimen used in these patients was exactly similar to that used for ABO compatible patients.

Treatment of Rejection

The protocol biopsies were performed at 12 months after transplantation, and donor-specific antibody was monitored every year. When acute rejection with elevation of serum creatinine was confirmed by biopsy, the first-line treatment was daily pulse steroid administration. Depending on the findings of biopsy examination, antithymocyte globulin or rituximab + plasma exchange was added for treatment. TMP-SMX prophylaxis was not restarted during treatment of rejection.

TABLE 1.

Patients demographics at onset of PCP (n = 48)

Age, y	48 ± 13 (21-74)
Male	45 (60%)
Time to onset from transplant, mo	29 (2–235)
Maintenance immunosuppression	
PSL + CNI + MMF	44 (92%)
PSL + MMF	3 (6%)
PSL + CNI + FTY	1 (2%)
Use of rituximab	5 (10%)
Contact with PCP	39 (81%)
Expected incubation period, d	70 (6–258)
Genotype of *P. jirovecii* (Bi/Eb/Ne)	14/3/10
β-D-glucan, pg/mL	722 ± 753 (13.2–3720)
Bronchoscopy for diagnosis	41 (85%)
P. jirovecii smear positive (BALF)	20 (49%)
P. jirovecii PCR positive (BALF)	41 (100%)
Coinfection with CMV	29 (60%)

Data are mean ± SD, median (range) values, or number (%).

PSL, prednisolone; CNI, calcineurin inhibitor; FTY, sphingosine 1-phosphate receptor agonist; CMV, cytomegalovirus.

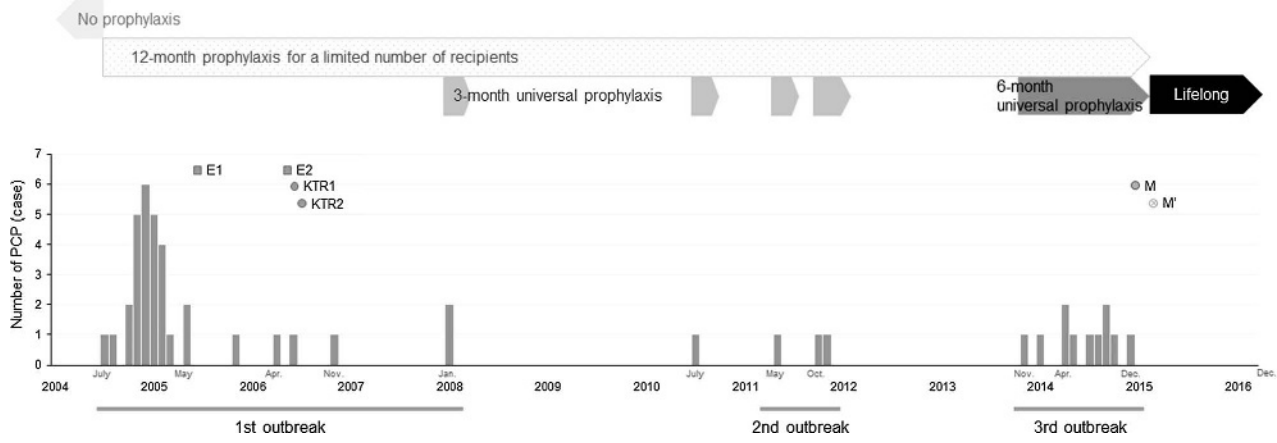

FIGURE 1. PCP outbreaks and prophylaxis regimens. TMP-SMX was administered 3 times a week for PCP prophylaxis. Surveillance was conducted of environmental (E1 and E2) and mouthwash samples obtained from asymptomatic kidney transplant recipients (KTR1 and KTR2) and medical staff (M). Genotypes Ip (E1), In (KTR1), and Bi (E2 and KTR2) were detected during the first outbreak. The mouthwash survey obtained from a medical staff member (M) demonstrated detection of type Ne at third outbreak, while *P. jirovecii* was not detected from the same medical staff member 3 months later (M′).

Genotyping of *P. jirovecii* and Phylogenetic Tree Analysis

The method of genotyping was described in detail previously.[4] Briefly, 530 bp of internal transcribed spacer (ITS) 1 and 2, each containing a region of the nuclear operon (5.8S) of *P. jirovecii*, was amplified by 2 rounds of PCR. The sequences of the primers used were as follows: N18FS (sense primer for the first PCR), 5′-GGT CTT CGG ACT GGC AGC-3′; N26SRX (antisense primer for the first PCR), 5′-TTA CTA AGG GAA TCC TTA-3′; ITSF3 (sense primer for the nested PCR), 5′-CTG CGG AAG GAT CAT TAG AAA-3′; and ITS2R3 (antisense primer for the nested PCR), 5′-GAT TTG AGA TTA AAA TTC TTG-3′′. Nested PCR products were directly sequenced by using an automated 3730 DNA Analyzer (Applied Biosystems). The genotype of *P. jirovecii* was determined using the obtained sequences according to the scores of combination of ITS1 and ITS2, as described by Lee et al.[15] The results of alignment were analyzed by the Clustal W program,[21] and the distance matrixes were generated by the neighbor-joining method. Bootstrap re-sampling (500 data sets) of multiple alignments was performed to test the statistical robustness of the tree. Phylogenetic analysis was conducted using Molecular Evolutionary Genetics Analysis software (version 6.0).[22]

Drug Resistance Against TMP-SMX Analysis

SMX inhibits dihydropteroate synthase (DHPS), an integral enzyme in folate synthesis, and TMP inhibits the subsequent step of dihydrofolate reductase (DHFR). DNA sequencing analysis of *DHPS* and *DHFR* genes was performed as described in detail previously.[23,24] The following mutations were recognized as resistance mutations: Thr55Ala and Pro57Ser in *DHPS* gene,[23] and Ala67Val and Cys166Tyr in *DHFR* gene.[24]

RESULTS

PCP Outbreaks at Our Kidney Transplant Unit

A total of 48 cases of PCP were diagnosed in the 3 outbreaks. One case was a sensitized pretransplant, and 10 had ABO incompatibility to donor blood type. HLA mismatches range was 2.3 ± 1.2. Forty received kidneys from living donors. The first outbreak involved 33 cases and lasted 42 months (July 2004 to January 2008). The genotype of *P. jirovecii* at the first outbreak was Bi. Details of the first outbreak have already been published.[4] A 6-month prophylaxis with TMP-SMX 3 times a week was recommended at that time; however, 3-month prophylaxis provided to all recipients (universal short-term prophylaxis) was sufficient to control the outbreak. The second outbreak consisted of 3 cases in 6 months (May 2011 to November 2011), and the third included 11 cases and lasted 14 months (November 2013 to December 2014). All 48 PCP patients received immunosuppressive therapy. Patient demographics at onset are listed in Table 1. The regimens for PCP prophylaxis changed over time (Figure 1). Basically, prophylaxis with TMP-SMX (80 mg TMP/400 mg SMX) was provided 3 times a week. The prophylaxis dose was reduced to twice a week in patients with serum creatinine greater than 2 mg/dL. In patients who developed side effects (eg, nephrotoxicity, hyperkalemia, or bone marrow suppression), the TMP-SMX dose was reduced to twice a week or once a week even in those with normal graft function. PCP prophylaxis was completed in almost all the recipients at our kidney transplant unit. Only 1 patient developed acute rejection before PCP and 1 patient required dose adjustment for renal insufficiency. Figure 2 showed time

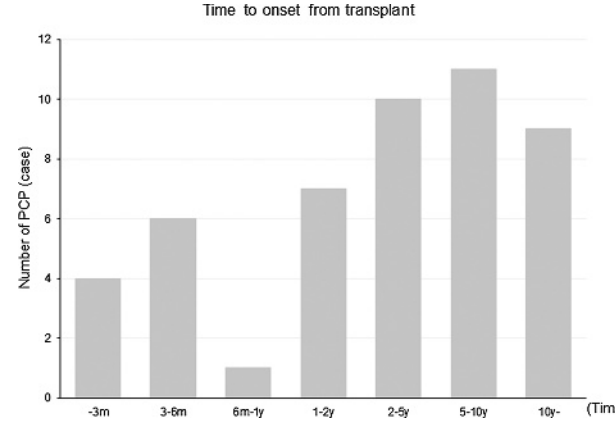

FIGURE 2. Time to onset of PCP after kidney transplantation. All PCP cases were not on prophylaxis at the onset of PCP.

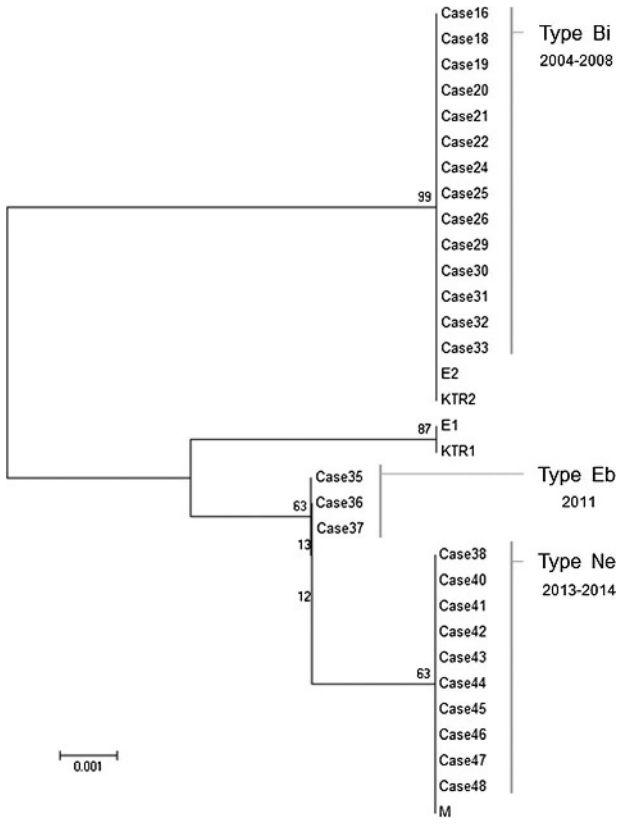

FIGURE 3. Results of phylogenetic analysis of *P. jirovecii* DNA. Tree based on the sequences of 530 bp of the *ITS1* + *5.8S* + *ITS2* gene region. The phylogenetic tree was determined by the neighbor-joining method, in which the numbers on the branches are bootstrap values with 500 bootstrap samples. Scale bar = 0.001 substitutions. E1 and E2: environment swabs; KTR1 and KTR2: mouthwash samples obtained from asymptomatic KTRs; M: mouthwash sample from the medical staff member.

to develop PCP after kidney transplantation. All 48 patients did not receive any prophylaxis at the onset of PCP. Among them, PCP occurred in 11 patients after the transplantation within 12 months or less when the period was at risk for PCP. It was surprising to note that 37 patients (77%) had PCP later than 12 months after the transplantation.

Case 34 (July 2010)

This case had been followed up at another hospital and was transferred to our emergency room after acute-onset dyspnea. None of the recipients, except those within 1 year of kidney transplantation, was under TMP-SMX prophylaxis at either hospital. Subsequently, after identification of case 34, all the 33 recipients received the 3-month prophylaxis. Unfortunately, the clinical sample was missing, and accordingly, genotype testing could not be performed in this case.

The Second Outbreak (Cases 35-37: May 2011 to November 2011)

Case 35 was diagnosed 10 months after case 34. None of the recipients, except those within 1 year of kidney transplantation, received prophylaxis at that time because the universal 3-month prophylaxis was completed after case 34. Case 35 became a trigger of the small second outbreak in 4 recipients. The genotype of *P. jirovecii* in this outbreak was Eb (Figure 3). It was controlled with a universal 3-month prophylaxis after each case of PCP.

The Third Outbreak (Cases 38-48; November 2013 to December 2014)

Case 38 was responsible for the third outbreak. Again, none of the recipients, except those within 1 year of kidney transplantation, was under prophylaxis because the universal 3-month prophylaxis was completed after the second outbreak. The genotype of *P. jirovecii* was Ne in all cases (Figure 3). This time, all kidney transplant recipients received a 6-month prophylaxis with TMP-SMX 3 times a week whenever a new case of PCP was identified. However, some of the recipients objected to the 6-month prophylaxis and, therefore, the third outbreak consisted of 11 cases and lasted 14 months. No drug resistance against TMP-SMX associated with amino acid mutations was identified in 11 of 13 cases that were successfully analyzed in the second and third outbreaks (data not shown). Finally, lifelong prophylaxis with TMP-SMX in all recipients started from December 2014. All recipients were advised to adhere to the prophylaxis regimen. None of the PCP cases who were followed up over 20-month period after a lifelong prophylaxis strategy developed PCP again.

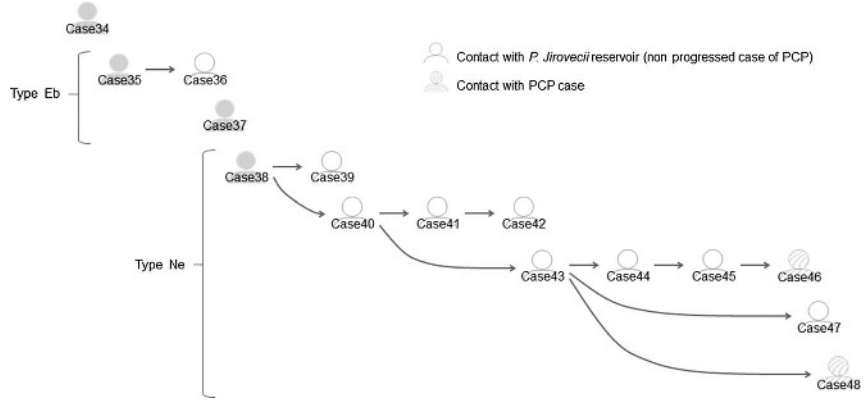

FIGURE 4. Transmission map of PCP in the second and third outbreaks. Recipients shared the same waiting room of our department. Human-to-human transmission was suggested in 11 cases based on dates of visits to the Outpatient Clinic. All cases, except case 37, had contact opportunity with a PCP carrier.

Outcome of PCP

There were 6 deaths (13%; 4 cases in the first outbreak and 2 cases in the third outbreak) and 3 graft losses (6%) in patients with PCP. Ten patients (21%) required treatment in the intensive care unit due to severe progression of PCP, including noninvasive positive pressure ventilation in 3 cases and endotracheal intubation in 7 cases.

DISCUSSION

We experienced 3 PCP outbreaks in a single kidney transplant center over a period of 10 years. The first and second outbreaks were controlled by a universal short-term prophylaxis, which effectively controlled each of the 2 outbreaks. However, at the onset of the second and third outbreaks, none of the patients, except those who received kidney transplantation within 1 year, was under the prophylaxis. Molecular analysis demonstrated that each intraoutbreak was caused by identical *P. jirovecii*; whereas interoutbreaks were by different genotypes, indicating that each outbreak was a completely separate event. Our experience presented here documented that it was impossible to prevent a new outbreak by a universal short-term prophylaxis. Based on our environmental surveys and patient-to-patient transmission map analysis (Figure 4),[4] *P. jirovecii* is ubiquitous and highly contagious for susceptible hosts, especially when PCP developed in a few patients. Currently, PCP prophylaxis for at least 4 months is recommended after transplantation in the European Renal Transplant Guidelines,[20] and for 3 to 6 months, or 6 to 12 months in the Kidney Disease Improving Global Outcomes guideline.[18,19] However, these guidelines are only directed at prevention of PCP in individuals soon after transplantation, not for prevention of a PCP outbreak.

Japanese kidney recipients have a greater chance of sharing time and space in outpatient clinics. This situation is similar to European outpatient clinics. The occurrence of a single case of PCP under such circumstances could easily result in a PCP outbreak. Several studies on PCP outbreak were reported from Europe. Only 1 study was reported recently from North America.[25] Based on the current situation and our experience, prolonged universal prophylaxis in kidney transplant recipients is applicable; otherwise, the PCP mortality rate likely increases in recipients in the future, as it did in past decades.

Our study has certain limitations. First, the observation period was too short to confirm the efficacy of lifelong prophylaxis with TMP-SMX 3 times a week for kidney transplant recipients. Only 2 years had passed since implementation; longer follow-up period should resolve this limitation. Though longer observation periods may provide stronger evidence, PCP outbreaks certainly occur in many kidney transplant units worldwide, exposing the affected recipients to risk of death or graft loss. Therefore, transplant nephrologists and transplant infectious disease physicians need to know our experience in spite of the limited evidence. The second limitation is that the exact dose of TMP-SMX used for prophylaxis has not yet been determined. Given that hyperkalemia may result from deterioration of graft function, lifelong prophylaxis with a lower dose of TMP-SMX may be required. Further studies should investigate renal safety issue in kidney transplant recipients.

We conclude that universal short-term prophylaxis did not prevent intermittent new PCP outbreaks caused by different genotypes, whereas no PCP outbreaks were observed over a 20-month period after a lifelong prophylaxis strategy was adopted. Therefore, we decided to implement lifelong prophylaxis in kidney transplant recipients under current immunosuppressive therapy in our unit.

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