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Pneumocystis jirovecii pneumonia in solid organ transplant recipients: a descriptive analysis for the Swiss Transplant Cohort

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Abstract

Background. Descriptive data on *Pneumocystis jirovecii* pneumonia (PJP) in solid organ transplant recipients (SOTr) in the era of routine *Pneumocystis*-prophylaxis are lacking.

Methods. All adult SOTr between 2008-2016 were included. PJP was diagnosed based on consensus guidelines. Early-onset PJP was defined as PJP within the 1st-year-post-transplant.

Results. 41/2842 SOTr (1.4%) developed PJP (incidence rate: 0.01/1000 person-days) at a mean of 493-days post-transplant: 21 (51.2%) early vs 20 (48.8%) late-onset PJP. 2465 (86.7%) SOTr received *Pneumocystis*-prophylaxis for a mean 316 days. PJP incidence was 0.001% and 0.003% (log-rank<0.001) in SOTr with and without *Pneumocystis*-prophylaxis, respectively. PJP was an early event in 10/12 (83.3%) SOTr who did not receive *Pneumocystis*-prophylaxis and developed PJP, compared to those patients who received prophylaxis (11/29, 37.9%; P-value: 0.008). Among late-onset PJP patients, most cases (13/20, 65%) were observed during the 2nd year post-transplant. Age ≥65 years (OR: 2.4, P-value: 0.03) and CMV infection during the first 6 months post-SOT (OR: 2.5, P-value: 0.006) were significant PJP predictors, while *Pneumocystis*-prophylaxis was protective for PJP (OR: 0.3, P-value: 0.006) in the overall population. Most patients (35, 85.4%) were treated with trimethoprim-sulfamethoxazole for a mean 20.6 days. 1-year mortality was 14.6%.

Conclusions. In the *Pneumocystis*-prophylaxis-era, PJP remains a rare post-transplant complication. Most cases occurred post-PJP-prophylaxis-discontinuation, particularly during the 2nd-year-post-transplant. Additional research may help identify indications for *Pneumocystis*-prophylaxis prolongation.

Background

Pneumocystis jirovecii pneumonia (PJP) is a rare complication in solid organ transplant recipients (SOTr), as a result of routine *Pneumocystis*-prophylaxis applied in most transplant centers (1-7). In the absence of effective prophylaxis, 5-15% SOTr may develop PJP post-transplant (2, 4, 8-10). Most experts and existing guidelines agree that *Pneumocystis*-prophylaxis should be administered for 12 months (Europe) to indefinitely (United States of America, USA) in lung and heart transplant recipients, and for 3-6 months (Europe) or 6-12 months post-transplant (USA) post-kidney or liver transplant (4, 8, 11, 12). Furthermore, reinstitution or prolongation of *Pneumocystis*-prophylaxis may be required in SOTr with persistently high immunosuppression, prior history of PJP, recent rejection episodes or cytomegalovirus (CMV) infection (4, 8, 13, 14). However, large variability in prophylactic strategies exists across different SOT centers worldwide, which may impact the epidemiology of this infection post-transplant (1, 7, 15). We performed a retrospective study to describe the

epidemiology, timing, risk factors and outcomes of PJP using the Swiss Transplant Cohort Study (STCS) between 2008 and 2016.

Methods

Study design and objectives. The STCS is a prospective national cohort, in which all SOTr in Switzerland, who sign a written informed consent are registered, representing >95% of SOTr (16). Transplant activity is shared between six main institutions: all centers perform kidney transplants, whereas heart, liver, lung and pancreas transplants are performed in three (Bern, Lausanne and Zürich), three (Bern, Geneva and Zürich), two (Lausanne and Zürich) and two (Geneva and Zürich) centers, respectively. We performed an observational retrospective cohort study to describe the incidence, risk factors and outcomes of PJP in this multicenter cohort of SOTr. All consecutive adult (≥18 years) patients who received a first SOT (heart, kidney, liver, lung, pancreas, or combined) between 01.05.2008 and 01.05.2016 were included in this study. Patients were censored for death, graft loss, loss-to-follow-up, or consent withdrawal. For patients without a censoring event, a minimum 6-month follow-up post-SOT was required for study inclusion. Patients were excluded if they received pancreatic islets or small bowel or had >1 SOT during the study period.

Pneumocystis prophylaxis. Although *Pneumocystis*-prophylaxis guidelines are not universal in Switzerland, prophylaxis is generally provided for 12 months to lifelong in lung recipients, 6 months in heart and kidney transplant recipients, and in case of severe immunosuppression for liver recipients. In case of rejection episodes, treatment with thymoglobulin or intensification of immunosuppression and/or CMV infection/disease prophylaxis is further prolonged.

Data collection. Data collection was performed in a two-step approach. All patients were identified using the STCS database. The following data were directly retrieved from the database: (i) demographics (age, gender), (ii) baseline SOT variables: SOT type, transplant center, year of transplant, type of donor (living vs. cadaveric), induction immunosuppression, cytomegalovirus (CMV), Epstein-Barr virus (EBV) and toxoplasmosis donor and recipient serology status, (iii) baseline (at the time of SOT) comorbidities: diabetes mellitus, chronic renal insufficiency, and body-mass index (BMI) at the time of transplantation, (iv) maintenance immunosuppression administered for ≥7 days and acute organ rejection during the first 6 months post-transplant, and (v) post-transplant complications: rejection (cellular or antibody-mediated) and CMV infection and/or disease requiring treatment initiation with a systemically administered CMV-active agent during the first year post-

SOT. *Pneumocystis*-prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX), atovaquone, dapsone, or pentamidine initiated during the first 3 months post-transplant and administered for ≥7 days was recorded. Additional data pertaining to PJP were retrospectively extracted from hospital charts using a case-report form, including: (i) radiological findings, (ii) histopathology, (iii) microbiology, including direct microscopy, polymerase-chain reaction (PCR) and serum b-D glucan, and (v) administered treatment.

Definitions. PJP diagnosis was based on consensus guidelines, requiring a positive direct microscopy by immunofluorescence on induced sputum or bronchoalveolar lavage (BAL) and/or a positive PCR assay on a BAL specimen (17). The day the first positive clinical sample for *Pneumocystis* spp. was obtained was considered as the day of PJP diagnosis. For the purposes of this study baseline chronic renal insufficiency was defined as serum creatinine ≥1.5mg/dl and/or requirement for hemodialysis. Primary *Pneumocystis*-prophylaxis was defined as any *Pneumocystis*-active prophylaxis initiated during the first 3 months post-transplant and administered for ≥7 days. Early- and late-onset PJP were defined as an infection diagnosed ≤ and >365 days post-transplant, respectively. CMV infection and disease were diagnosed based on prior published guidelines (18, 19). Due to the small number of cases of CMV disease, CMV infection and disease were considered together in all analyses performed for the purposes of this study. Acute rejection was defined for each organ following standard international guidelines (20).

Statistical analysis. Standard descriptive statistics were used to summarize the study population characteristics. The Fisher's exact or chi-square tests were used for categorical variables and Student's t -test for continuous variables. Continuous variables are presented as mean, with standard deviation (SD) and range. Cumulative incidences of PJP among different SOT categories, transplant centers and year of transplantation were estimated from first day post-transplantation to PJP during the study period, censoring for death, graft loss, and loss of follow-up. Logistic regression was used to identify risk factors for PJP. Independent variables with $P<0.12$ in univariable analyses were subsequently entered in a backward stepwise fashion into a multivariable logistic regression model with mixed effect. Results are presented as odds ratios (OR) with 95% confidence intervals (CI). Collinearity among independent variables was assessed using variance inflation factors (VIFs), with VIF values >3 suggesting the presence of significant collinearity. The Pearson correlation coefficient was additionally used to determine the strength of possible correlations between independent variables. The overall mortality was analyzed using Kaplan-Meier survival curves. The log-rank test was used to compare survival distribution between groups. A two sided test was

performed and a P-value<0.05 was considered to be statistically significant. Data were analyzed using STATA 14 statistical software.

Results

Incidence. The study population of 2842 SOTr included 1667 (58.7%) kidney, 567 (20.0%) liver, 251 (8.8%) lung, 204 (7.2%) heart, 85 (3.0%) kidney-pancreas, and 68 (2.4%) combined transplant recipients. Forty-one (1.4%) patients developed PJP with an overall incidence rate of 0.01/1000 person-days (95% confidence interval (CI): 0.009, 0.02). The baseline patient characteristics are shown in **Table 1**. Incidence rates for PJP were 0.02/1000 person-days (95% CI 0.01, 0.06) for heart, 0.01 (95% CI 0.01, 0.02) for kidney, 0.009/1000 person-days (95% CI 0.001, 0.07) for kidney-pancreas, 0.006/1000 person-days (95% CI 0.002, 0.02) for liver, and 0.004/1000 person-days (95% CI 0.0005, 0.03) for lung SOTr, respectively (log-rank: 0.08) (**Figure 1a** and **Table 2**).

Incidence rate was significantly higher at center-2 (0.06/1000 person-days, 95% CI 0.04, 0.09) compared to all other centers (0.005, 95% CI 0.003, 0.008/1000 person-days; log-rank<0.001; **Figure 1b**). Notably, more than half (27/41, 65.8%) of PJP cases occurred at center-2 compared to other centers (14/41, 34.2%; P-value: <0.001). Fewer patients (328/395, 83.0%) at center-2 received *Pneumocystis*-prophylaxis compared to other centers (2137/2447, 87.3%; P-value: 0.02). Whereas administration of primary *Pneumocystis*-prophylaxis did not significantly change in all other centers during the study period (P-value: 0.64), there was an increase in the proportion of patients who received primary *Pneumocystis*-prophylaxis at center-2 from 77.2% (200/259 patients) in 2008-2012 to 94.1% (128/136 patients) in 2012-2016 (P-value<0.001; **Figure 1c**). The number of PJP cases at center-2 dropped from 10.0% (26/259 patients) in 2008-2012 to 0.7% (1/136; P-value<0.001) in 2013-2016, reaching the level of other centers (**Figures 1c** and **d**).

Pneumocystis prophylaxis. The vast majority (2465, 86.7%) of SOTr received primary *Pneumocystis*-prophylaxis for a mean duration of 316 days (SD: 526, median: 176 days, range 0, 5944). Primary *Pneumocystis*-prophylaxis was not administered in 377 (13.3%) patients: 7 (1.8%) combined, 35 (9.3%) heart, 112 (29.7%) kidney, 4 (1.1%) kidney-pancreas, 213 (56.5%) liver, and 6 (1.6%) lung transplant recipients. PJP occurred more frequently among them (12/377, 3.2%) compared to SOTr who received *Pneumocystis*-prophylaxis (29/2465, 1.2%; P-value=0.002). PJP incidence rate was

0.01/1000 person-days (95% CI 0.007, 0.01) in patients with and 0.03/1000 person-days (95% CI: 0.02, 0.05) in patients without primary *Pneumocystis*-prophylaxis (log-rank<0.001: **Figure 2a**). PJP was an early event, with most events occurring during the six months following SOT in the majority of SOTr who did not receive primary *Pneumocystis*-prophylaxis and developed PJP (10/12, 83.3%), compared to those patients who received prophylaxis (11/29, 37.9%; P-value: 0.008; **Figure 2b**). Among patients with late-onset PJP, most cases (13/20, 65%) were observed during the 2nd year post-transplant. There were no breakthrough PJP cases during administration of *Pneumocystis*-prophylaxis.

Duration of prophylaxis was longer in lung (mean: 1117 days, SD: 52, 95% CI 1015, 1218) compared to other SOTr (mean: 238, SD: 8, 95% CI 222, 254; P-value<0.001; **Figure 2c).** Mean duration of primary *Pneumocystis*-prophylaxis was 671 days (SD: 716, median: 376, range: 0, 3791) in heart transplant recipients, 226 days (SD: 373, median: 177, range: 0, 5444) in kidney and 117 days (SD: 279, median: 65, range: 0, 3410) in liver transplant recipients, respectively. Prophylaxis was stopped in the vast majority of kidney (943/1655, 57%) and liver (482/563, 85.6%) transplant recipients by 6-months post-transplant. By 1-year post-transplant, prophylaxis was stopped in almost all recipients of a combined, kidney, kidney-pancreas and liver transplant. In contrast, more than half of heart (105/199, 52.8%) and lung (191/251, 76.1%) transplant recipients remained on prophylaxis by 1-year post-transplant (log-rank<0.001; **Figure 2d**).

Timing, Diagnosis and Treatment of PJP **(Table 2).** PJP occurred at a mean of 493 days (SD: 438, median: 363, range: 67, 1915) post-transplant. Twenty-one (of 41, 51.2%) cases of PJP occurred during the 1st year post-transplant vs 20 (48.8%) thereafter. Induced sputum and BAL specimens were obtained in 4 (9.8%) and 37 (90.2%) patients, respectively. Histopathology was performed in only 3 (7.3%) patients. Direct microscopy by immunofluorescence was positive in 37 (of 41, 90.2%) patients. A PCR was performed and found positive in 8 (of 41, 19.5%) patients: 4 (of 8, 50%) patients were diagnosed based on a positive PCR result only and 4 (50%) patients had a concomitant positive immunofluorescence test (one patient had a histopathology positive result as well). A total of 34 (82.9%) patients had a chest-computed tomography (CT) performed and 8 (19.5%) patients underwent a chest XR for the diagnosis of PJP. Ground-glass opacities were the most frequently identified radiologic finding (29, 70.7%), followed by non-specific infiltrates (17, 41.5%), nodular lesions (11, 26.8%) and pleural effusions (4, 9.8%). Among 35 patients with known absolute

lymphocyte count (ALC) within 7 days of PJP diagnosis, the mean ALC was 548 cells/mm^3 (SD: 411, median: 440, range: 50, 1930). The vast majority of patients (35, 85.4%) were treated with TMP-SMX, followed by clindamycine-primaquine (5, 12.2%) and pentamidine (1, 2.4%). Treatment was changed in 14 (34.2%) patients: in 11 (of 14, 78.6%) patients from intravenous to orally administered TMP-SMX and in 3 (21.4%) patients from TMP-SMX to another agent. Treatment duration was at a mean of 20.6 days (SD: 5.1, median: 21 days, range 5, 34). Twenty-five (of 34 with available data, 73.5%) patients received concomitant treatment with corticosteroids for a mean duration of 26.3 days (SD: 20.1, median: 21 days, range: 10, 90).

Risk factor analysis for PJP. Multivariable analyses on the overall patient population identified transplantation at center-2 (OR: 11.0, 95% CI 4.6, 26.1, P-value<0.001) and kidney transplant (OR: 3.5, 95% CI 1.3, 9.3, P-value: 0.01) as significant risk factors for PJP (**Table 3**). Transplantation during 2013-2016 (OR: 0.14, 95% CI 0.03, 0.6, P-value: 0.008) was protective for PJP. There was no significant collinearity between independent variables, with a mean VIF=1.0. However, the Pearson correlation coefficient identified possible associations between (i) SOT-type and: age (r=0.11, P-value<0.001), SOT-center (r=0.10, P-value<0.001), mTOR-inhibitor administration (r=-0.18, P-value<0.001), and *Pneumocystis*-prophylaxis (r=0.23, P-value<0.001), and (ii) SOT-center and mTOR-inhibitor administration (r=0.19, P-value<0.001). In addition, administration of primary PJP prophylaxis (particularly for center 2) changed during the study period. Hence, another model was created excluding SOT-center, SOT-type and SOT-year. In this model, age ≥65 year was a significant predictor for PJP (OR: 2.4, 95% CI 1.1, 5.5, P-value 0.03), followed by CMV infection/disease (OR: 2.5, 95% CI 1.2, 5.4, P-value 0.006). Administration of primary *Pneumocystis*-prophylaxis (OR: 0.3, 95% CI 0.14, 0.7, P-value: 0.006) was protective for PJP.

Considering the significantly more cases of PJP at center-2, separate risk factor analyses were performed for all other centers after excluding center-2 and for center-2 alone (**Table 4**). When patients from center-2 were excluded, there was a trend for age ≥65 year (OR: 2.9, 95%CI 0.9, 1.1, P-value: 0.07) and rejection episode during the first 6 months post-transplant (OR: 2.7, 95%CI 0.9, 7.8, P-value: 0.07) to be PJP predictors in multivariable analyses. Univariable analyses performed on patients enrolled at center-2 alone revealed age ≥65 year and a kidney transplant as significant PJP predictors, while an SOT during 2013-2016 and administration of *Pneumocystis*-prophylaxis during the first 3 months post-transplant appeared to be protective. In multivariable analyses, a kidney transplant (OR: 5.6, 95%CI 2.1, 15.1, P-value: 0.001) was the most significant PJP predictor, while

transplantation in 2013-2016 was protective (OR: 0.08, 95%CI 0.01, 0.6, P-value: 0.02). Considering the lack of *Pneumocystis*-prophylaxis administration during the first part of the study (2008-2012) at center-2, mainly observed in kidney SOTr, SOT type and time were subsequently removed from the model. In these repeat analyses (multivariable analysis-II), only age ≥65 year (OR: 2.4, 95%CI 1.0. 5.6, P-value: 0.05) remained a significant PJP predictor, while administration of primary *Pneumocystis*-prophylaxis was protective for PJP (OR: 0.4, 95%CI 0.17, 0.9, P-value: 0.04).

Outcomes. Two (of 41, 4.9%) patients with PJP died by 12-weeks post-PJP diagnosis, with 1-year overall mortality of 14.6% (6/41 patients). One patient died during treatment for PJP with uncontrolled infection, while the other patient died after completion of PJP treatment, without evidence of active infection at the time of death. Overall, 11 (of 41, 26.8%) patients with PJP vs 443 (of 2801, 15.8%) patients without PJP (log-rank: 0.36) were dead at the end of the study-follow-up. Survival did not significantly differ between centers (log-rank: 0.17) or year of transplantation (log-rank: 0.32). Investigation of mortality predictors among the 41 SOTr with PJP failed to identify any significant associations between independent variables (including antibiotic treatment type and duration or administration of corticosteroids) and survival in univariable analyses (data not shown).

Discussion

In this large multi-center 8-year cohort of SOTr between 2008 and 2016 PJP remains a relatively rare event post-transplant with a wide time-distribution, depending on the administration of primary *Pneumocystis*-prophylaxis.

Effective prophylaxis has significantly decreased the incidence of PJP in SOTr from 5-15% to 0.3-2.5% (1, 3, 6, 7, 9, 15). We report an overall incidence rate of 0.01/1000 person-days for PJP in a cohort of almost 3000 SOTr with a mean follow-up time of 3 years. This set of data from the 2000-2010's confirm that PJP has become a rare event post-SOT, mostly due to administration of effective prophylaxis. Indeed, our data provide a "snapshot" of current primary *Pneumocystis*-prophylaxis administration practices and its effects. More than 85% of patients in this cohort received primary *Pneumocystis*-prophylaxis for variable duration. Consistent with current recommendations, administration of prophylaxis was longer for heart and lung transplant recipients and discontinued in >90% of non-cardiothoracic SOTr by 1-year post-transplant (3, 5, 6, 21). Among SOTr who received

primary *Pneumocystis*-prophylaxis most cases were observed after the 1st year post-transplant, predominately clustering during the 2nd year post-transplant after prophylaxis was discontinued. A similar shift in the timing of PJP in SOTr from the 1st to the 2nd year post-transplant has been recently described by Iriart *et al* and been attributed to administration of effective early-post-transplant *Pneumocystis*-prophylaxis (2, 3). Furthermore, multivariable analyses identified transplantation in the first part of the study-period (2008-2012) as a significant predictor for PJP. Hence, the longer SOTr are alive post-transplant the higher the likelihood they may develop PJP. Further studies are required to identify risk factors that may help stratify SOTr in need for prolongation or re-institution of *Pneumocystis*-prophylaxis later post-transplant (2, 3, 15).

Lack or inconsistent administration of *Pneumocystis*-prophylaxis have been, prior, associated with high rates of PJP (22-29). Almost 15% of SOTr in this cohort did not receive *Pneumocystis*-prophylaxis. Significantly higher rates of PJP occurring predominately during the 1st year post-transplant were observed in this patient-group. The importance of *Pneumocystis*-prophylaxis was nicely illustrated in the case of center-2. During the first part of the study-period, PJP incidence was significantly higher at that center, where primary prophylaxis was not routinely administered. When prophylactic strategies changed to include >90% of SOTr, the incidence of PJP at center-2 rapidly dropped to the level of other centers. In an era, during which PJP-prophylaxis has become the standard of care, these data represent a valuable reminder of the importance of timely administration of effective *Pneumocystis*-prophylaxis in SOTr. Indeed, primary P*neumocystis*-prophylaxis was identified as a protective factor against PJP in multivariable analyses, specifically for center-2 (**Table 4**).

Consistent with prior reports, older age (particularly **≥**65 year-old patients) emerged as a significant risk factor for PJP (2, 3, 6, 30). As previously described, CMV infection/disease was also identified as an independent predictor of PJP in multivariable analyses on the overall study population (2, 3, 6, 7). However, the effect of CMV infection/disease on PJP was diluted in the separate analyses performed based on different SOT-centers (**Table 4**). There was a trend for administration of mTOR-inhibitors as maintenance immunosuppression during the first 6 months post-transplant as a predictor of PJP. A probable association between administration of sirolimus and infectious complications, including PJP, has been previously described (10, 30-33). This may, in part, be attributed to the impact of mTOR inhibition on T- and B-cell immunity (34, 35). Moreover, a recently published meta-analysis

suggested that administration of sirolimus may be associated with higher mortality rates due to infections in kidney transplant recipients (36). Although intriguing, these observations need to be further investigated to make any more meaningful conclusions. Lymphopenia has been previously identified as a significant predictor of PJP among SOTr (3, 10). Most SOTr with PJP in this cohort had an ALC<500 cells/mL. Iriart *et al* have suggested that the triad of age ≥65 years, 2nd year post-transplant, and lymphopenia could potentially identify patients at higher risk for PJP (3). Similarly, our observations suggest that high clinical suspicion for PJP could be applied in ≥65 year-old lymphopenic SOTr -treated with mTOR-inhibitors, who present with a syndrome compatible with PJP, particularly during the 2nd year post-transplant.

Considering the lower organism burden in HIV-negative patients with PJP, current guidelines recommend shorter treatment courses for SOTr with PJP (14-21 days) (4, 37). Most patients in this cohort received treatment with TMP-SMX for a duration of 21 days and co-administration of corticosteroids. As PJP has become a rare complication in SOTr, it is very unlikely that prospective, randomized clinical trials on the treatment of PJP in this patient population will be performed. Our data provide an update on real-life management of PJP in SOTr, suggesting that clinicians are more likely to use longer treatment courses and corticosteroids to treat SOTr with PJP. In contrast to previously reported high mortality rates (27–60%) in HIV-negative immunocompromised patients with PJP, 1-year mortality was <15% in this cohort (2, 4, 33, 38). This may reflect the progress attained in the field of PJP diagnosis, with the routine use of PCR and b-D-glucan at most centers, leading to earlier diagnosis and timely treatment initiation. In a small number of cases the diagnosis of PJP was solely based on PCR, with the potential for over-diagnosis of colonization. However, their treating physicians were convinced enough to treat these patients with full-courses of antibiotic treatment, frequently co-administered with corticosteroids.

Notably, one of the major limitations of the study was that the PCR cycle threshold (C_t) was not available for cases diagnosed based on a positive PCR, although only 4 cases were diagnosed based on a positive *Pneumocystis* PCR only. Finally, we were not able to exclude a possible outbreak at center-2, as molecular typing was performed but due to fragmented DNA results were non-interpretable. In conclusion, in the *Pneumocystis*-prophylaxis era PJP appears to be a rare, albeit associated with favorable outcomes, post-transplant complication, with most cases occurring post-discontinuation of PJP prophylaxis particularly during the 2nd year post-transplant in older patients

with lymphopenia. Additional research may help us identify indications for prolongation or reinstitution of *Pneumocystis*-prophylaxis later post-transplant in specific patient categories.

References

1. Fillatre P, Decaux O, Jouneau S, Revest M, Gacouin A, Robert-Gangneux F, et al. Incidence of Pneumocystis jiroveci pneumonia among groups at risk in HIV-negative patients. Am J Med. 2014;127(12):1242 e11-7.

2. Iriart X, Bouar ML, Kamar N, Berry A. Pneumocystis Pneumonia in Solid-Organ Transplant Recipients. J Fungi (Basel). 2015;1(3):293-331.

3. Iriart X, Challan Belval T, Fillaux J, Esposito L, Lavergne RA, Cardeau-Desangles I, et al. Risk factors of Pneumocystis pneumonia in solid organ recipients in the era of the common use of posttransplantation prophylaxis. Am J Transplant. 2015;15(1):190-9.

4. Martin SI, Fishman JA, Practice ASTIDCo. Pneumocystis pneumonia in solid organ transplantation. Am J Transplant. 2013;13 Suppl 4:272-9.

5. Muhlethaler K, Bogli-Stuber K, Wasmer S, von Garnier C, Dumont P, Rauch A, et al. Quantitative PCR to diagnose Pneumocystis pneumonia in immunocompromised non-HIV patients. The European respiratory journal. 2012;39(4):971-8.

6. de Boer MG, Kroon FP, le Cessie S, de Fijter JW, van Dissel JT. Risk factors for Pneumocystis jirovecii pneumonia in kidney transplant recipients and appraisal of strategies for selective use of chemoprophylaxis. Transpl Infect Dis. 2011;13(6):559-69.

7. Wang EH, Partovi N, Levy RD, Shapiro RJ, Yoshida EM, Greanya ED. Pneumocystis pneumonia in solid organ transplant recipients: not yet an infection of the past. Transpl Infect Dis. 2012;14(5):519-25.

8. Fishman JA. Prevention of infection caused by Pneumocystis carinii in transplant recipients. Clin Infect Dis. 2001;33(8):1397-405.

9. Anand S, Samaniego M, Kaul DR. Pneumocystis jirovecii pneumonia is rare in renal transplant recipients receiving only one month of prophylaxis. Transpl Infect Dis. 2011;13(6):570-4.

10. De Castro N, Xu F, Porcher R, Pavie J, Molina JM, Peraldi MN. Pneumocystis jirovecii pneumonia in renal transplant recipients occurring after discontinuation of prophylaxis: a case-control study. Clin Microbiol Infect. 2010;16(9):1375-7.

11. Kasiske BL, Zeier MG, Chapman JR, Craig JC, Ekberg H, Garvey CA, et al. KDIGO clinical practice guideline for the care of kidney transplant recipients: a summary. Kidney international. 2010;77(4):299-311.

12. Transplantation EEGoR. European best practice guidelines for renal transplantation. Section IV: Long-term management of the transplant recipient. IV.7.1 Late infections. Pneumocystis carinii pneumonia. Nephrol Dial Transplant. 2002;17 Suppl 4:36-9.

13. Gordon SM, LaRosa SP, Kalmadi S, Arroliga AC, Avery RK, Truesdell-LaRosa L, et al. Should prophylaxis for Pneumocystis carinii pneumonia in solid organ transplant recipients ever be discontinued? Clin Infect Dis. 1999;28(2):240-6.

14. Green H, Paul M, Vidal L, Leibovici L. Prophylaxis for Pneumocystis pneumonia (PCP) in non-HIV immunocompromised patients. The Cochrane database of systematic reviews. 2007(3):CD005590.

15. Borstnar S, Lindic J, Tomazic J, Kandus A, Pikelj A, Prah J, et al. Pneumocystis jirovecii pneumonia in renal transplant recipients: a national center experience. Transplant Proc. 2013;45(4):1614-7.

16. Koller MT, van Delden C, Muller NJ, Baumann P, Lovis C, Marti HP, et al. Design and methodology of the Swiss Transplant Cohort Study (STCS): a comprehensive prospective nationwide long-term follow-up cohort. Eur J Epidemiol. 2013;28(4):347-55.

17. Alanio A, Hauser PM, Lagrou K, Melchers WJ, Helweg-Larsen J, Matos O, et al. ECIL guidelines for the diagnosis of Pneumocystis jirovecii pneumonia in patients with haematological malignancies and stem cell transplant recipients. J Antimicrob Chemother. 2016;71(9):2386-96.

18. Humar A, Michaels M, Monitoring AIWGoID. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. Am J Transplant. 2006;6(2):262-74.

19. Ljungman P, Boeckh M, Hirsch HH, Josephson F, Lundgren J, Nichols G, et al. Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials. Clin Infect Dis. 2017;64(1):87-91.

20. Golshayan D, Wojtowicz A, Bibert S, Pyndiah N, Manuel O, Binet I, et al. Polymorphisms in the lectin pathway of complement activation influence the incidence of acute rejection and graft outcome after kidney transplantation. Kidney international. 2016;89(4):927-38.

21. Pliquett RU, Asbe-Vollkopf A, Hauser PM, Presti LL, Hunfeld KP, Berger A, et al. A Pneumocystis jirovecii pneumonia outbreak in a single kidney-transplant center: role of cytomegalovirus co-infection. Eur J Clin Microbiol Infect Dis. 2012;31(9):2429-37.

22. Sassi M, Ripamonti C, Mueller NJ, Yazaki H, Kutty G, Ma L, et al. Outbreaks of Pneumocystis pneumonia in 2 renal transplant centers linked to a single strain of Pneumocystis: implications for transmission and virulence. Clin Infect Dis. 2012;54(10):1437-44.

23. Arend SM, Westendorp RG, Kroon FP, van't Wout JW, Vandenbroucke JP, van Es LA, et al. Rejection treatment and cytomegalovirus infection as risk factors for Pneumocystis carinii pneumonia in renal transplant recipients. Clin Infect Dis. 1996;22(6):920-5.

24. Branten AJ, Beckers PJ, Tiggeler RG, Hoitsma AJ. Pneumocystis carinii pneumonia in renal transplant recipients. Nephrol Dial Transplant. 1995;10(7):1194-7.

25. Dummer JS. Pneumocystis carinii infections in transplant recipients. Seminars in respiratory infections. 1990;5(1):50-7.

26. Elinder CG, Andersson J, Bolinder G, Tyden G. Effectiveness of low-dose cotrimoxazole prophylaxis against Pneumocystis carinii pneumonia after renal and/or pancreas transplantation. Transpl Int. 1992;5(2):81-4.

27. Hardy AM, Wajszczuk CP, Suffredini AF, Hakala TR, Ho M. Pneumocystis carinii pneumonia in renal-transplant recipients treated with cyclosporine and steroids. J Infect Dis. 1984;149(2):143-7.

28. Hennequin C, Page B, Roux P, Legendre C, Kreis H. Outbreak of Pneumocystis carinii pneumonia in a renal transplant unit. Eur J Clin Microbiol Infect Dis. 1995;14(2):122-6.

29. Lufft V, Kliem V, Behrend M, Pichlmayr R, Koch KM, Brunkhorst R. Incidence of Pneumocystis carinii pneumonia after renal transplantation. Impact of immunosuppression. Transplantation. 1996;62(3):421-3.

30. Neff RT, Jindal RM, Yoo DY, Hurst FP, Agodoa LY, Abbott KC. Analysis of USRDS: incidence and risk factors for Pneumocystis jiroveci pneumonia. Transplantation. 2009;88(1):135-41.

31. Dominguez J, Mahalati K, Kiberd B, McAlister VC, MacDonald AS. Conversion to rapamycin immunosuppression in renal transplant recipients: report of an initial experience. Transplantation. 2000;70(8):1244-7.

32. Kahan BD, Wong RL, Carter C, Katz SH, Von Fellenberg J, Van Buren CT, et al. A phase I study of a 4-week course of SDZ-RAD (RAD) quiescent cyclosporine-prednisone-treated renal transplant recipients. Transplantation. 1999;68(8):1100-6.

33. Eitner F, Hauser IA, Rettkowski O, Rath T, Lopau K, Pliquett RU, et al. Risk factors for Pneumocystis jiroveci pneumonia (PcP) in renal transplant recipients. Nephrol Dial Transplant. 2011;26(6):2013-7.

34. Eiden AM, Zhang S, Gary JM, Simmons JK, Mock BA. Molecular Pathways: Increased Susceptibility to Infection Is a Complication of mTOR Inhibitor Use in Cancer Therapy. Clin Cancer Res. 2016;22(2):277-83.

35. Yang H, Wang X, Zhang Y, Liu H, Liao J, Shao K, et al. Modulation of TSC-mTOR signaling on immune cells in immunity and autoimmunity. J Cell Physiol. 2014;229(1):17-26.

36. Knoll GA, Kokolo MB, Mallick R, Beck A, Buenaventura CD, Ducharme R, et al. Effect of sirolimus on malignancy and survival after kidney transplantation: systematic review and meta-analysis of individual patient data. BMJ. 2014;349:g6679.

37. Kovacs JA, Hiemenz JW, Macher AM, Stover D, Murray HW, Shelhamer J, et al. Pneumocystis carinii pneumonia: a comparison between patients with the acquired

immunodeficiency syndrome and patients with other immunodeficiencies. Ann Intern Med. 1984;100(5):663-71.

38. Roux A, Canet E, Valade S, Gangneux-Robert F, Hamane S, Lafabrie A, et al. Pneumocystis jirovecii pneumonia in patients with or without AIDS, France. Emerg Infect Dis. 2014;20(9):1490-7.

Figure legends

Figure 1. Incidence of *Pneumocystis jirovecii* pneumonia (PJP).

(a) PJP incidence based on the type of transplanted organ, log-rank: 0.08 between kidney and other SOT. (b) PJP incidence between center-2 and other transplant centers, log-rank<0.001. (c) Proportion of patients who received *Pneumocystis* prophylaxis by year of transplantation between center-2 and other centers. (d) Incidence of PJP at center-2 among patients transplanted between 2008 and 2012 compared to patients at the same center transplanted in 2013-2016 and other centers (log-rank<0.001).

Figure 2. Administration of *Pneumocystis* prophylaxis.

(a) Overall incidence of *Pneumocystis jirovecii* pneumonia (PJP) among 377 patients who did not receive primary *Pneumocystis*-prophylaxis (incidence rate 0.003%) compared to those who did (incidence rate 0.001%; log-rank<0.001). (b) One-year post-transplant incidence of PJP between patients who did and those who did not receive *Pneumocystis*-prophylaxis (log-rank<0.001). (c) Duration of *Pneumocystis*-prophylaxis based on the type of transplanted organ presented as box-plots. (d) Duration of *Pneumocystis*-prophylaxis during the first year post-transplant among different organ types.

Table 1. Baseline patient characteristics.

	PJP N: 41 (%)	No PJP N: 2801 (%)
Demographics		
Age, Mean Years (SD; Range)	56.9 (12.1; 18.9-76.9)	52.2 (13.1; 18, 80.7)
Gender, Female	17 (41.5)	994 (35.5)
Transplant Characteristics		
SOT type		
Combined	0	68 (2.4)
Heart	5 (12.2)	199 (7.1)
Kidney	30 (73.2)	1637 (58.4)
Kidney-Pancreas	1 (2.4)	84 (3.0)
Liver	4 (9.8)	563 (20.1)
Lung	1 (2.4)	250 (8.9)
SOT center		
Center 1	2 (4.9)	452 (16.1)
Center 2	27 (65.8)	368 (13.1)
Center 3	2 (4.9)	418 (14.9)
Center 4	2 (4.9)	496 (17.7)
Center 5	2 (4.9)	139 (5.0)
Center 6	6 (14.6)	928 (33.1)
SOT year		
2008	6 (14.3)	263 (9.4)
2009	11 (26.8)	384 (13.7)
2010	7 (17.1)	406 (14.5)
2011	9 (21.5)	397 (14.2)
2012	5 (12.9)	365 (13.0)
2013	2 (4.9)	368 (13.1)
2014	1 (2.4)	354 (12.6)
2015	0	224 (8.0)
2016	0	40 (1.4)
Donor type		
Dead	30 (73.2)	1969 (70.3)

Living	11 (26.8)	746 (26.6)
NHBD	0	86 (3.1)
Induction Immunosuppression[1]		
Thymoglobulin	6 (14.6)	688 (24.6)
Basiliximab/Daclizumab	41 (75.6)	1914 (68.3)
IVIG	0	169 (6.0)
Rituximab	2 (4.8)	111 (4.0)
Maintenance Immunosuppression[2]		
Calcineurin Inhibitor	40 (97.6)	2727 (97.4)
Mycophenolate Mofetil	40 (97.6)	2648 (94.5)
mTOR-inhibitor	8 (19.5)	231 (8.3)
Steroids	41 (100)	2637 (94.1)
Serologies		
CMV, D+R-[3]	9 (21.9)	533 (19.2)
EBV, D+R-[4]	0	120 (4.4)
Toxoplasmosis, D+R-[5]	2 (7.4)	497 (23.3)
Comorbidities at Time of SOT		
Diabetes Mellitus	10 (24.4)	534 (19.1)
Chronic Renal Insufficiency	25 (61.0)	1845 (65.9)
Body Mass Index, Mean (SD; Range)	25.7 (4.8; 18.2, 36.2)	24.8 (9.2; 14.1, 35.7)
***Pneumocystis* Prophylaxis**		
First 3 months for >7 days	29 (70.7)	2436 (87.0)
Duration of Prophylaxis, Mean Days (SD; Range)	245 (372; 123, 367)	317 (528 ; 297, 336)

PJP: *Pneumocystis jirovecii* pneumonia, SD: Standard Deviation, SOT: Solid Organ Transplant, NHBD: Non-heart beating donor, IVIG: Intravenous Immunoglobulin, CMV: Cytomegalovirus, D: Donor, R: Recipient, EBV: Epstein-Barr Virus.

[1] Induction immunosuppression agents were not mutually exclusive. One patient might have received more than 1 agents.

[2] Maintenance immunosuppression agents were not mutually exclusive. One patient might have received more than 1 agents. Only agents administered for >7 days during the first 6 months post-transplant are reported.

[3] CMV D/R status was available for 41 and 2779 patients with and without PJP.

[4] EBV D/R status was available for 40 and 2744 patients with and without PJP.

[5] Toxoplasmosis D/R status was available for 27 and 2135 patients with and without PJP.

Table 2. Characteristics of patients with *Pneumocystis jirovecii* pneumonia.

	Heart N=5 (%)	Kidney N=30 (%)	Kidney-Pancreas N=1	Liver N=4 (%)	Lung N=1
Demographics					
Age, Mean Years (SD; Range)	54.7 (6.4 ; 49.8, 65.6)	59.4 (11.8 ; 22, 75)	43.2	63.2 (8.5 ; 51.6, 70)	40.3
Gender, Female	3 (60)	12 (40)	0	1 (25)	1
SOT center					
Center 1	0	2 (6.7)	0	1 (25)	0
Center 2	4 (80)	22 (73.3)	0	0	0
Center 3	0	1 (3.3)	0	1 (25)	0
Center 4	0	0	0	1 (25)	1
Center 5	0	2 (6.7)	0	0	0
Center 6	1 (20)	3 (10)	1	1 (25)	0
SOT year, 2008-2012	5 (100)	27 (90)	1	4 (100)	1
2008	1	3	1	0	1
2009	1	9	0	1	0
2010	1	6	0	0	0
2011	2	5	0	2	0
2012	0	4	0	1	0
2013	0	2	0	0	0
2014	0	1	0	0	0
2015	0	0	0	0	0
2016	0	0	0	0	0
***Pneumocystis* Primary Prophylaxis**	3 (60)	21 (70)	1	3 (75)	1
Duration of Primary Prophylaxis, Mean Days (SD; Range)	555 (726 ; 0, 1745)	149 (160 ; 0, 725)	179	95.7 (106 ; 0, 235)	1495
PJP Incidence	0.002%	0.001%	0.001%	0.0006%	0.0004%
PJP Diagnosis					
Timing post-SOT, Mean Days (SD; Range)	407 (316 ; 67, 844)	527 (469 ; 80, 1915)	240	440 (499 ; 71, 1163)	363
Early PJP	2 (40)	8 (26.7)	0	2 (50)	0
Specimen					

Sputum	0	3 (10)	1	0	1
BAL	5 (100)	27 (90)	0	4 (100)	0
Microbiology					
Direct microscopy	5 (100)	26 (100)	1	4 (100)	1
PCR	0	6 (20.0)	0	1 (25.0)	1
Histopathology, Done	1 (100)	0	0	2 (100)	0
Positive	0	0	0	2 (100)	0
Radiology					
Chest CT	4 (80)	26 (86.7)	1	3 (75.0)	0
Chest Radiography	1 (20)	4 (13.3)	1	1 (25.0)	1
Radiographic Findings					
Ground-Glass Opacities	3 (60)	23 (76.7)	0	3 (75.0)	0
Nodular lesions	2 (40.0)	9 (30.0)	0	0	0
Infiltrates	1 (20.0)	14 (46.7)	0	2 (50.0)	0
Pleural effusion	1 (20.0)	2 (6.7)	0	1 (25.0)	0
Laboratory Fndings					
WBC, $x10^9$ cells/mm^3, Mean (SD; Range)	3.7 (5.5 ; 2.2, 7.4)	7.8 (4.1 ; 2.4, 19.6)	13.9	5.5 (3.3 ; 3, 9.2)	3.8
ALC cells/mm^3, Mean (SD; Range)	252.5 (220 ; 70, 570)	520 (308 ; 50, 1240)	1930	742.5 (521 ; 310, 1500)	270

SD: Standard Deviation, SOT: Solid Organ Transplant, PJP: *Pneumocystis jirovecii* pneumonia, IF: Immunofluorescence, BAL: Bronchoalveolar Lavage, PCR: Polymerase Chain Reaction, CT: Computed Tomography, WBC: White Blood Count, ALC: Absolute Lymphocyte Count

Table 3. Univariable and multivariable risk factor analyses for *Pneumocystis jirovecii* pneumonia.

	Univariable analysis			Multivariable analysis-I*			Multivariable analysis-II*		
	OR	**95%CI**	**P**	**OR**	**95%CI**	**P**	**OR**	**95%CI**	**P**
Demographics									
Age	1.03	1, 1.06	0.03	Not applicable			Not applicable		
Age groups, ≥65 vs <65 Years	2.6	1.3, 5.0	0.004	2.1	0.9, 5.0	0.10	2.4	1.1, 5.5	0.03
Gender, Male vs Female	1.3	0.7, 2.4	0.43						
Transplant-Associated									
SOT-type, Kidney vs other	1.9	0.9, 3.9	0.06	3.5	1.3, 9.3	0.01	Not applicable		
SOT-center, 2 vs other	12.7	6.6, 23	<0.001	11.0	4.6, 26	<0.001	Not applicable		
SOT-year, 2013-16 vs 2008-12	0.14	0.04, 0.5	0.001	0.14	0.03, 0.6	0.008	Not applicable		
Induction IS									
Thymoglobulin	0.5	0.2, 1.3	0.15						
Basiliximab	1.4	0.7, 2.9	0.32						
Rituximab	1.2	0.3, 5.2	0.77						
Maintenance IS									
Calcineurin Inhibitors	1.2	0.1, 8.0	0.94						
Mycophenolate Mofetil	2.3	0.3, 17	0.41						
mTOR-inhibitors	2.7	1.2, 6.0	0.01	2.1	0.7, 6.9	0.2	2.5	0.9, 6.9	0.07
Steroids	1								
Post-transplant complications									
Rejection[1]	1.5	0.8, 3.0	0.21						
CMV infection/disease[1]	2.6	1.4, 4.9	0.002	1.7	0.8, 3.8	0.2	2.5	1.2, 5.4	0.006
Serologies									
CMV, D+R- vs other	1.2	0.5, 2.0	0.66						
Toxoplasmosis, D+R- vs other	0.26	0.1, 1.1	0.07	0.3	0.07, 1.4	0.12	0.3	0.06, 1.2	0.08
Comorbidities at SOT									
Diabetes Mellitus	1.4	0.7, 2.8	0.39						
Chronic Renal Insufficiency	0.8	0.4, 1.5	0.51						
BMI at SOT	1.0	0.9, 1.1	0.60						
Primary *Pneumocystis*-prophylaxis	0.36	0.2, 0.7	0.003	0.51	0.2, 1.2	0.14	0.3	0.14, 0.7	0.006

OR: Odds Ratio, 95% CI: 95% Confidence Interval, P: P-value, SOT: Solid Organ Transplant, IS: Immunosuppression, CMV: Cytomegalovirus, D: Donor, R: Recipient, BMI: Body Mass Index.

[1] Rejection included the first episode of any type of acute rejection (cell- or antibody-mediated) and CMV infection/disease that required treatment during the first year post-transplant.

* The Pearson correlation coefficient identified possible correlations between (i) SOT-type and: age (r=0.11, P-value<0.001), SOT-center (r=0.10, P-value<0.001), mTOR-inhibitors (r=0.18, P-value<0.001), CMV infection/disease (r=0.11, P-value<0.001) and *Pneumocystis*-prophylaxis (r=0.23, P-value<0.001) and (ii) SOT-center and mTOR-inhibitor administration (r=0.19, P-value<0.001). In addition, administration of primary PJP prophylaxis (particularly for Center 2) changed during the study period. Hence, model-II was constructed, after excluding SOT-center, SOT-year, and SOT-type from independent variables.

Table 4. Univariable and multivariable risk factor analyses for *Pneumocystis jirovecii* pneumonia in: (i) All centers, excluding center-2, and (ii) for center-2.

	All centers, excluding center-2						Center-2								
	Univariable analysis			Multivariable analysis			Univariable analysis			Multivariable analysis-I[2]			Multivariable analysis-II[2]		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Demographics															
Age	1.0	0.9, 1.1	0.25	Not applicable			1.0	0.9, 1.1	0.07	Not applicable					
Age groups, ≥65 vs <65 Years	2.8	0.9, 8.3	0.07	2.9	0.9, 8.9	0.06	2.6	1.1, 6.0	0.03	2.3	0.9, 5.6	0.07	2.4	1.0, 5.6	0.05
Gender, Male vs Female	1.3	0.5, 24.0	0.55				1.1	0.5, 2.5	0.74						
Transplant-Associated															
SOT-type, Kidney vs other	0.9	0.3, 2.5	0.80				5.6	2.1, 15.1	0.001	5.6	2.0, 15.3	0.001			
SOT-year, 2013-16 vs 2008-12	0.3	0.07, 1.4	0.13				0.07	0.008, 0.5	0.008	0.08	0.01, 0.6	0.02			
Induction IS															
Thymoglobulin	0.5	0.1, 2.2	0.34				0.86	0.3, 2.6	0.78						
Basiliximab	0.9	0.3, 2.7	0.88				0.9	0.3, 2.5	0.88						
Rituximab	1.6	0.2, 12.6	0.64				7.0	0.6, 80.2	0.12						
Maintenance IS															
Calcineurin Inhibitors	1						1.4	0.2, 11.0	0.74						
Mycophenolate Mofetil	0.7	0.09, 5.5	0.74				1								
mTOR-inhibitors	2.5	0.5, 11.2	0.24				1.06	0.4, 2.7	0.90						
Steroids	1						1								
Post-transplant complications															
Rejection[1]	2.6	0.9, 7.3	0.09	2.7	0.9, 7.8	0.07	1.2	0.5, 2.9	0.62						
CMV infection/disease[1]	2.4	0.8, 7.3	0.11	2.2	0.7, 6.6	0.16	1.8	0.8, 3.9	0.15						
Serologies															
CMV, D+R- vs other	0.7	0.2, 3.2	0.66				1.4	0.6, 3.4	0.50						
Toxoplasmosis, D+R- vs other	0.3	0.04, 2.6	0.29				0.2	0.03, 1.9	0.17						
Comorbidities at SOT															
Diabetes Mellitus	2.4	0.8, 7.1	0.12				0.9	0.3, 2.6	0.90						
Chronic Renal	0.	0.2,	0.4				1.4	0.6,	0.42						

Insufficiency	6	1.8	1				3.1							
BMI at SOT	0.9	0.9, 1.1	0.85			1.0	0.9, 1.1	0.72						
Primary *Pneumocystis*-prophylaxis	0.5	0.15, 1.9	0.33			0.4	0.2, 0.9	0.02	0.6	0.2, 1.4	0.20	0.4	0.17, 0.9	0.04

OR: Odds Ratio, 95% CI: 95% Confidence Interval, P: P-value, SOT: Solid Organ Transplant, IS: Immunosuppression, CMV: Cytomegalovirus, D: Donor, R: Recipient, BMI: Body Mass Index.

Only variables with P-value>0.12 in univariable analyses were considered in the multivariable analyses.

[1] Rejection included the first episode of any type of acute rejection (cell- or antibody-mediated) and CMV infection/disease that required treatment during the first 6 months post-transplant.

[2] A second multivariable analysis-II was performed after excluding the variables associated with SOT type and year, to avoid potential interactions between these independent variables and administration of prophylaxis.