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## **Risk factors and new markers of pulmonary fungal infection**

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# Chapter 4

## **RISK FACTORS FOR *PNEUMOCYSTIS JIROVECI* PNEUMONIA IN KIDNEY TRANSPLANT RECIPIENTS AND APPRAISAL OF STRATEGIES FOR SELECTIVE USE OF CHEMOPROPHYLAXIS**

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## Abstract

Differentiated use of Trimethoprim-Sulfamethoxazole (TMP-SMX) chemoprophylaxis to prevent *Pneumocystis pneumonia* (PCP) in kidney transplant recipients based on risk factor analysis is not a universally adapted strategy and supporting evidence based sources are limited. We performed a large retrospective study to identify risk factors for PCP in kidney transplant recipients and to define parameters for use in clinical prophylaxis guidelines. Fifty consecutive patients with confirmed PCP and two time-matched controls per case were enrolled. Potential risk factors were compared between groups by uni- and multivariate matched analyses. At transplantation, age >55 years and not receiving basiliximab induction therapy predicted development of PCP. In addition, CMV infection (OR 3.0 95%CI 1.2-7.9) and rejection treatment (OR 5.8 95%CI 1.9-18) were independently associated with PCP. Using the variables identified by the multivariate analyses, effects of different hypothetical chemoprophylaxis strategies were systematically evaluated. Exploring different scenarios showed that chemoprophylaxis in the first 6 months for all- and during the first year post-transplantation for patients >55 years or those treated for rejection would result in very low PCP incidence and optimal avoidance of TMP-SMX toxicity. The clinical approach towards application of PCP chemoprophylaxis may be refined accordingly by adopting a both time and risk factor based strategy.

## Introduction

*Pneumocystis pneumonia* (PCP), caused by *Pneumocystis jirovecii* is a recognized cause of morbidity and mortality in kidney- and other solid organ transplant recipients [1, 2]. Recently issued kidney transplantation guidelines recommend the prescription of chemoprophylaxis for at least 3-6 months after transplantation, whereas other sources or expert opinions may advise other time based schedules [3-5]. Trimethoprim-Sulfamethoxazole (TMP-SMX) is the drug of choice and has been proven highly effective in preventing PCP in solid organ transplant recipients [6, 7]. However, although in general the use of PCP chemoprophylaxis after kidney transplantation is a widely accepted practice, a definitive more individualized approach towards the prescription of chemoprophylaxis has not been established [8]. Incentives to abstain from a prophylactic strategy using TMP-SMX include adverse effects e.g. increase in serum creatinine, severe hyperkalemia, gastro-intestinal complaints, Stephen-Johnson's syndrome, interstitial nephritis and interactions with other medication [9-12]. Weighing the incidence and impact of these side effects against the overall morbidity and mortality of PCP among kidney transplant recipients, local renal transplantation program committees or individual physicians may decide when to – or not to – prescribe PCP chemoprophylaxis [13, 14]. Nonetheless, individual cases and 'outbreaks' of PCP that occur in the absence of adequate chemoprophylaxis are reported with some regularity [15-18]. Hence, the need for selective prescription of chemoprophylaxis for patients with an increased risk profile is an important issue to be considered, but few evidence-based sources exist [19]. Two small case-controlled studies with less than 20 cases each, indicated that treatment for graft rejection and Cytomegalovirus virus (CMV) infection are important risk factors [20, 21]. From case series other risk factors were suggested including smoking behavior, use of specific immunosuppressive compounds e.g. Cyclosporine A (CsA) or Tacrolimus (TCR), concurrent Hepatitis C infection, or active tuberculosis [22-26]. In this larger retrospective case-control study the potential risk factors for PCP in kidney transplant recipients are evaluated with the emphasis on the appraisal of previously attributed risks and on the definition of the parameters that could enable future guidelines to contain a differentiated, more individualized approach towards the prescription of PCP chemoprophylaxis in this population.

## Methods

### Study population

Case patients were identified from the database of the Department of Infectious Diseases of the Leiden University Medical Center, a tertiary care and teaching hospital in the Netherlands with an extensive transplantation program. All consecutive kidney- and combined

kidney-pancreas transplant recipients with clinical signs and symptoms compatible with PCP and with confirmed presence of *P. jirovecii* by direct microscopy (giemsa- and/or silver staining) and/or PCR between January 1983 and July 2008 were included. A standard PCP prophylaxis policy (comprising the first year post transplantation for all patients) was only properly implemented after the end of this period. Control patients were recruited from the transplantation database of the Department of Nephrology. To prevent time period related bias in the analysis, the patients receiving their graft directly before- and after the patient that finally developed PCP were selected as controls. Control patients had to have an at least equal time of immunosuppression as their matched case. Also, they had to be negative for suspicion of PCP throughout their complete follow-up. The use of TMP-SMX (e.g. if prescribed for other indications) or other antibiotics effective as PCP chemoprophylaxis and infection with HIV-1/2 were exclusion criteria for both case- and control patients. If a control patient was excluded, he or she was replaced by the patient transplanted directly before or after this individual. When this method failed, a patient transplanted within a 5 year period was randomly selected as control.

### **Patient data**

Clinical data about mortality, demographic characteristics and the following potential risk factors for development of PCP was collected from the hospital's electronic- and paper patient records and the Nephrology Department's transplantation database: underlying renal and infectious diseases, type of transplant (kidney vs. combined kidney-pancreas), graft origin, immunosuppressive regimen, treatment for graft rejection, smoking behavior and CMV-status. Routine pre-transplantation evaluation included serologic screening for CMV, HIV (from 1985 onward), Hepatitis B and C, and a Mantoux test for the detection of latent or active tuberculosis. Observed time in case patients ranged from transplantation to the day of diagnosis, defined as the day that microbiological evidence of PCP was obtained by broncho-alveolar lavage. For control patients the observed time window ranged from transplantation to the corresponding day of diagnosis in their matched case. Data about the immunosuppressive regimen was obtained at 3 months post-transplantation and at the time of diagnosis (and at corresponding times for control patients). Pre-emptive treatment with the monoclonal antibody basiliximab at the time of transplantation as well as specifics concerning rejection treatment(s) (number, timing and type of medication) within the observed time window was recorded. To investigate the association between CMV infection and PCP, the 3 month period prior to diagnosis of PCP was evaluated. Imperative due to the time period spanned by this study, prove of CMV infection was defined by presence of either a positive pp65 antigen test, CMV seroconversion, rising (4-fold) CMV titers or a positive PCR test for CMV. Surveillance and a low threshold for performing diagnostics in case of suspected CMV infection was the standard of care throughout the period of study. CMV replication as detected by these meth-

ods is referred to as CMV infection throughout the article without discriminating between reactivation and primo infections of CMV. If no test to detect CMV was performed in the time window of interest, this was processed in the analysis as missing data.

## Statistical Analysis

Uni- and multivariate (conditional logistic regression for matched data) analyses were performed to assess the association of each of the variables with respect to the risk for development of PCP. Binary variables were incorporated in the multivariate model if the univariate p-value was <0.10 or when incorporation was deemed necessary for logical reasons. Relative risks were approximated by odds ratio's (OR) with 95% confidence intervals (95%CI). A p-value of <0.05 was considered statistically significant. Continuous variables are expressed as medians with interquartile ranges, for binary variables numbers and percentages are given. Using risk factors, identified by the multivariate analysis, the effect of different strategies for prescribing chemoprophylaxis were evaluated (see supplement 1 for details). STATA version 10.0 and SPSS version 17.0 were used to perform all analyses.

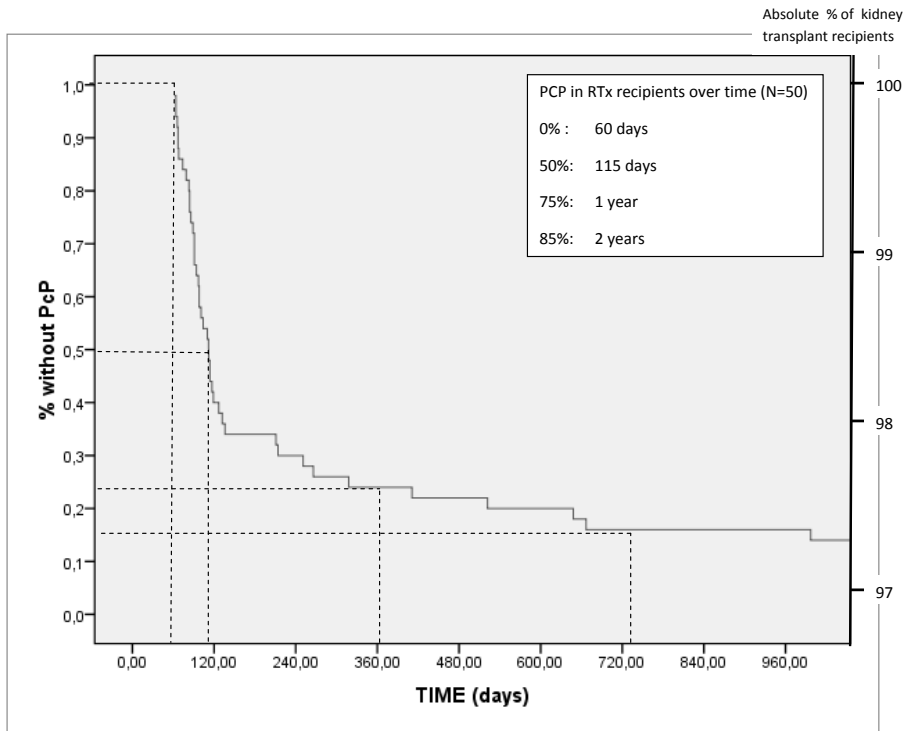
## Results

### Study population characteristics

Fifty-two kidney transplant recipients with PCP were identified and the overall incidence of PCP was 27 per 1000 transplantation procedures (i.e. 2.7%) in the period of study. None had used SMX-TMP chemoprophylaxis. Two cases were excluded from the analysis due to incomplete data and absence of suitable controls as defined previously. ICU admission and need for mechanical ventilation developed in 4/50 cases and overall 30-day mortality was 6%. Two out of the three patients that died suffered from severe co-morbidity (necrotizing pancreatitis, heart failure). PCP did not occur in the first 60 days post-transplantation despite the absence of prophylaxis; 85% of cases were diagnosed within 24 months after transplantation (figure 1). Some clustering of cases occurred in 2005, but this had no influence on the study outcomes. Uni- and multivariate comparisons of baseline characteristics between case- and control patients are showed in tables 1, 2 and 4A. Due to very low incidences of Hepatitis C infection and active tuberculosis, possible associations could not be established.

### Immunosuppressive regimen

At 3 months post transplantation the immunosuppressive regimen generally constituted out of low dose prednisone (5-10 mg/day) in combination with one or two additional compounds.

**Figure 1.** Development of *Pneumocystis pneumonia* in (study-cohort) kidney transplant recipients over time.

CsA and Mofetyl mycophenolate (MMF) were predominantly used. PCP occurred more frequent when three instead of two drugs, including prednisone, were used at 3 months post transplantation, but this difference was not statistically significant (OR 1.5 95%CI 0.4-4.9). Use of CsA within the regimen at 3 months showed a trend towards an association with the development of PCP; but these findings were not confirmed at the time of diagnosis nor in the multivariate analysis at baseline (tables 2 and 4A). In case patients, median daily doses of medication at time of diagnosis were 1500 mg (range 1000-2000 mg) for MMF, 75 mg (range 50-100 mg) for Azathioprine (AZA), 3.1 mg/kg (range 1.1-8.4 mg/kg) for CsA and 3 mg (range 2-4 mg) for TCR. Everolimus or Sirolimus were prescribed to less than 5 cases per group. A difference with regard to dosage was found for CsA only, with higher dosages in the group of control patients (median 4.3, range 1.7-12.2 mg/kg;  $p=0.03$ ).

### Treatment for graft rejection and CMV infection

Table 3 summarizes the frequency, number and type of rejection treatments for patients and controls with the corresponding OR's and 95%CI. The standard first treatment was Solumedrol 1000 mg for 3 days. If rejection was steroid resistant, subsequent treatments consisted of anti-thymocyte globulin (ATG) or, if contraindicated, a repeated course of Solumedrol. The

**Table 1.** Baseline characteristics of kidney transplant recipients with and without development of *Pneumocystis* pneumonia

Variable	Cases (PCP+) No. (%)	Controls (PCP-) No. (%)	OR 95%CI	p-value <sup>‡</sup>
<b>Total No.</b>	<b>50</b>	<b>99</b>		
<b>Age (years), median (IQR)</b>	57.4 (46-62)	52.1 (42-60)		<b>0.03</b>
Age 55+	31 (62)	39 (39)	2.6 (1.3-5.1)	<b>0.01</b>
Age 60+	19 (38)	26 (26)	1.9 (0.7-1.7)	0.90
<b>Sex (male)</b>	19 (38)	54 (55)	0.5 (0.2-1.0)	0.05
<b>BMI, median (IQR),</b>	25.7 (23-28)	24.0 (22-26)		0.06
>30 (vs reference category 20-30)	3 (6)	2 (2)	2.6 (0.4-16)	0.29
<b>Type of Transplantation</b>				
Simultaneous Kidney and Pancreas	6 (12)	13 (13)	0.9 (0.3-2.7)	0.86
Kidney	44 (88)	86 (87)	-	-
<b>Donor origin</b>				
Deceased donor	40 (80)	77 (78)	1.1 (0.5-2.7)	0.77
Living donor	10 (20)	22 (22)	-	-
<b>&gt;1 transplantation episode</b>	2 (4)	11 (11)	0.3 (0.1-1.5)	0.15
<b>Primary underlying disease</b>				
ADPKD	8 (16)	20 (20)	0.8 (0.3-1.9)	0.56
Hypertension	11 (22)	19 (19)	1.2 (0.5-2.7)	0.67
DM	14 (28)	23 (23)	1.2 (0.5-2.5)	0.70
IgA nephropathy	2 (4)	5 (5)	0.8 (0.1-4.5)	0.77
Auto-immune diseases <sup>§</sup>	9 (18)	10 (10)	1.7 (0.7-4.3)	0.23
Recurrent pyelonephritis/other	7 (14)	15 (15)	0.9 (0.3-2.5)	0.87
Unknown cause of renal failure	6 (12)	10 (10)	-	-
<b>Smoking status</b>				
Smoking	7/48 (15)	24/97 (25)	1.1 (0.5-2.6)	0.83
<b>Pre-emptive use of basiliximab at Transplantation</b>	15 (30)	39 (39)	0.3 (0.1-1.1)	0.08

PCP denotes *Pneumocystis* pneumonia; OR: odds ratio; 95%CI: 95% confidence interval; IQR: inter-quartile range; BMI: body mass index; ADPKD: autosomal dominant polycystic kidney disease; DM: diabetes mellitus; ATG: anti-thymocyte globulin. fl: systemic vasculitis e.g. Wegener's granulomatosis, Systemic Lupus Erythematoses etc.; ‡: p-value's determined by univariate matched analysis (binary variables) or Student-t test for continues variables. When a difference was found (p-value cut-off <0.10) variables were included in a binary logistic multivariate model (see table 4).

median duration from transplantation to the first and last rejection treatment given were 16 and 48 days respectively, with 95% of treatments administered within 6 months post transplantation for patients with PCP. These numbers were 15, 36 days and 7 months in the group of control patients. The median interval between the first rejection treatment and PCP was 67 days (IQR 53-81 days). The time to development of PCP inversely correlated with the number of rejection treatments given. If no rejection treatment (either Solumedrol, ATG or both) was prescribed, the median time from transplantation to PCP was 114 days (IQR 90-242 days) and decreased to 104 days (IQR 68-216 days) after one rejection treatment, 98 days (IQR 79-199 days) after two and 87 days (IQR 67-117 days) after  $\geq 3$  rejection treatments.



**Table 2.** Immunosuppressive regimen: maintenance at 3 months and at time of diagnosis in patients with and without development of *Pneumocystis pneumonia*.

Immunosuppressive regimen <sup>†</sup>	Cases (PCP+) No. (%)	Controls (PCP-) No. (%)	OR (95%CI)	p-value
<i>At 3 months post Tx:</i>				
1) CNI + PUR	30 (60)	58 (60)	1.2 (0.4-3.3)	0.79
2) CNI	15 (30)	28 (29)	1.1 (0.4-3.0)	0.87
3) PUR	5 (10)	10 (10)	0.7 (0.2-2.6)	0.60
<i>regimen containing:</i>				
MMF	30 (60)	54 (56)	2.7 (0.6-12)	0.19
CsA	44 (88)	73 (76)	2.9 (1.0-8.3)	0.05
<i>At time of PCP diagnosis:</i>				
1) CNI + PUR	25 (50)	54 (56)	0.7 (0.3-1.8)	0.42
2) CNI	14 (28)	29 (30)	0.9 (0.3-2.4)	0.80
3) PUR	10 (20)	12 (13)	1.7 (0.6-4.7)	0.34
<i>regimen containing:</i>				
MMF	30 (60)	52 (53)	2.4 (0.7-8.5)	0.16
CsA	37 (74)	68 (69)	1.3 (0.6-3.1)	0.46

†: data available for 50 cases and 96 control patients, all used oral prednisolon (median daily dose of 10 mg). PCP denotes *Pneumocystis pneumonia*; OR: odds ratio; 95%CI: 95% confidence interval; MMF: Mofetyl mycophenolate; CsA: cyclosporine A; AZA: Azathioprine; TCR: tacrolimus; CNI: calcineurin inhibitor (i.e. CsA or TCR); PUR: purine synthese inhibitor (i.e. MMF or AZA); p-values determined by univariate matched analysis (see methods section). Sirolimus and Everolimus were used only incidentally.

**Table 3.** Treatment for graft rejection as a risk factor for development of *Pneumocystis pneumonia* in kidney transplant recipients.

Variable	Cases (PCP+) No. (%)	Controls (PCP-) No. (%)	OR (95%CI)	p-value
No. of patients in the analysis <sup>‡</sup>	42	84		
<b>Total No. of treatments:</b>				
1	6 (14)	10 (12)	2.5 (0.7-8.8)	0.163
2	9 (21)	11 (13)	4.9 (1.3-18.4)	<b>0.019</b>
≥3	11 (26)	5 (6)	12.9 (3.0-56.3)	<b>0.001</b>
<b>Type of Treatment:</b>				
-Solumedrol only :	8 (19)	12 (14)	1.3 (0.52-3.2)	0.55
-ATG use in at least 1 episode	18 (43)	14 (17)	7.1 (2.0-25) <sup>μ</sup>	<b>0.002<sup>μ</sup></b>

‡: the analysis was restricted to all patients with PCP < 2years post transplantation. PCP: *Pneumocystis pneumonia*; OR: odds ratio; 95%CI: 95% confidence interval; ATG: anti-thymocyte globulin; OR: odds ratio; 95%CI: 95% confidence interval; OR, 95%CI and p-value as determined by matched approach of the data; μ: corrected for the number of treatments given by use of binary conditional logistic regression with matched approach of data using STATA.

The CMV donor/acceptor serostatus at transplantation was not significantly associated with PCP, even if the donor was CMV positive and the acceptor CMV negative (OR 1.2 95%CI 0.5-2.7). In case patients, CMV infection was more frequently present prior to PCP diagnosis (unadjusted OR 2.7 95%CI 1.2-6.2). In the multivariate analysis both CMV infection (OR 3.0 95%CI 1.2-7.9) and rejection treatment (OR 5.8 95%CI 1.9-18) were independently associated with development of PCP (table 4B).

**Table 4.** Multivariate analysis of baseline and contemporary risk factors for development of *Pneumocystis* pneumonia in kidney transplant recipients

<b>A) Risk factors for development of PCP at baseline</b>			
<b>Variable</b>	<b>Adjusted Odds Ratio<sup>‡</sup></b>	<b>95% Confidence Interval</b>	<b>p-value</b>
Sex (male)	0.4	0.2-1.0	0.06
Age 55+	2.7	1.3-5.9	<b>0.01</b>
CsA containing regimen first 3 months post-transplantation	2.1	0.5-8.5	0.30
TCR containing regimen first 3 months post-transplantation <sup>‡</sup>	0.2	0.2-2.3	0.22
Basiliximab induction therapy	0.2	0.1-0.9	<b>0.04</b>
<b>B) Baseline and contemporary risk factors for development of PCP</b>			
<b>Variable</b>	<b>Adjusted Odds Ratio<sup>‡</sup></b>	<b>95% Confidence Interval</b>	<b>p-value</b>
Sex (male)	0.5	0.2-1.3	0.17
Age 55+ at transplantation	1.9	0.8-4.7	0.18
Treatment for rejection (≥1 vs none) <sup>†</sup>	5.8	1.9-17.5	<b>0.002</b>
CMV infection	3.0	1.2-7.9	<b>0.02</b>
Basiliximab induction therapy	0.4	0.1-1.3	0.10

‡ OR's are adjusted for all other variables in the same section of the table. ‡: use of TCR at 3 months post transplantation was added to the analysis to correct for an alternate choice of calcineurine inhibitors with respect to CsA. †: If treatment for rejection was replaced by 'use of ATG' the OR was 4.0 95%CI 1.3-12.5, p=0.015.

### Exploring the need for chemoprophylaxis: calculated estimations

The relevant variables from the multivariate analyses were used to describe several strategies for selective use of PCP chemoprophylaxis. The effect of each of the different strategies is described in table 5. Some strategies - depending on the expected incidence - resulted in incidences <1% and a substantially reduced use of chemoprophylaxis. Assuming a 100% effect of TMP-SMX chemoprophylaxis, prescribing PCP chemoprophylaxis between the 2nd and 6th month prevents approximately 80% of cases occurring within 2 years post transplantation and reduces the use of TMP-SMX more than 5-fold as compared to its use for the whole 2-year period. Continuing chemoprophylaxis between the 2nd and 12th month is more effective (prevention of 91% of cases) but results in a higher number of patients (11-50) needed to treat to prevent one case (NNTP). Effective use of TMP-SMX was also predicted if all patients used chemoprophylaxis between the 2nd and 6th month post transplantation and if patients older than 55 years of age and/or patients treated for graft rejection continued this prophylaxis until 1 year post transplantation (prevention of 83-88% of cases, NNTP at incidences 1-5%: 37-7). Adding CMV infection into the strategy rules did not improve the results.

**Table 5.** Estimated effects of implementation of different selective prophylactic strategies on PCP rate and prescription of TMP-SMX prophylaxis in kidney transplant recipients in the first 2 years post transplantation.

Prophylaxis strategy	Estimated proportion of patients with PCP prevented	Estimated proportion of patients treated	residual frequency   NNTP					
			frequency <sup>f</sup>					
			1.0%	2.5%	5.0%	1.0%	2.5%	5.0%
#0 No prophylaxis	<b>0.00</b>	<b>0</b>	1.00	0	2.50	0	5.00	0
#1 All patients 2-24 mo.	<b>1</b>	<b>1</b>	0.00	100	0.00	40	0.00	20
#2 All patients 2-12 mo.	<b>0.91</b>	<b>0.45</b>	0.09	50	0.23	21	0.45	11
#3 All patients 2-6 mo.	<b>0.79</b>	<b>0.18</b>	0.21	24	0.53	10	1.05	6
#4 Age 55+	<b>0.64</b>	<b>0.32</b>	0.36	51	0.90	21	1.80	11
#5 Treatment for rejection	<b>0.62</b>	<b>0.31</b>	0.38	51	0.95	21	1.90	11
#6 Age 55+ OR Treatment for rejection	<b>0.88</b>	<b>0.52</b>	0.12	60	0.30	25	0.60	13
#7 Age 55+ OR Treatment for rejection OR CMV infection	<b>0.93</b>	<b>0.71</b>	0.07	77	0.18	31	0.35	16
#8 All patients 2-6 mo. & 55+ → 1yr post Tx	<b>0.83</b>	<b>0.27</b>	0.17	33	0.43	14	0.85	7
#9 All patients 2-6 mo. & 55+ → 2yrs post Tx	<b>0.86</b>	<b>0.44</b>	0.14	52	0.35	21	0.70	11
#10 All patients 2-6 mo. & RejRx → 1yr post Tx	<b>0.83</b>	<b>0.26</b>	0.17	32	0.43	13	0.85	7
#11 All patients 2-6 mo. & RejRx → 2yrs post Tx	<b>0.88</b>	<b>0.43</b>	0.12	50	0.30	20	0.60	11
#12 All patients 2-6 mo. & RejRx or 55+ → 1yr post Tx	<b>0.88</b>	<b>0.32</b>	0.12	37	0.30	16	0.60	8
#13 All patients 2-6 mo. & RejRx or 55+ → 2yrs post Tx	<b>0.95</b>	<b>0.61</b>	0.05	65	0.13	27	0.25	14

f: frequency of PCP as percentage of the total No. of transplantation procedures; Tx denotes transplantation; mo.: months; RejRx: treatment for rejection; yr(s): year(s); 55+: above 55 years of age; NNTP: number needed to treat to prevent one case; → 1 yr post Tx: when TMP-SMX is prescribed until 1 year post transplantation. Grey bars highlight prophylactic strategies that result in a relatively high proportion of cases prevented (>0.8) and a relatively low NNTP.

## Discussion

The main findings of this study are that age older than 55 years at the time of transplantation, CMV infection and treatment for rejection were independent risk factors for development of PCP in kidney transplant recipients. No specific immune-suppressive compound was associated with PCP. The vast majority (85%) of PCP cases developed within two years post transplantation. Furthermore, within the first 60 days post transplantation no PCP case was observed. This may be explained by the incubation period as well as by the cumulative suppressive effect on T-cell related immunity. The fact that no cases occurred within these first 2 months post transplantation strongly suggests that the probability that PCP develops in this time window is very low. The observed attributable mortality in this cohort due to PCP was estimated less than 6%. Although higher fatality rates, up to 50%, were previously reported, in more recent publications similar low rates were observed [16, 27]. This may indicate that, due to a multitude of factors e.g. improved post transplantation care and increased awareness among physicians mortality due to PCP in this solid organ transplant population can be diminished.

### Analysis of Risk factors for PCP

The study has some limitations due to the retrospective design and the size of the study cohort, although it is to our knowledge the largest study published on this topic up to date. Robust aspects of our study include the time matched approach of both the selection of control patients and the statistical analysis, which prevented skewed results due to changes in e.g. the first choice of immunosuppressive regimen and alterations of diagnostic strategies to diagnose CMV infection over time. Also, next to microbiological ascertainment, the case definition required clinical signs and symptoms compatible with PCP, thereby preventing inclusion of asymptomatic carriers of *Pneumocystis jirovecii* [28].

The occurrence of PCP in renal transplant recipients has previously been linked to the type of immunosuppressive regimen. It was suggested by case series and experimental studies that CsA in particular increased the risk for PCP in contrast to Azathioprine or MMF [22, 29, 30]. In our study more cases than controls used CsA, but this difference did not reach significance. Noteworthy, CsA was used in a lower dosage in cases as compared to controls, conferring a protective mode of action. This finding remains unexplained by the current knowledge of its effects on either the immune system or *P. jirovecii*.

In concordance with prior observations, we found treatment for graft rejection to be the most important risk factor [20, 21]. A 'dose-dependent' effect was noted, with an OR of more than 10 in patients who received more than 3 rejection treatments compared to patients without rejection. Basiliximab induction therapy at transplantation appeared to be protective which was associated with a decreased need for the use of ATG (data not shown). In the multivariate model to correct for this confounding, basiliximab use as factor that prevented PCP was no longer significant. Presence of CMV infection was independently associated with PCP in uni- and multivariate analyses, suggesting that it is not only an epiphenomenon caused by increased levels of immune suppression. Other clinical studies and in-vitro experiments indicated that CMV may have a direct effect on the cellular immune response [31]. The association we found may be partly ascribed to a decreased immune status due to treatment for rejection, and at least in part to a direct effect of CMV infection on cellular immune responses.

### Appraisal of prophylactic strategies for PCP

In modeling the risk for development of PCP, knowledge of exposure rates or risk per patient could increase our understanding of managing the issue of chemoprophylaxis. However, exposure rates for kidney transplant recipients or other patient groups at risk are, for obvious reasons, not available. Although the mode of transmission of *Pneumocystis jirovecii* is heavily debated, increasing consensus exist about asymptomatic carriership in humans as the primary source (in healthy- and immunocompromised individuals) and about interhuman transmission [32-35]. Since it is known from serologic and microbiologic evidence that

more than 80% of infants is exposed within 2 years after birth, it is unlikely that exposure can be avoided long term by kidney transplant recipients [36]. From a clinical practice point of view, the impact of 13 simple selective chemoprophylactic strategies - based on the outcome of this study - was explored at different PCP incidence rates (table 5). Without chemoprophylaxis, the estimated burden of hospital admissions, need for treatment at an ICU and mortality at an incidence <1% is less than 10, 0.8 and 0.5 cases per 1000 transplantation procedures respectively. To maintain these desirable low rates at higher PCP incidences and at the same time avoiding unnecessary use and side effects, several chemoprophylactic strategies seemed feasible. In addition to solely time-based schedules, combining timing with risk factors from the multivariate analysis predicted a more effective use of TMP-SMX and avoidance of unneeded use (60-70%) and subsequent adverse events. Of note, a selective chemoprophylactic strategy may be rolled out only when regular follow-up visits, easy access to high standards of care and awareness of attending physicians is warranted. Next to chemoprophylaxis, other preventive measures, e.g. avoiding contact of patients with PCP with kidney transplant recipients when hospitalized must be considered.

## **Summary and conclusions**

The results of this study provide substantial support for a risk factor based, differentiated approach towards PCP chemoprophylaxis, comprising the first 6 months for all- and for a prolonged period (e.g. during the first year) post transplantation for patients over 55 years of age and those treated for graft rejection. This is partly in line with the recently updated KDIGO guidelines but adds considerably to European guidelines issued in 2002 [5]. As for PCP, chemoprophylaxis may be delayed 4-6 weeks post transplantation. However, depending on local circumstances, other indications (e.g. the risk for Toxoplasmosis) may necessitate the prophylactic use of TMP-SMX in this period [37]. Physicians should also be aware that prolonged prescription of prophylaxis, even more than 2 years post transplantation, sometimes is necessary for those patients at increased risk due to accompanying, conditions (e.g. treatment for lymphoma). Since PCP in kidney transplant recipients remains relatively rare, the safety and effectiveness of the above mentioned strategic approach for managing PCP chemoprophylaxis should be confirmed by long term prospective evaluation of their use in clinical practice.

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# Chapter 4 | Supplement I

## CALCULATION OF THE DIFFERENT PARAMETERS FOR THE EFFECTIVENESS OF SELECTIVE PROPHYLAXIS FOR PNEUMOCYSTIS PNEUMONIA (SUPPLEMENT TO TABLE 5)

### Proportion of prevented PCP cases

The effect of a strategy to prevent PCP can be described by the proportion of prevented PCP cases by this strategy ( $P_{prev}$ ) within a certain time period post transplantation (in this study between the 2<sup>nd</sup> month and the first 2 years post-transplantation). The (estimated) proportion of prevented cases  $P_{prev}$  is calculated by dividing the number of cases that would have prevented ( $N_{prev}$ ) by a strategy (e.g. strategy X) by the number of cases that would have occurred without this strategy ( $N_{norm}$ ):

If no strategy is applied  $P_{prev} = 0 / N_{norm} = 0$

If a strategy is applied that prevents all cases:

$N_{prev} = N_{norm}$  and thus  $P_{prev} = N_{norm} / N_{norm} = 1$

$P_{prev} = N_{prev} / N_{norm}$

In the current described model, the data from the cases (PCP patients) were used to calculate  $P_{prev}$ . For example: suppose strategy X was to prescribe prophylaxis from the 2<sup>nd</sup> month to the first 2 years post-transplantation to all patients older than 55 years. There were 42 patients who experienced a PCP in this period, of whom 27 were older than 55 years at the time of diagnosis. Assuming that the prophylaxis is 100% succesful, this means that  $N_{prev} = 27$  and  $N_{norm} = 42$  and filling in the formula:  $P_{prev} = N_{prev} / N_{norm} = 0.64$ . This implies that 64% of cases , could be prevented by strategy X.

### Residual frequency

Assume that  $I$  is the incidence (the frequency of occurrence) of PCP in the general population before applying any strategy. The incidence (residual frequency) after applying strategy X ( $I_{res}$ ) can be calculated as:

$$I_{res} = (1 - P_{prev}) \times I$$

**Proportion of patients treated unnecessary**

From the data of control patients, the proportion who would have received unneeded prophylaxis can be calculated (Proportion of persons treated,  $P_{pt}$ ) under a strategy X :

$$P_{pt} = \frac{\text{Number of controls receiving prophylaxis under strategy X}}{\text{total Number of controls}}$$

In this model the control group serves as a representative of the total population of kidney transplant recipients. At low incidences of the disease, the need for correction due to the number of 'would be case' patients receiving prophylaxis in this model can be neglected.

**The number needed to treat to prevent one case (NNTP)**

To assess the efficacy of a strategy X, we calculated the number needed to treat to prevent one case (NNTP). The NNTP for strategy X is equal to the total number of patients that would receive prophylaxis (i.e. the number of cases that would have been prevented ( $P_{prev} \times I$ ) plus the number of controls that would receive prophylaxis ( $(1-I) \times P_{pt}$ ), divided by the number of cases that would have been prevented ( $P_{prev} \times I$ ):

$$NNTP = \frac{I \times P_{prev} + (1 - I)P_{pt}}{I \times P_{prev}}$$

**Note:**

To work with a both useful and practical model, only the most relevant variables from the multivariate analyses (in this study: age >55 years and treatment for rejection, as well as the time windows of interest ) should be incorporated in the set of simple prediction rules to explore the effect of different strategies for selective use of prophylaxis as demonstrated in table 5.



