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Frequent occurrence of late-onset pneumocystis pneumonia in renal transplant recipients with recurrence in the absence of secondary prophylaxis

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Frequent occurrence of late-onset pneumocystis pneumonia in renal transplant recipients with recurrence in the absence of secondary prophylaxis

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Institutional Review Board approval for the project was obtained.

Abstract

Background:

Pneumocystis jirovecii pneumonia (PCP) is a potentially life-threatening infection in renal transplant recipients (RTRs), usually occurring early after transplantation. A high proportion of late-onset PCP was observed at our centre. The aim of this study was to evaluate risk factors for late PCP after kidney transplantation.

Materials/methods:

Thirty-six PCP cases were identified in our cohort between 01/2009–12/2014. We analysed clinical, laboratory and radiological data from all confirmed PCP cases. Molecular genotyping of PCP was performed in a subset of patients.

Results:

Among 36 cases (26/36, 72% males) of PCP 30 showed late-onset presentation (30/36, 84% >6 months, 25/36, 69% >12 months after transplantation), with a median indwelling time of 27 months (range 3–205 months). Clinical symptoms were rather unspecific (cough/dyspnoea ~70%, SpO2 <90% ~60%, and fever ~30%), but radiological findings (>85%) and biochemical pattern at PCP diagnosis were rather specific: a significantly lower total lymphocyte count and lower serum sodium together with higher lactate dehydrogenase (LDH) and serum calcium levels than before diagnosis. None of the patients were on PCP prophylaxis at diagnosis. No specific risk factors (e.g. increased daily prednisone dose) for PCP reactivation were found in two-thirds (23/36, 64%) of the late-onset cases.

The outpatient consultations map showed several possible encounters between PCP and RTR patients, including 26 cases (72%) with at least one contact before diagnosis, suggestive of interhuman transmission, and the same genotype in a subset of cases tested.

Secondary prophylaxis was established in 32/36 patients for a median duration of 15.7 months.

Conclusion:

In conclusion, we confirm that under the current immunosuppression regiments PCP manifests late after transplantation with distinct laboratory and radiological patterns. Interhuman transmission seems to be the major driver in patients without prophylaxis. Interventions covering the entire population at risk are therefore needed.

Outline

Abbreviations

ADH	Antidiuretic hormone
AUC	Area under the curve
BAL	Broncho-alveolar lavage
CMV	Cytomegalovirus
CRP	C-reactive protein
CT scan	Computed tomography scan
HIV	Human immunodeficiency virus
ICU	Intensive care unit
IQR	Interquartile range
LDH	Lactate dehydrogenase
MMF	Mycophenolate mofetil
mTOR	Mechanistic target of rapamycin
N	Number
NSTEMI	Non-ST segment elevation myocardial infarction
P. jirovecii	*Pneumocystis jirovecii*
PCO_2	Carbon dioxide partial pressure
PCP	*Pneumocystis jirovecii* pneumonia
PCR	Polymerase chain reaction
PO_2	Oxygen partial pressure
ROC	Receiver operating characteristic
RTR	Renal transplant recipient
RTx	Renal transplantation
SOT	Solid-organ transplant
TMP/SMX	Trimethoprim/sulfamethoxazole

Introduction

Pneumocystis pneumonia (PCP) caused by *Pneumocystis jirovecii (P. jirovecii)* is one of the most frequent opportunistic infections, with marked morbidity and mortality in immunocompromised patients[1]. Renal transplant recipients (RTRs) are at the highest risk of developing PCP early after transplantation[2]. The introduction of *P. jirovecii* prophylaxis after transplantation has reduced the incidence of PCP among solid-organ transplant recipients (SOT)[3]. Despite universal post-transplantation prophylaxis and the implementation of steroid-sparing immunosuppressive regiments over the last decades, several clusters of PCP outbreaks, predominantly among RTRs, have been reported worldwide[4-20].

Most clusters of RTRs occurred late (>12 months) after renal transplantation (RTx), and therefore beyond the currently recommended prophylaxis period[9,11,21]. Recommendations as to when to maintain or reintroduce individual prophylaxis after the early post-transplantation period are lacking. Risk factors for PCP after six months post-transplantation may include age, cytomegalovirus (CMV) infection, immunosuppressive regimen, treatment of rejection episodes, and low lymphocyte count[9,17,21,22]. Probably the most important risk factor in developing late-onset PCP is exposure to other patients with PCP, since most cases of late-onset PCP occur in cluster outbreaks[9,17,21,22,23]. The transmission mode of *P. jirovecii* is strongly suspected to be airborne; patient-to-patient transmission is well described[17,24]. Colonization by *P. jirovecii* and reactivation may also play a role in the development of PCP infections and outbreaks[18,25]. Laboratory changes associated with PCP are lactate dehydrogenase (LDH) elevation[22] and hypercalcemia[26]. Outbreaks are mostly terminated by establishing chemoprophylaxis in the entire patient cohort affected[27].

We recently reported on radiological differences in PCP between RTRs and patients with human immunodeficiency virus (HIV) infection[28]. In this subsequent study, we explored the prolonged cluster of cases of PCP that occurred at our institution from January 2009 through

December 2014.

The primary aim of this study was to investigate the incidence, time of onset, potential acquisition, and transmission routes of PCP and to identify risk factors associated with late PCP manifestation, defined as infections occurring beyond 6 months post transplantation.

Patients and methods

Patient inclusion

This retrospective case study analysis included all RTRs in the local adult transplant recipient cohort who presented with clinical/radiological suspicion of PCP at admission. One of the following confirmative criteria was necessary in order for the cases to be enrolled: 1) microbiological confirmation of *P. jirovecii* by staining or polymerase chain reaction (PCR) in sputum or broncho-alveolar lavage (BAL) sample; 2) successful antimicrobial PCP treatment.

Ethics

All transplantations were performed according to the criteria of the Swiss National Transplant program based on strict national laws and regulations for cell, tissue, and organ donation (https://www.admin.ch/opc/de/classified-compilation/20051806/index.html, Swiss national law 810.211,) and conformed with international standards. None of the transplant donors was from a vulnerable population and all (living) donors or next of kin provided freely given, written informed consent. All research involving human participants is approved by the local Cantonal Ethics Commission of Berne (Kantonale Ethikkommission Bern). All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Patient data

Data on the use of PCP prophylaxis, treatment, and secondary prophylaxis, medication, laboratory parameters, and dates of inpatient stays and visits to the outpatient department, underlying disease, clinical condition, and demographic data were obtained from the hospital information system (i-pdos/phoenix).

Laboratory analysis

Laboratory parameters (sodium, potassium, calcium, phosphate, C-reactive protein (CRP), creatinine, urea, glomerular filtration rate, LDH, leukocytes, lymphocytes, neutrophilic granulocytes, oxygen partial pressure (PO_2), carbon dioxide partial pressure (PCO_2), and pH) were analysed at the time of admission and three months, two months, and one month before and after PCP diagnosis. Laboratory parameters at the different timepoints were compared using a paired t-test to establish differences that might be associated with PCP. Distribution was assumed to be non-normal due to the cohort size and *p*-values <0.05 were considered statistically significant. Statistics was done with GraphPad Software Inc. Version 6.

Results

Incidence

47 PCP cases were identified in the local transplant cohort from 2002–2014. We included 36 cases occurring after 1 January 2009, after the computerized hospital information system was implemented. The incidence of PCP during the study period ranged from 2.61 to 20.87/1000 patient years at risk (Figure 1). The cumulative incidence was 4% over the 6-year period. The median time between transplantation and PCP diagnosis was 27 months (interquartile range (IQR) 11–69). Late-onset PCP (>6 months after RTx) occurred in 30/36 cases (83%). Figure 2 shows the indwelling time from transplantation to PCP diagnosis. 75% of the cases of PCP occurred after the first 11 months (27/36), and 50% (18/36) later than 22 months after transplantation.

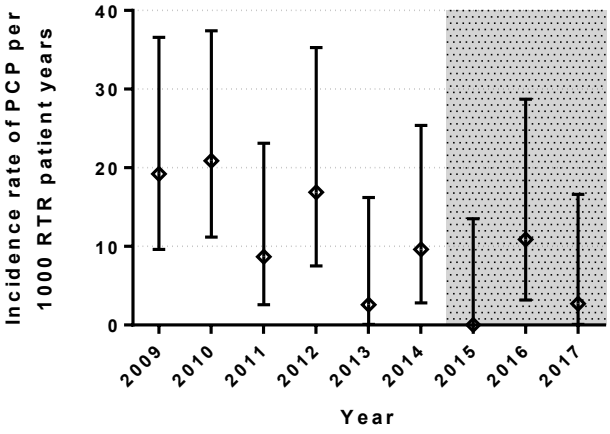

Figure 1. Y-axis: incidence of *Pneumocystis jirovecii* pneumonia (PCP) per 1000 patient years of renal transplant recipients (RTR). Vertical bars, 95% confidence interval. The greyish highlighted area shows the further course after the study period (2009–2014), which showed a tendency to decline. Prolongation of PCP prophylaxis had taken place due to a high incidence of PCP. 6 months of post-transplantation prophylaxis was given from March 2013 onward, and in May 2014 was increased to 12 months.

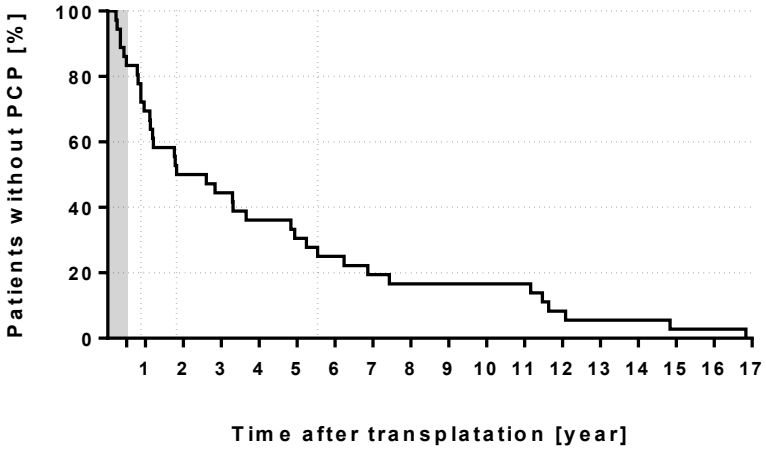

Figure 2. Distribution of 36 *Pneumocystis jirovecii* pneumonia (PCP) cases according to the time between transplantation and PCP diagnosis. The grey area is the early-onset period of 6 months after renal transplantation. The first vertical line is the timepoint by which 25% of all PCP cases had been diagnosed; the second vertical line is the timepoint at which 50% of all PCP cases had been diagnosed. The third vertical line is the timepoint at which 75% of all PCP cases had been diagnosed.

Clinical, radiological, and laboratory characteristics

Clinical features, patient data, and outcome are shown in Table 1. During the study period, 36 consecutive PCP cases were identified. Most patients were male (72%, 26/36) and had received a cadaveric allograft (81%, 29/36). In 34/36 (94%) patients, the PCP diagnosis was established by isolation of *P. jirovecii* from BAL specimens: 79% (27/34) with immunofluorescence, and 7/34 by qualitative PCR alone. In the remaining two cases, clinical manifestations, computed tomography scan (CT scan) findings, and resolution under trimethoprim/sulfamethoxazole (TMP/SMX) were indicative for PCP. Determination of 1,3 β-d-Glucan was not regularly performed. The duration from symptom onset to PCP diagnosis was 10 days (IQR 5–14). The most common symptoms were unspecific, such as cough and dyspnoea (both >80%). Fever was only present in 1/3 of all cases (35%, 11/31). Specific PCP-related radiological findings with predilection sites in the upper lobes; subpleural sparing, and asymmetric ground glass opacities were documented in the majority of cases in CT scans (87%, 27/31). Conventional chest radiographs were unremarkable in 11/30 (37%) patients.

Relevant laboratory values are shown in Figure 3. Statistically significant changes in serum sodium, calcium, LDH, and lymphocyte count were observed between the preceding period and on diagnosis of PCP. Serum sodium showed a declining course with hyponatremia at the time of diagnosis, while serum calcium showed an increase towards the diagnosis. LDH as a marker of cellular damage increased during the course of the disease and lymphocyte count decreased. Figure 4 shows the receiver operating characteristic (ROC) curves of laboratory characteristics at PCP diagnosis and three months before. In contrast to serum calcium, the serum sodium, LDH levels and lymphocytes reached accuracy (Area under the curve (AUC) >0.70).

Table 1. Characteristics of 36 cases of P. jirovecii pneumonia (PCP) among renal transplant recipients (RTRs)[a]

	Median (IQR)	*N*=36	%
Age, years (range)	58 (22–77)		
Male		26/36	72.2
Time between RTx and PCP, months	27 (11–69)		
Type of renal donor			
Cadaveric		29/36	80.6
Living related		7/36	19.4
Early-onset PCP[b]		6/36	16.7
Late-onset PCP[c]		30/36	83.3
Clinical presentation at PCP diagnosis			
Fever		11/31	35.5
Cough		23/32	71.9
Chills		5/32	15.6
Dyspnoea		23/33	69.7
Acute respiratory failure		20/34	58.8
Time between symptom onset and PCP, days (IQR)	10 (5–14)		
Radiological findings suspicious for PCP on chest radiograph		19/30	63.3
Radiological findings suspicious for PCP on CT-scan		27/31	87.1
Length of hospital stay, days (IQR)	12 (7–24)		
ICU stay		5/36	13.9
Length of ICU stay, days (IQR)	5 (3–5)		
Chemoprophylaxis at PCP diagnosis		0	0
Immunosuppressive agents at PCP diagnosis			
Calcineurin inhibitors		25/35	71.4
Ciclosporin		19/35	54.3
Tacrolimus		6/35	17.1
mTOR inhibitors		6/35	17.1
Sirolimus		1/35	2.9
Everolimus		5/35	14.3
Antimetabolites		29/35	82.9
Mycophenolate mofetil		28/35	80
Azathioprine		1/35	2.9
Prednisone		32/35	91.4
Recurrence of PCP		1/36	2.8
Time until recurrence of PCP, months	19		
Death		6/36	16.7
During hospitalization		2/36	5.6
NSTEMI		1/36	2.8
Pulmonary embolism		1/36	2.8
After hospitalization		4/36	11.1
Time between PCP and death, months (IQR)	23 (3–38)		

[a] Abbreviations: PCP, *Pneumocystis jirovecii* pneumonia; RTx, renal transplantation; IQR, interquartile range; N, Number; CT-scan, computed tomography scan; ICU, intensive care unit; mTOR inhibitor, mechanistic target of Rapamycin inhibitor; NSTEMI, non-ST segment elevation myocardial infarction.

[b] PCP Diagnosis within 6 months after RTx.

[c] PCP Diagnosis beyond 6 months after RTx.

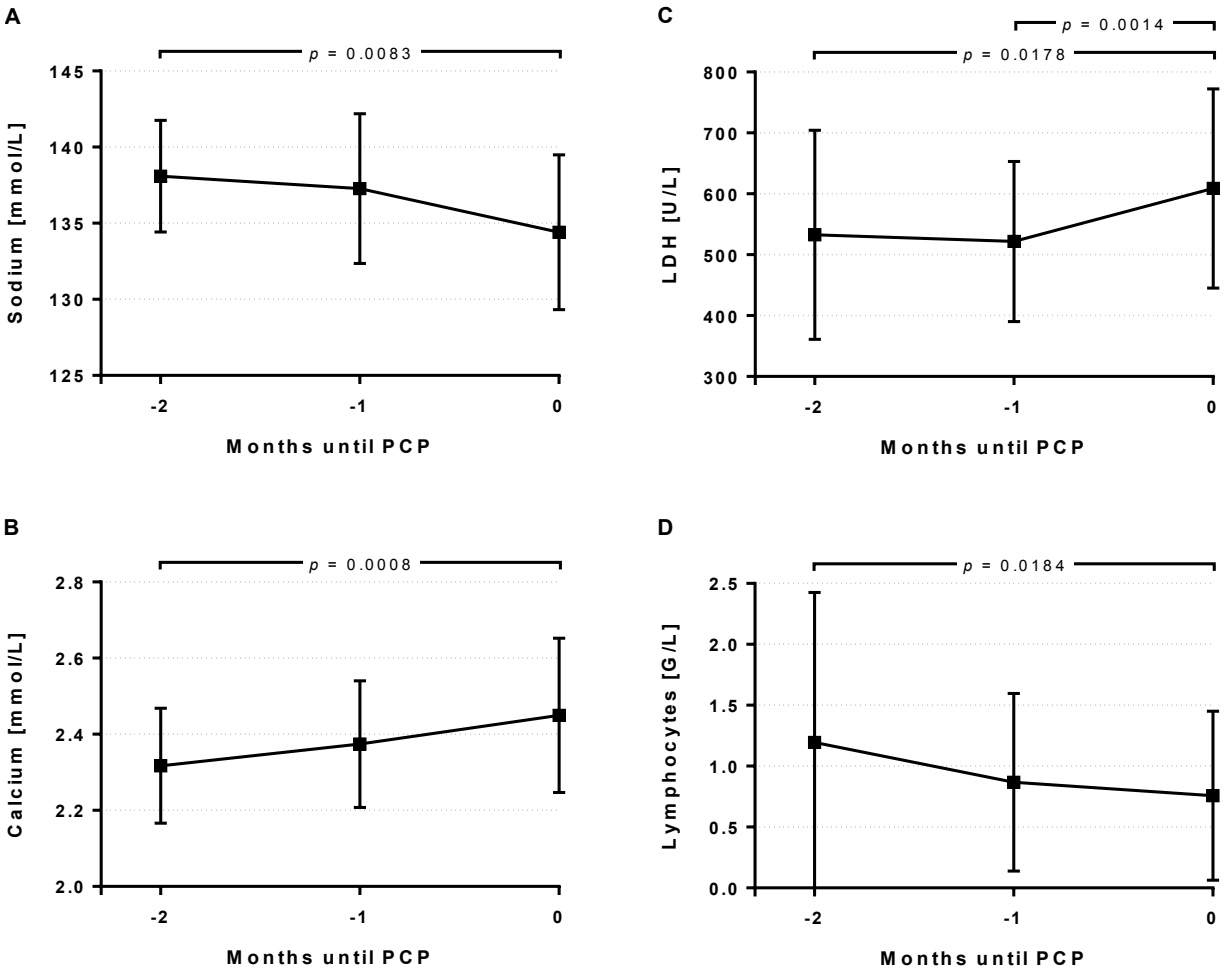

Figure 3. Course of sodium (a), calcium (b), lactate dehydrogenase (LDH) (c), and lymphocyte count (d) in renal transplant recipients (RTRs) two months and one month before *Pneumocystis jirovecii* pneumonia (PCP) diagnosis, and at the time of admission. Sodium and lymphocyte count showing a decreasing trend towards diagnosis, and calcium and LDH an increasing trend.

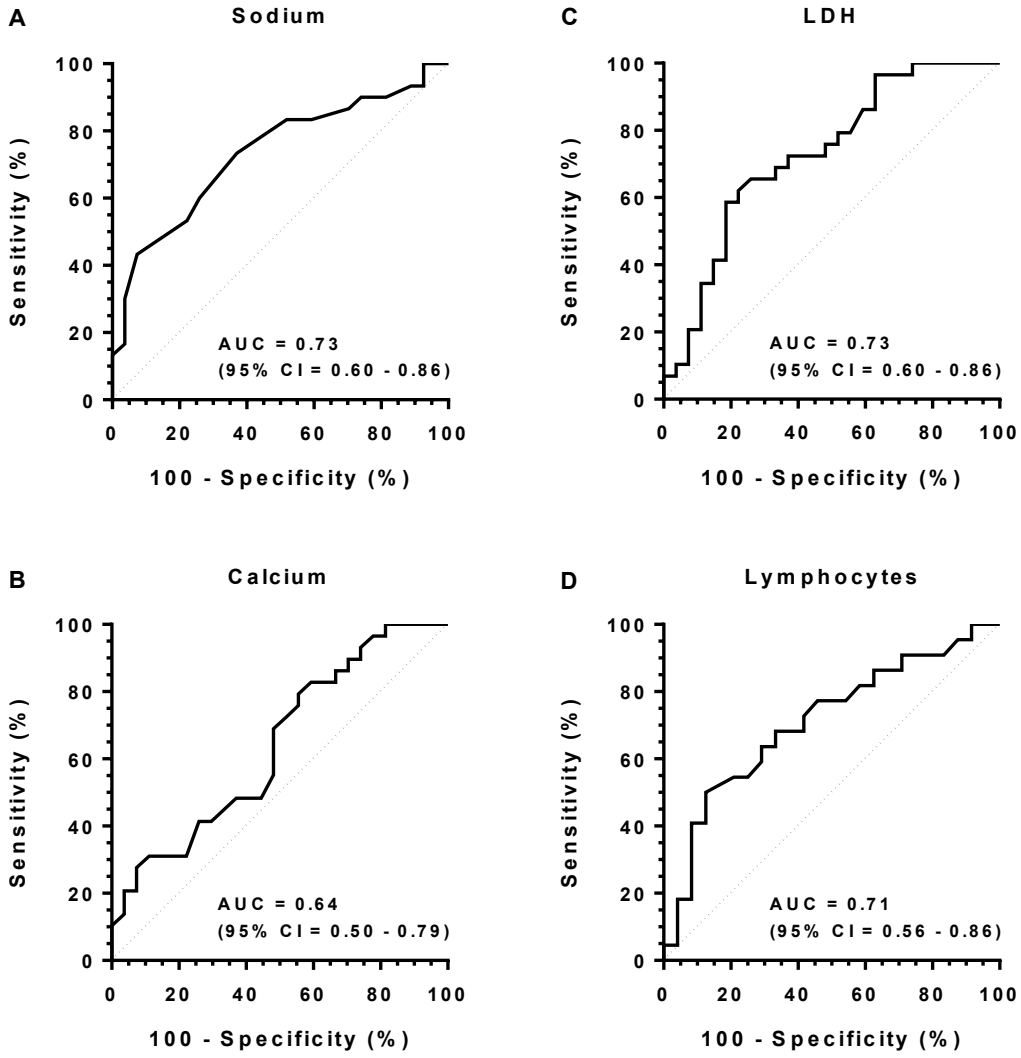

Figure 4. Receiver operating characteristic (ROC) curves of several laboratory parameters in renal transplant recipients (RTRs) three months before the diagnosis of *Pneumocystis jirovecii* pneumonia (PCP) compared to the laboratory parameters at time of diagnosis; sodium (a), calcium (b), lactate dehydrogenase (LDH) (c), and lymphocyte count (d). AUC stands for area under the curve.

Risk factors and prophylaxis

The standard immunosuppressive regimen at our centre consists of triple therapy with calcineurin inhibitors (primarily ciclosporin or tacrolimus), antimetabolites (primarily mycophenolate mofetil), and steroids, which are tapered slowly. Our induction regime also includes basiliximab on days 0 and 2 after transplantation. At PCP diagnosis, about three-quarters of patients (71%, 25/35) were receiving triple-therapy immunosuppression including a calcineurin- or an mechanistic target of rapamycin (mTOR) inhibitor, 8/35 (23%) were on dual immunosuppression, and 2/35 patients were on corticosteroids only. The regime for one

patient was unknown. 32/35 (91%) patients were receiving corticosteroids, 29/35 (83%) antimetabolites, 25/35 (71%) calcineurin inhibitors, 6/35 (17%) mTOR inhibitors (Table 1).

In 6/36, PCP occurred in the first 6 months after transplantation. In the six patients who developed early PCP, prophylaxis had not been given due to allergy (3/6), transient allograft dysfunction (1/6), and compliance issues (2/6).

In 26 patients (72%), at least one kidney biopsy was performed due to suspected rejection between transplantation and the onset of PCP (Figure 5), in most cases with presumed corticosteroid pulse therapy. The median indwelling time between corticosteroid pulse therapy and PCP diagnosis was 12 (IQR 4–36) months. In one third of all patients (12/36), kidney biopsy had been performed within the last 12 months, in four cases (11%) within the last three months before PCP diagnosis, but none of the patients experienced acute rejection. In the last 3 months before PCP diagnosis, corticosteroid doses >20mg/day for >4 weeks had been given in 22% (8/36) of cases. CMV reactivation was detected in 3/36 patients. PCP prophylaxis had not been established in 13/36 (36%) patients, despite previously mentioned possible indications. TMP-SMX was the most frequent prophylaxis.

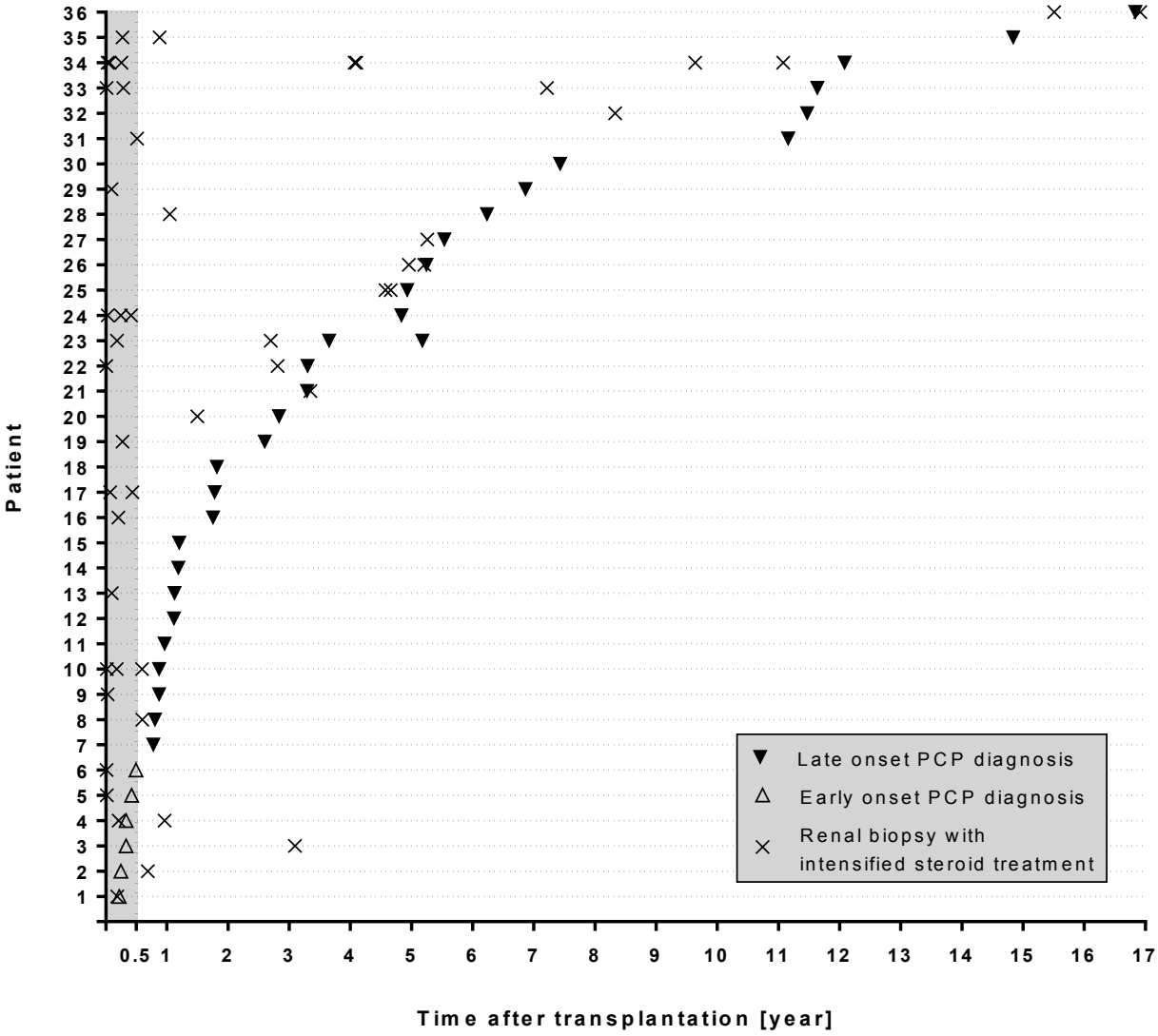

Figure 5. Occurrence of *Pneumocystis jirovecii* pneumonia (PCP) after transplantation and correlation between corticosteroid pulse therapy and PCP onset. Renal biopsy had been performed and corticosteroid pulse therapy was being given due to suspected rejection.

PCP Transmission

Figure 6 shows the occurrence of PCP over time. In 20/36 (55%) patients, PCP occurred over a period of 22 months (01/2009–11/2010), suggestive of a cluster. The remaining 16/36 (44%) cases occurred over a period twice as long, of 46 months (03/2011–12/2014). Patients diagnosed with PCP who visited the outpatient clinic on the same day as another patient who was later diagnosed with PCP were noted. 26 patients had contact with at least one other PCP patient before PCP onset. The median time between outpatient contact and PCP diagnosis was 27 (1–59) days. Genotyping of *Pneumocystis* strains was performed in 21

BAL samples from 21 different patients. Seven samples were suitable for analysis and all were of the same genotype.

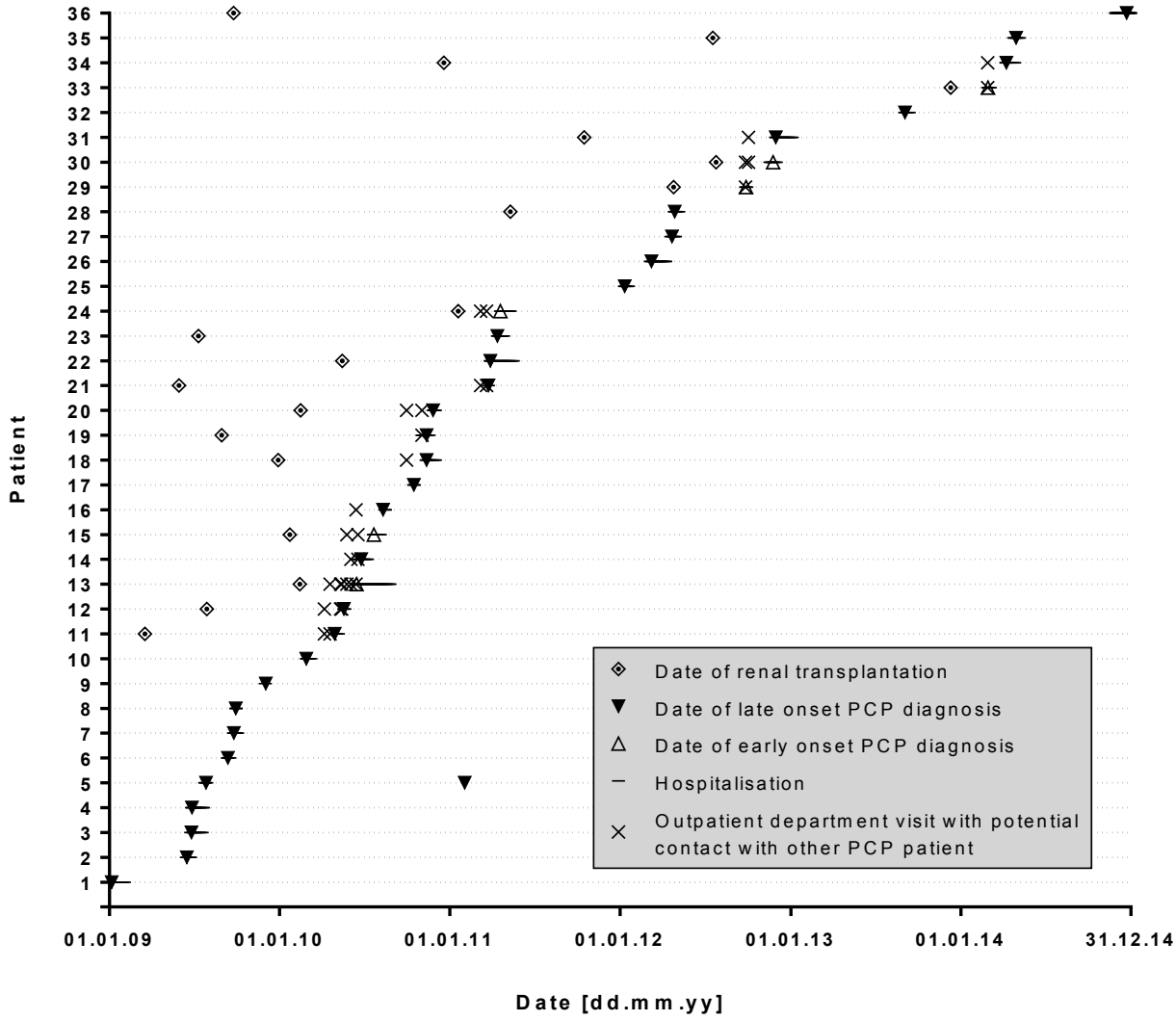

Figure 6. Transmission map of 36 *P. jirovecii* pneumonia (PCP) cases. Each day when at least two PCP-patients attended the nephrology outpatient department is marked with an X.

General Prophylaxis

PCP prophylaxis was usually given for 3–6 months at the start of the study. During follow-up, awareness was raised, and from 03/2013 onwards, PCP prophylaxis was prolonged to 6 months after transplantation and then later increased to 12 month after transplantation as secondary prophylaxis in 05/2014. Blanket prophylaxis for the entire population at risk was given. Incidence of PCP decreased over time but was never completely stopped (Figure 1).

Secondary Prophylaxis

Secondary prophylaxis was started in 32/34 (94%) patients and was given for a median of 15.7 months. Permanent prophylaxis was in place at the end of follow-up in only 4 patients. PCP recurred in 1 patient (1/36) 19 months after the initial PCP infection (02/2011); secondary prophylaxis was given for 12 months.

Outcome

Two patients died during hospitalization for PCP, but death was not associated with the infection. One patient died due to myocardial infarction and one due to pulmonary embolism. Six patients died during the follow-up period, but none of the deaths were associated with PCP. Median follow-up was 5.26 (0.03–7.99) years.

Discussion

Despite primary PCP prophylaxis, an elevated incidence of late-onset PCP (>80% later than 6 months after transplantation) was found in our RTR cohort, similar to other centres[9,15,17,21,23,29]. In two-thirds of patients (64%), no individual risk factors (acute rejection, recent CMV infection, or elevated steroid dosage) requiring restart of PCP prophylaxis were present. However, encounters with other transplant recipients involved in the cluster, indicative for interhuman transmission as probably the most important driver for late PCP occurrence, did occur[22,27].

Other risk factors associated with late-onset PCP, including age, lymphocyte count and immunosuppressive regimen (e.g. high doses of calcineurin inhibitors and steroid maintenance), interfere with the net status of immunosuppression[9,17,22]. These parameters are highly indicative of a risk of developing PCP in clinical practice, but are not statistically robust. Restarting prophylaxis in all patients experiencing one or more of the above factors would be complicated and would probably lead to a large number-needed-to-treat to prevent one case of PCP. Nevertheless, in a case-control study[22] in patients aged >65 years with a lymphocyte count <750/mm^3 and detectable CMV viremia, these factors together would have led to 70% of patients with PCP versus 30% of the control patients benefiting from PCP prophylaxis in the 180 days before diagnosis of PCP infection. In our cohort, 74% would have been covered.

Clinical presentation in our patients was unspecific (cough and dyspnoea >80%) and oligosymptomatic (fever in only 30%), reflecting a slowly progressing, subtle clinical course, difficult to distinguish from other respiratory infections. Interestingly, however, specific laboratory and radiological (CT scan) changes were found (Figures 3 and 4). A decline in serum sodium was seen, as described in other lower respiratory infections (e.g. Legionnaire's disease), where hyponatremia has traditionally been assumed to be the result

of increased production of antidiuretic hormone (ADH), although this has been recently challenged[30]. The median lymphocyte count was 1.03 G/l three months before the onset of PCP and 0.75 G/l one month before. Although the rate of patients with a lymphocyte count <0.75 G/l remained stable (7/22 vs. 9/18), lymphopenia is an emerging risk factor for late-onset PCP infection[9,17,21,22], with a relatively sharp decrease in levels in the period immediately before PCP infection (Figure 3). This was also seen in a French case-control study where lymphopenia was significantly more often present in PCP-cases than controls in the 50 days before onset of PCP, but not in the 200 days before[22]. In addition, in a retrospective study, increased serum LDH was seen around the time of onset of PCP reflecting cellular damage, with a good positive and negative predictive value for PCP[31]. Interestingly, ROC curves for the above-mentioned values showed accuracy. In addition, we observed an increase in serum calcium, possibly associated with extended 1,25-dihydroxyvitamin D levels in fungal infections[22,26]. In most patients (27/31, 87%), CT scans showed findings suggestive of PCP, as already described[28]. The role of conventional chest radiography in the diagnosis of PCP seems very limited (11/30, 37% with suggestive findings). In summary, in patients with unspecific symptoms together with the above laboratory findings, PCP should be suspected and confirmed using adequate diagnostic procedures (CT, BAL). The abovementioned markers should also be evaluated in prospective cohorts to rule out or confirm PCP in this vulnerable population, as is done for other respiratory infections[32].

In our cohort – in contrast to the older literature[33] – the outcome was favourable for most patients. Only a few patients had to be admitted to the intensive care unit (ICU) for mechanical ventilation (14%, 5/36). No patients died of PCP.

In our patients, the median indwelling time from transplantation to PCP onset was 27 months. This was in broad agreement with other studies reporting periods from 19 to 32 months in patients receiving 6 months of prophylaxis after transplantation[17,21]. Accordingly,

the second year after RTx has been described as the new period with the highest risk of developing PCP[22].

Because of the abovementioned changing tendency for PCP to become a late onset infection – with PCP cases being described in all post-transplantation periods – the appropriate duration of antimicrobial prophylaxis is controversial. PCP outbreaks have even been described[9] in centres offering long-term prophylaxis of 12 months after transplantation. Lifelong prophylaxis is therefore given at some renal transplant centres[34]. Studies evaluating the benefits and disadvantages of prolonged prophylaxis should be conducted, since, as yet, no explicit recommendations have been published regarding the prolongation of PCP prophylaxis, and lifelong PCP prophylaxis has been proposed to be cost effective in an unpublished modelling study[34].

During our cluster, primary prophylaxis was prolonged to 6 months and then to 12 months to protect the most susceptible patients, and was given thereafter for shorter periods. In addition, finite secondary prophylaxis was established in the majority of patients. Immunosuppression was reduced in 12 patients (in five patients Mycophenolat-Mofetil (MMF), and in five patients the ciclosporin dose was reduced; two patients even had a reduction of both MMF and ciclosporin dosage).

It seems that an inconsistent approach to the restarting of PCP prophylaxis in cases where immunosuppression was increased (e.g. rejection treatment or higher steroid doses) and any secondary prophylaxis given for finite periods may play a significant role. Similarly, inappropriate PCP prophylaxis has been detected in most clusters[27]. Blanket prophylaxis was not established in our patients to prevent further PCP cases. To end outbreaks, blanket prophylaxis for the whole population at risk has been successfully given for 6–12 months beyond the last case of PCP[9,14]. In addition, in a centre reporting adverse events with this strategy, a high number of complications leading to termination of prophylaxis in 38% of

stable renal transplant recipients was reported. Relevant adverse events were an acute rise in creatinine levels (20%, >20%), gastrointestinal symptoms (5%), and leukopenia (2%)[35].

Approaches to improve the uptake of TMP-SMX prophylaxis in patients with previous adverse events led to an improvement in uptake of first-line PCP prophylaxis[36].

The role of person-to-person transmission versus an unidentified common environmental source of exposure was not elucidated in any outbreaks[3] and no formal infection control policies had been introduced at the hospitals or healthcare clinics concerned, even though the importance of such measures is underlined by the large number of reported outbreaks with presumed nosocomial acquisition and by possible person-to-person transmission[27]. Droplet precautions have therefore also been suggested by some authors[27].

The large number of cases and the long follow-up period with collection of detailed information are strengths of our study. Our study has several limitations, however, due to its retrospective and observational nature. In addition, due to technical issues, sequencing was only successful in a limited number of patients. Common characteristics were therefore mainly based on similar outbreaks from the literature, the large number of PCP cases, and encounters between the cases, mainly in the outpatient clinic. A further limitation is the lack of data on secondary prophylaxis and strategies other than blanket prophylaxis to end PCP outbreaks.

In conclusion, late-onset PCP occurred in our patients despite post-transplantation prophylaxis. Individual factors indicating that prophylaxis should be restarted were not present in most patients, but we did observe encounters with PCP cases supporting interhuman transmission as a main driver in the occurrence of late-onset PCP. Unlike blanket prophylaxis, prolongation of post-transplantation prophylaxis failed to stop ongoing PCP transmission.

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