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Lessons learned from a pneumocystis pneumonia outbreak at a Scottish renal transplant centre

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SUMMARY

Background: Pneumocystis pneumonia (PCP) is an opportunistic infection occurring in renal transplant patients. Over a 14-month period an increase in PCP cases was identified among our renal transplant cohort.

Aim: The outbreak population was studied to identify potential risk factors for the development of PCP.

Methods: A retrospective analysis of hospital records was carried out, with each case being matched with two case-linked controls. Information was collected on patient demographics, laboratory tests, and hospital visits pre and post development of infection.

Findings: No patients were receiving PCP prophylaxis at the time of infection and mean time from transplantation to developing PCP was 4.7 years (range: 0.51–14.5). The PCP group had a significantly lower mean estimated glomerular filtration rate than the control group (29.3 mL/min/1.73 m^2 vs 70 mL/min^{-1} ($P = 0.0007$)). Three patients were treated for active cytomegalovirus (CMV) infection prior to PCP diagnosis and two had active CMV at the time of diagnosis compared to none in the control group ($P = 0.001$). Those who developed PCP were more likely to have shared a hospital visit with another patient who went on to develop PCP; 37% of clinic visits vs 19% ($P = 0.014$).

Conclusion: This study highlights the ongoing risk of opportunistic infection several years after transplantation and adds weight to potential person-to-person *Pneumocystis jirovecii* transmission. Risk factors have been identified which may highlight those most at risk, enabling targeted rather than blanket long-term PCP prophylaxis.

Introduction

Pneumocystis jirovecii is an opportunistic fungal pathogen capable of causing severe pneumonia in immunocompromised individuals, leading to considerable morbidity and mortality. Pneumocystis pneumonia (PCP) gained medical prominence during the human immunodeficiency virus (HIV) pandemic of the 1980s. PCP was often the indicant diagnosis in HIV-positive

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patients when CD4 counts declined to <200 cells/mm^3 [1]. Solid organ transplant (SOT) recipients represent a large and growing cohort of iatrogenically immunosuppressed patients. Whereas immunosuppression is critical to preserve allograft function, a reliable biomarker of this functional immunosuppression is lacking. CD4$^+$ T-cell counts are an informative and widely used marker of immune function in HIV-positive patients, but not SOT patients [2,3]. To make matters more complex, therapeutic drug monitoring of immunosuppressive agents even within the reference range does not necessarily correlate with equal degrees of functional immunosuppression between individuals, making the simple identification of those who are at most risk of the complications of over-immunosuppression difficult [4].

The risk of developing PCP in SOT recipients was traditionally considered to be highest in the 12 months immediately post transplantation. In the absence of prophylaxis, PCP occurs in 5–15% of renal transplant recipients with mortality rates of up to 50% reported [5,6]. It is on this basis that many guidelines recommend a period of PCP prophylaxis ranging between three and 12 months post transplantation. Despite this, reports of PCP cluster outbreaks have emerged worldwide, particularly in renal transplant recipients, leading to significant morbidity and mortality [7–9]. Importantly, the demographic of those presenting with PCP appears to be changing, often presenting many years from initial transplantation and therefore beyond the period where routine PCP prophylaxis is employed. This represents a worrying trend, calling current recommendations regarding prophylaxis and ongoing management into question and encouraging practitioners to seek out those who are at greatest risk to target prevention strategies.

Between November 2014 and January 2016 we had such an outbreak among our renal transplant recipients. Over the year preceding this outbreak the rate of presentation of cases of PCP in all patients (including those with HIV infection and other causes of immunosuppression) had been 0.02 per 1000 bed-days in our hospital. This rate rose to 0.038 per 1000 bed-days during the outbreak, falling to 0.014 per 1000 bed-days since the investigation. Here we present a case–control study of our experience of this PCP outbreak.

Methods

A retrospective analysis of cases was performed using case notes and our electronic patient records (Vital Data (Vital Pulse) Version 1.6.0.7771 and Trakcare (Intersystems) Version 2016.2). Each case was matched with two case-linked controls in order to examine potential risk determinants for developing PCP. Patients and controls were randomly matched for age, sex, transplant type, and time post transplantation ±2 years. Controls were excluded if they were deceased or no longer followed up by our transplant centre.

Cases of suspected PCP were those patients with clinical symptoms in-keeping with PCP, including cough, sputum, shortness of breath, or fever with/without hypoxia. PCP diagnosis was confirmed using PCR analysis of the single-copy dihydropteroate synthetase gene in either induced sputum or broncho-alveolar lavage and genotyping where possible [10,11]. Only confirmed diagnoses were included as cases. Estimated glomerular filtration rate (eGFR) was obtained using the six-variable modified diet in renal disease (MDRD) equation calculated by vital data. eGFR data were calculated as the mean of the previous four measurements recorded prior to PCP diagnosis in cases. Total lymphocyte counts were recorded at time of PCP diagnosis and at approximately three months and six months prior to this. Corresponding eGFR and lymphocyte counts were captured in controls at equivalent times post transplantation of PCP diagnosis in cases.

Standard practice with strongly suspected or confirmed PCP diagnosis was isolation in side-room accommodation on the general ward with barrier nursing and respiratory droplet precautions in place. This was continued until patients were asymptomatic and clinically improved, death, or discharge. Those requiring intensive care unit (ICU) care were not universally isolated in side-room accommodation. All transplant clinic consultations were carried out in a private room, though there are communal waiting area facilities and consultation rooms are not routinely cleaned between patient visits. Ward attendees share communal areas with hospitalized inpatients. Inpatients prior to a confirmed diagnosis of PCP were accommodated in a mixture of side rooms and open bays.

The electronic patient record was used to identify transplant clinic visits, ward attendance, and transplant ward admissions in the 12 months prior to PCP diagnosis. The same exercise was conducted relating to control patients at the equivalent time post transplant. Data were maintained on secure MS Excel 2010 databases held on the hospital-shared drive. Data were analysed and statistical analysis performed using the Graph Pad Prism 7.0 analysis software. One patient required exclusion from statistical analysis as there were no case-linked controls available. Data for this investigation of a possible upturn in PCP patients were managed in accordance with the Data Protection Act and NHS Lothian Caldicott Guidelines.

Results

Nine cases of PCP were identified in our transplant population, between November 2014 and January 2016. Eight were solitary kidney transplant recipients and one was a simultaneous pancreas and kidney (SPK) transplant recipient. Eight patients presented with shortness of breath, six were hypoxic, five had cough, and five had pyrexia. One patient's only manifest symptom at time of presentation was pyrexia. The presence or absence of cough or pyrexia was not documented for two patients, respectively. Eight patients presented with chest X-ray appearances consistent with PCP; one patient was noted to have a normal chest X-ray on admission. Four patients required admission to the ICU; two required renal replacement therapy. Five patients died within two months of PCP diagnosis, four as a direct consequence of PCP. The fifth patient had graft failure with the reinstitution of regular outpatient haemodialysis prior to death. The four patients who survived beyond two months remain dialysis independent.

All patients had received PCP prophylaxis with co-trimoxazole for three months post transplantation, as per our unit protocol. Patients who had previously received intensification of immunosuppression for acute allograft rejection had also received co-trimoxazole for three months post treatment. No patients were receiving PCP prophylaxis at the time of diagnosis. The mean time from transplantation to

PCP diagnosis was 4.7 years (range: 0.51–14.5) ($N = 9$). Table I highlights the well-matched characteristics of both case and control groups ($N = 8$).

There was no significant difference in the prevalence of common co-morbidities nor immunosuppressive regimens at the time of diagnosis between the two groups. Three patients who developed PCP had an episode of acute rejection in the past compared to zero patients in the control group ($P = 0.088$) (Table II). Eight of the nine patients who developed PCP were cytomegalovirus (CMV) immunoglobulin (IgG) positive at the time of transplantation. Six received CMV-seronegative donor kidneys, including the CMV-seronegative recipient. Of the control population, six out of 16 were CMV IgG positive at the time of transplantation. One of these CMV-seronegative recipients received a CMV-seropositive donor kidney.

In the PCP cohort, mean eGFR at diagnosis was 29.3 mL/min/1.73 m^2, significantly lower than the control group with a mean eGFR at 70 mL/min^{-1} ($P = 0.0007$) (Figure 1).

Three patients were treated for active CMV infection prior to PCP diagnosis, at 51, 84, and 1178 days preceding diagnosis. A further two patients were found to have active CMV infection concurrent with diagnosis of PCP. In comparison, no control patients had been diagnosed with CMV viraemia or disease post transplant ($P = 0.001$). Mean lymphocyte count at presentation was 0.39×10^9 in the cases and 0.9×10^9 in the control group ($P = 0.028$) (Figure 2). There was no significant difference in lymphocyte count at six months pre-PCP diagnosis ($P = 0.76$) (Figure 3).

Table I
Matching criteria

Variable	Cases ($N = 8$)	Controls ($N = 16$)	*P*-value
Mean age (years)	65 (±17)	52.5 (±20)	0.73
Female	4/8	8/16	>0.99
Time from transplant (days), mean (SD)	1140 (±788)	3119 (±9274)	0.88

One patient was excluded from matching owing to the lack of availability of a suitable control.

Table II
Past medical history and immunosuppression regimens

Variable	Cases ($N = 8$)	Controls ($N = 16$)	*P*-value
Hypertension	4 (50%)	9 (56.2%)	0.77
Diabetes	2 (25%)	2 (12.5%)	0.44
Chronic–obstructive pulmonary disease	0	2 (16.67%)	0.22
IgA nephropathy	1 (12.5%)	3 (18.75%)	0.67
Autosomal Dominant Polycystic Kidney Disease	3 (37.5%)	5 (31.25%)	0.76
Calcineurin inhibitor	7 (87.5%)	14 (87.5%)	>0.99
Mycophenolate mofetil	8 (100%)	11 (68.75%)	0.07
Prednisolone	8 (100%)	12 (75%)	0.12
Acute rejection	3 (37.5%)	0	0.088
Delayed graft function	2 (25%)	2 (11.1%)	0.36
Previous or current CMV viraemia	5 (62.5%)	0	0.001
Previous plasma exchange	2 (25%)	0	0.037

CMV, cytomegalovirus.

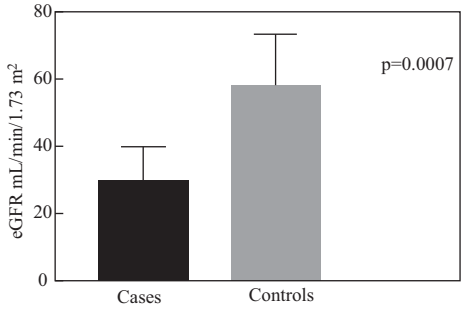

Figure 1. Mean of the four recorded estimated glomerular filtration rates (eGFRs) prior to the date of pneumocystis pneumonia diagnosis.

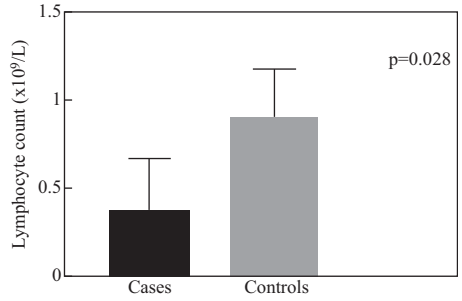

Figure 2. Mean lymphocyte count at approximately the date of pneumocystis pneumonia diagnosis.

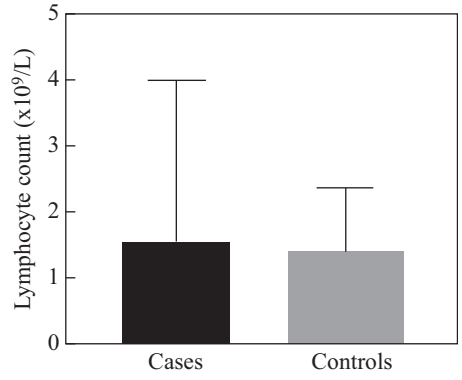

Figure 3. Mean lymphocyte count at approximately six months prior to the date of pneumocystis pneumonia diagnosis.

Timeline analysis of healthcare contacts

Following a detailed review of the healthcare interactions of PCP cases, all but one of these patients had overlapped with another affected patient prior to diagnosis. Between April 2014 and December 2015 those affected had an average of 7.9 transplant clinic visits each, compared to the cumulatively greater 10.2 for the control group. In this period, 37% (26/71) of cases' visits overlapped with a patient who went on to develop PCP, compared to 19% (20/105) clinic visits for the control group ($P = 0.014$). Also within this time-period, those who went on to develop PCP had an average of 18 transplant ward admission days prior to PCP diagnosis compared with 7.2 days

for the control group. Of the total 164 pre-diagnosis ward admission days in those diagnosed with PCP, 57% of these were spent in the presence of another case who went on to develop PCP. By contrast with the 115 ward admission days for the control group, fewer (47%) were spent in the presence of a pre-diagnosis PCP patient ($P \leq 0.0001$).

Figure 4 details an interaction map between those diagnosed with PCP. For simplicity only healthcare interactions that resulted in an overlap in time and place with another affected patient are included.

Pneumocystis genotyping was carried out for five of the nine cases who had sufficient material available. All five specimens demonstrated the presence of mt26SRNA allele 8.

Discussion

Several cluster outbreaks of PCP have been reported over the last decade, particularly among solid organ transplant patients [12]. This study highlights the ongoing threat of opportunistic infection in the transplant population with a shift to 'late' infectious morbidity and considerable mortality, often many years from initial transplantation. We have confirmed potential risk factors for the late development of PCP post renal transplantation, including low eGFR, CMV viraemia, and previous acute rejection. Additionally, in our cohort we demonstrated that contact with a person who goes on to develop PCP is a risk factor for these susceptible hosts to develop clinical disease in the future. These could be used to risk-stratify patients for the potential to develop PCP disease in the future. A decrease in incidence was observed after infection prevention and control interventions had been implemented, with surveillance identifying one further case occurring since January 2016, in December 2017. No blanket prophylaxis was instituted during or after the outbreak. This patient had been treated for rejection but had not received further PCP prophylaxis.

Impaired renal function in patients who develop PCP has previously been described, and this is echoed in our study [13]. The potential mechanisms for this are unclear, though it is postulated that the uraemic milieu impairs T-lymphocyte function [14]. Decreased excretion of mycophenolate mofetil and its metabolites at lower GFR increases the burden of immunosuppression at standard therapeutic doses [15]. In our study, we found that patients diagnosed with PCP were more likely to have had active CMV viraemia prior to diagnosis, in keeping with previous reports [5]. It remains unclear whether active CMV replication simply reflects a more immunocompromised state or whether it is directly implicated in the pathogenesis of PCP.

T-Lymphocytes are essential in the host defence against PCP. Lymphopenia has previously been documented at ~50 days preceding the diagnosis of PCP disease, with Struijk *et al.* noting depletion of $CD4^+$ T-cells prior to PCP diagnosis [4,5]. Lymphocyte counts were found to be significantly lower in our PCP cases at the time of presentation, although this was not evident at three, six, nor 12 months prior to presentation. It can be argued that lymphocyte counts will have declined in our case population as a consequence of an acute intercurrent infection such as PCP, or, in two cases, acute CMV co-infection. Furthermore, due to the retrospective nature of this study, preceding lymphopenia may not have been identified due to the interval nature of full blood count checking. As previously discussed, the use of lymphopenia in these immunosuppressed patients is a rather crude measure of immunosuppression and is not considered a reliable marker.

The precise route of acquisition of *Pneumocystis jirovecii* and the manner in which disease develops are debated. Transmission is accepted to be airborne and PCP disease was traditionally thought to be due to reactivation of latent infection in immunocompromised hosts. Indeed Pifer *et al.* demonstrated that two-thirds of children exposed to pneumocystis via the aerosol route had developed antibodies to it by four years of age [16]. The theory of reactivation was later called into question by the lack of *Pneumocystis* species present at autopsy in the lungs of previously seropositive hosts [17]. Moreover, animals previously infected with pneumocystis did not go on to develop the disease following immunosuppression [18]. This suggests possible immune clearance following initial infection, pointing towards acquisition later in life. Further weight has been given to de-novo acquisition by the finding that the genotype of an infecting species is more likely to resemble that of the area of acquisition rather than the patient's place of birth [19].

Moreover, Choukri *et al.* demonstrated the presence of *Pneumocystis jirovecii* in air samples collected 1 m from an infected patient's head in 15 out of 19 cases, providing evidence of an aerosol route of transmission [20]. Furthermore, Yazaki *et al.* demonstrated identical infecting strains during an outbreak with the presence of asymptomatic carriers identified within their studied cohort [21]. Indeed a recent systematic review of PCP outbreaks by Yiannakis *et al.* revealed that a common organism could be identified in 81% of outbreaks where genotyping was carried out [12]. In the current era, whole genome sequencing of *Pneumocystis* strains has allowed improved characterization of the organism's demographics. From our limited genotyping of infecting PCP strains, the mt26SRNA allele 8 was present in all five patients typed, which is consistent with the experience of a similar outbreak in the west of Scotland [11].

Since the median incubation period of PCP may be in the region of 60 days, transmission could occur during the prodromal phase of the illness [21]. An investigative timeline of cases demonstrated considerable pre-diagnosis overlap in those diagnosed. Our experience adds to the compelling case for person-to-person transmission occurring between susceptible hosts, and it questions the theory regarding re-activation of latent infection.

PCP prophylaxis for entire transplant programmes or individually to those who have been exposed have previously been documented [22,23]. After multi-disciplinary advisory group discussion, we elected not to institute blanket prophylaxis within our programme. We continued isolation of possible cases with droplet precautions and increased awareness among clinicians to the possibility of PCP infection. There was also individualization of prophylaxis extension for SOT recipients deemed at higher risk of infection.

Limitations of our study are that we had a small number of patients with controls not available for all. Additionally, analysis was retrospective and genotyping of PCP was incomplete. Currently, there are no international guidelines regarding the prevention and management of an outbreak of PCP. Raising awareness of the possibility of PCP should be encouraged and appropriate chemoprophylaxis supported as per a recent

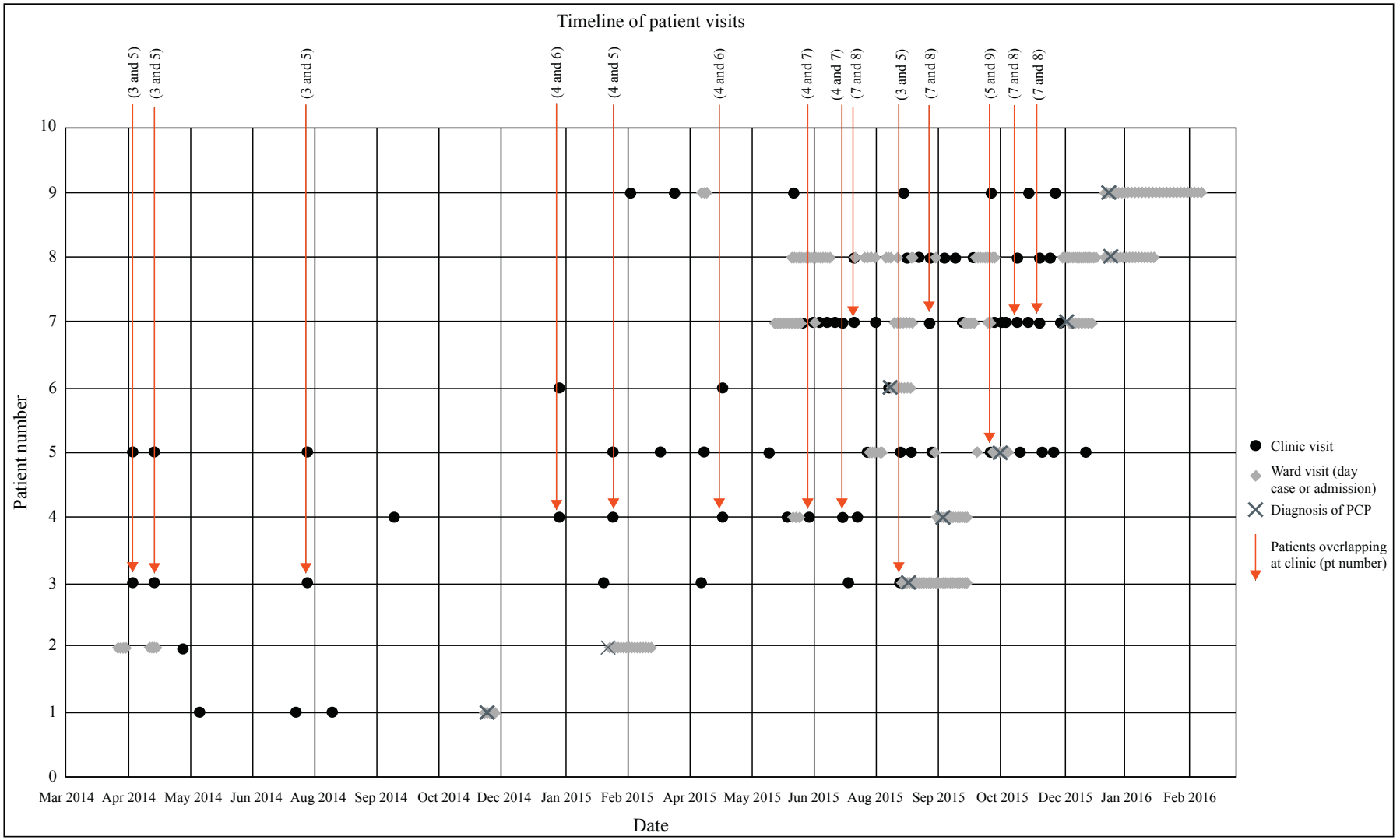

Figure 4. Contact tracing map for the affected patients in the period prior to pneumocystis pneumonia (PCP) diagnosis.

Cochrane review, with expert guidance suggesting three to 12 months of use post transplantation.

In conclusion, this study highlights the ongoing risk of opportunistic infection in renal transplant recipients many years from transplantation. Clinical and epidemiological features have been suggested, including age, cumulative burden of immunosuppression, lower eGFR, and CMV infection. These could help to risk-stratify future patients for extended chemoprophylaxis. We believe that stratification would rely on a view of these factors in combination. Person-to-person transmission in susceptible patients remains a possibility and we identify means via co-ordinated responses to mitigate risk both to individuals and more widely within transplant programmes, without instituting blanket prophylaxis.

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Conflict of interest statement
None declared.

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References

[1] Stansell JD, Osmond DH, Charlebois E, LaVange L, Wallace JM, Alexander BV, et al. Predictors of *Pneumocystis carinii* pneumonia in HIV-infected persons. Pulmonary Complications of HIV Infection Study Group. Am J Respir Crit Care Med 1997;155:60—6.

[2] Kaplan JE, Hanson D, Dworkin MS, Frederick T, Bertolli J, Lindegren ML, et al. Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. Clin Infect Dis 2000;30(Suppl 1):S5—14.

[3] Hutchinson P, Chadban SJ, Atkins RC, Holdsworth SR. Laboratory assessment of immune function in renal transplant patients. Nephrol Dial Transplant 2003;18:983—9.

[4] Struijk GH, Gijsen AF, La Yong S, Zwinderman AH, Geerlings SE, Lettinga KD, et al. Risk of *Pneumocystis jiroveci* pneumonia in patients long after renal transplantation. Nephrol Dial Transplant 2011;26:3391—8.

[5] 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;15:190—9.

[6] Elinder CG, Andersson J, Bolinder G, Tydén G. Effectiveness of low-dose cotrimoxazole prophylaxis against *Pneumocystis carinii* pneumonia after renal and/or pancreas transplantation. Transpl Int 1992;5:81—4.

[7] Le Gal S, Damiani C, Rouillé A, Grall A, Tréguer L, Virmaux M, et al. A cluster of pneumocystis infections among renal transplant recipients: molecular evidence of colonized patients as potential infectious sources of *Pneumocystis jirovecii*. Clin Infect Dis 2012;54.

[8] de Boer MGJ, Bruijnesteijn van Coppenraet LES, Gaasbeek A, Berger SP, Gelinck LBS, van Houwelingen HC, et al. An outbreak of *Pneumocystis jiroveci* pneumonia with 1 predominant genotype among renal transplant recipients: interhuman transmission or a common environmental source? Clin Infect Dis 2007;44:1143—9.

[9] Mulpuru S, Knoll G, Weir C, Desjardins M, Johnson D, Gorn I, et al. *Pneumocystis pneumonia* outbreak among renal transplant recipients at a North American transplant center: risk factors and implications for infection control. Am J Infect Control 2016;44:425—31.

[10] Linssen CFM, Jacobs JA, Beckers P, Templeton KE, Bakkers J, Kuijper EJ, et al. Inter-laboratory comparison of three different real-time PCR assays for the detection of *Pneumocystis jiroveci* in bronchoalveolar lavage fluid samples. J Med Microbiol 2006:1229—35.

[11] Inkster T, Dodd S, Gunson R, Imrie L, Spalding E, Packer S, et al. Investigation of outbreaks of *Pneumocystis jirovecii* pneumonia in two Scottish renal units. J Hosp Infect 2017;96:151—6.

[12] Yiannakis EP, Boswell TC. Systematic review of outbreaks of *Pneumocystis jirovecii* pneumonia: evidence that *P. jirovecii* is a transmissible organism and the implications for healthcare infection control. J Hosp Infect 2016;93:1—8.

[13] Radisic M, Lattes R, Chapman JF, del Carmen Rial M, Guardia O, Seu F, et al. Risk factors for *Pneumocystis carinii* pneumonia in kidney transplant recipients: a case—control study. Transpl Infect Dis 2003;5:84—93.

[14] 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;26:2013—7.

[15] Naesens M, de Loor H, Vanrenterghem Y, Kuypers DRJ. The impact of renal allograft function on exposure and elimination of mycophenolic acid (MPA) and its metabolite MPA 7-O-glucuronide. Transplantation 2007;84:362—73.

[16] Pifer LL, Hughes WT, Stagno S, Woods D. *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. Pediatrics 1978;61. 35 LP-41.

[17] Peters SE, Wakefield AE, Sinclair K, Millard PR, Hopkin JM. A search for *Pneumocystis carinii* in post-mortem lungs by DNA amplification. J Pathol 1992;166:195—8.

[18] Chen W, Gigliotti F, Harmsen AG. Latency is not an inevitable outcome of infection with *Pneumocystis carinii*. Infect Immun 1993;61:5406—9.

[19] Miller RF, Lindley AR, Copas A, Ambrose HE, Davies RJ, Wakefield AE. Genotypic variation in *Pneumocystis jirovecii* isolates in Britain. Thorax 2005;60:679—82.

[20] Choukri F, Menotti J, Sarfati C, Lucet J-C, Nevez G, Garin YJF, et al. Quantification and spread of *Pneumocystis jirovecii* in the surrounding air of patients with *Pneumocystis* pneumonia. Clin Infect Dis 2010;51:259—65.

[21] Yazaki H, Goto N, Uchida K, Kobayashi T, Gatanaga H, Oka S. Outbreak of *Pneumocystis jiroveci* pneumonia in renal transplant recipients: *P. jiroveci* is contagious to the susceptible host. Transplantation 2009;88:380—5.

[22] Chapman JR, Marriott DJ, Chen SC, MacDonald PS. Post-transplant *Pneumocystis jirovecii* pneumonia — a re-emerged public health problem? Kidney Int 2013;84:240—3.

[23] Phipps LM, Chen SC, Kable K, Halliday CL, Firacative C, Meyer W, et al. Nosocomial *Pneumocystis jirovecii* pneumonia: lessons from a cluster in kidney transplant recipients. Transplantation 2011;92:1327—34.