



ORIGINAL ARTICLE

Hypercalcaemia preceding diagnosis of *Pneumocystis jirovecii* pneumonia in renal transplant recipients

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Abstract

Background: The overall incidence of *Pneumocystis jirovecii* pneumonia (PJP) in solid organ transplant recipients is 5–15%. A timely diagnosis of PJP is difficult and relies on imaging and detection of the organism.

Methods: We present a case series of four patients displaying hypercalcaemia with an eventual diagnosis of PJP and document the management of the outbreak with a multidisciplinary team approach. We discuss the underlying pathophysiology and previous reports of hypercalcaemia preceding a diagnosis of PJP. We also reviewed the evidence concerning PJP diagnosis and treatment.

Results: Within our renal transplant cohort, four patients presented within 7 months with hypercalcaemia followed by an eventual diagnosis of PJP. We measured their corrected calcium, parathyroid hormone (PTH), 1,25-dihydroxycholecalciferol [1,25-(OH)₂D₃] and 25-hydroxycholecalciferol [25(OH)D] levels at admission and following treatment of PJP. All four patients diagnosed with PJP were 4–20 years post-transplantation. Three of the four patients demonstrated PTH-independent hypercalcaemia (corrected calcium >3.0 mmol/L). The presence of high 1,25(OH)₂D₃ and low 25(OH)D levels suggest negation of the negative feedback mechanism possibly due to an extrarenal source; in this case, the alveolar macrophages. All four patients had resolution of their hypercalcaemia after treatment of PJP.

Conclusions: Given the outbreak of PJP in our renal transplant cohort, and based on previous experience from other units nationally, we implemented cohort-wide prophylaxis with trimethoprim-sulphamethoxazole for 12 months in consultation with our local infectious diseases unit. Within this period there have been no further local cases of PJP.

Key words: hypercalcaemia, immunosuppression, kidney transplantation, *Pneumocystis jirovecii*, transplant, trimethoprim-sulphamethoxazole

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Background

Pneumocystis jirovecii is a human-specific ascomycetous fungal organism discovered in the 1900s [1]. *Pneumocystis* infection is thought to involve aerosolized particle transmission [1]. *Pneumocystis* colonization may not manifest clinically in immunocompetent humans [1, 2], yet in an immunocompromised patient, *Pneumocystis* has lung tropism and can cause opportunistic pneumonia [1, 2].

In renal transplant recipients (RTRs), the incidence of *P. jirovecii* pneumonia (PJP) is 5–15% in patients without prophylaxis, with a greater relative risk up to 6 months post-transplant [3, 4]. Mortality rates are 13–38% in this population [5]. Cytomegalovirus (CMV) infection, glucocorticoid use and recurrent rejection are independent risk factors for PJP [6, 7]. Trimethoprim–sulphamethoxazole (TMP-SMX) prophylaxis [8] is recommended for 3–6 months post-transplant [9–11], which is when most infections occur [3, 10]. Beyond the first year post-transplant, risk factors for PJP include increased levels of immunosuppression [12], allograft rejection and abnormal renal function [13].

While PJP in RTRs is relatively uncommon, recent PJP outbreaks in Australian renal transplant units [9, 14] have refocused attention on the current evidence behind PJP prophylaxis. An uncommon feature of PJP is hypercalcaemia, often preceding radiographic findings [15–21]. We present four RTRs with hypercalcaemia refractory to medical management preceding the diagnosis of PJP. We hypothesize that PJP contributed to the preceding hypercalcaemia in three of the cases. Biochemical and haematological results are summarized in Table 1. Immunosuppression post-transplant and at diagnosis are recorded in Table 2. The trend of hypercalcaemia is depicted in Figure 1.

Case 1 (January 2014)

A 45-year-old restaurant worker with a living-related ABO-incompatible renal transplant 4 years prior presented with exertional dyspnoea and hypercalcaemia of 3.43 mmol/L. She had 6 months of TMP-SMX prophylaxis post-transplant. She was on tacrolimus, mycophenolate and prednisolone without vitamin D or calcium supplementation at presentation (Tables 1 and 2). She previously had CMV viraemia (treated with currently undetectable levels) and cellular-mediated graft rejection (diagnosed November 2011). This was treated with pulsed methylprednisolone and increased immunosuppression. A biopsy post-treatment showed no further rejection. Bactrim was not restarted during treatment for graft rejection.

Auscultation revealed fine crackles bilaterally and she was afebrile. Her parathyroid hormone (PTH)-independent hypercalcaemia (PTH <0.32 pmol/L) remained high after intravenous crystalloids and calcitonin. Bisphosphonates were avoided due to impaired renal function (creatinine 258 µmol/L). Other tests showed elevated 1,25-dihydroxycholecalciferol [1,25(OH)₂D₃] 450 pmol/L, low 25-hydroxycholecalciferol [25(OH)D] 28 nmol/L, serum angiotensin-converting enzyme (ACE) 37.7 U/L and PTH-related protein of 2.7 pmol/L. She was not leucopenic on admission.

Chest x-Ray (CXR) showed interstitial infiltrates. A high-resolution CT scan of the chest (HRCT) showed ground-glass opacities bilaterally. TMP-SMX was commenced at the treatment dose for presumed PJP. Bronchoalveolar lavage (BAL) revealed *P. jirovecii* on polymerase chain reaction (PCR) testing. Following PJP treatment, she recovered and her serum corrected

Table 1. Serum creatinine, corrected calcium, PTH, 25-(OH)D and 1,25(OH)₂D pre-transplant, at diagnosis and post-treatment and any calcium/vitamin D supplementation (in bold) at diagnosis

Case	Corrected calcium post-transplant (2.1–2.6 mmol/L)	PTH post-transplant (1.6–6.9 pmol/L)	Serum creatinine post-transplant (µmol/L)	Serum creatinine at diagnosis	Vitamin D or calcium supplements at diagnosis	Corrected calcium at diagnosis (2.1–2.6 mmol/L)	25-(OH)D at diagnosis (>50 nmol/L)	1,25-(OH) ₂ D at diagnosis (72–225 pmol/L)	PTH at diagnosis (1.6–6.9 pmol/L)	Serum creatinine at diagnosis (µmol/L)	Corrected calcium post-treatment (2.1–2.6 mmol/L)	PTH post-treatment (1.6–6.9 pmol/L)	Serum creatinine post-treatment (µmol/L)
1	2.3	7.52	175	175	Nil	3.43	28	450	<0.32	290	2.20	11	350
2	2.38	N/A	160	160	Nil	3.04	83	588	<0.32	208	2.53	5.6	180
3	2.41	N/A	170	170	Vitamin D 2 tabs daily + calcitriol 0.25mcg daily	3.05	116	N/A	2.38	455	2.48	2.38	Dialysis dependent
4	2.46	12.8	145	145	Calcitriol 0.25mcg alternate days at diagnosis	2.68	28	75	18.8	159	2.49	18.8	170

N/A, not available (sample not collected).

Table 2. Demographics of cases, time from transplantation, immunosuppression doses after renal transplant and at time of diagnosis and white cell, lymphocyte and neutrophil counts at diagnosis

Case	Age (years) and gender	Time from transplant (years)	Immunosuppression post-transplant			Immunosuppression at diagnosis			White cell count at diagnosis	Lymphocyte count at diagnosis	Neutrophil count at diagnosis
			Calcineurin inhibitors	Antiproliferative agents	Prednisolone	Calcineurin inhibitors	Antiproliferative agents	Prednisolone			
1	45 F	4	Tac 4 mg twice daily	Myfortic 720 mg twice daily	20 mg daily	Tac 3.5 mg once daily (trough level 3.2 ng/ml)	Myfortic 360 mg twice daily	5 mg once daily	7.5	0.9	6.6
2	57 M	11 years	CYC 200 mg twice daily	Cellcept 1 g twice daily	20 mg daily	None	Cellcept 1 g twice daily	6 mg daily	8.9	1.1	6.7
3	45 F	30 years	CYC 450 mg twice daily	Azathioprine 50 mg daily	5 mg daily	None	Cellcept 250 mg twice daily	5 mg daily	12.3	0.9	11.0
4	52 M	6 years	Tac 5.5 mg twice daily	Cellcept 1 g three times daily	25 mg daily	Tac 0.5 mg twice daily (trough level 2.5 ng/ml)	Cellcept 750 mg twice daily	5 mg daily	8.9	1.4	5.9

Cellcept, mycophenolate mofetil; Myfortic, mycophenolate sodium; CYC, cyclosporin; Tac, tacrolimus.

calcium and PTH improved to 2.2 mmol/L and 11 pmol/L, respectively, on discharge (Table 1, Figure 1).

Case 2 (March 2014)

A 57-year-old policeman with a deceased-donor renal transplant 11 years before with no previous allograft rejection presented with exertional dyspnoea over the preceding month. He was on mycophenolate and prednisolone without vitamin D or calcium supplementation (Tables 1 and 2). Auscultation revealed bilateral fine crackles and he was afebrile.

At presentation, he had PTH-independent hypercalcaemia (corrected calcium 3.04 mmol/L, PTH <0.32 pmol/L) with acute-on-chronic renal failure (creatinine 208 µmol/L from a baseline of 170 µmol/L in 2013). Other tests showed 1,25(OH)₂D₃ of 588 pmol/L, PTH-related protein <2.0 pmol/L and serum ACE <5.0 U/L. Serum protein electrophoresis and urine electrophoresis were normal. An HRCT showed diffuse ground-glass changes. BAL was negative for *P. jirovecii* oocysts and other infections.

Management with intravenous fluids, increased prednisolone dose and intravenous bisphosphonate was commenced. His dyspnoea improved and his corrected calcium improved to 2.89 mmol/L and PTH to 4.04 pmol/L at discharge. He was readmitted the following week with worsening dyspnoea and persisting hypercalcaemia of 3.19 mmol/L. He had a normal transthoracic echocardiogram and a negative septic screen, but a repeat HRCT showed worsening ground-glass infiltrates. A repeat bronchoscopy revealed *P. jirovecii* PCR positive on BAL. TMP-SMX was commenced; this was briefly changed to atovaquone while his renal function recovered. At discharge, his corrected calcium and PTH were 2.53 mmol/L and 5.6 pmol/L, respectively (Table 1, Figure 1).

Case 3 (May 2014)

A 45-year-old woman with a living-related renal transplant 30 years before presented with worsening exertional dyspnoea. Her comorbidities included ischaemic heart disease and no previous allograft rejection. She was on mycophenolate and prednisolone as well as vitamin D and calcitriol (Tables 1 and 2). Auscultation revealed fine crackles, worse on the left, and she was afebrile.

A CXR was clear. Empirical treatment for presumed atypical pneumonia with ceftriaxone and azithromycin was started. An HRCT showed ground-glass opacities bilaterally. She was hypercalcaemic (corrected calcium 3.05 pmol/L) and had renal failure (creatinine 455 µmol/L). Other tests showed serum ACE <5.0 U/L, serum PTH of 2.38 pmol/L and a normal serum/urine electrophoresis and autoimmune screen. Viral PCR on blood was positive for CMV. Induced sputum was tested for PJP PCR. Calcitriol and vitamin D were ceased.

Septic shock necessitated intensive care unit (ICU) admission and intubation while awaiting a bronchoscopy. A broad-spectrum antibiotic (piperacillin-tazobactam) was started. PJP PCR from admission was positive and intravenous TMP-SMX was commenced. Intravenous ganciclovir for CMV viraemia was withheld, as she clinically improved. She was extubated and discharged from the ICU after 3 days; however, her renal function continued to deteriorate, necessitating dialysis.

Respiratory distress prompted a return to the ICU for reintubation. Antibiotics were changed to meropenem and doxycycline, while intravenous TMP-SMX was continued. Bronchoscopy was negative for *P. jirovecii* but positive for CMV

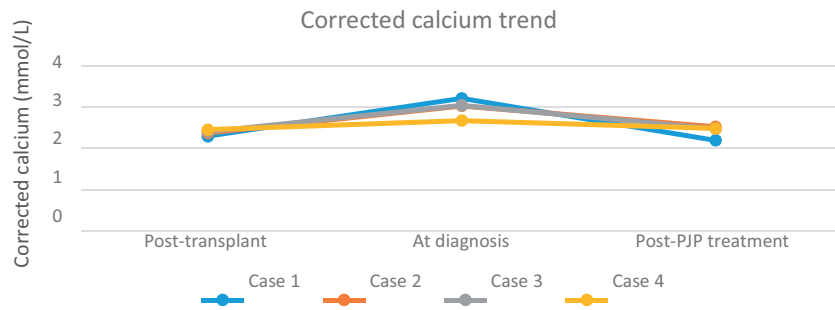


Fig. 1. Trend of serum corrected calcium post-transplant, at diagnosis and post-PJP treatment.

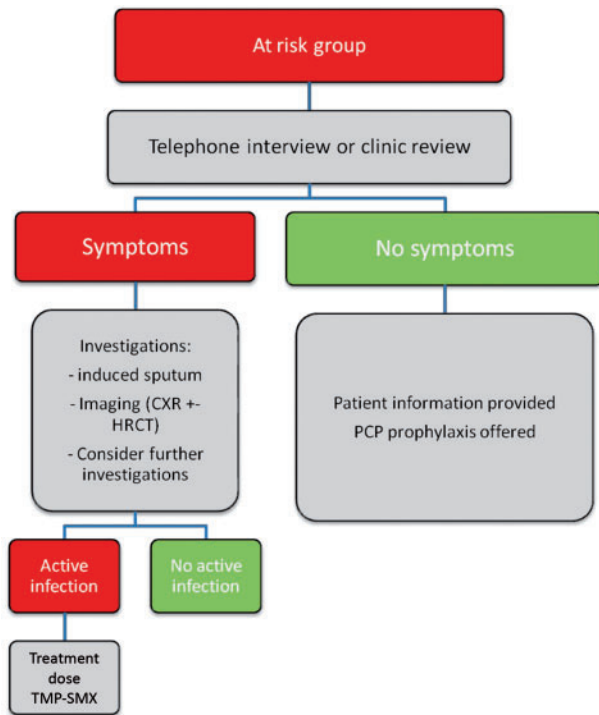


Fig. 2. Pathway used to contain outbreak in our renal transplant cohort.

PCR (titre 9471 copies/mL). Intravenous ganciclovir was added. Meropenem and doxycycline were ceased after 8 days while ganciclovir and TMP-SMX were continued (14 and 21 days, respectively). She returned to the ward after an 18-day ICU stay. Prophylactic oral valganciclovir and oral TMP-SMX were commenced after her initial treatment. Her corrected calcium was 2.48 mmol/L on discharge (Table 1, Figure 1). She remained dialysis dependent.

Case 4 (July 2014)

A 52-year-old salesman with a deceased-donor renal transplant 6 years before with no previous allograft rejection was admitted with worsening exertional dyspnoea. He had pre-existing primary hyperparathyroidism with a subtotal parathyroidectomy in 2004. Subsequent PTH levels were 10–44 pmol/L. He was on tacrolimus, mycophenolate and prednisolone as well as calcitriol (Tables 1 and 2). Respiratory examination was unremarkable and he was afebrile.

Treatment for presumed atypical pneumonia with ceftriaxone and azithromycin was commenced after a normal CXR and septic screen. His corrected calcium was 2.68 mmol/L. Other

tests showed PTH of 18.8 pmol/L, 1,25(OH)₂D₃ of 75 pmol/L, serum creatinine of 159 µmol/L and serum ACE of 29.4 U/L. An HRCT showed small patchy areas of ground-glass opacity. Other viral PCRs, including CMV, were negative. Calcitriol was ceased.

Given our prior cases, we started TMP-SMX at a lower dose in consultation with the infectious diseases unit. A bronchoscopy was done 3 days after starting TMP-SMX, as he failed to improve. This was negative for fungi, tuberculosis, bacteria and PJP PCR; however, PJP PCR from an induced sputum sample on admission returned positive. He was commenced on TMP-SMX at the treatment dose with a brief change to atovaquone for renal impairment. Interestingly, his hypercalcaemia had peaked and returned to normal (corrected calcium 2.49 mmol/L) pre-bronchoscopy (Table 1, Figure 1).

PJP outbreak

At the time of the outbreak, our transplant cohort consisted of 146 RTRs (80 public RTRs and 66 RTRs with private follow-up). With no previous cases in our renal transplant cohort, the case series represented an outbreak of PJP as defined by the World Health Organization (WHO) [22]. After Cases 1 and 2 were identified, a working group (comprising the nephrology, infectious diseases, respiratory and physiotherapy departments) was formed with the aims of early recognition of at-risk patients, institution of universal prophylaxis and minimizing patient-to-patient transmission. Outbreak management was developed according to Phipps et al. [14].

Contact tracing identified our outpatient waiting area for public RTRs as a source of transmission. A protocol (Figure 2) involving induced sputum testing, CXR and HRCT was designed to screen all 80 public RTRs for signs and symptoms of PJP. To minimize further transmission, public RTRs were designated their own waiting room and N95 masks were provided to patients with respiratory symptoms. An isolation room staffed by physiotherapists was designated for induced sputum testing, which was fast-tracked by the pathology laboratory. RTRs were invited to appointments where they were given information about the PJP outbreak. RTRs with respiratory symptoms received a CXR and induced sputum testing, with progression to HRCT if any infiltrates were present.

All public RTRs were commenced on a 6-month course of TMP-SMX at a prophylactic dose of 160/800 mg half-tablet daily and adjusted for renal function. This was empirically extended to 1 year based on previous experience [14] of new infections within a 6-month prophylaxis period. Throughout this period, eight patients were intolerant of TMP-SMX and received alternative agents. The major side effect of TMP-SMX in our cohort was a transient increase in serum creatinine that resolved on cessation of TMP-SMX. Cases 3 and 4 presented to the hospital

before starting prophylaxis. In all four cases, treatment of PJP with TMP-SMX was dosed at TMP 15–20 mg/kg/day and SMX 75–100 mg/kg/day in three divided doses for 3 weeks with dose adjustment for renal impairment. Private RTRs were not exposed (private rooms located in another building) and were therefore excluded from cohort prophylaxis. None of these patients were ultimately infected with PJP.

Discussion

Hypercalcaemia and PJP

PJP was originally described as a reactivation of latent infection acquired during childhood; however, current evidence points towards *de novo* infection from environmental sources and infected individuals as other methods of transmission [23, 24]. Five case reports [15–20] and one case series [21] describe similar presentations of hypercalcaemia in RTRs eventually leading to a diagnosis of PJP. The underlying pathophysiology has been linked with alveolar macrophage activity in PJP clearance [25, 26].

There is a high prevalence of hypercalcaemia after renal transplant (up to 25% in the first year post-transplant [27]), which was reflected in our cohort (21.6% at the time of the outbreak). Our cases had relatively normal corrected calcium levels post-transplant (Table 1). At presentation, only Cases 3 and 4 were on calcium and/or vitamin D supplementation, which may have potentiated hypercalcaemia. Cases 1, 2 and 3 had significant PTH-independent hypercalcaemia (corrected calcium >3.0 mmol/L) that was resistant to medical management. Case 4 technically had primary hyperparathyroidism causing hypercalcaemia, however, he was included as part of the PJP outbreak. With increased 1,25-(OH)₂D₃ levels, we postulate increased hydroxylation via 25-hydroxyvitamin D 1-alpha hydroxylase from alveolar macrophages could be responsible for the hypercalcaemia [28]. While negative feedback of this enzyme usually occurs in hypercalcaemia, extra-renal synthesis and metabolism of 1,25-(OH)₂D₃ is less tightly regulated by PTH and calcium, and calcium intake does not lower serum 1,25-(OH)₂D₃ in such patients [29]. Increased 1,25-(OH)₂D₃ production by alveolar macrophages occurs in sarcoidosis [28–31], and a similar process has been demonstrated post-nephrectomy [32]. The high 1,25-(OH)₂D₃ levels were present in Cases 1, 2 and 4 (no results were recorded for Case 3). Low 25-(OH)D levels were present in Cases 1 and 4 [Cases 2 and 3 were taking cholecalciferol at the time of diagnosis, contributing to a high 25-(OH)D level]. The low 25-(OH)D levels may have been caused by ongoing 1-alpha hydroxylation with consumption of 25-(OH)D. The eventual normalization of 1,25-(OH)₂D₃ could correlate with decreased alveolar macrophage activity with treatment of PJP. However, low 25-(OH)D levels are also common post-transplantation [33] for various reasons [34] including renal impairment and increased levels of fibroblast growth factor 23.

1,25-(OH)₂D₃ production *in vitro* has been demonstrated in pulmonary alveolar macrophages (PAMs) from patients with sarcoidosis [30]. A similar response occurs with normal PAMs incubated with recombinant human interferon-gamma; however, exogenous 1,25-(OH)₂D₃ was able to decrease endogenous hormone production in normal PAMs to a greater degree than in sarcoid PAMs [31]. The increased ability of sarcoid PAMs to produce 1,25-(OH)₂D₃ compared with normal PAMs has been documented in other granulomatous diseases [35] but has not been studied at a molecular level in infections such as PJP. Hypercalcaemia associated with elevated 1,25(OH)₂D₃ has also

been documented in fungal infections in immunocompromised patients (*Cryptococcus* and *Coccidioides*) [36, 37].

PJP prophylaxis regimens, risk factors and difficulties in diagnosis

Our case series illustrates that PJP can present late post-transplant. It also raises the question of the optimal duration of PJP prophylaxis post-transplant and the efficacy of TMP-SMX as prophylaxis. Prevention is important, as PJP presentations are difficult to differentiate from other respiratory infections [1], as seen in our series. Cases 3 and 4 in particular highlight how PJP can be misdiagnosed as atypical pneumonia. Further diagnostic issues include direct microscopy being less sensitive in HIV-negative patients [38] and PCR being positive in patients colonized with *P. jirovecii* [39]. While new methods of diagnosing PJP are being developed [39, 40], PJP diagnosis remains reliant on the correlation of clinical and radiological findings with microbiological investigations [1].

While PJP usually occurs within the first year post-transplant [3, 4, 15, 17, 19, 21], presentations up to 17–40 months post-transplant have been noted (5, 41–45). In these groups, risk factors of PJP included early discontinuation of TMP-SMX prophylaxis within the first year post-transplant (or complete lack of prophylaxis), number and type of rejection treatments, age ≥65 years, lymphocyte count <750/mm³ and CMV infection. The use of mycophenolate mofetil was also a risk factor [46]. In our cohort, only Case 1 had previous allograft rejection preceding PJP diagnosis. Our unit policy at the time did not include restarting PJP prophylaxis during rejection treatment. Regarding age as a risk, all our patients were <65 years [45]. Cases 1–3 had varying levels of renal impairment at diagnosis; a risk factor identified by Eitner *et al.* [13]. All cases had a minimum of 6 months TMP-SMX prophylaxis post-transplantation. Case 1 had prior CMV viraemia that was treated (viral load <400 copies/mL at PJP outbreak). None of our cases were lymphopaenic at presentation (Table 2).

The dosage and type of immunosuppression may also influence the risk of PJP [13, 47–49]. Increased immunosuppression during rejection treatment increases PJP risk [13]. Retrospective studies highlight high-dose corticosteroids over a prolonged period [47] and tacrolimus-based regimens [48] (compared with cyclosporin) as causing a higher PJP incidence. The US Renal Data System (USRDS) cohort showed no association between induction immunosuppression levels and PJP risk, but did show that regimens containing sirolimus were associated with PJP infection [49]. None of our cases were on sirolimus. At transplantation, Cases 1, 2 and 4 had standard immunosuppression doses that were decreased to appropriately lower levels at the time of diagnosis. Case 3 had a lower immunosuppression dose at transplantation and Cases 2 and 3 had ceased tacrolimus and were only on mycophenolate and prednisolone at presentation (Table 2).

The recommended duration of PJP prophylaxis is 3–12 months after solid organ transplantation [9]. The Kidney Disease: Improving Global Outcomes (KDIGO) 2009 clinical practice guidelines recommend 3–6 months post-transplant prophylaxis, and at least 6 weeks during and after treatment for acute rejection [50]. The European Best Practice guidelines recommend at least 4 months of prophylaxis post-transplant, with a further 3–4 months of prophylaxis during rejection [11]. There is worldwide variation in the use of TMP-SMX prophylaxis [5, 41] with PJP outbreaks in Japan, the UK and Switzerland occurring in units that did not offer routine PJP prophylaxis [51].

No prophylaxis following heart, liver and kidney–pancreas transplant was associated with a higher incidence of PJP compared with either a low-dose or high-dose TMP-SMX prophylaxis regimen [52].

There was no difference in PJP incidence between the low-dose TMP-SMX (80/400 mg/day or 160/800 mg/alternate days) and high-dose TMP-SMX (160/800 mg/day) groups. Second-line prophylaxis options include dapsone, atovaquone and pentamidine. Dapsone [53] and atovaquone [54] have been compared with TMP-SMX in two separate retrospective case-matched studies of RTRs, with no PJP cases documented in either arm over a 12-month period. In the dapsone study, a higher rate of infections occurred in the dapsone group, while in the atovaquone study, patients on TMP-SMX prophylaxis had a higher rate of treatment discontinuation due to adverse events.

Six months has been suggested as the optimal duration of PJP prophylaxis post-transplant, as it correlates with the estimated median incubation period of PJP (53 days with a range of 7–188 days) [55]. Without randomized controlled trial evidence for both PJP prophylaxis and treatment in RTRs [24], current prophylaxis guidelines such as from KDIGO are the best guide and are certainly preferable to no prophylaxis [5]. While previous reviews mention treatment of PJP outbreaks with TMP-SMX and instituting universal prophylaxis in RTRs, prophylaxis duration from that point on is unclear [41–42, 44]. Post-PJP outbreak, Phipps et al. [14] and Mitsides et al. [56] instituted a 12-month TMP-SMX prophylaxis regimen across their transplant cohorts, with no further cases diagnosed apart from patients who were not compliant with prophylaxis.

In total, 83 cases of PJP occurred in Australia from 2010 to 2012, comprising a total of 14 outbreaks, with the majority being in RTRs [9]. Our case series adds to the literature of hypercalcaemia presenting as a feature of PJP in the renal transplant population and demonstrates the need for ongoing vigilance against post-transplant infections and minimizing the risk factors thereof. We highlight the importance of a multidisciplinary approach in managing an outbreak. Measures such as contact tracing and a 12-month prophylaxis strategy were important in limiting further infections. To date, there have been no further PJP cases in this cohort. We have also instituted PJP prophylaxis in patients treated for graft rejection as part of our unit policy.

Conclusion

In conclusion, we submit that hypercalcaemia of an unclear aetiology and refractory to treatment in a renal transplant recipient can be a feature of impending PJP. An early bronchoscopy is essential to aid the diagnosis. Early recognition and cohort prophylaxis for 12 months is a proven strategy that can limit PJP progression, with a multidisciplinary approach representing a crucial component of any outbreak containment strategy.

Conflict of interest statement

None declared.

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