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CASE REPORT

Hypercalcemia Heralding *Pneumocystis jirovecii* Pneumonia in an HIV-Seronegative Patient with Diffuse Cutaneous Systemic Sclerosis

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Abstract *Pneumocystis* pneumonia (PCP) is a life-threatening fungal infection occurring in immunocompromised patients such as HIV-positive patients with low CD4 cell count or patients under heavy immunosuppressive therapy. We report the case of a 59-year-old male with severe diffuse cutaneous systemic sclerosis presenting with asthenia, dry cough and worsening shortness of breath for the last 15 days. Biological studies were remarkable for PTH-independent severe hypercalcemia with low 25-hydroxyvitamin D and a paradoxically elevated 1,25-dihydroxyvitamin D. Early bronchoalveolar lavage allowed for PCP diagnosis and targeted treatment. We discuss the underlying physiopathology and difficulties regarding prophylaxis and treatment.

Keywords Hypercalcemia · *Pneumocystis* pneumonia · Systemic sclerosis · Immunocompromised

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Introduction

Pneumocystis jirovecii is a ubiquitous fungus causing relatively common, although life-threatening, opportunistic pulmonary infections in severely immunocompromised human hosts by means of aerosolized particle transmission [1, 2]. Significant infection risk factors include low lymphocyte count (CD4+ $< 200/\mu l$), immunosuppressant treatment, and underlying interstitial lung disease.

Pneumocystis pneumonia (PCP) is known as a rare cause of hypercalcemia via macrophage-related 1-alpha hydroxylation of vitamin D. It was first described in an AIDS patient in 1993 [3], in a leukemic patient in 1999 [4] and in a few renal transplant recipients since 2002 [5–15]. We report herein the first—to the best of our knowledge—case of hypercalcemia heralding PCP in a patient with native kidneys suffering from an autoimmune disorder, in this case severe diffuse cutaneous systemic sclerosis (dcSSc).

Case Report

We report the case of a 59-year-old male patient presenting with asthenia, dry cough and worsening shortness of breath over the last 15 days associated with objective hypoxemia. There was neither fever nor night sweats. His relevant personal history includes severe dcSSc diagnosed 18 months ago and currently treated with oral methylprednisolone 6 mg once daily and subcutaneous methotrexate 20 mg once weekly. He also received a first course of rituximab (2 × 1 g at 14 days interval) 5 months ago.

Physical examination was remarkable for cachexia, hardened and scarred skin, sclerodactyly, Raynaud phenomenon and bilateral pleural friction rubs.

Biological studies revealed severe hypercalcemia (13.5 [8.4–10.5] mg/dl) with normal albumin levels (3.2 [3.0–5.0] g/dl), accounting for a corrected calcium level of 14.3 mg/dl. The patient also presented moderate inflammatory syndrome (CRP 103.3 [< 5] mg/l) and acute renal failure. Lymphocyte count was 510/µL, of which 484/µL was CD4+ and none was CD19+. LDH was elevated to 344 [125–243] U/L. Further tests showed an undetectable parathyroid hormone (PTH) and normal phosphate. PTH-related protein, vitamin A, thyroid tests, morning cortisol and protein electrophoresis and immunofixation returned normal. Low blood levels of 25-hydroxy vitamin D contrasted with paradoxically elevated 1,25-dihydroxy vitamin D levels (87.2 [19.9–79.3] pg/ml). Lysozyme levels were high (16,1 [2.7–9.3] mg/l).

Complementary tests included (1) a chest radiography showing an interstitial pneumopathy, emphysema bullae at both apices and blunting of the right costodiaphragmatic sinus (Fig. 1), (2) a chest computed tomography confirming aspecific diffuse pulmonary infiltrate with centrilobular emphysema predominantly at the apices, (3) pulmonary functional tests remarkable for a very severe restrictive syndrome with FEV1 at 30% and FVC at 31% of expected values, (4) a bone scan showing no lytic lesions, (5) a PET scan revealing rectal hyperfixation and (6) a colonoscopy with single simple rectal polyp resection.

The increase in 1,25-dihydroxyvitamin D and the diffuse pulmonary infiltrate evoked granuloma-related vitamin D 1-alpha hydroxylation, which can be seen in sarcoidosis or infectious granulomatosis. Angiotensin converting enzyme returned negative although lysozyme levels were elevated. Bronchoalveolar lavage

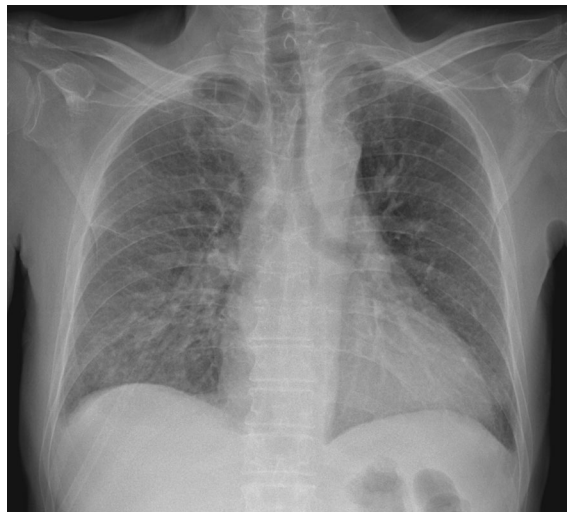

Fig. 1 Chest X-ray showing an interstitial pneumopathy, emphysema bullae at both apices and blunting of the right costodiaphragmatic sinus

(BAL) showed a mixed neutrophilic (51%) and eosinophilic (4%) formula. Direct examination using Diff-Quik stain (modified Wright–Giemsa) revealed *Pneumocystis jirovecii* infection, which is known to be a rare cause of hypercalcemia via macrophage-related vitamin D 1-alpha hydroxylation. Routine testing and cultures for viruses, bacteria, fungi, mycobacteria and other organisms yielded negative results.

Treatment consisted of oral and intravenous hyperhydration, conventional dose oral trimethoprim–sulfamethoxazole (TMP 18 mg/kg/24 h–SMX 90 mg/kg/24 h) and high-dose corticosteroids (twice daily methylprednisolone 32 mg orally on days 1–5, 32 mg once daily on days 6–10 and 12 mg once daily on days 11–21). Hematologic tolerance was poor because of recent administration of methotrexate and the combined anti-folate mechanism with TMP–SMX. Because of OMS grade IV neutropenia on day six, we administered high-dose folate and GM-CSF and switched to second-line anti-*Pneumocystis* therapy (three times daily clindamycin 900 mg plus once daily primaquine 15 mg orally). Primaquine was introduced with 1 week delay, that is, after we tested the patient for G6PD-deficiency. Combined treatment was maintained for 3 weeks with a favorable biological (Fig. 2: rapid normalization of calcemia and renal function, slow decrease in CRP) and clinical evolution. The choice of second-line treatment was mainly justified by the possibility of outpatient care (pentamidine

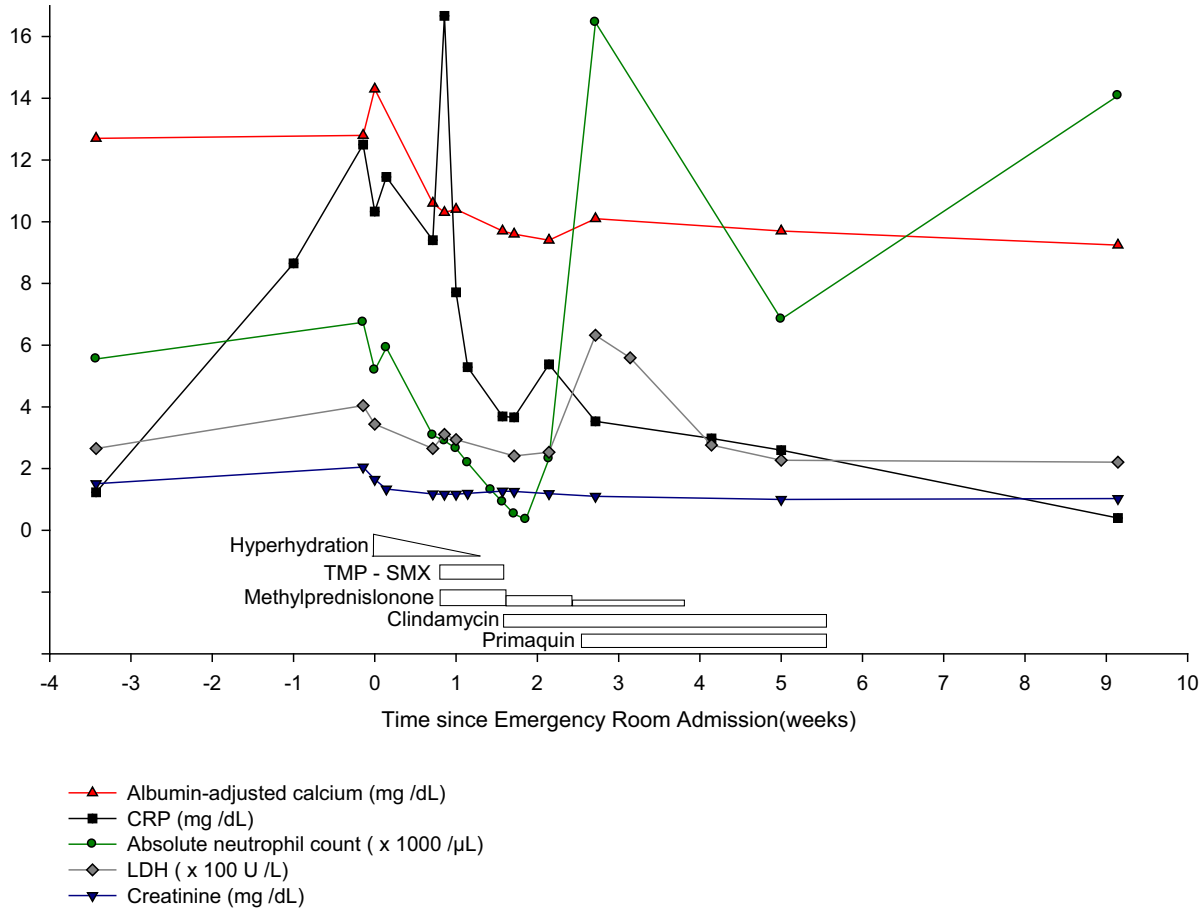

Fig. 2 Biological development before and after initial presentation in the emergency room. The bottom boxes represent the various treatment regimens in a timeline

requires intravenous administration for 3 weeks) and cost considerations (atovaquone is more expensive and not reimbursed by health insurance in Belgium).

Discussion

The differential diagnosis for hypercalcemia is broad. In roughly 85% of the cases, it is explained by hyperparathyroidism or malignancy. Less frequent etiologies include hyperthyroidism, sarcoidosis, drugs (e.g. vitamin D, thiazide diuretics, lithium and vitamin A) or infections (e.g. tuberculosis, coccidioidomycosis, histoplasmosis, and candidiasis). Our patient presented with suppressed PTH. The increase in 1,25-dihydroxyvitamin D and lysozyme levels and the diffuse pulmonary infiltrate on high-resolution computed tomography scan evoked granuloma-related vitamin D 1-alpha hydroxylation, which can be seen in sarcoidosis or infectious granulomatosis. Chest CT was not in favor of sarcoidosis or malignancy. Early BAL showed a mixed neutrophilic (51%) and eosinophilic (4%) cellular pattern, which is compatible with bacterial or fungal infections, and particularly PCP. Direct examination using Diff-Quik stain by a highly trained and accustomed biologist further confirmed the presence of *Pneumocystis jirovecii*. Conversely, a lymphocytic cellular pattern would have raised suspicion for sarcoidosis or drug-induced pneumonitis (e.g. due to methotrexate) [16]. In this case, the severe restrictive syndrome on pulmonary functional tests was attributed to chronic intrinsic lung and chest wall involvement of diffuse cutaneous systemic sclerosis, which was acutely exacerbated by PCP.

PCP is considered a main AIDS-defining illness but thanks to widespread use of prophylaxis and highly active antiretroviral therapy (HAART); the incidence in HIV-infected patients dropped. Conversely, the increasing use of immunosuppressants to treat patients with rheumatologic diseases and following bone marrow or solid organ transplantation put these patients at significantly high risk for infection [17]. As a result, the incidence in HIV-seronegative patients currently outnumbers that of HIV-positive patients [18]. Moreover, the severity and mortality are significantly higher in HIV-seronegative patients because of a delayed diagnosis and a higher inflammatory response impairing gas exchange [18, 19].

The typical histopathological presentation of PCP consists of a foamy eosinophilic intra-alveolar exudate [20]. *Pneumocystis jirovecii* asci can be seen within the exudate by means of usual stains but also specific stains such as Grocott's methenamine silver or toluidine blue O. Trophic forms, usually more substantial during PCP, can be identified with modified Papanicolaou, Wright–Giemsa, or Gram–Weigert stains [21]. However, hypercalcemia is thought to occur in granulomatous PCP, which is a rare but well-known pathological finding encountered in the setting of immunosuppression. Other unusual presentations include parenchymal cavities, vascular invasion and vasculitis. In a review of atypical histological features in 123 lung biopsies from patients with AIDS and PCP, granulomatous inflammation was found in 5% of the cases [22]. Because granulomas are tissue-bound structures, they are rarely described in BAL and the definite diagnosis is therefore histopathological. We did not perform an invasive lung biopsy in our patient as it would not have changed the therapeutic approach.

The reasons for developing a granulomatous reaction to PCP are speculative. Granuloma formation is a delayed-type hypersensitivity reaction including activation of T-lymphocytes, Th1-cytokine production, e.g., interferon gamma and expression of adhesion molecules. Such histological reaction may reflect a better immune status, which could explain the subacute clinical presentation observed in granulomatous PCP [23, 24] in contrast to the abrupt respiratory insufficiency commonly seen in non-HIV patients.

The underlying mechanism for hypercalcemia in granulomatous PCP is the endogenous extrarenal overproduction of 1,25-dihydroxyvitamin D by disease-activated macrophages through production of lipopolysaccharide substances. Studies have indeed shown that 1,25-dihydroxyvitamin D synthesis by disease-activated macrophages is poorly regulated because inflammatory cytokines antagonize the negative feedback on 1-alpha hydroxylase [25]. PTH secretion is secondarily suppressed.

A similar mechanism has been largely studied in sarcoidosis where the incidence of hypercalcemia is more frequent than in fungal infections, which may reflect fluctuations in granuloma formation or other involved mechanisms such as osteoclast activation via osteopontin or receptor activator of NF-κB ligand (RANKL) generation [26].

PCP-induced hypercalcemia has been previously described in renal transplant recipients [5–15] but also in an AIDS patient [3] and in a patient in leukemic remission [4]. In those previously published cases, PTH-independent hypercalcemia was systematically highlighted, while 1,25-dihydroxyvitamin D levels could not always be measured at diagnosis [6, 7]. In some cases, hypercalcemia has been shown to be a prodromal feature of indolent PCP [9, 10]. In a patient with the previous total parathyroidectomy, hypercalcemia induced by PCP was aggravated by calcium and vitamin D oral supplementation [10]. In all cases, treatment for PCP resulted in a return within the normal range of calcium levels, confirming the idea of a positive relationship between PCP and hypercalcemia. However, the kinetics of calcium levels differ in our paper compared to some of the previously published reports [5, 9] as there was an early decrease in calcemia thanks to aggressive hyperhydration, before initiation of PCP treatment. Our patient presented acute renal insufficiency on admission. Biological features suggested pre-renal (plasmatic urea/creatinine levels > 40) or intrinsic (fractional excretion of sodium 2.9% and urea 52.1%) renal failure. However, history, physical exam and favorable response to hyperhydration accounted for a greater suspicion of pre-renal acute kidney injury, which could be the consequence of hypercalcemia, which is known to cause dehydration by inducing renal resistance to vasopressin, leading to nephrogenic diabetes insipidus. Dehydration, in turn, leads to a corresponding further increase in serum calcium concentration [27–29]. The kinetics of calcemia cannot question the role of PCP in hypercalcemia presented by our patient because of the high serum concentration in lysozyme and 1,25-dihydroxy

vitamin D which are both biomarkers of granulomatosis associated with hypercalcemia and are not compatible with hypercalcemia secondary to dehydration.

This is the first reported case of hypercalcemia heralding PCP in a patient with an autoimmune disorder such as systemic sclerosis. PCP is an uncommon but often fatal occurrence in patients with connective tissue disease. Ward and colleagues showed that diagnostic suspicion is an important factor in the correct identification of affected patients and consequently prompt administration of a targeted treatment [30]. Hypercalcemia may therefore prove to be a significant clue to PCP diagnosis since it usually precedes radiographic findings in published reports.

PCP carries important morbimortality yet could be easily avoided as chemoprophylaxis with TMP–SMX proves highly effective in both HIV-positive and seronegative patients [31]. In HIV-positive patients, the risk of PCP is strongly correlated with decreased CD4 cell count ($< 200/\mu L$). However, the role of CD4 cell count in the pathogenesis of PCP in non-HIV immunocompromised patients is still controversial. In a systematic literature review on HIV-seronegative patients with a variety of immunosuppressive conditions, Messiaen et al. [32] showed only 73% of patients to have CD4 cell counts $< 200/\mu L$. In patients under treatment for rheumatic disease, prophylaxis should be considered for patients exceeding the daily 20 mg prednisone threshold and those receiving cyclophosphamide [33]. Some authors suggest a possible benefit of prophylaxis in the 3–6 months period following rituximab infusions, which is the time of maximal B cell depletion [34]. CD20+ B-lymphocytes are indeed critical for generating protective CD4+ T cell immune responses against *Pneumocystis jirovecii* [35]. Besides rituximab infusion 5 months earlier (and a subsequent complete CD19 depletion), our patient did not meet any of the current recognized major risk factors for PCP (he was indeed on low-dose corticosteroids and had a CD4 cell count of $> 450/\mu L$) and therefore did not benefit from prophylaxis. All in all, great demand exists for a more consistent prevention strategy and prospective studies are warranted. Although usually well tolerated, long-term TMP–SMX prophylaxis is associated with inherent costs, threat of emergent antibiotic resistance and non-negligible side effects. In a meta-analysis in HIV-negative patients, 3.1% of patients had to discontinue prophylaxis because of side effects [36]. However, a conventional dose treatment (TMP 15–20 mg/kg/day) carries more frequent and potentially severe adverse effects (41.7% of HIV-negative patients) [37]. Adverse effects include potentially life-threatening skin rashes (Stevens–Johnson syndrome), fever, hepatitis, renal failure, hyperkalemia and myelosuppression. For example, our patient developed grade IV OMS neutropenia within 10 days, facilitated by the recent administration of methotrexate and its combined anti-folate activity with TMP–SMX. Recent retrospective studies suggest that lower doses of TMP–SMX could deliver similar benefits along with better tolerance [37, 38].

Conclusion

PCP is a rare but severe cause of hypercalcemia and must be kept in mind in HIV-seronegative immunocompromised patients with respiratory complaints, even with a CD4 cell count $> 200/\mu L$. Hypercalcemia can precede typical radiographic findings, and early BAL is therefore essential to facilitate diagnosis. The underlying mechanism is the 1-alpha hydroxylation of vitamin D by alveolar macrophages responsible for PCP clearance. PCP treatment can be challenging, and this case report emphasizes potentially serious adverse effects.

Compliance with Ethical Standards

Conflict of interest The authors hereby certify that there is no conflict of interest regarding the publication of this paper. It has been written taking into account the checklist implemented by Mycopathologia to ensure consistency and quality across case reports [39].

References

1. Fréalle E, Valade S, Guigue N, Hamane S, Chabé M, Le Gal S, et al. Diffusion of *Pneumocystis jirovecii* in the surrounding air of patients with *Pneumocystis* colonization: frequency and putative risk factors. Med Mycol. 2017;55(5):568–72.
2. Choukri F, Menotti J, Sarfati C, Lucet JC, Nevez G, Garin YJ, Derouin F, Totet A. Quantification and spread of *Pneumocystis jirovecii* in the surrounding air of patients with *Pneumocystis* pneumonia. Clin Infect Dis. 2010;51(3):259–65.
3. Ahmed B, Jaspan JB. Case report: hypercalcemia in a patient with AIDS and *Pneumocystis carinii* pneumonia. Am J Med Sci. 1993;306(5):313–6.

4. Mills AK, Wright SJ, Taylor KM, McCormack JG. Hypercalcaemia caused by *Pneumocystis carinii* pneumonia while in leukaemic remission. Aust N Z J Med. 1999;29(1):102–3.
5. Chen WC, Chang SC, Wu TH, Yang WC, Tarng DC. Hypercalcemia in a renal transplant recipient suffering with *Pneumocystis carinii* pneumonia. Am J Kidney Dis. 2002;39(2):E8.
6. Hung YM. *Pneumocystis carinii* pneumonia with hypercalcemia and suppressed parathyroid hormone levels in a renal transplant patient. Transplantation. 2006;81(4):639.
7. Aguirre AR, Balbo BE, Ianhez LE, da Costa MC, Andrade L. Hypercalcemia and suppressed PTH levels in a renal transplant patient infected with *Pneumocystis carinii*. Ren Fail. 2007;29(4):513–6.
8. Hajji K, Dalle F, Harzallah A, Tanter Y, Rifle G, Mousson C. Vitamin D metabolite–mediated hypercalcemia with suppressed parathormone concentration in *Pneumocystis jiroveci* pneumonia after kidney transplantation. Transplant Proc. 2009;41(8):3320–2.
9. Bency R, Roger SD, Elder GJ. Hypercalcaemia as a prodromal feature of indolent *Pneumocystis jivorecii* after renal transplantation. Nephrol Dial Transplant. 2011;26(5):1740–2.
10. Chatzikyrkou C, Clajus C, Haubitz M, Hafer C. Hypercalcemia and *pneumocystis* pneumonia after kidney transplantation: report of an exceptional case and literature review. Transpl Infect Dis. 2011;13(5):496–500.
11. Ramalho J, Bacelar Marques ID, Aguirre AR, Pierrotti LC, de Paula FJ, Nahas WC, David-Neto E. *Pneumocystis jirovecii* pneumonia with an atypical granulomatous response after kidney transplantation. Transpl Infect Dis. 2014;16(2):315–9.
12. Dubrofsky L, Lipman ML, Nessim SJ. The casel hypercalcemia in a renal transplant recipient. Kidney Int. 2015;88(5):1207–8.
13. Ling J, Anderson T, Warren S, Kirkland G, Jose M, Yu R, et al. Hypercalcaemia preceding diagnosis of *Pneumocystis jirovecii* pneumonia in renal transplant recipients. Clin Kidney J. 2017;10(6):845–51.
14. El-Reshaid K, Al-Bader S. Hypercalcemic crisis as a prodromal feature of *Pneumcystis jirovecii* pneumonia. Saudi J Kidney Dis Transpl. 2018;29(4):993–6.
15. Taylor LN, Aesif SW, Matson KM. A case of *Pneumocystis* pneumonia, with a granulomatous response and Vitamin D-mediated hypercalcemia, presenting 13 years after renal transplantation. Transpl Infect Dis. 2019;20:e13081.
16. Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, Drent M, Haslam PL, Kim DS, Nagai S, Rottoli P, Saltini C, Selman M, Strange C, Wood B, American Thoracic Society Committee on BAL in Interstitial Lung Disease. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med. 2012;185(9):1004–14.
17. Carmona EM, Limper AH. Update on the diagnosis and treatment of *Pneumocystis* pneumonia. Ther Adv Respir Dis. 2011;5(1):41–59.
18. Roux A, Canet E, Valade S, et al. *Pneumocystis jirovecii* pneumonia in patients with or without AIDS. Fr Emerg Infect Dis. 2014;20:1490–7.
19. Li M-C, Lee N-Y, Lee C-C, Lee H-C, Chang C-M, Ko W-C. *Pneumocystis jiroveci* pneumonia in immunocompromised patients: delayed diagnosis and poor outcomes in non-HIV-infected individuals. J Microbiol Immunol Infect. 2014;47:42–7.
20. Kadakia J, Kiyabu M, Sharma OP, Boylen T. Granulomatous response to *Pneumocystis carinii* in patients infected with HIV. Sarcoidosis. 1993;10(1):44–9.
21. Thomas CF, Limper AH. *Pneumocystis* pneumonia. N Engl J Med. 2004;350(24):2487–98.
22. Travis WD, Pittaluga S, Lipschik GY, et al. Atypical pathologic manifestations of *Pneumocystis carinii* pneumonia in the acquired immune deficiency syndrome. Review of 123 lung biopsies from 76 patients with emphasis on cysts, vascular invasion, vasculitis, and granulomas. Am J Surg Pathol. 1990;14(7):615–25.
23. Bondoc AY, White DA. Granulomatous *Pneumocystis carinii* pneumonia in patients with malignancy. Thorax. 2002;57(5):435–7.
24. Lauffer L, Kini JA, Costello P, Godleski J. Granulomatous *Pneumocystis carinii* pneumonia in a non-AIDS patient: an atypical presentation. J Thorac Imaging. 2004;19(3):196–9.
25. Dusso AS, Kamimura S, Gallieni M, et al. Gamma-interferon-induced resistance to 1,25-(OH)2 D3 in human monocytes and macrophages: a mechanism for the hypercalcemia of various granulomatoses. J Clin Endocrinol Metab. 1997;82(7):2222–32.
26. Lionakis MS, Samonis G, Kontoyiannis DP. Endocrine and metabolic manifestations of invasive fungal infections and systemic antifungal treatment. Mayo Clin Proc. 2008;83(9):1046–60.
27. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. Clin J Am Soc Nephrol. 2015;10(7):1257–72. https://doi.org/10.2215/CJN.09750913.
28. Baylis PH, Milles JJ, Wilkinson R, Heath DA. Vasopressin function in hypercalcaemia. Clin Endocrinol. 1981;15:343–51.
29. Garofeanu CG, Weir M, Rosas-Arellano MP, et al. Causes of reversible nephrogenic diabetes insipidus: a systematic review. Am J Kidney Dis. 2005;45:626–37.
30. Ward MM, Donald F. *Pneumocystis carinii* pneumonia in patients with connective tissue diseases: the role of hospital experience in diagnosis and mortality. Arthritis Rheum. 1999;42(4):780–9.
31. Stern A, Green H, Paul M, Vidal L, Leibovici L. Prophylaxis for *Pneumocystis* pneumonia (PCP) in non-HIV immunocompromised patients. Cochrane Database Syst Rev. 2014;10:CD005590.
32. Messiaen PE, Cuyx S, Dejagere T, van der Hilst JC. The role of CD4 cell count as discriminatory measure to guide chemoprophylaxis against *Pneumocystis jirovecii* pneumonia in human immunodeficiency virus-negative immunocompromised patients: a systematic review. Transpl Infect Dis. 2017;19(2):e12651.
33. Mecoli CA, Saylor D, Gelber AC, Christopher-Stine L. *Pneumocystis jiroveci* pneumonia in rheumatic disease: a 20-year single-centre experience. Clin Exp Rheumatol. 2017;35(4):671–3.
34. Alexandre K, Ingen-Housz-Oro S, Versini M, Sailler L, Benhamou Y. *Pneumocystis jirovecii* pneumonia in patients

treated with rituximab for systemic diseases: report of 11 cases and review of the literature. Eur J Intern Med. 2018;50:e23–4.
35. Elsegeiny W, Eddens T, Chen K, Kolls JK. Anti-CD20 antibody therapy and susceptibility to *Pneumocystis* pneumonia. Infect Immun. 2015;83(5):2043–52.
36. Green H, Paul M, Vidal L, Leibovici L. Prophylaxis of *Pneumocystis* pneumonia in immunocompromised non-HIV-infected patients: systematic review and meta-analysis of randomized controlled trials. Mayo Clin Proc. 2007;82:1052–9.
37. Kosaka M, Ushiki A, Ikuyama Y, Hirai K, Matsuo A, Hachiya T, Hanaoka M. A four-center retrospective study of the efficacy and toxicity of low-dose trimethoprim-sulfamethoxazole for the treatment of *Pneumocystis* pneumonia in patients without HIV infection. Antimicrob Agents Chemother. 2017;61(12):e01173-17.
38. Shibata T, Tonooka K, Tsuchida K, Mitomi H, Shibata T, Katsuyama N. Retrospective investigation of side effects and prognoses of moderate-dose trimethoprim-sulfamethoxazole treatment for *pneumocystis* pneumonia that developed in patients with autoimmune diseases. Nihon Rinsho Meneki Gakkai Kaishi. 2016;39(3):213–8.
39. Bouchara JP, Chaturvedi V. The curious case of "case report" of infections caused by human and animal fungal pathogens: an educational tool, an online archive, or a format in need of retooling. Mycopathologia. 2018;183(6):879–91.