ERJ Express. Published on April 12, 2018 as doi: 10.1183/13993003.01960-2017

EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Research letter

Pulmonary alveolar proteinosis and *Mycobacterium abscessus* lung infection related to ruxolitinib after allogeneic stem cell transplantation

H. Salvator, E. Berti, E. Catherinot, E. Rivaud, A. Chabrol, S. Nguyen, L. Zemoura, E. Cardot, C. Tcherakian, L.J. Couderc

Please cite this article as: Salvator H, Berti E, Catherinot E, *et al*. Pulmonary alveolar proteinosis and *Mycobacterium abscessus* lung infection related to ruxolitinib after allogeneic stem cell transplantation. *Eur Respir J* 2018; in press (https://doi.org/10.1183/13993003.01960-2017).

Pulmonary alveolar proteinosis and *Mycobacterium abscessus* lung infection related to ruxolitinib after allogeneic stem cell transplantation

SALVATOR H.[1,2,3], BERTI E.[1], CATHERINOT E. [1], RIVAUD E.[1], CHABROL A.[1,2] NGUYEN S.[4], ZEMOURA L. [5], CARDOT E.[6], TCHERAKIAN C.[1,3], COUDERC LJ.[1,2,3]
and The Foch Hospital Lung Immune Deficiencies Study Group

Affiliations:
1- Foch Hospital, Respiratory Diseases Department, Suresnes, France
2- Versailles Saint Quentin University, Montigny-le-Bretonneux, France
3- UPRES EA 220, Suresnes, France
4- APHP, Hôpital Pitié-Salpétrière Hospital, Haematology Department, Paris, France
5- Foch Hospital, Pathology Department, Suresnes, France
6- Foch Hospital, Microbiology Department, Suresnes, France

Ruxolitinib may induce pulmonary alveolar proteinosis and favour respiratory non-tuberculous mycobacterial infections.

Non-infectious pulmonary complications are frequent after allogeneic haematopoietic stem cell transplantation (HSCT). They are mainly represented by bronchiolitis obliterans and interstitial pneumonia in the setting of chronic graft-versus-host disease (cGVHD) (1).

Pulmonary alveolar proteinosis (PAP) is a rare disorder characterised by accumulation of lipoproteinaceous material into alveolar spaces due to alveolar macrophage dysfunction (2). It has been been described as a rare early complication after HSCT (3).

We report here the case of a patient who developed a PAP associated with *Mycobacterium abscessus* infection several years after an allogeneic HSCT. This case is very unusual because of its late onset. We suggest these manifestations to be drug-induced, related to the functional deficiency of the alveolar macrophages induced by ruxolitinib.

Case report

A 66-year-old female was diagnosed with a myelodysplasic syndrome with blastic transformation in 2011. After having received daunorubicin and azacitidine, she underwent a sibling donor HSCT in January 2013. Conditioning regimen consisted in fludarabine, busulfan and anti-thymocyte globulins.

She developed severe chronic cutaneous cGVHD from August 2013. Several therapies were unsuccessful, until ruxolinitib (20mg/d) was added in October 2015. The extensive sclerodermic cutaneous cGVHD was stabilised in six months with a combination of oral corticosteroids (15mg/d), ciclosporin A, methotrexate, extracorporeal photochemotherapy and ruxolitinib. Anti-infectious prophylaxis consisted in posaconazole, valaciclovir, trimethoprim-sulfamethoxazole and azithromycin.

On May 2016, she began to complain of slowly increasing non-febrile dyspnea. Pulmonary function tests (PFT) showed normal values except for a recent decreased lung diffusion capacity (DLCOc) at 45% of predicted value. A thoracic CT-scan revealed bilateral ground glass opacities predominant in the upper lobes and thickened interlobular septa. Bronchoalveolar lavage (BAL) fluid analysis displayed 50 000 cells/ml, with 88% macrophages, 8% lymphocytes and 4% neutrophils. Specific stains (Ziehl, Grocott and Periodic acid–Schiff) and extensive microbiological tests were negative. Despite methotrexate interruption and antibiotic therapy, pulmonary infiltrates extended on thoracic CT-scan.

In January 2017, video-assisted biopsies were performed on all three lobes of the right lung. Histological analysis showed an intra-alveolar accumulation of eosinophilic proteinaceous granular material, positive with the Periodic acid–Schiff (PAS) stain. This proteinaceous material was extensive but patchy, with areas of normal lung, without inflammation, granuloma, necrosis or interstitial fibrosis. The Ziehl stain demonstrated extracellular acid-fast bacilli in alveolar spaces. Cultures in liquid medium were positive after six days for

Mycobacterium abscessus. Susceptibility testing showed inducible resistance to clarithromycin (T28 sequovar) and usual weak sensitivity to imipenem and amikacin (minimum inhibitory concentration of 32 and 16 mg/l respectively).

The patient worsened rapidly with oxygen requirement and fever. Blood analysis showed: haemoglobin 7.8g/dL, neutrophils 5700/mm^3, lymphocytes 600/mm^3, platelets 75 000/mm3, C-Reactive Protein 30mg/L. Thoracic CT-scan revealed diffuse ground glass opacities, crazy paving and areas of pulmonary consolidation. Antibiotics against *M. abscessus* (imipenem 750mg tid, amikacin 600 mg three times per week and azithromycin 500 mg/d) were started. Because of increased thrombocytopenia, ruxolinitib was withdrawn.

Chimerism remained 100% donor and a myelogram did not show any sign of myelodysplasia. No serum anti-granulocyte monocyte colony-stimulating factor (GM-CSF) antibodies were detected. There was no GATA2 mutation.

The clinical course was rapidly favorable. Six days after admission, the fever had abated and the patient was weaned from oxygen.

We pursued antibiotics for seven months and replaced ruxolitinib with mycophenolate mofetil from February 2017. Four months later, she felt significantly better, thoracic CT scan and PFT were greatly improved (figure 1B) and DLCOc increased to 51% of predicted values. Cutaneous GVHD worsened but only to a small extent after ruxolitinib cessation. Respiratory improvement is maintained one year after PAP and mycobacterial infection management, with only discrete persistent radiological infiltrates on thoracic CT-scan.

Discussion:

We report, to our knowledge, the first case of a late onset secondary PAP occurring after allogeneic HSCT and an unusual alveolar *M. abscesssus* infection, which may both be related to ruxolitinib.

Three distinct aetiologic forms of PAP have been described: congenital due to mutations in genes encoding surfactant proteins, acquired PAP characterised by the acquisition of anti granulocyte-macrophage-colony-stimulating factor (GM-CSF) antibodies, and secondary PAP associated with various conditions including myeloid malignancies.

Although PAP occurring after allogeneic HSCT is well known, the case of our patient exhibits some interesting features:

1- Previous reports described PAP as an event occurring early after HSCT, before the immune reconstitution is effective (4) (5). In contrast, the PAP in our patient occurred more than three years after allogeneic HSCT. Only one case of late-onset PAP occurring 18 months after HSCT had been previously reported but only partially explored because of untimely death (6).

2- PAP diagnosis required surgical lung biopsy. Negativity of PAS staining on BAL fluid has previously been noted in reports of post HSCT PAP (6).

3- None of the usual causes of PAP were detected. No anti GM-CSF antibodies were detected. We eliminated a relapse of the haematological malignancy and GATA2 deficiency, here evoked because of the association of alveolar proteinosis, myelodysplasic disorder and mycobacterial infection. The role of *M. abscessus* infection in the occurrence of PAP seems unlikely, as pulmonary involvement occurred several months before mycobacteria isolation and the patient improved despite partially active antibiotic treatment.

This led us to suspect the role of ruxolitinib, introduced six months before the occurrence of these two complications. Ruxolitinib is a selective Janus kinase (JAK) 1/2 inhibitor, approved for the treatment of myelofibrosis and refractory cGVHD (7) (8). Interestingly, the GM-CSF receptor is coupled with a Janus Kinase. The first step of intracellular signalling in macrophages depends on JAK2 activation and STAT3 phosphorylation. By disrupting the GM-CSF intracellular signalling, ruloxitinib may have led to the onset of alveolar proteinosis.

Another striking feature of our case report is the occurrence of an alveolar *M. abscessus*

infection without any granuloma in the interstitial tissue or bronchial wall. Non-tuberculous mycobacteria infections are rare after allogeneic HSCT, their incidence varying between 0.4 and 4.9% of the graft recipients (9). Catheter-related infections are the most commonly encountered NTM complications followed by pulmonary and cutaneous infections (9). Infections by mycobacteria are a known complication of PAP (10). Alveolar macrophages have a dual role in lung immune response, phagocyting intra-alveolar pathogens and guiding adaptative immunity. They are deficient in PAP. Moreover, the abundance of a lipoproteinaceous substrate favours pathogen adhesion and growth within the alveolar space. In our case, bacilli were observed exclusively in alveolar spaces within lipo-proteic material. It could be related to the ability of *M. abscessus* to obtain energy from the degradation of host-derived lipids with numerous lipase-encoding genes (11). According to these mechanisms, ruxolitinib may also have favoured the occurrence of *M. abscessus* infection. Ruxolitinib is known to be associated with an increased risk of pneumonia (incidence of 9% at 12 months and 16.4% at 48 months in myelofibrosis) (12) and *M. tuberculosis* reactivation (13). Inhibiting the JAK-STAT pathway may increase the risk of mycobacterial infection by impairing the production and response to IFN-gamma, the function of dendritic cells and by reducing TNFα production which plays a crucial role in granuloma formation (14). This hypothesis is supported by the fact that no granuloma was observed in the lung biopsy of our patient, as reported in HIV-infected patients with low CD4+ cell count or in patients with complete IFN-gamma receptor deficiency (15).

In conclusion, physicians should be aware that ruxolitinib may be associated with severe pulmonary complications. We suggest that all patients should have a thoracic CT-scan before initiation of ruxolitinib and be monitored for respiratory manifestations. Although it is impossible to indicate precise guidelines, we think that ruxolitinib should be stopped in case

of severe pulmonary infection and/or PAP occurrence. It is important to report these respiratory manifestations to the health authorities for worldwide surveillance.

Acknowledgments

The authors thank all the members of Foch Hospital Lung Immune Deficiencies study group for their dedication and their daily work with patients
Thoracic Surgery: Pr Alain Chapelier, Dr Edouard Sage, Dr Pierre Bonnette, Dr Philippe Puyo
Intensive Care Unit: Dr François Parquin, Dr Gregoire Trebbia
Radiology: Dr François Mellot, Dr Axel Guth, Dr Fabrice Staub, Dr Marie-Laure Chabi
Microbiology: Dr Eric Farfour, Dr Emilie Cardot, Dr Pierre Cahen

Contribution
H.S: analysed clinical data, wrote the manuscript
E.B: analysed clinical data, wrote the manuscript
E.C: analysed clinical data, wrote the manuscript
E.R: analysed data, reviewed the manuscript
A.C: analysed data, reviewed the manuscript
S.N: addressed patient, reviewed the manuscript
L.Z: performed pathological analyses, reviewed the manuscript
E.C: designed microbiological analyses, reviewed the manuscript
C.T: analysed data, reviewed the manuscript
LJ.C: analysed clinical data, wrote the manuscript

Conflict of interest Disclosure: The authors declare no competing financial interests.

Correspondence: Hélène Salvator Hopital Foch Service de Pneumologie, 40 rue Worth 92150 Suresnes, France. Email : h.salvator@hopital-foch.com

References

1. Guihot A, Becquemin M-H, Couderc L-J, Randrianarivelo O, Rivaud E, Philippe B, Sutton L, Neveu H, Tanguy ML, Vernant JP, Dhédin N. Telemetric monitoring of pulmonary function after allogeneic hematopoietic stem cell transplantation. Transplantation. 2007; 83: 554‑560.
2. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. N Engl J Med. 2003; 349: 2527‑2539.
3. Cordonnier C, Fleury-Feith J, Escudier E, Atassi K, Bernaudin JF. Secondary alveolar proteinosis is a reversible cause of respiratory failure in leukemic patients. Am J Respir Crit Care Med.1994; 149: 788‑794.
4. Ansari M, Rougemont A-L, Le Deist F, Ozsahin H, Duval M, Champagne MA, fournet JC. Secondary pulmonary alveolar proteinosis after unrelated cord blood hematopoietic cell transplantation. Pediatr Transplant. 2012; 16: E146-149.
5. Tomonari A, Shirafuji N, Iseki T, Ooi J, Nagayama H, Masunaga A, Tojo A, Tani K, Asano S. Acquired pulmonary alveolar proteinosis after umbilical cord blood transplantation for acute myeloid leukemia. Am J Hematol. 2002; 70: 154‑157.
6. Pidala J, Khalil F, Fernandez H. Pulmonary alveolar proteinosis following allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2011; 46: 1480‑1483.
7. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, Catalano JV, Deininger M, Miller C, Silver RT, Talpaz M, Winton EF, Harvey JH Jr, Arcasoy MO, Hexner E, Lyons RM, Paquette R, Raza A, Vaddi K, Erickson-Viitanen S, Koumenis IL, Sun W, Sandor V, Kantarjian HM. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012; 366: 799‑807.
8. Zeiser R, Burchert A, Lengerke C, Verbeek M, Maas-Bauer K, Metzelder SK, Spoerl S, Ditschkowski M, Ecsedi M, Sockel K, Ayuk F, Ajib S, de Fontbrune FS, Na IK, Penter L, Holtick U, Wolf D, Schuler E, Meyer E, Apostolova P, Bertz H, Marks R, Lübbert M, Wäsch R, Scheid C, Stölzel F, Ordemann R, Bug G, Kobbe G, Negrin R, Brune M, Spyridonidis A, Schmitt-Gräff A, van der Velden W, Huls G, Mielke S, Grigoleit GU, Kuball J, Flynn R, Ihorst G, Du J, Blazar BR, Arnold R, Kröger N, Passweg J, Halter J, Socié G, Beelen D, Peschel C, Neubauer A, Finke J, Duyster J, von Bubnoff N. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. Leukemia. 2015; 29: 2062‑2068.
9. Al-Anazi KA, Al-Jasser AM, Al-Anazi WK. Infections caused by non-tuberculous mycobacteria in recipients of hematopoietic stem cell transplantation. Front Oncol. 2014; 4: 311.
10. Punatar AD, Kusne S, Blair JE, Seville MT, Vikram HR. Opportunistic infections in patients with pulmonary alveolar proteinosis. J Infect. 2012; 65: 173‑179.
11. Ripoll F, Pasek S, Schenowitz C, Dossat C, Barbe V, Rottman M, Macheras E, Heym B, Herrmann JL, Daffé M, Brosch R, Risler JL, Gaillard JL. Non mycobacterial virulence genes in the genome of the emerging pathogen Mycobacterium abscessus. PloS One. 2009; 4: e5660.
12. Verstovsek S, Mesa RA, Gotlib J, Gupta V, DiPersio JF, Catalano JV, Deininger MW, Miller CB, Silver RT, Talpaz M, Winton EF, Harvey JH Jr, Arcasoy MO, Hexner EO, Lyons RM, Paquette R, Raza A, Jones M, Kornacki D, Sun K, Kantarjian H; COMFORT-I investigators. Long-term treatment with ruxolitinib for patients with myelofibrosis: 5-year update from the randomized, double-blind, placebo-controlled, phase 3 COMFORT-I trial. J Hematol OncolJ Hematol Oncol. 2017; 10:55.

13. Abidi MZ, Haque J, Varma P, Olteanu H, Guru Murthy GS, Dhakal B, Hari P. Reactivation of Pulmonary Tuberculosis following Treatment of Myelofibrosis with Ruxolitinib. Case Rep Hematol. 2016; 2016: 2389038
14. Heine A, Held SAE, Daecke SN, Wallner S, Yajnanarayana SP, Kurts C, Wolf D, Brossart P. The JAK-inhibitor ruxolitinib impairs dendritic cell function in vitro and in vivo. Blood. 2013; 122: 1192‑1202.
15. Lammas DA, De Heer E, Edgar JD, Novelli V, Ben-Smith A, Baretto R, Drysdale P, Binch J, MacLennan C, Kumararatne DS, Panchalingam S, Ottenhoff TH, Casanova JL, Emile JF. Heterogeneity in the granulomatous response to mycobacterial infection in patients with defined genetic mutations in the interleukin 12-dependent interferon-gamma production pathway. Int J Exp Pathol. 2002; 83: 1‑20.

Figure 1

A. CT-scan at time of PAP and mycobacterial infection diagnosis, highlighting ground glass opacities, thickened interlobular septa and patchy condensations

B. Radiological evolution four months after beginning anti-mycobacterial treatment and withdrawal of ruxolitinib.