



Expansion of ST2-expressing macrophages in a patient with bronchiolitis obliterans syndrome

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To the Editor:

Bronchiolitis obliterans syndrome (BOS) is a major complication of haematopoietic stem cell transplantation (HSCT), which affects the lungs and has limited treatment options [1]. BOS after HSCT is considered a manifestation of chronic graft-versus-host disease (GVHD). T-cells play a crucial role in its onset [2], while the contribution of myeloid cells has been largely unknown.

Here, we report a case of a 29-year-old woman with acute myeloid leukaemia (AML-M2) who developed BOS 5 months after receiving an allogeneic peripheral blood stem cell transplant. The patient presented with symptoms of dry cough and dyspnoea, and a chest computed tomography scan revealed mosaic attenuation, mild bronchial thickening, small nodules and faint ground-glass opacity with hyperinflation in both lungs (figure 1a). Pulmonary function tests indicated severe obstructive disease compared to the results before HSCT, with a forced expiratory volume in 1 s (FEV₁) of 0.98 L (33.6% predicted), a forced vital capacity (FVC) of 1.90 L (56.4% pred), and an FEV₁/FVC ratio of 0.52, as well as diffusing capacity of the lungs for carbon monoxide of 62.4% pred (figure 1b). Bronchoalveolar lavage was conducted in the right B4b lung area using 150 mL of saline solution, resulting in the collection of 87 mL of bronchoalveolar lavage fluid (BALF). Analysis of BALF revealed 95.2% macrophages, 0.6% neutrophils and 4.2% lymphocytes, with no evidence of infection. Transbronchial lung biopsy showed mild chronic inflammation without any signs of specific infection or malignancy. The patient was subsequently diagnosed with BOS.

Mass cytometry analysis of BALF cells revealed distinct patterns of immune cell populations compared to other lung diseases, such as sarcoidosis, connective tissue disease (CTD)-related interstitial lung diseases (ILDs) and cytotoxic drug-induced ILDs (figure 1c and d). The macrophage population was prevalent, as indicated by the cytological data, and there was a significantly increased proportion of ST2⁺CD64⁺ macrophages in the BALF of the patient with BOS. These ST2⁺CD64⁺ macrophages were CD11b⁺CD11c⁺CD16⁺HLA-DR^{lo}CCR2⁺CCR5⁺CD36⁺CD163^{hi}CD223⁺, possibly representing an alternatively activated macrophage phenotype [4]. This is the first demonstration of the increased proportion of ST2⁺ macrophages in BALF from a patient with BOS. ST2 is a receptor for interleukin (IL)-33, a cytokine released by damaged epithelial cells, and is expressed on various cell types, including type 2 innate lymphoid cells and macrophages [5]. During epithelial damage, airway macrophages are activated to support epithelial repair and display IL-33–ST2 activation during their differentiation [6]. Continuous epithelial damage by alloreactive T-cells [2] may aberrantly stimulate these ST2⁺ macrophages to produce excessive growth factors and extracellular matrix, resulting in airway remodelling and obstruction. Soluble ST2 levels in plasma have been identified as a marker for the risk of therapy-resistant GVHD [7], suggesting a link to our hypothesis, although we did not assess plasma ST2 levels in the patient. Thus, ST2⁺ macrophages may be a potential therapeutic target, particularly for treatment-refractory BOS [1].

We also observed a reduction in the proportion of CCR2⁺CD14⁺ monocytes compared to other lung disorders. These monocytes were characterised as CD64⁺CD11b⁺CD11c⁺CD16⁺HLA-DR⁺CCR5⁺CD32⁺CD36⁺CD163⁺CD206^{lo}. Recent evidence suggests that CCR2⁺ monocytes accumulating in the lung can stimulate tissue-resident CD8⁺ T-cell activation, leading to airway epithelial cell apoptosis in a mouse model of BOS after lung transplantation [8]. The precise reason for the reduction of CCR2⁺CD14⁺



Shareable abstract (@ERSpublications)

This case study of a patient with BOS after HSCT found increased ST2⁺CD64⁺ macrophages in BALF, a potential therapeutic target for treatment-refractory BOS, and reduced CCR2⁺CD14⁺ monocytes compared to other lung disorders <https://bit.ly/406Uyy9>

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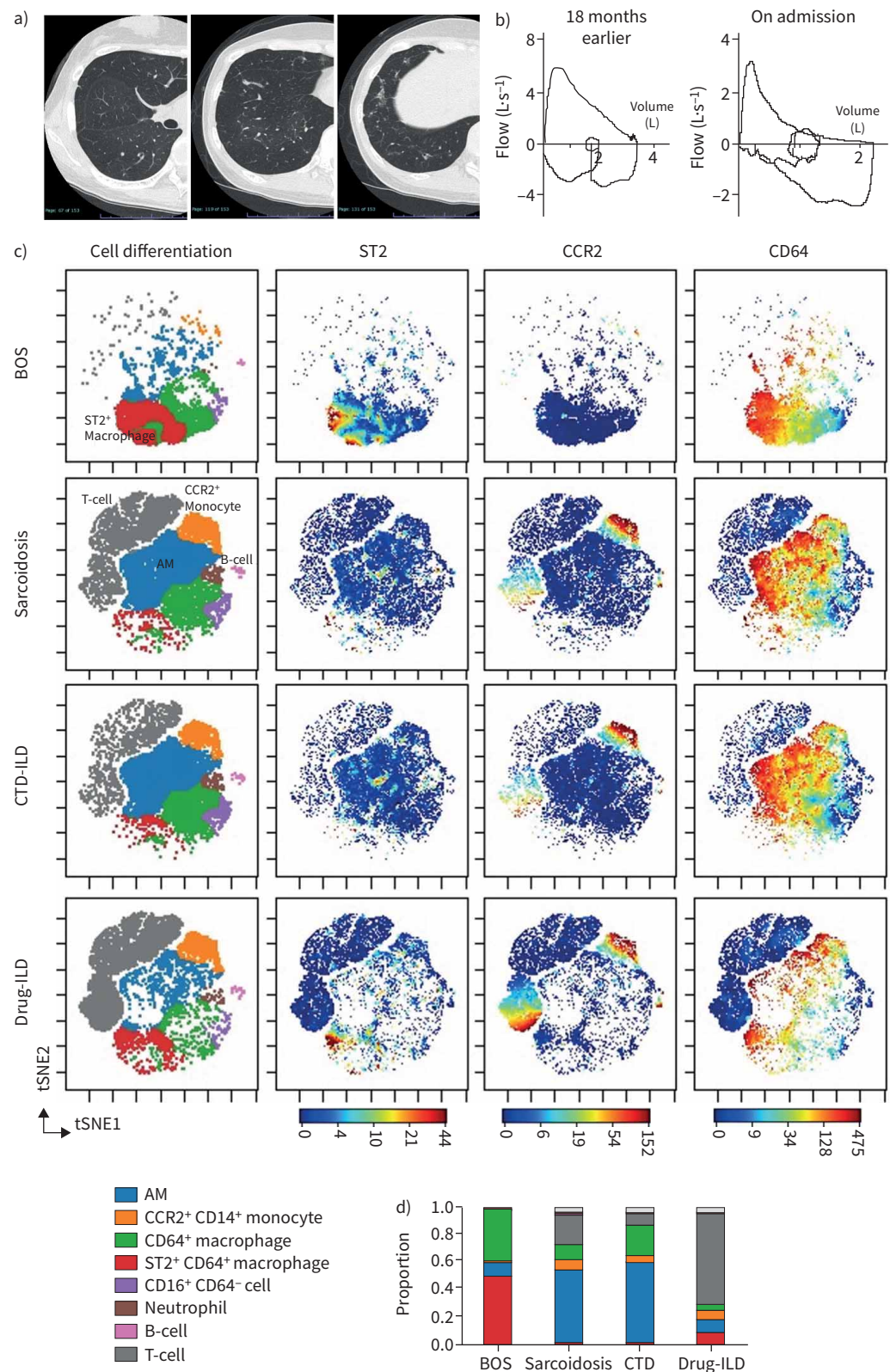


FIGURE 1 a) A chest computed tomography image and b) flow-volume curves on spirometry of the patient. c) t-Stochastic neighbourhood embedding (tSNE) plots visualising cell population, distribution and intensity of ST2, CCR2 and CD64 in CD45⁺-gated bronchoalveolar lavage fluid (BALF) cells from patients with bronchiolitis obliterans syndrome (BOS) (n=1), sarcoidosis (n=11), connective tissue disease (CTD)-associated interstitial lung disease (ILD) (n=8) and cytotoxic drug-ILD (n=5). For sarcoidosis, CTD-ILD and cytotoxic drug-ILD, individual

data were concatenated into one file per disease group. Alveolar macrophages are characterised by CD64⁺CD14⁺CD11b^{hi}CD11c^{hi}HLA-DR^{hi} expression, neutrophils by CD64⁺CD11b⁺CD16⁺CD11c⁺HLA-DR⁺ expression, B-cells by CD19⁺ expression and T-cells defined by CD3⁺ expression. **d**) The proportion of cell populations in CD45⁺-gated BALF cells as determined by tSNE analysis. The methodology has been described previously [3]. AM: alveolar macrophage.

monocytes (which were previously observed to be increased in a mouse model of BOS) in the present case remains unclear. Possible explanations include variations in the conditions of BOS (lung transplantation *versus* HSCT), limitations in model replication or disparities in timing from disease onset.

The study has limitations. First, only one case was investigated due to its rarity, and validation is required. Second, we have used BALF samples from sarcoidosis, CTD-ILDs and cytotoxic drug induced-ILDs as disease controls. Given that BOS mainly affects “small airways”, it would have been ideal to use other airway diseases, such as bronchial asthma or COPD, that exhibit small airway lesions as disease controls. The remaining BALF obtained as part of clinical testing was used in our study. It is not common to perform bronchoalveolar lavage in these patients as part of clinical practice, which led to the unavailability of BALF samples from small airway diseases as disease controls. Last, the BALF analysis lacks spatial information on the lung. Recently, MURANUSHI *et al.* [9] reported monocyte and lymphocyte accumulation around bronchioles in human GVHD characterised by bronchiolitis obliterans, although precise analysis of peribronchial macrophages was not conducted. Since BALF can reflect peribronchial components as well as alveolar ones, these ST2⁺ macrophages could be from the peribronchiole, the main inflammation site of BOS. Immunohistochemistry analysis of these macrophages in BOS would provide additional insights into the pathogenesis of BOS. Further research for validation, spatial investigation and measurement of plasma ST2 levels is warranted to fully understand the precise mechanisms of immune dysregulation in BOS.

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