Analysis of Cytokines in Serum and Bronchoalveolar Lavage Fluid in Patients with Immune-Checkpoint Inhibitor Associated Pneumonitis: a Parallel Cohort Study

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Research

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Abstract

Background

Immune-checkpoint inhibitors (ICI) present a new treatment for malignancies by boosting the immune system. This has led to a variety of immune-related adverse events, including ICI-associated pneumonitis (ICIaP). Diagnosis thereof is often challenging, and its pathogenesis has not yet been fully understood. The aim of this parallel cohort study was to investigate cytokines in serum and bronchoalveolar lavage fluid (BALF) expressed in patients with ICI-associated pneumonitis compared to healthy individuals.

Methods

From January 2018 until June 2019, 401 adult patients with various lung diseases were prospectively enrolled in a BALF- and serum biobank, called BALOTHEK. Of these, 12 patients were diagnosed with ICIaP (Pembrolizumab, Ipilimumab, or both, and Durvalumab) and included in this parallel cohort study. Additionally, 12 healthy subjects from the biobank served as matched control group. The following 11 cytokines were simultaneously analyzed in BALF and serum of each study participant: interferon gamma, tumor necrosis factor alpha, interleukin (IL) 1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12p70, IL-13 and IL-17A. This study was approved by the local ethic review committee (BASEC-ID 2017-02307 and 2018-01724).

Results

Absolute number and percentage of lymphocytes in BALF of patients with ICIaP were significantly higher compared to control group. For the investigated cytokines in serum and BALF, a significant increase of IL-6 levels was shown for patients with ICIaP ($p=0.044$, adjusted for multiple comparisons).

Conclusion

Cytokine profile assessed in BALF shows promising potential for facilitating diagnosis and understanding of pathophysiology of ICIaP. IL-6 may not only contribute to better understanding of pathophysiology but also herald therapeutic implications for Tocilizumab.

Background

Both, chemo- and radiation therapy used to be the common approach for cancer treatment throughout decades. Recently, immune-checkpoint inhibitors (ICI) made of monoclonal antibodies (mAB) against receptors on T-lymphocytes have been introduced as a new therapeutic ideology in fighting cancer. Ipilimumab was the first ICI to be approved, representing a breakthrough in the treatment of metastatic melanoma [1]. Since then, clinical practice has changed [2] and new ICIs allowed treatment of various malignancies other than melanoma. Currently, there are two targetable immune checkpoints: cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and the axis programmed cell death protein 1 (PD-1) and PD-1 ligand (PD-L1). Instead of repeated exposure to cytotoxic agents in traditional chemotherapy, ICIs boost the immune system by “inhibiting the inhibition” and, thus, helping it to tackle neoplasia effectively [3].

Compared to chemotherapy, the side effects of ICIs are different. Whereas adverse events from chemotherapy are partially caused by a compromised immune system, those caused by immunotherapy are mainly due to immune reinforcement. Thus, autoimmunity and excessive inflammatory responses referred to as immune-related adverse events (irAE) may be induced, leading to gastrointestinal, cutaneous, endocrinal and pulmonary manifestations, among others [4]. Pneumonitis is an uncommon, but potentially life threatening irAE. The reported incidence for pneumonitis is 1.3–11% for ICI monotherapy [5] and 6.6% for combination therapy of Ipilimumab with Pembrolizumab or Nivolumab [6].

The clinical presentation of ICI-associated pneumonitis is non-specific and heterogeneous, ranging from asymptomatic, only radiological manifestations to mild or moderate symptoms with cough and dyspnea, and, eventually, to very severe cases of acute respiratory distress syndrome (ARDS) [7–9]. While computed tomography (CT) is indispensable for diagnosis, imaging of pneumonitis is also unspecific and includes various patterns of interstitial lung disease (ILD), such as organizing pneumonia (OP), non-specific interstitial pneumonia (NSIP), hypersensitivity pneumonitis (HP), acute interstitial pneumonia (AIP) and usual interstitial pneumonia (UIP) [10, 9, 11]. Median onset has been observed at 2.5 months after initiation of ICI therapy, with a time window ranging between 2 and 24 months [4, 9]. All the above mentioned factors may contribute to delayed diagnosis of ICI-associated pneumonitis, while pathogenesis has not yet been fully understood.

Since ICI-associated pneumonitis is thought to result from immune driven over-reaction, changes in cytokines as immunomodulatory proteins produced by immune cells among others can be expected. While a recent study [12] successfully identified 11 cytokines in serum to predict irAE, data about cytokines expressed in pneumonitis are scarce. However, cytokines measured only in serum might be misleading in determining type and degree of inflammation in a specific organ. Various studies suggest organ specific sample collection as the best method for exact and targeted assessment [13–17]. Flexible bronchoscopy is an established diagnostic tool for a broad range of pulmonary diseases, since it enables minimally invasive biopsies at low risk for various techniques including (BAL) [18–20]. Therefore, BAL appears suitable to provide more detailed information on the lung tissue by obtaining bronchoalveolar lavage fluid (BALF).

The aim of this parallel cohort study was to investigate cytokines in serum and BALF expressed in patients with ICI-associated pneumonitis compared to healthy individuals. To the best of our knowledge, there is only one study [21] investigating cytokine expression in BALF in patients with ICI-associated pneumonitis.

Materials And Methods

Patients

The present study is part of a prospective multicenter study, which aims to establish a biobank ("*BALOTHEK*") using blood serum and BALF for the research of various lung diseases. The samples were

acquired from patients in whom BAL was performed for purpose of routine clinical evaluation (BASEC-ID 2017–02307 and 2018 – 01724). Enrolled patients were retrospectively clustered in five groups according to clinical and radiological presentation, confirmed by histology: lung cancer, sarcoidosis, ILD, drug-related pneumonitis and chronic cough. Patients in the latter group served as healthy controls, when there were no pathological findings in the chest CT during a follow-up time of six months. Patients were excluded in case of precedent lung transplantation, general patient vulnerability such as emergencies or pregnancies and errors in sampling or processing of the samples, e.g. BAL to processing time exceeding 60 minutes [22].

From January 2018 until June 2019, a total of 401 adult patients were enrolled in *BALOTHEK*. Simultaneously, 240 patients were treated with ICIs at the Departments of Dermatology and Medical Oncology from University Hospital Zurich because of various malignancies. Of these 240 patients, 16 developed typical symptoms (i.e. cough, fever, dyspnea) and CT findings (i.e. COP, NSIP, HP, AIP) suggestive for ICI-associated pneumonitis. After conducting BAL however, in four patients an alternative diagnosis other than ICI-associated pneumonitis had been made (one patient with Melphalan-induced pulmonary toxicity, two patients with acute bronchitis and one patient with chronic cough of unknown origin). Thus, the remaining 12 patients were eventually included with the diagnosis of ICI-associated pneumonitis confirmed by BAL. From these patients BALF could be harvested for purpose of *BALOTHEK* and for the present study, respectively. In addition, 12 subjects from the control group of *BALOTHEK*, matched according to gender and age (range +/- five years) were used as healthy controls.

This parallel cohort study was approved by the local ethic review committee (BASEC-ID 2017–02307 and 2018 – 01724).

Blood specimens and processing

All blood samples were collected by nurses proficient in blood drawing as part of the routinely performed pre-interventional peripheral venous access. For differential blood count and whole blood count 10 ml BD Vacutainer K2E tubes (EDTA, Plus Blood Collection Tubes, Becton Dickinson, Plymouth, UK) were used. To gain serum samples, whole blood was collected in 10 ml BD Vacutainer Clot Activator Tube (CAT, Plus Blood Collection Tubes, Becton Dickinson, Plymouth, UK) and centrifuged at 3500 rounds per minute (rpm) at room temperature. Thereafter, the supernatant was aliquoted and eventually stored at − 80 °C for later analyses, according to Valaperti et al. [22]. Once thawed for analysis, the samples were not frozen again.

Bronchoscopy, BAL and processing of BALF

Bronchoscopy was performed in moderate sedation with propofol using Olympus (Tokyo, Japan) flexible bronchoscopes (190 series). BALF was obtained conforming to official recommendations [23, 24] by instillation of isotonic saline solution in four times 50 ml portions into the wedged pulmonary segment

that showed the most prominent finding in the most recent chest CT. Through gentle suction of the same syringe that injected the solution, BALF was yielded and filled in designated tubes, absent any further substances such as anticoagulants and preservatives. The recovered BALF was quantitatively expressed in absolute values (ml) and in percent of the instilled volume. BALF was routinely used for cytological and microbiological analyses, whereas the rest served the purpose of this study. For processing, BALF was centrifuged at 1’000 rpm at room temperature, the supernatant was aliquoted and stored at -80 °C in accordance to Valaperti et al. [22]. Once thawed for analysis, the samples were not frozen again. The routinely performed analysis of BALF for cell differentiation was performed by ADVIA 2120i (Siemens Healthcare AG, Zurich, Switzerland) via peroxidase staining. Cell differentiation included cell count, macrophages, lymphocytes, neutrophils, eosinophils, mast cells, and plasma cells.

Cytokine analysis

A Milliplex MAP kit (human high sensitivity T cell magnetic bead panel) customized by Merk Millipore (Darmstadt, Germany) was used to analyze cytokines applying MAGPIX system (Luminex Corporation, Austin, TX, USA). The array contained the following 11 cytokines: interferone-gamma (IFN-gamma), interleukin (IL)-1B, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12p70, IL-13, IL-17, and tumor necrosis factor alpha (TNF-alpha). This selection of cytokines based on several publications [25–27, 12, 28] investigating inflammatory biomarkers in drug-induced pneumonitis as well as specifically ICI-associated pneumonitis. The preparation of standards was composed of serial dilution 1:4 of each stock standard to generate seven standard concentrations which were used to create a five-parameter logistic curve-fit standard curve with the xPONENT software (Luminex Corporation, Austin, TX, USA). Before quantifying cytokines, the high sensitivity bead panel was successfully validated and calibrated, showing a correct standard curve for each cytokine. Cytokines were determined in BALF as well as in serum.

Statistical analysis

Continuous data are reported as median ± interquartile range (IQR) or as mean ± standard deviation (SD), as appropriate. Normal distribution was tested using the Shapiro-Wilk test. To express comparisons between groups, Chi-squared test or Fisher’s exact test was used for categorical variables and the Mann-Whitney U test or independent t-test for continuous variables. The Bonferroni correction was used adjust p-values for multiple comparisons to avoid the risk of a type I error. *P* values of less than 0.05 were considered to be statistically significant and were based on two-sided hypothesis. All analyses were conducted using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corporation, Armonk, NY, USA) and R Core Team, 2013; R version 4.0.3 (2020-10-10).

Results

Totally, 24 patients were included in this parallel cohort study. From one patient with pneumonitis, BALF could not be yielded due to bronchial collapse, and from one patient in the control group the serum

sample was lost. Demographic characteristics and bronchoscopy data are summarized in Table 1. The absolute volume of recovered BALF in the pneumonitis compared to the control group was 65.0 ± 75.0 ml and 110.0 ± 100.0 ml (p = 0.304), respectively, referring to a recovery rate of 38.2 ± 11.3% and 52.2 ± 15.8% (p = 0.112), respectively. Absolute cell count and differentiation in serum and in BALF are summarized in Table 2 and Table 3, respectively. Expectedly, the absolute number and percentage of lymphocytes in BALF were significantly higher in the pneumonitis compared to control group (each p = 0.004). However, CD4/CD8-ratios were comparable between groups (p = 0.436). Inversely, the portion of macrophages was significantly higher in the control group (p = 0.008). In serum of the pneumonitis group, C-reactive protein (CRP) was significantly higher (p = 0.039). Likewise, the blood leukocytes count was higher in the pneumonitis group compared to controls, albeit this difference was not statistically significant. Analysis of cytokines in serum and BALF are presented in Table 4 and Table 5, respectively. Whereas all investigated cytokines in serum were equally distributed between the groups, analysis of BALF revealed a significantly increased portion of IL-6 in the pneumonitis compared to the control group (adjusted p = 0.044). All other investigated cytokines in BALF did not discriminate between the groups.

Table 1
Baseline characteristics of study population

	Pneumonitis	Controls	p-value
Number	12 (50.0)	12 (50.0)	
Demographics			
Female	5 (41.7)	4 (33.3)	
Age	70.6 (61.5–76.1)	73.8 (61.6–75.3)	0.755
Smoking			
Never smoker	3 (25.0)	6 (50.0)	
Current smoker	1 (8.3)	1 (8.3)	
Ex-smoker	8 (66.7)	5 (41.7)	
Pack years	10.0 (0.0–40.0)	3 (0.0–13.8)	0.260
Medical history			
Adenocarcinoma of the lung	5 (41.7)	0	-
Metastasized melanoma	7 (58.3)	0	-
Administered ICI			
Pembrolizumab	6 (50.0)	0	-
Nivolumab	2 (16.7)	0	-
Durvalumab	1 (8.3)	0	-
Ipilimumab + Pembrolizumab	3 (25.0)	0	-
Bronchoscopy data			
BAL in [ml]	200.0 (15.1)	200.0 (28.9)	1.000
BAL ex [ml]	65.0 (21.3)	110.0 (32.4)	0.304
BAL recovery [%]	32.5 (11.4)	60.0 (15.8)	0.112
BAL cell count [cells/µl]	195.8 (201.0)	75.5 (80.8)	0.528
Data are presented as n (%), median (IQR) or mean (SD), as appropriate			
Abbreviations: BAL = bronchoalveolar lavage			

Table 2
Cell count and differentiation in serum

	Pneumonitis	Controls	p-value
CRP [mg/l]	42.5 (80.1)	4.3 (37.0)	0.039*
Leukocytes [G/l]	7.0 (2.5)	6.4 (2.7)	0.487
Neutrophils [G/l]	4.8 (2.2)	3.9 (2.4)	0.314
Monocytes [G/l]	0.7 (0.3)	0.6 (0.2)	0.203
Eosinophils [G/l]	0.1 (0.2)	0.1 (0.2)	1.000
Basophils [G/l]	0.0 (0.0)	0.0 (0.0)	0.346
Lymphocytes [G/l]	1.1 (0.4)	1.5 (0.6)	0.080
Neutrophils [%]	73.3 (9.4)	64.6 (10.5)	0.295
Monocytes [%]	9.3 (5.5)	10.4 (2.7)	1.000
Eosinophils [%]	1.9 (3.2)	2.2 (1.4)	1.000
Basophils [%]	0.3 (0.3)	0.5 (0.4)	1.000
Lymphocytes [%]	14.5 (5.3)	23.1 (10.3)	0.085
* = $p < 0.05$, adjusted for multiple comparisons using the Bonferroni correction method.			
Data are presented as mean (SD).			
Abbreviations: CRP = C-reactive protein			

Table 3
Cell count and cell differentiation in BALF

	Pneumonitis	Control	p-value
Macrophages [/µl]	74.7 (18.4–139.0)	52.5 (40.3–113.0)	1.000
Lymphocytes [/µl]	66.9 (30.8–152.9)	4.0 (3.4–25.4)	0.004*
Neutrophils [/µl]	10.0 (4.7–14.8)	1.0 (0.6–15.3)	0.244
Eosinophils [/µl]	0.4 (0.0–3.7)	0.3 (0.0–2.4)	1.000
$CD4^+$ T-cells [/µl]	31.8 (7.6–100.7)	0.0 (0.0–8.4)	0.038*
$CD8^+$ T-cells [/µl]	18.9 (8.0–52.6)	0.0 (0.0–6.5)	0.006*
$CD4^+/CD8^+$ ratio	1.4 (0.5–3.5)	5.8 (1.3–5.8)	0.436
Macrophages [%]	49.5 (27.5–62.5)	88.0 (63.3–95.3)	0.008*
Lymphocytes [%]	45.5 (132.0–64.0)	7.3 (3.9–13.4)	0.004*
Neutrophils [%]	5.5 (3.0–7.5)	1.5 (0.5–10.3)	0.608
Eosinophils [%]	0.8 (0.4–1.8)	1.0 (0.0–3.0)	0.813
$CD4^+$ T-cells [%]	54.3 (27.4–69.2)	60.1 (33.4–60.1)	0.758
$CD8^+$ T-cells [%]	21.7 (19.0–63.2)	17.2 (8.5–17.2)	0.436
* = $p < 0.05$, adjusted for multiple comparisons using the Bonferroni correction method.			
Data are presented as median (IQR).			
Abbreviations: BALF = bronchoalveolar lavage fluid			

Table 4
Analysis of cytokines in serum

	Pneumonitis	Control	p-value
IFN-γ [pg/ml]	11.4 (2.7–18.5	4.9 (1.8–9.4)	0.316
IL-1B [pg/ml]	0.5 (0.2–0.8)	0.2 (0.1–0.6)	0.413
IL-2 [pg/ml]	1.9 (0.9–4.2)	1.4 (1.1–1.9)	0.697
IL-4 [pg/ml]	9.0 (4.0–14.7)	6.4 (2.2–11.6)	0.260
IL-5 [pg/ml]	3.8 (1.8–5.2)	3.1 (1.3–5.7)	0.833
IL-6 [pg/ml]	5.8 (1.8–11.1)	3.5 (1.8–7.1)	0.379
IL-8 [pg/ml]	9.4 (7.8–12.8)	7.1 (6.5–10.3)	0.833
IL-12p70 [pg/ml]	2.9 (0.6–6.2)	2.2 (1.0–4.6)	0.786
IL-13 [pg/ml]	5.5 (2.2–10.1)	3.7 (1.2–7.6)	0.316
IL-17 [pg/ml]	3.5 (0.7–12.9)	2.4 (0.6–8.6)	0.695
TNF-α [pg/ml]	5.9 (4.5–11.1)	7.0 (4.6–8.9)	0.833
* = $p < 0.05$			
Data are presented as median (IQR).			
Abbreviations: IFN-γ = Interferon gamma, IL = interleukin, TNF- α = tumor necrosis factor alpha			

Table 5
Analysis of cytokines in BALF

	Pneumonitis	Control	p-value
IFN-γ [pg/ml]	0.8 (0.0–1.3)	0.1 (0.0–1.2)	1.000
IL-1B [pg/ml]	0.2 (0.1–0.3)	0.2 (0.1–0.8)	1.000
IL-2 [pg/ml]	0.4 (0.3–0.8)	0.6 (0.3–1.3)	1.000
IL-4 [pg/ml]	0.0 (0.0–0.4)	0.0 (0.0–0.3)	1.000
IL-5 [pg/ml]	0.4 (0.0–0.8)	0.2 (0.0–0.6)	1.000
IL-6 [pg/ml]	126.0 (14.6–248.9)	1.9 (0.5–4.5)	0.044*
IL-8 [pg/ml]	40.4 (18.5–77.5)	39.0 (9.2–68.9)	1.000
IL-12p70 [pg/ml]	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1.000
IL-13 [pg/ml]	0.0 (0.0–0.2)	0.3 (0.0–0.8)	1.000
IL-17 [pg/ml]	0.5 (0.5–0.7)	0.7 (0.3–0.7)	1.000
TNF-α [pg/ml]	3.0 (0.8–8.8)	0.8 (0.6–2.1)	0.759
* = $p < 0.05$, adjusted for multiple comparisons using the Bonferroni correction method			
Data are presented as median (IQR).			
Abbreviations: IFN-γ = Interferon gamma, IL = interleukin, TNF- α = tumor necrosis factor alpha			

Discussion

After introducing ICIs in modern cancer treatment regimens, a new entity of irAEs has emerged including ICI-associated pneumonitis [4]. Since the immunological mechanism is not fully understood and, thus, diagnosis of ICI-associated pneumonitis is not straight forward due to several differential diagnoses, the focus of this study was to assess cytokine profile in BALF and serum of patients with ICI-associated pneumonitis compared to a matched healthy control group. In serum, both cell counts and all investigated cytokines were expressed similarly between the groups. However, in BALF of patients with ICI-associated pneumonitis there was a significantly higher lymphocytes count and a significantly higher expression of IL-6 compared to the control group.

Expressed by Th2-cells, monocytes, macrophages, dendritic cells and bone marrow stroma, IL-6 is an important cytokine for vast inflammatory responses including induction of acute phase proteins (APP) and enhancement of T-cell proliferation as well as polarization of Th17-cells. Th17-cells are a subset of CD4-cells known to trigger massive inflammatory diseases with a tendency for autoimmune reactions [29]. Notably, in the present study, CD4/CD8 ratio in BALF was comparable between the groups, although one might have expected a lower ratio in the pneumonitis group. Possibly, the sample size was too small

to show a significant difference. Th17-cells reside mostly in tissues exposed to the external environment such as gastrointestinal tract, skin and respiratory tract, and express themselves cytokines, notably IL-17A, IL-21 and IL-22. Both, autoimmune diseases and chronic inflammatory disorders of the lung are suspected to arise from dysregulation of Th17-cells such as chronic obstructive pulmonary disease (COPD), bronchial asthma, rheumatoid arthritis (RA), chronic hypersensitivity pneumonitis and other forms of interstitial fibrosis [30–33]. In addition, in a mice model, recovery from acute lung injury in absence of Th17-cells was faster compared to those with Th17-positive cellular immune reaction [34]. It seems plausible that Th17-cells stimulated by IL-6 play an important role in the still vague pathogenesis of ICI-associated pneumonitis.

Although IL-6 levels in BALF of the pneumonitis group were significantly increased compared to healthy subjects in our study, their origin remains uncertain. According to a recent study by our group IL-6 was not significantly increased in BALF of patients with untreated lung cancer and other lung diseases (sarcoidosis, interstitial lung disease), deeming IL-6 appropriate as biomarker of unspecific inflammation [35]. Therefore, increased IL-6 levels in our study could originate from inflammation due to ICI-associated pneumonitis. However, enhanced and polarized through IL-6 expression, Th17-cells may serve as a more precise biomarker. Determining Th17-cell count, IL-17A, IL-21, and IL-22 in BALF and serum, respectively, might be indispensable to gain further knowledge about their role in immune-related pneumonitis. At least, there is one study showing significant association of elevated levels of IL-17 in BALF and serum of patients with ICI-associated pneumonitis [21].

Owed to the broad inflammatory response of IL-6, there is little use for IL-6 as single diagnostic tool. However, as important element of pathogenesis in several diseases, antibodies against IL-6 might be used as therapeutic approach. Initially used for treatment of RA, the anti-IL-6R antibody Tocilizumab has since been successfully approved for therapy of systemic juvenile idiopathic arthritis and polyarticular juvenile idiopathic arthritis [36]. Due to the importance of IL-6 in immune response, Tocilizumab may have the potential to play a similarly important role in regulation thereof, as there are numerous preclinical and clinical studies investigating further example of application such as giant cell arteritis, polymyalgia rheumatica and large vessel vasculitis [36]. In light of the significantly increased IL-6 levels in our study and potential starting point for therapy, one study in particular showed promising results. Patients with steroid-refractory ICI-associated pneumonitis showed significant clinical improvement upon administration of Tocilizumab [37], underlining our findings and the potential relevance of IL-6 in ICI-associated pneumonitis.

There are several limitations to this study. First, a relatively small number of patients treated with ICI paired with low incidence of ICI-associated pneumonitis resulted in a small sample size. Consequently, general validity of results is limited. Second, albeit BAL is widely accepted as valid tool for assessing the cellular composition of the alveolar compartment and, thus, for contributing to diagnosis of various lung diseases [38], it presents constraints owed to dilution necessary to acquire BALF. Therefore, lower cytokine readings in BALF may go undetected, which could skew data. Lastly, while comparing healthy

individuals and patients with ICI-associated pneumonitis, further distinctions of ICI-associated pneumonitis to other pulmonary diseases remain to be demonstrated.

Conclusion

Cytokine profile assessed through BAL shows promising potential for facilitating diagnosis and understanding of the pathophysiology of ICI-associated pneumonitis. As such, IL-6 seems to play a relevant role, which may herald also therapeutic implications for the use of Tocilizumab. In addition, importance of Th17-cells in pathogenesis of ICI-associated pneumonitis appears worthy of future investigations. Further studies have to be conducted to determine indications of cytokine analysis in BALF in patients with suspected ICI-associated pneumonitis.

Abbreviations

AIP = acute interstitial pneumonia

APP = acute phase protein

ARDS = acute respiratory distress syndrome

BAL = bronchoalveolar lavage

BALF = bronchoalveolar lavage fluid

COPD = chronic obstructive pulmonary disease

CRP = C-reactive protein

CT = computed tomography

CTLA-4 = cytotoxic T-lymphocyte-associated antigen 4

HP = hypersensitivity pneumonitis

ICI = immune-checkpoint inhibitor

IFN-gamma = interferon gamma

IL = interleukin

ILD = interstitial lung disease

IQR = interquartile range

irAE = immune-related adverse events

mAB = monoclonal antibodies

NSIP = non-specific interstitial pneumonia

OP = organizing pneumonia

PD-1 = programmed cell death protein 1

PD-L1 = PD-1 ligand

RA = rheumatoid arthritis

SD = standard deviation

TNF-alpha = tumor necrosis factor alpha

UIP = usual interstitial pneumonia

Declarations

Ethics approval

This study followed guidelines and protocol of Swiss Ethics and was approved by the local ethic review committee (BASEC-ID 2017-02307 and 2018-01724).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests regarding this study.

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Authors’ contributions

Study design (BK, AV, US, PB, MEV, DF), data collection (BK, AV, DF, DS, SW, PB, MEV, AW), data analysis (BK, PB, MEV, AV), drafting of the manuscript (BK, DF), approval of the final version of the manuscript (all authors)

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