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Prognostic value of bronchoalveolar lavage fluid CD103+CD4+/CD4+ ratio in sarcoidosis

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Abstract

The CD103+ integrin is an adhesion molecule found on > 95% of intra-epithelial mucosal lymphocytes, but on <2% of circulating lymphocytes. Several studies have shown that the proportion of CD4+ lymphocytes expressing the CD103+ integrin (CD103+CD4+/CD4+ ratio) in bronchoalveolar lavage fluid (BALF) was low in sarcoidosis compared to other interstitial lung diseases, but we previously demonstrated that the difference was insufficient to allow a confident diagnosis of sarcoidosis without tissue biopsy. Whether BALF CD103+CD4+/CD4+ ratio could have prognostic value in sarcoidosis is unknown. To address this issue, we examined the associations between BALF CD103+CD4+/CD4+ ratio and clinical and imaging characteristics in a retrospective series of 115 patients with sarcoidosis. 63% were men, with a median age of 48 years. 47% had radiographic stage I, 49% had stage II, and 4% had stage III or IV. The median BALF lymphocyte count was 43% and the median CD103+CD4+/CD4+ ratio was 17%. A higher CD103+CD4+/CD4+ ratio was significantly associated with a higher radiographic stage at diagnosis ($p=0.017$) and last visit ($p<0.0001$), as well as the presence of fibrosis at imaging at diagnosis ($p=0.043$) and last visit ($p=0.032$). Besides an inverse correlation with forced vital capacity at diagnosis ($\beta=-0.16$, $p=0.031$), no consistent association was found between the BALF CD103+CD4+/CD4+ ratio at diagnosis and lung function parameters at diagnosis and last visit. We conclude that BALF CD103+CD4+/CD4+ ratio may have prognostic value in sarcoidosis.

Introduction

Sarcoidosis is a multisystemic disease, characterized by the formation of non-caseating granulomas in various organs [1], and involves the lungs in more than 90% of cases [2, 3]. The disease causes remain unknown, but genetic and environmental factors play a role [4-6]. The clinical presentation is heterogeneous, and symptoms are variable according to the affected organs. There are two main types of onsets in thoracic sarcoidosis: an acute onset associated with better prognostic and high rate of spontaneous remission, and a chronic onset characterized by more insidious organ involvement, less systemic symptoms and more relapses [3]. The overall mortality of sarcoidosis is 1-5%, most of the time due to pulmonary failure or secondary to cardiac or neurological involvement [1].

The diagnosis of thoracic sarcoidosis is based on a compatible clinical picture and chest imaging, the presence of non-caseating granulomas at tissue biopsy or cytology, and the exclusion of other diseases with similar histological or clinical presentation. Patients with pathognomonic Löfgren syndrome characterized by bilateral hilar lymphadenopathy, ankle arthritis and erythema nodosum do not need a histological confirmation [3, 7]. The bronchoalveolar lavage fluid (BALF) is characterized by lymphocytic alveolitis with CD4+ T-cell predominance [8, 9]. A CD4/CD8 ratio > 3.5 has been reported to have sensitivity of 94% for the diagnosis of sarcoidosis [10].

The CD103+ integrin is an adhesion molecule found on > 95% of intra-epithelial mucosal lymphocytes, but <2% of circulating lymphocytes [11, 12]. Previous studies have suggested that the CD103+CD4+/CD4+ ratio in BALF is lower in sarcoidosis compared to other interstitial lung diseases (ILD) [13-15], which was attributed to a redistribution of peripheral blood CD4 lymphocytes to the lungs [16]. It was further suggested that a CD103+CD4+/CD4+ ratio below 31% could allow to confidently diagnose pulmonary sarcoidosis in the absence of biopsy [15], but we previously demonstrated that this ratio is insufficient to accurately discriminate between sarcoidosis and other ILD [13]. However, in exploratory analyses, we found an inverse relationship between the CD103+CD4+/CD4+ ratio and forced vital capacity (FVC), total lung capacity (TLC) and the carbon monoxide diffusing capacity (DLCO) at last visit, suggesting that the CD103+CD4+/CD4+ ratio could have a prognostic value. Another study by Lohmeyer [17] also found that patients with stage 1 sarcoidosis had a lower CD103+CD4+/CD4+ ratio than patients with stages 2, 3, and 4.

The aim of this study was therefore to determine whether BALF CD103+CD4+/CD4+ ratio at diagnosis could have prognostic value in sarcoidosis.

Methods

Case recruitment and data collection

Results of BALF flow cytometry performed between January 2006 and October 2018 at the Institute of Pathology of the Lausanne university hospital were retrospectively reviewed. These analyses have been routinely performed since 2006 on all BALF samples having >20% lymphocytes on differential cell count, and on samples with <20% lymphocytes in patients with immunosuppression or hematological malignancies. Cases with mention of poor technical quality (< 10'000 events at flow cytometry) were discarded. For the remaining cases, health records were reviewed for diagnostic information. Cases without diagnostic information or with diagnoses other than sarcoidosis were removed, and only cases with a well-defined diagnosis of sarcoidosis were included. The diagnosis of sarcoidosis required compatible clinical and imaging features and the presence of non-caseating granulomas on tissue biopsy or transbronchial needle aspiration cytology of mediastinal lymph nodes. Patient with a pathognomonic Löfgren's syndrome in the absence of histological or cytological sample were also included. For cases with BALF flow cytometry performed at our institution but followed outside, we contacted the respiratory physician who performed the bronchoscopy to retrieve clinical information.

Collected data included demographic information, BALF cytology, radiographic stage at diagnosis and at last visit, the presence of parenchymal fibrosis at chest computed tomography at diagnosis and last visit, lung function at diagnosis and last visit, extrapulmonary organ involvement, the need for systemic treatment, and the clinical phenotype according to the 6 categories proposed by Prasse et al. [18].

Bronchoalveolar lavage fluid analysis and flow cytometry

The method of BALF analysis has been previously described [13]. In brief, BALF was collected in sterile bottles during regular diagnostic work-up and filtered through gauze. Ten milliliters of sample were cytocentrifuged, then smeared and stained with May-Grünwald, Giemsa, modified Papanicolaou, Prussian Blue, Gram-Weigert, and Ziehl-Neelsen as needed. For differential cell count, a sample corresponding to 250'000-300'000 cells was passed through a multipore filter. After staining with modified Papanicolaou, a minimum of 2x100 cells were counted. For flow cytometry, 10-30 ml of native sample were centrifuged, decanted and resuspended in NH4Cl or Roswell Park Memorial Institute medium several times until reaching a cellularity of 2 x 10^6. The cells were stained with fluorochrome-labelel monoclonal antibodies (Dako Denmark A/S, Glostrup, Denmark) for CD45 (FITC clone T29/33), CD4 (RPE, clone MT310), CD8 (FITC, clone DK25), and CD103 (FITC, clone Ber-ACT8)

following a standard protocol, and 5-color flow cytometric analysis was performed (Navios Beckman Coulter flow cytometer).

Statistical analyses

Continuous variables were expressed as mean, median, standard deviation (SD) and interquartile range (iqr), and categorical variables were described as percentage. Six explanatory variables (age at diagnosis, sex, radiographic stage at diagnosis, percentage of CD103+CD4+/CD4+, CD4/CD8 ratio, and percentage of BALF lymphocytes) were correlated with the following outcome variables, both at diagnosis and at last visit: forced expiratory volume in one second (FEV1), FVC, total lung capacity (TLC), residual volume (RV), DLCO, presence of fibrosis at chest high-resolution computed tomography (HRCT), and radiographic stage. Correlations were also made with the 6-category disease phenotype as proposed by Prasse et al. [18]. Robust regression and logistic regression were used for these analyses. Statistics were performed with Stata. Patient's consent was waived. The study was approved be the Ethics committee of canton Vaud (CER-VD 2017-01784).

Results

Study population

Case selection is summarized in Figure 1. Seven hundred and twenty-six BALF were analyzed by flow cytometry at the Institute of Pathology between 1.1.2006 and 30.10.2018. One hundred and sixty of them were of insufficient quality and were excluded. For the remaining 566 cases, electronic health records were reviewed for diagnostic information: 274 patients had a disorder other than sarcoidosis, and 104 had a diagnosis of sarcoidosis. However, 15 of them had neither histological confirmation nor Löfgren's syndrome and were excluded, leaving 89 cases of proven sarcoidosis. For 188 out of 566 patients, diagnostic information was not available in electronic health records as they had only BALF analysis performed at the Institute of Pathology but were followed outside the institution. Diagnostic information could be retrieved by contacting their physician in 78/188 (41%), and 26 of them had sarcoidosis with sufficient clinical data to allow inclusion, and were added to the 89 institutional cases. Thus, 115 patients constituted the study population.

Patient characteristics

Table 1 summarizes patient characteristics at diagnosis and at last visit. The median age at diagnosis was 48 years and 73/115 (63%) were men. The median CD103+CD4+/CD4+ ratio was 17%, the median

CD4/CD8 ratio was 4, and the median BALF lymphocyte count was 43%. At diagnosis, 47% had radiographic stage 1, 49% had stage 2, and 4% had stages 3 or 4. A last visit, 30% had normal chest X-ray, 37% had stage 1, and only 20% had stage 2, but the proportion of stages 3 or 4 increased to 13%. The percentage of patients with fibrosis at HRCT increased from 9% at diagnosis to 14% at last visit.

The most common phenotype according to Prasse, found in 44%, was phenotype 4 (subacute onset and no need for systemic treatment). The second most common phenotype, found in 22%, was phenotype 6 (subacute onset, and need for several courses of therapy or treatment lasting more than one year). Twenty-four percent had acute disease onset (phenotypes 1, 2 and 3) and 76% had subacute onset (phenotypes 4, 5 and 6). Fifty-five percent did not require corticosteroid therapy (phenotypes 1 and 4), whereas 14% required one period of treatment for no longer than one year (phenotypes 2 and 5), and 31% required several course of therapy or a treatment longer than one year (phenotypes 3 and 6).

Analysis of variables at diagnosis

The CD103+CD4+/CD4+ ratio was significantly associated with age (b=0.493, p=0.001), whereas the CD4/CD8 ratio, and BALF lymphocyte count were not (data not shown).

Table 2 shows the associations between explanatory variables and lung function at diagnosis. Age and sex were not associated with any lung function parameter, except for a positive correlation between age and residual volume at diagnosis (p=0.042). Higher CD103+CD4+/CD4+ ratio was only associated with lower FVC (p=0.031), but no association was found with other lung function parameters at diagnosis. The CD103+CD4+/CD4+ ratio was also positively correlated with the age at diagnosis (p=0.001). No significant association was found between the CD4/CD8 ratio and initial lung function. An inverse relationship was found between BALF lymphocytes and both FEV_1 (p=0.01) and DLCO (p=0.014). The most consistent associations were found between radiographic stage and lung function at diagnosis. Indeed, higher radiographic stages were significantly correlated with lower FEV1, lower FVC, lower TLC, and lower DLCO (table 2).

Relationships between explanatory variables and radiographic stage at diagnosis are shown in table 3. Higher CD103+CD4+/CD4+ ratio was associated with higher radiographic stage (p=0.017), whereas no such association was found for CD4/CD8 ratio, BALF lymphocytes count, age and sex.

Correlations between explanatory variables and presence of fibrosis at HRCT at diagnosis are shown in table 4. Presence of fibrosis was associated with higher CD103+CD4+/CD4+ ratio (p=0.043), whereas CD4/CD8 ratio, BALF lymphocyte count, age and sex did not correlate with the presence of fibrosis.

Radiographic stage at diagnosis was strongly associated with the presence of fibrosis at HRCT (table 4).

No association was found between the CD103+CD4+/CD4+ ratio and type of disease onset according to Prasse, either acute (phenotypes 1, 2 and 3) or subacute (phenotypes 4, 5 and 6) (table 5). Acute disease onset was associated with higher CD4/CD8 ratio (median 5.0 vs 3.5, p=0.014) and younger age (42.9 vs 50.0, p=0.007).

Analysis of variables at last visit

Table 6 shows the associations between explanatory variables at diagnosis and lung function at last visit. Men had significantly higher DLCO at last visit (p=0.012), and younger age at diagnosis was associated with higher DLCO (p=0.045) at last visit. The CD103+CD4+/CD4+ ratio at diagnosis was not associated with any lung function parameter at last visit. Lower BALF lymphocyte count at diagnosis was correlated with higher FEV1 at last visit (p=0.018) but there was no association with other lung function parameters. Initial radiographic stage was not significantly correlated with lung function at last visit.

Associations between explanatory variables at diagnosis and radiographic stage at last visit are shown in table 7. The CD103+CD4+/CD4+ ratio at diagnosis was strongly associated with the radiographic stage at last visit (p<0.0001). The CD4/CD8 ratio was negatively correlated with the radiographic stage (p=0.003).

Associations between explanatory variables at diagnosis and presence of fibrosis at last visit are shown on table 8. Higher CD103+CD4+/CD4+ ratio at diagnosis was associated with presence of fibrosis at last visit (p=0.032), whereas no such relationship was found for CD4/CD8 ratio, BALF lymphocyte count, age and sex. Higher radiographic stage at diagnosis was significantly associated with presence of fibrosis (table 8).

Discussion

The main finding of the present study is that, in sarcoidosis, the BALF CD103+CD4/CD4+ ratio at diagnosis is associated with radiographic stage and the presence of parenchymal fibrosis at chest imaging, both at diagnosis and at last visit. This suggests that this ratio has prognostic value in sarcoidosis, and might be a parameter of interest during the initial work-up of patients with this disease.

From our previous study [13], we expected to find a correlation between the values of the lung functions at the last visit and the CD103+CD4/CD4+ ratio in the initial BALF. This present study showed a deterioration in the lung function in patients with higher CD103+CD4+/CD4+ ratio but the inverse correlation was not significant. As the first study was done on a smaller amount of patients, it was necessary to verify this finding on a larger pool of patients. Another explanation could be the gap between the BALF and the last visit, since there was no fixed interval and some of the patients had lung functions ten years after the BALF. Moreover, no distinction was made between patients receiving treatment and the ones with spontaneous remission. According to Judson [19] the lung functions are improved with time and therapy, thus, it could be an explanation for the differences in the results. The FVC at diagnosis is the only lung function parameter significantly correlated with the CD103+CD4+/CD4+ ratio. This isolated correlation is hard to explain.

However, as expected from the study of Lohmeyer [17], a significant correlation was found between the CD103+CD4+/CD4+ ratio and the radiological stage, not only at diagnosis but also at the last visit –the correlation being even stronger at the last visit. This means that patients with a low CD103+CD4+/CD4+ ratio have a better radiological stage at diagnosis but also an improvement of the radiological impairment during the following years. As the radiological stage is known to be a prognostic marker [20], the CD103+CD4+/CD4+ ratio, being lower with lower radiological stage, should also have a prognostic value. This could also explain the disagreements found in the different studies about the diagnostic value of the CD103+CD4+/CD4+ ratio. Studies having a high proportion of patients with radiological stage I had a higher probability of finding lower CD103+CD4+/CD4+ ratio in patients with sarcoidosis and use it for a discriminative diagnosis purpose. For example, Heron [14] in his study had 49% of patient with sarcoidosis with a radiological stage I.

Several studies tried to understand the role of the CD103 integrin in inflammatory and pro-fibrotic processes and its link with TGF-beta, but the phenomenon is not yet fully understood. Rhis [21] showed that the presence of the TGF-beta, known to be implicated in fibrosis [22], increases the expression of the CD103 integrin, especially on CD8 lymphocytes. On CD4 lymphocytes, undetermined factors seem to play an additional role in the increase of CD103. The article written by Zissel [23] confirms the correlation between the level found of TGF-beta in the BALF and the induction of a spontaneous remission in patient with sarcoidosis. The level of TGF-beta seems also to increase after the beginning of corticosteroid therapy. Both speaking for a positive effect of the TGF-beta and in contradiction with the fibrotic effect previously described. Zissel offers different explanations for these differences : the level of TGF-beta found in the BALF do not reflect the active level of the TGF-beta in the lung, the TGF-beta impact depend on the fibroblast, the TGF-beta may be necessary for

the induction of fibrosis but not sufficient – which joins the observation of Rhis described above about the necessity of additional mechanisms to induce the CD103 on CD4 lymphocytes.

As for the CD103 integrin itself, several studies explored the pro-inflammatory role of the CD103+CD4+/CD4+ ratio in BALF and its link with the presence of fibrosis in lung diseases, and observed a higher ratio in patients with fibrotic lung diseases as compared to other lung diseases [17, 21]. These findings are consistent with our present study, which shows a significantly higher CD103+CD4+/CD4+ ratio in patients with signs of fibrosis at chest imaging. Therefore, our study confirms a link between the BALF CD103+CD4+/CD4+ ratio at diagnosis and occurrence of fibrosis in pulmonary sarcoidosis, and suggests that this ratio has a prognostic value.

Our study also confirms the prognostic value of the radiographic stage at diagnosis, which is now well established in the literature [20, 24, 25]. Indeed, patients with radiographic stages II and III-IV at diagnosis had significantly higher risk of fibrosis at last visit than patients with stage I.

Unsurprisingly, lung function parameters at diagnosis were lower in patients with radiographic stage II or III-IV compared to stage I, except for RV (table 2). One possible explanation for the lack of effect on RV could be air trapping, which was found to be common in sarcoidosis [26] and could counteract the restrictive effect of lung parenchyma infiltration by sarcoidosis on RV. Higher initial radiographic stages at diagnosis tended to result in lower lung function parameters at last visit, but the differences did not reach statistical significance, which could be attributed to the effect of therapy.

The prognostic role of the CD4/CD8 ratio has been much debated in the literature [27-29]. In our study, the ratio is correlated to the radiological stage at the last visit. The association between an high ratio and an improvement of the radiological stage was previously observed by Verstraeten [28]. The correlation was no longer significant when patients with radiological stage III were excluded from the analysis. This exclusion was not done in our study. What emerges from different studies is the observation of a high CD4/CD8 ratio in patients with Löfgren's syndrome. This is explained by the activities of the disease. When the disease is acutely active the CD4/CD8 ratio is higher. This observation is confirmed in our study, indeed, patients with an acute onset had a significant higher CD4/CD8 ratio than patient with a subacute onset. In the literature, the ratio seems also to have a link with the response to corticosteroid therapy, patient with a high ratio have a better response to treatment [27]. This was not analyzed in our study.

The last analytic variable in the BALF was the percentage of lymphocytes. The role of the lymphocytosis as prognostic factor is debated and the percentage seems to depend on the interval of time between the onset of the disease and the BALF [27, 30, 31]. In our study we found a correlation

between the percentage of lymphocytosis and the FEV_1 at diagnosis and at the last visit as well as a diminution of the DLCO but we did not search in our data a link between this percentage and a spontaneous remission, which could be interesting for further analyze.

The age is surprisingly correlated with lung functions, which is hard to explain given that the lung functions are expressed in percentage of the value expected for the age. An older age correlates also with a subacute onset of the disease. Older patients have a more insidious disease and the impairments of the lung function become bigger.

Conclusion

This present study suggests that the CD103+CD4+/CD4+ ratio has prognostic value in sarcoidosis. It would be interesting to do additional analyses to search a correlation between the ratio and the need of therapy. It might make sense to treat patients with a high ratio in order to limit fibrosis but more analyzes need to be done.

Limitation

The interpretations of the findings at the last visit need to be analyzed with caution, as explained above the interval is different from one patient to another. It would have made more sense to predefine a time interval between the analyses at diagnosis and the comparison values.

The study showing a correlation between the age and the CD103+CD4+/CD4+ ratio, more analyzes are necessary to confirm that the findings are linked to the ratio itself and not influenced by age.

The last interesting point is the conclusion made by Ward [31] to explain the contradictory results found in the diverse studies on BALF : the results are influenced by the different lavage protocols, genetics and racial characteristics of the patients, the onset of the symptoms and the clinical presentation. To assert our finding, the analyze should be done in subgroups with theses specific criteria.

Figure 1

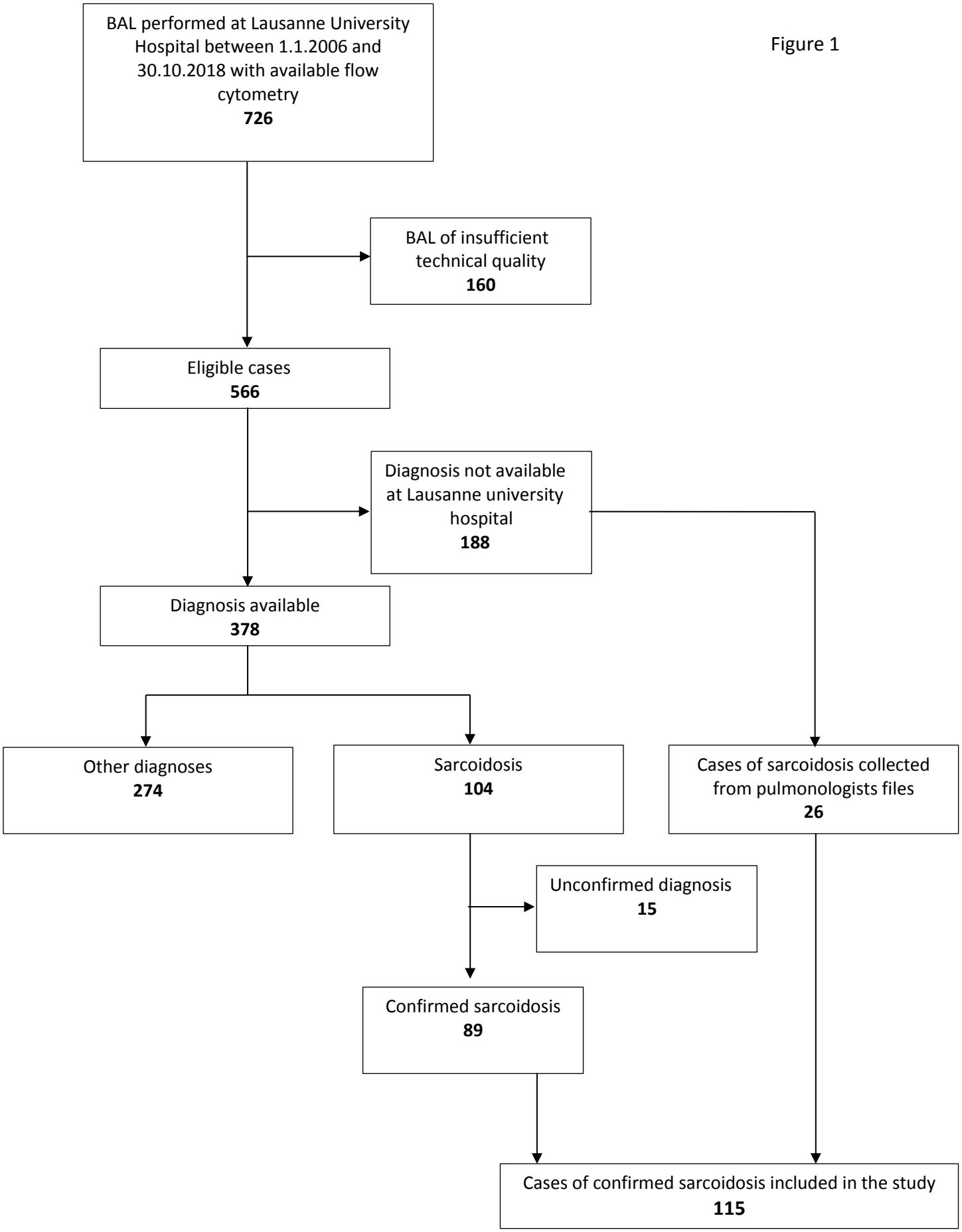
BAL performed at Lausanne University Hospital between 1.1.2006 and 30.10.2018 with available flow cytometry
726
BAL of insufficient technical quality
160
Eligible cases
566
Diagnosis not available at Lausanne university hospital
188
Diagnosis available
378
Other diagnoses
274
Sarcoidosis
104
Cases of sarcoidosis collected from pulmonologists files
26
Unconfirmed diagnosis
15
Confirmed sarcoidosis
89
Cases of confirmed sarcoidosis included in the study
115

Table 1. Patient characteristics at diagnosis and last visit										
	At diagnosis					At last visit				
	n	mean	SD	median	n (%)	n	mean	SD	median	n (%)
age	115	48	12	48						
men	115				73 (63)					
FEV_1, % predicted	102	91	16	94		84	91	18	94	
FVC, % predicted	101	98	17	100		84	96	18	97	
TLC, % predicted	94	91	16	90		77	92	14	92	
RV, % predicted	93	82	34	78		76	86	24	82	
DLCO, % predicted	96	82	18	84		78	85	22	89	
Radiological stage:										
normal CXR	114				0 (0)	91				27 (30)
I	114				53 (47)	91				34 (37)
II	114				56 (49)	91				18 (20)
III-IV	114				5 (4)	91				12 (13)
%CD103/CD4	115	24	21	17						
CD4/CD8 ratio	115	5	5	4						
% BAL lymphocytes	115	43	18	43						
Fibrosis at imaging	115				10 (9)	92				13 (14)
Phenotype*										
1						110				12 (11)
2						110				4 (4)
3						110				10 (9)
4						110				49 (44)
5						110				11 (10)
6						110				24 (22)
*according to Prasse. CXR: chest X-ray										

Table 2. Associations between explanatory variables at diagnosis and initial lung function*

Variable at diagnosis	FEV_1 at diagnosis		FVC at diagnosis		TLC at diagnosis		RV at diagnosis		DLCO at diagnosis	
	b	p	b	p	b	p	b	p	b	p
male sex	-3.33	0.307	-4.37	0.198	-5.60	0.080	0.89	0.901	6.35	0.114
age	0.017	0.895	0.155	0.255	0.098	0.434	0.57	**0.042**	-0.22	0.143
BAL CD103/CD4, %	-0.097	0.185	-0.16	**0.031**	-0.109	0.122	0.025	0.874	-0.05	0.566
BAL CD4/CD8 ratio	-0.013	0.971	0.20	0.593	0.136	0.698	-0.183	0.817	-0.65	0.141
BAL lymphocytes, %	-0.208	**0.010**	-0.14	0.109	-0.145	0.084	-0.121	0.527	-0.26	**0.015**
radiographic stage:										
I	reference		reference		reference		reference		reference	
II	-8.66	**0.006**	-7.28	**0.033**	-7.18	**0.025**	-1.87	0.79	-14.06	**<0.0001**
III-IV	-18.18	**0.013**	-14.9	0.056	-17.97	**0.022**	-19.43	0.28	-21.78	**0.018**
*robust regression										

Table 3. Associations between explanatory variables and radiographic stage at diagnosis				
	stage I (n=53)	stage II (n=56)	OR (95% CI)	p*
%CD103/CD4, median (iqr)	11 (17)	25 (29)	1.025 (1.004-1.046)	**0.017**
CD4/CD8, median (iqr)	4.0 (5.2)	3.6 (3.5)	0.932 (0.854-1.018)	0.12
% of lymphocytes, median (iqr)	41 (27)	45 (33)	1.013 (0.992-1.034)	0.213
age, median (iqr)	45.2 (20.1)	50.6 (16.9)	1.007 (0.976-1.039)	0.622
male sex, n (%)	34 (64)	34 (61)	0.863 (0.397-1.877)	0.711
*logistic regression				

Table 4. Associations between explanatory variables and fibrosis at diagnosis				
	fibrosis absent (n=105)	fibrosis present (n=10)	OR (95% CI)	p*
%CD103/CD4, median (iqr)	15 (27)	42 (40)	1.028 (1.001-1.056)	**0.04**
CD4/CD8, median (iqr)	4.0 (4.3)	2.9 (4.1)	0.887 (0.711-1.105)	0.29
% of lymphocytes, median (iqr)	43 (29)	38 (20)	0.995 (0.960-1.032)	0.8
age, median (iqr)	45.7 (17.4)	53.8 (10.1)	1.033 (0.978-1.090)	0.235
male sex, n (%)	65 (62)	8 (80)	2.461 (0.497-12.177)	0.269
Radiographic stage:				
stage I	53 (51)	0 (0)	reference	
stage II	50 (48)	6 (60)	0.03 (0.0028-0.31)	**0.003**
stage III-IV	1 (1)	4 (40)	**	**
*logistic regression. **radiographic stage I predicts perfectly the outcome				

Table 5. Associations between explanatory variables and disease onset				
	acute onset[a] (n=26)	subacute onset[b] (n=84)	OR (95% CI)	p*
%CD103/CD4, median (iqr)	11 (23)	20 (29)	1.018 (0.994-1.044)	0.14
CD4/CD8, median (iqr)	5.0 (6.1)	3.5 (4.1)	0.895 (0.820-0.978)	**0.014**
% lymphocytes, median (iqr)	44 (30)	41 (29)	0.999 (0.976-1.023)	0.984
age, median (iqr)	42.9 (13.6)	50.0 (17.3)	1.059 (1.015-1.105)	**0.007**
mlae sex, n (%)	15 (58)	55 (66)	1.390 (0.566-3.416)	0.472
radiographic stage at diagnosis:				
I	14 (54)	38 (46)	reference	
II	12 (46)	41 (49)	1.258 (0.517-3.060)	0.612
III-IV	0 (0)	4 (5)	**	**

[a]phenotypes 1, 2, and 3 according to Prasse. [b]phenotypes 4, 5, and 6 according to Prasse.
*logistic regression. **radiographic stage III-IV predicts perfectly the outcome

Table 6. Associations between explanatory variables at diagnosis and lung function at last visit*

Variable at diagnosis	FEV_1 last visit		FVC last visit		TLC last visit		RV last visit		DLCO last visit	
	b	p	b	p	b	p	b	p	b	p
male sex	-2.19	0.538	-3.03	0.44	-0.64	0.855	7.85	0.225	13.29	**0.012**
age	-0.25	0.062	-0.25	0.080	-0.19	0.139	0.21	0.382	-0.43	**0.045**
BAL CD103/CD4, %	-0.05	0.542	-0.14	0.099	-0.09	0.213	0.08	0.553	-0.07	0.550
BAL CD4/CD8 ratio	0.04	0.912	0.47	0.23	0.19	0.579	-0.031	0.961	-0.76	0.149
BAL lymphocytes, %	-0.21	**0.018**	-0.10	0.304	-0.11	0.247	0.048	0.775	-0.24	0.102
radiographic stage at diagnosis:										
I	reference		reference		reference		reference		reference	
II	-3.33	0.29	-4.13	0.27	-1.59	0.64	5.90	0.33	-8.42	0.09
III-IV	5.37	0.60	-10.26	0.31	-11.02	0.21	-6.84	0.66	-15.25	0.23

*robust regression

Table 7. Associations between explanatory variables and radiographic stage at last visit						
	normal CXR (n=27)	stage I (n=34)	stage II (n=18)	stage III-IV (n=12)	OR (95% CI)	p*
%CD103/CD4, median (iqr)	10 (26)	18 (31)	31 (28)	45 (32)	1.033 (1.014-1.053)	**<0.0001**
CD4/CD8, median (iqr)	5.6 (6.3)	3.5 (4.1)	3.2 (3.5)	2.6 (3.9)	0.856 (0.772-0.950)	**0.003**
% of lymphocytes, median (iqr)	44 (39)	44 (24)	48 (20)	32 (15)	0.994 (0.974-1.015)	0.592
age, median (iqr)	45.2 (21.3)	45.5 (25.7)	48.9 (17.2)	53.8 (6.4)	1.005 (0.975-1.035)	0.736
male sex, n (%)	14 (52)	24 (71)	12 (67)	9 (75)	1.812 (0.814-4.031)	0.142
*ordinal logistic regression. CXR: chest X-ray						

Table 8. Associations between explanatory variables and fibrosis at last visit

		fibrosis absent (n=79)	fibrosis present (n=13)	OR (95% CI)	p*
%CD103/CD4, median (iqr)		17 (30)	34 (46)	1.028 (1.002-1.055)	**0.032**
CD4/CD8, median (iqr)		4.0 (4.0)	2.3 (3.7)	0.859 (0.689-1.071)	0.18
% of lymphocytes, median (iqr)		45 (31)	36 (16)	0.977 (0.943-1.012)	0.19
age, median (iqr)		45.7 (17.3)	53.1 (8.8)	1.020 (0.973-1.070)	0.40
male sex, n (%)		50 (63)	10 (77)	1.933 (0.491-7.600)	0.34
radiographic stage at diagnosis					
	I	40 (52)	1 (8)	reference	
	II	37 (47)	8 (61)	8.648 (1.031-72.514)	**0.047**
	III-IV	1 (1)	4 (31)	160 (8.322-3076)	**0.001**

*logistic regression

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