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Clinical differences in sarcoidosis patients with and without lymphoma: a single-center retrospective cohort analysis

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Abstract:	

Dear Prof. Martin Kolb, Editor in Chief
Dear Prof. James D. Chalmers, Deputy Editor
Dear Prof. Joachim Muller-Quernheim, Associate Editor
European Respiratory Journal

Thank you very much indeed for giving us the opportunity to submit a revised version of our manuscript “Clinical differences in sarcoidosis patients with and without lymphoma: a single-center retrospective cohort analysis” (Manuscript ID ERJ-02470-2018). We have addressed all the points very reasonably raised by the Reviewers and amended our manuscript accordingly. We believe this has increased considerably the quality of our article.
Please find below a point-by-point reply to the Reviewers and Associate Editor comments, as requested.
We hope our revised manuscript will be considered for publication in the *European Respiratory Journal* in its present form
Sincerely yours

Fabrizio Luppi (for the Authors)

Associate Editor

AE.C1

ACE measurement is not standardized. Cut-off values vary from laboratory to laboratory. Please check whether your lab kept the system constant over the observation time.

AE.R1

We thank the Associate Editor for this insightful and reasonable comment. All of the angiotensin-converting enzyme (ACE) assays have been performed in the laboratory of our University Hospital, which provides certified and standardized analysis and is a referral center for the entire county (i.e., approximately 1 million people population). Specifically, the methodology of ACE measurement has remained the same throughout the study period, and certified internal as well as external quality control has been regularly performed over time. Therefore, we confirm that ACE measurements have been performed using a consistent methodology and have provided highly reliable values.

AE.C2

You mention a correlation between BAL cytology CD4/CD8 ratio and lymphoma. This of practical interest since BAL is done quite frequently in Europe. Please give some more details.

AE.R2

We agree with the Associate Editor that the correlation between BAL CD4/CD8 and lymphoma, if confirmed, could potentially be important and clinically relevant. However, in our study, BAL was performed in only 5 out of 10 patients affected by sarcoidosis and lymphoma. Although an increased CD4/CD8 ratio was observed in the BAL fluid of all 5 patients, we decided not to include these data due to the limited number of patients and the possibility that these may represent spurious results. We hope the Associate Editor agrees with this decision.

Reviewer 1

R1.C1

In the methods, the authors state that comparisons were performed with the two-samples Student´s T test, assuming equal variance for all the variables tested in the 2 groups. Variance should be tested, and a non-parametric test should be used when appropriate. Furthermore, the authors compare also categorical variables, and other tests should be used to compare these variables in the 2 groups. Please clarify and adjust the analysis as requested

R1.R1

We thank the Reviewer for his/her comment. T test was used in variables assumed to be normally distributed and for which the assumption of equal variances was not rejected. This has now been clarified. Please, see text for details.

R1.C2

The authors state that the occurrence of lymphoma is usually considered more frequent among cases of chronic sarcoidosis; however, 50% of sarcoidosis-lymphoma in their cohort presented with a stage I, and no patients with sarcoidosis-lymphoma experienced a relapse of sarcoidosis. These facts raise 2 concerns: 1) the sequence of the events: the authors do not differentiate cases presenting first with lymphoma and subsequently developing sarcoidosis and vice versa; please provide this information; 2) which where the diagnostic criteria to diagnose sarcoidosis and lymphoma in these cases? Did the

patients undergo EBUS or surgical biopsies of the nodes to verify both the diseases? Was the diagnosis of sarcoidosis based on BAL and clinical-radiological features alone? Was the diagnosis of lymphoma based on biopsies from organs other than the lung/mediastinal nodes?

R1.R2

This is a crucial point and we thank the Reviewer for raising it. As he/she points out, most cases of lymphoma tend to occur in chronic sarcoidosis, which was not the case in our study population.
With regard to the two specific points raised:

1. Five out of ten patients affected by sarcoidosis subsequently developed lymphoma. Conversely, in the remaining five cases, lymphoma preceded sarcoidosis.
2. In 183 patients enrolled in our study, the diagnosis of sarcoidosis was based on a compatible clinical radiological picture, histological evidence of noncaseating granulomatous inflammation and after careful exclusion of other diseases that may present with a similar clinical, radiological, or histological features. In the remaining 26 cases, the diagnosis was based on a compatible clinical and/or radiological picture, as occurred in patients presenting with Lofgren's syndrome, BAL cell count and CD4/CD8 ratio >3.5 and/or nuclear medicine data, after careful exclusion of alternative diagnoses. The diagnosis of lymphoma was always confirmed histologically, performing skin (two patients), bone marrow (four patients) and lymph node biopsy in various sites, such as abdomen (one patient), latero-cervical area (two patients) and mediastinoscopy (one patient).

R1.C3

Length of the follow-up and survival: the authors state that there was no difference in survival between the 2 groups; on the other hand, they report only data on the follow-up time at their centre; was the survival obtained from a death registry or the comparison is performed using the follow-up time? Please clarify.

R1.R3

We thank the Reviewer for this comment. The death/survival status of all patients included in the study was obtained from a death registry that is linked to a software utilized in our University Hospital for collecting clinical information.

Reviewer 2

Major comments

R2.C1

It will be useful to have more detailed information about the SA diagnosis (how many patients performed a BAL/biopsy?), the kind of lymphoma, clinical onset of SA and LY.

R2.R1

We thank the Reviewer for this comment, which mirrors one of Reviewer's 1 comments. As already clarified, in 183 patients enrolled in the study, the diagnosis of sarcoidosis was formulated based on a compatible clinical and/or radiological picture, histological evidence of noncaseating granulomas and exclusion of other diseases able to present with a similar histological or clinical picture. In the remaining 26 patients, the diagnosis was based on a compatible clinical and/or radiological picture, as is the case in patients affected by Lofgren's syndrome, on BAL cellular count together with an

increased CD4/CD8 ratio and/or nuclear medicine investigations, as well as on the exclusion of other diseases able to present with a similar histological or clinical picture.
Regarding the type of lymphoma (10 cases) observed in our series, they were as follows:

- Two diffuse large B-cell lymphomas
- Four follicular lymphomas
- One Hodgkin lymphoma
- One Burkitt's lymphoma
- One lymphoplasmacytic lymphoma (also known as Waldenstrom's disease)
- One anaplastic large cell lymphoma

Regarding the clinical onset of sarcoidosis, 31 patients were diagnosed incidentally, 105 patients were affected by respiratory symptoms, mainly dry cough and dyspnea either at rest or on exertion, 42 patients were diagnosed because of systemic symptoms, including fever, malaise, fatigue, weight loss and sweats, while 31 patients showed extrapulmonary manifestations, including abdominal pain, cutaneous lesions, neurological symptoms and ear, nose and throat manifestations.

Regarding the clinical onset of lymphoma, the presentation was the following:

- Diffuse large B-cell lymphomas (n=2): in one patient, during follow-up for his sarcoidosis, enlarged mediastinal lymph nodes were detected during steroid treatment. The patient underwent mediastinoscopy that allowed the diagnosis of lymphoma to be made; in the second patient the diagnosis of lymphoma was made following the occurrence of thrombocytopenia and fever.

- Follicular lymphomas (n=4): one patient developed a thoracic consolidation, subsequently diagnosed as lymphoma; two patients developed enlarged lymph-nodes (latero-cervical and inguinal). In the fourth patient, lymphoma presented as subcutaneous mass.

- Hodgkin lymphoma (n=1): the patient complained of systemic symptoms, such as fever, malaise and weight loss.

- Burkitt's lymphoma (n=1): the patient developed a submandibular mass.

- Lymphoplasmacytic lymphoma (also known as Waldenstrom disease) (n=1): the diagnosis was made following the occurrence of monoclonal gammopathy (IgM)

- Anaplastic large cell lymphoma (n=1): the patient developed severe leukopenia and sepsis.

R2.C2

The X-ray staging is referred to the time of diagnosis of SA or at the time of LY diagnosis? This point is unclear and appears to be crucial for this study. Accepting that the lung staging was made at the time of SA diagnosis, it is possible to describe a trend of less severe presentation in SA-LY (but not with statistical significance). For this reason it could be useful to know the staging at the time of LY onset. It can result similar, considering the longer FU for SA-LY group. Or as an alternative, the lung involvement can be less severe. In this latter case, the LY treatment could have stopped/slowed down the lung damage. Please, clarify and discuss about this.

R2.R2

We thank the Reviewer for his/her comment. In our study, the X-ray stage was collected at the time of the diagnosis of sarcoidosis. Regarding the radiographic staging at the time of lymphoma diagnosis (5 cases) - with the exception of one patient whose X-ray stage worsened – in all other cases the radiographic stage at the time of the diagnosis of lymphoma was the same as that observed at the time of sarcoidosis diagnosis. Therefore, we are not in a position to speculate on the potential association between improved/worsened radiographic stage of disease and development of lymphoma.

R2.C3

The value of ACE: Regarding the first determination of ACE levels, these were evaluated at the time of the diagnosis of SA, but how many patients in the SA-LY group had already both the disease together at the diagnosis? Regarding the second determination, it was reported an unclear "last follow up" with a mean difference of about 3 years in the FU between the two groups. So, the second determination should be corrected for the disease duration. Furthermore, have you tried to perform a ROC curve for ACE to describe a reasonable cut off in terms of sensitivity, specificity and accuracy between the two groups? Have you available the ACE levels temporally close to the diagnosis of LY?

R2.R3

We thank the Referee for these comments. Five patients (corresponding to 50% of those affected by both sarcoidosis and lymphoma) developed the two diseases simultaneously.

In our opinion, the statistically significant increase in serum ACE levels in patients with both sarcoidosis and lymphoma suggests that serum ACE might potentially represent a clinical marker at the time of the diagnosis. We agree with the Reviewer that the meaning of the measurement performed "at the last follow up" is less clear, particularly given the lack of serum ACE data temporally close to the diagnosis of lymphoma. Yet, persistently elevated serum ACE levels at the second measurement, performed in the same laboratory in both groups at the time of the diagnosis and at the last follow-up, support our argument that serum ACE levels may represent a risk factor for the development of lymphoma in patients with sarcoidosis, whereas it does not seem to predict response to treatment in patients with lymphoma.

R2.C4

Considering the retrospective nature of the work and the large amount of data (mainly regarding the organ involvement), a clear quantification of the missing data is needed

R2.R4

As suggested, please find below a detailed list of missing data in our study:

Smoking habit: 173/209 (82%): missing data 18%

Serum ACE data were available for 161/209 patients (77%): missing data 23%

Lung function tests were available for 157/209 patients (75%): missing data 25%

Radiological imaging data were available for 193/209 patients (93%): missing data 7%

Clinical symptoms were known for 152/209 patients (73%): missing data 27%

Pathology data for sarcoidosis diagnosis were available for 183/209 patients (88% - missing data 12%), while pathology data were available for 10/10 patients with lymphoma (100%): no missing data

R2.C5

Page 5 line 14: please, check reference. You wrote about ACE as a biological marker of disease progression during follow up citing ref 12 (Miyoshi s et al Chest 2010): in this work ACE NOT correlated with BALF regarding total cells, lymphocytes, CD4+ or CD4/CD8 ratio, no difference between SA with and without parenchymal infiltration regarding ACE, AUC of only 0.61. In reference 13 the macrophages rather than CD4+ cells are supposed as the cell source of ACE. Furthermore, ACE levels seem to be related with an acute onset of the disease rather than a chronic involvement (10.12659/PJR.897708).

R2.R5

The Reviewer is correct. The references he/she is alluding to are misplaced here or at least they do not support the statements they refer to. Accordingly, we have removed them from the reference list.

R2.C6

Page 5 line 15: If the principal sources of ACE are the lung endothelium and the macrophages, I have some difficult to explain why LY alone should have less serum ACE level than the control group (ref 15 you cited), SA alone higher levels than the control group (ref 13 you cited) and your SA-LY group higher level respect to SA alone. Lymphocytic activation during LY is reduced? In this case, why SA-LY have higher levels than SA? This point should be discussed.

R2.R6

We thank the Reviewer for this comment. The mechanisms by which SA-LY patients have higher levels of ACE can only be speculated upon. One possibility is that the degree of lymphocytic activation of sarcoidosis might differ from the aberrant lymphocytic proliferation that occurs in lymphomas. In sarcoidosis, granuloma formation appears to be driven by alveolar macrophages that recruit Th-lymphocytes thus triggering a vicious circle in which lymphocytes promote granuloma formation and maintenance through the production of cytokines and stimulation and recruitment of macrophages and B-cells. In other words, macrophages, that are the main source of ACE, at the same time stimulate lymphocytes and are stimulated by the lymphocytes themselves. In this scenario, ACE levels could reflect an exaggerated stimulation and activity of lymphocytes. On the other hand, the reduced T-reg activity found in sarcoidosis but not in lymphoma might contribute to their neoplastic transformation in patients in whom lymphoma occurs after sarcoidosis. More studies are needed to clarify the association, if any, between serum ACE levels and lymphoma development.

Minor comments

Page 3 line 7: it can be useful to produce a definition of disease activity for SA.

Thank you. We have now clarified that in sarcoidosis, the term “disease activity” refers to the fact that the disease is still active and may undergo clinical, radiological or functional change as a consequence of the persistence of the inciting antigen, which remains unknown.

Page 3 lines 27-28: p=0.344 is very far from a statistical significance. No gender differences in SA-LY syndrome.

We thank the Reviewer for this comment. While traditionally a preponderance of female patients has been reported in sarcoidosis, more recent data suggest this may not be necessarily the case. In our study, sarcoidosis was more frequent among females whereas combined sarcoidosis and

lymphoma was more common in males, although this difference only mildly trended towards statistical significance. The small number of individuals in our study, however, does not allow us to draw firm conclusions.

Page 4 line 3: please, define "relapse" in sarcoidosis

We thank again the Reviewer for the comment. According to the definition given by Baughman et al. (Eur Respir J 2014), which we favor, the term relapse (or exacerbation) of sarcoidosis refers to: 1) Significant increase in systemic medication; 2) Worsening of chest imaging; 3) Worsening of pulmonary function status; 4) Worsening of dyspnea. The presence of any one or more of these features is considered progression of the disease.

Page 4 lines 12-14: No statistical difference between the severity of lung involvement between the two groups, a trend of significance in FVC liters but not in FVC% predicted. Please, reformulate and discuss why in your opinion only FVC liters differ from the two group but not FVC% predicted

We agree with the Reviewer that these inconsistent data may look a bit odd. However, we believe the differences between absolute FVC expressed in liters and FVC % predicted may be accounted for by the retrospective nature of our study, wherein different spirometers were used over a period of time of about 20 years.

Page 5 line 18: a higher CD4/CD8 ratio in SA-LY cohort appears to be a very interesting data. Why it was not reported?

Thank you. As already clarified in our response to the AE, we did not report these data because BAL was performed only in 5 out of 10 patients with both sarcoidosis and lymphoma. However, we are ready to reconsider this decision if the Referee believes these data may provide meaningful information.

Clinical differences in sarcoidosis patients with and without lymphoma: a single-center retrospective cohort analysis

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Word count: 11652~~1~~ (excluding references)

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Sarcoidosis is a systemic disease of unknown origin, characterized by the presence of non-caseating granulomas at disease sites[1]. A relevant clinical problem in the management of this disease is the co-existence of other clinical conditions, such as solid tumors or lymphomas, that may occur before or following the diagnosis of sarcoidosis as well as simultaneously[2]. Particularly, the association of sarcoidosis and lymphoma is well established and was named the "sarcoidosis-lymphoma syndrome" by Brincker and colleagues in 1986[3]. In this syndrome, lymphoma occurs mainly in patients with a chronic active form of sarcoidosis, suggesting that chronic disease could be a risk factor for lymphoma. However, the distinctive clinical features of patients with sarcoidosis and lymphoma, and the precise mechanism underlying this association ~~are still~~remain unclear.

We retrospectively reviewed the database of the "Center for Rare Lung Diseases" at the University Hospital of Modena to identify all subjects with a diagnosis of sarcoidosis between 1990 and 2013, with the aim to evaluate clinical, functional and serological differences related to the presence of lymphoma in sarcoidosis patients, as well as differences in survival.

We recorded the following clinical data~~set~~: gender, age, radiographic (i.e., Scadding) disease staging, organ involvement and treatment of both conditions, stage of lymphoma, sarcoidosis and lymphoma relapses, pulmonary function tests (PFTs), serology and haematology data as well as serum angiotensin-converting enzyme (ACE) levels at the time of diagnosis. These parameters were compared between ~~the two groups of~~ patients with and~~or~~ without lymphoma (~~see in~~ Table 1).
We retrieved 209 sarcoidosis patients and found 10 cases (4,8%) with a previous or subsequent diagnosis of lymphoproliferative disorder and a mean follow-up of 6.7 years and 9.5 years, respectively.

Differences between groups were tested with a two-sample Student's T test in continuous variables normally distributed with equal variances. The Chi-squared test was used to compare the distribution of categorical variables between the two groups, and the Fisher exact test when appropriate. Survival was estimated using the Kaplan-Meier method and compared between groups with the log-rank test.

~~Differences between groups were tested with a two-sample Student's T test assuming equal variance between groups, whereas group survival was estimated using the Kaplan-Meier method with the log-rank test.~~

There was no difference in patients' median age between the sarcoidosis and the sarcoidosis-lymphoma syndrome group (48.7 *vs* 46 years, p=0.578); the majority of subjects within

the sarcoidosis group were females; in contrast, a slightly, not significant male predominance was observed in the sarcoidosis-lymphoma syndrome group (p=0.344).

Most of the patients in the two groups were non-smokers (60.7% and 85.7% in the sarcoidosis and sarcoidosis-lymphoma groups, respectively). A difference was found in terms of chest X-ray staging, specifically a ~~lower prevalence of respiratory involvement~~milder disease extent/severity in patients with lymphoma. In fact, stage II was the most common stage in the sarcoidosis group, whilst stage I was more frequently ~~represented~~ observed in the sarcoidosis-lymphoma ~~syndrome~~group. PFTs trended towards worsening in the sarcoidosis group, wherein functional abnormalities were more likely to be present, although this difference did not reach statistical significance ~~a pulmonary involvement was markedly more likely~~ (FVC: 3,3 vs 4,2 liters, p=0.052). In addition, 36 relapses (18,9%) of sarcoidosis were reported in the sarcoidosis group, while no relapse was observed in the sarcoidosis-lymphoma group.

We also detected a statistically significant difference in ACE serum levels between groups. Indeed, in the sarcoidosis-lymphoma syndrome group, serum ACE level was significantly higher compared to patients without lymphoma, both at the time of the diagnosis of sarcoidosis (94.9 UI/L *vs* 55.8 UI/L, p = 0.02) and at the last measurement available (83.3 UI/L *vs* 50.7 UI/L, p = 0.047).

~~Finally~~However, survival did not differ between the two groups (log-rank test p=0.3724).

In the present study, we investigated whether serological, clinical, functional or radiological features may help differentiate patients with sarcoidosis from those with sarcoidosis-lymphoma syndrome. We showed that lung involvement as assessed by chest X-ray (Scadding radiographic stage II), ~~and~~ a restrictive ventilatory defect and a higher rate of relapse were more common~~ly associated with~~among patients with sarcoidosis alone. ~~(radiological stage II), also showing a markedly higher rate of disease relapse.~~ Furthermore, and perhaps more ~~importantly~~interestingly, serum ACE levels were~~as~~ higher in patients with sarcoidosis-lymphoma syndrome both at the time of the diagnosis and at the last follow-up measurement available, indicating that ~~those~~ patients with persistently elevated serum ACE levels should probably be carefully monitored over longer periods, despite the similar outcomes in term of mortality observed ~~between~~ in the two groups.

Previous epidemiological studies showed an increased incidence of various types of cancers in patients with sarcoidosis[4]~~. Although~~ , but this association does not seem to be cancer-specific. However, breast and testicular tumors are more frequently described in association with sarcoidosis[5], and may occur either before, concurrently or after onset of sarcoidosis[2,6]. I~~Furthermore, i~~n patients with either hematological malignancies or solid tumors, ~~who do not fulfill~~

~~the criteria for systemic sarcoidosis,~~ granuloma~~tous~~ inflammation ~~may be found as a "sarcoid reaction", that~~ is a ~~usually~~ frequent finding~~observed~~ mainly in the local lymph nodes draining the cancer site[7]. However, these "sarcoid-like reactions" have limited clinical relevance and should not be regarded as sarcoidosis.

Coexistence of sarcoidosis and lymphoma ~~has been reported previously~~is well known. Indeed, patients with sarcoidosis are up to 11 times more likely to develop lymphoma as compared to the general population[8]. Specifically, an increased risk of Hodgkin lymphoma was observed in patients with sarcoidosis in a population-based case-control study in Scandinavia[4]. In the majority of cases, lymphoma occurred after sarcoidosis, usually within a short time interval ~~of time~~[9]. P~~The evidence also suggests that p~~atients with sarcoidosis-lymphoma syndrome ~~are generally~~tend to be significantly older than unselected individuals with sarcoidosis[10]. In contrast, in our study, no differences were observed in the median age of patients with or without lymphoma. Different genetic background or environmental exposures may account for this ~~controversial~~ inconsistent finding[1].

Similar~~ly~~ to our study, *Blank and colleagues*[11] performed a retrospective study analyzing the incidence and ~~the~~ type of malignancies in a large cohort of patients with sarcoidosis, showing a similar rate of lymphoproliferative disorders (4.1% *vs* 4.8 % in our study), thus confirming the generalizability of our findings.

In our study, a number of limitations should also be acknowledged, t~~T~~he main ~~limitations of~~being the ~~present study is the~~ small ~~sample~~ number of patients with sarcoidosis-lymphoma syndrome ~~included~~, although, reassuringly, a comparable ~~rate~~ prevalence of sarcoidosis-lymphoma syndrome was found in previous similar studies~~observations of patients with sarcoidosis~~[11]. Moreover, the retrospective design is prone to incomplete or missing data. Finally, the lack of a control population without sarcoidosis followed ~~for~~ over the same period of time precludes us from identifying sarcoidosis as a risk factor for malignancy. These limitations n

~~N~~otwithstanding, our data suggest that in patients with sarcoidosis persistently elevated serum ACE levels ~~could be important in evaluating the~~ should raise the suspicion of supervening ~~risk to develop~~ lymphoma ~~in patients with sarcoidosis~~. ~~Indeed,~~ ACE is generally ~~even more~~ used ~~as a biological marker of disease progression~~to monitor disease behaviour during follow-up[1,~~12,13~~]. Thus, we ~~might~~ argue ~~speculate~~ that serum ACE levels may reflect the intensity of the lymphocytic activation occurring in patients with sarcoidosis, and that an exuberant and uncontrolled lymphocytic activation, perhaps not counterbalanced by T-regulatory cell activity[12], may trigger

predispose to an increased risk forthe development of lymphoma development. In support of our hypothesis, eEvidence suggests that serum ACE is lower in malignant patients with lymphoma alone[135]. As a side observation, we iInterestingly, we also observed that the CD4/CD8 lymphocyte ratio in bronco-alveolar lavage was higher among patients with sarcoidosis-lymphoma syndrome (data not shown), further supporting the hypothesis of an aberrant lymphocytic activation (T-helper) in patients with both conditions.

In conclusion, our study suggests the existence of clinical, radiological and serological differences in sarcoidosis with or without lymphoma syndrome. The knowledge of these differences seems important for a timely diagnosis and treatment. However, furtherLarger prospective studies are required to confirm present and expand on these observations.

We acknowledge that this research was partially supported by the Italian Ministry of University and Research (MIUR) - Department of Excellence project PREMIA (PREcision MedIcine Approach: bringing biomarker research to clinic)

Table 1. Demographic and clinical characteristics of the study population

	Sarcoidosis	**Sarcoidosis + Lymphoma**	**P**
Patients, n	199	10	
Age, years	48.7 (± 14.8)	46 (± 12)	0.578
Gender			
Male	89 (44.7)	6 (60)	0.344
Female	110 (55.3)	4 (40)	
Smokers:			
No	105 (60.7)	6 (85.7)	
Ex	26 (15)	0 (0)	0.630
C	42 (24.3)	1 (14.3)	
FVC, litres	3.3 (± 1,1)	4.2 (± 0,8)	0.052
FVC, % predicted	96.2 (± 18.0)	100.1 (± 19,2)	0.583
FEV1, litres	2.7 (± 0,9)	3.3 (± 0,6)	0.92
FEV1, % predicted	94.4 (± 19,1)	97.1 (± 18.0)	0.720
DLCO, ml min kPa	6.2 (± 2,6)	7.4 (± 1,8)	0.234
DLCO, % predicted	73.9 (± 23,8)	75.3 (± 15,3)	0.881
Chest X-rays stage (%)			
Stage 0	15 (7.5)	0 (0)	
Stage 1	59 (29.7)	5 (50)	
Stage II	89 (44.7)	3 (30)	0.671
Stage III	30 (15.1)	2 (20)	
Stage IV	6 (3.0)	0 (0)	
ACE (U/L)			
At diagnosis	55.1 (± 36,7)	94.9 (± 43,9)	0.02
First relapse	86.0 (± 56,4)	-	N/A
Last follow up	50.7 (± 41,7)	83.3 (± 42,5)	0.04
Relapses	36 (18.9%)	0 (0%)	N/A
Organ involvement			
Mediastinum	159 (79.9)	8 (80)	0.994
Lungs	137 (68.8)	7 (70)	0.939
Skin	53 (26.6)	4 (40)	0.354
Lymph-nodes	34 (17.6)	3 (30)	0.321
Eyes	12 (6.0)	1 (10)	0.612
Spleen	12 (6.0)	1 (10)	0.612
Liver	17 (8.5)	0 (0)	0.335
Salivary glands	5 (2.5)	0 (0)	0.999
Nervous system	7 (3.5)	0 (0)	0.999
Hypercalcemia	1 (0.5)	0 (0)	0.999
Bones	2 (1.0)	1 (10)	0.137
ORL	1 (0.5)	0 (0)	0.999
Gastrointestinal	2 (1.0)	0 (0)	0.999
Lacrimal glands	1 (0.5)	1 (10)	0.094
Bone marrow	2 (1.0)	0 (0)	0.999
Heart	1 (0.5)	0 (0)	0.999
Testis	1 (0.5)	0 (0)	0.999

Continuous variables are expressed as mean±SD if normally distributed, as median (min, max) if non-normally distributed; categorical variables are expressed as absolute numbers and percentages
N/A = not applicable

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Clinical differences in sarcoidosis patients with and without lymphoma: a single-center retrospective cohort analysis

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Sarcoidosis is a systemic disease of unknown origin, characterized by the presence of non-caseating granulomas at disease sites[1]. A relevant clinical problem in the management of this disease is the co-existence of other clinical conditions, such as solid tumors or lymphomas, that may occur before or following the diagnosis of sarcoidosis as well as simultaneously[2]. Particularly, the association of sarcoidosis and lymphoma is well established and was named the "sarcoidosis-lymphoma syndrome" by Brincker and colleagues in 1986[3]. In this syndrome, lymphoma occurs mainly in patients with a chronic active form of sarcoidosis, suggesting that chronic disease could be a risk factor for lymphoma. However, the distinctive clinical features of patients with sarcoidosis and lymphoma, and the precise mechanism underlying this association remain unclear.

We retrospectively reviewed the database of the "Center for Rare Lung Diseases" at the University Hospital of Modena to identify all subjects with a diagnosis of sarcoidosis between 1990 and 2013, with the aim to evaluate clinical, functional and serological differences related to the presence of lymphoma in sarcoidosis patients, as well as differences in survival.

We recorded the following clinical data: gender, age, radiographic (i.e., Scadding) disease staging, organ involvement and treatment of both conditions, stage of lymphoma, sarcoidosis and lymphoma relapses, pulmonary function tests (PFTs), serology and haematology data as well as serum angiotensin-converting enzyme (ACE) levels at the time of diagnosis. These parameters were compared between patients with and without lymphoma (Table 1).

We retrieved 209 sarcoidosis patients and found 10 cases (4,8%) with a previous or subsequent diagnosis of lymphoproliferative disorder and a mean follow-up of 6.7 years and 9.5 years, respectively.

Differences between groups were tested with a two-sample Student's T test in continuous variables normally distributed with equal variances. The Chi-squared test was used to compare the distribution of categorical variables between the two groups, and the Fisher exact test when appropriate. Survival was estimated using the Kaplan-Meier method and compared between groups with the log-rank test.

There was no difference in patients' median age between the sarcoidosis and the sarcoidosis-lymphoma syndrome group (48.7 *vs* 46 years, p=0.578); the majority of subjects within the sarcoidosis group were females; in contrast, a slightly, not significant male predominance was observed in the sarcoidosis-lymphoma syndrome group (p=0.344).

Most of the patients in the two groups were non-smokers (60.7% and 85.7% in the sarcoidosis and sarcoidosis-lymphoma groups, respectively). A difference was found in terms of

chest X-ray staging, specifically a milder disease extent/severity in patients with lymphoma. In fact, stage II was the most common stage in the sarcoidosis group, whilst stage I was more frequently observed in the sarcoidosis-lymphoma group. PFTs trended towards worsening in the sarcoidosis group, wherein functional abnormalities were more likely to be present, although this difference did not reach statistical significance (FVC: 3,3 vs 4,2 liters, p=0.052). In addition, 36 relapses (18,9%) of sarcoidosis were reported in the sarcoidosis group, while no relapse was observed in the sarcoidosis-lymphoma group.

We also detected a statistically significant difference in ACE serum levels between groups. Indeed, in the sarcoidosis-lymphoma syndrome group, serum ACE level was significantly higher compared to patients without lymphoma, both at the time of the diagnosis of sarcoidosis (94.9 UI/L *vs* 55.8 UI/L, p = 0.02) and at the last measurement available (83.3 UI/L *vs* 50.7 UI/L, p = 0.047).

However, survival did not differ between the two groups (log-rank test p=0.3724).

In the present study, we investigated whether serological, clinical, functional or radiological features may help differentiate patients with sarcoidosis from those with sarcoidosis-lymphoma syndrome. We showed that lung involvement as assessed by chest X-ray (Scadding radiographic stage II), a restrictive ventilatory defect and a higher rate of relapse were more common among patients with sarcoidosis alone. Furthermore, and perhaps more interestingly, serum ACE levels were higher in patients with sarcoidosis-lymphoma syndrome both at the time of the diagnosis and at the last follow-up measurement available, indicating that patients with persistently elevated serum ACE levels should probably be carefully monitored over longer periods, despite the similar outcomes in term of mortality observed in the two groups.

Previous epidemiological studies showed an increased incidence of various types of cancers in patients with sarcoidosis[4], but this association does not seem to be cancer-specific. However, breast and testicular tumors are more frequently described in association with sarcoidosis[5], and may occur either before, concurrently or after onset of sarcoidosis[2,6]. In patients with either hematological malignancies or solid tumors, granulomatous inflammation is a frequent finding mainly in the local lymph nodes draining the cancer site[7]. However, these “sarcoid-like reactions” have limited clinical relevance and should not be regarded as sarcoidosis.

Coexistence of sarcoidosis and lymphoma is well known. Indeed, patients with sarcoidosis are up to 11 times more likely to develop lymphoma as compared to the general population[8]. Specifically, an increased risk of Hodgkin lymphoma was observed in patients with sarcoidosis in a population-based case-control study in Scandinavia[4]. In the majority of cases, lymphoma occurred

after sarcoidosis, usually within a short time interval[9]. Patients with sarcoidosis-lymphoma syndrome tend to be significantly older than unselected individuals with sarcoidosis[10]. In contrast, in our study, no differences were observed in the median age of patients with or without lymphoma. Different genetic background or environmental exposures may account for this inconsistent finding[1]. Similar to our study, *Blank and colleagues*[11] performed a retrospective study analyzing the incidence and type of malignancies in a large cohort of patients with sarcoidosis, showing a similar rate of lymphoproliferative disorders (4.1% *vs* 4.8 % in our study), thus confirming the generalizability of our findings.

In our study, a number of limitations should also be acknowledged, the main being the small number of patients with sarcoidosis-lymphoma syndrome, although, reassuringly, a comparable prevalence of sarcoidosis-lymphoma syndrome was found in previous similar studies[11]. Moreover, the retrospective design is prone to incomplete or missing data. Finally, the lack of a control population without sarcoidosis followed over the same period of time precludes us from identifying sarcoidosis as a risk factor for malignancy. These limitations notwithstanding, our data suggest that in patients with sarcoidosis persistently elevated serum ACE levels should raise the suspicion of supervening lymphoma. ACE is generally used to monitor disease behaviour during follow-up[1]. Thus, we argue that serum ACE levels may reflect the intensity of the lymphocytic activation occurring in patients with sarcoidosis, and that an exuberant and uncontrolled lymphocytic activation, perhaps not counterbalanced by T-regulatory cell activity[12], may trigger the development of lymphoma. In support of our hypothesis, evidence suggests that serum ACE is lower in patients with lymphoma alone[13]. Interestingly, we also observed that the CD4/CD8 lymphocyte ratio in bronco-alveolar lavage was higher among patients with sarcoidosis-lymphoma syndrome (data not shown), further supporting the hypothesis of an aberrant lymphocytic activation (T-helper) in patients with both conditions.

In conclusion, our study suggests the existence of clinical, radiological and serological differences in sarcoidosis with or without lymphoma syndrome. Larger prospective studies are required to confirm and expand on these observations.

We acknowledge that this research was partially supported by the Italian Ministry of University and Research (MIUR) - Department of Excellence project PREMIA (PREcision MedIcine Approach: bringing biomarker research to clinic)

Table 1. Demographic and clinical characteristics of the study population

	Sarcoidosis	**Sarcoidosis + Lymphoma**	**P**
Patients, n	199	10	
Age, years	48.7 (± 14.8)	46 (± 12)	0.578
Gender			
Male	89 (44.7)	6 (60)	0.344
Female	110 (55.3)	4 (40)	
Smokers:			
No	105 (60.7)	6 (85.7)	
Ex	26 (15)	0 (0)	0.630
C	42 (24.3)	1 (14.3)	
FVC, litres	3.3 (± 1,1)	4.2 (± 0,8)	0.052
FVC, % predicted	96.2 (± 18.0)	100.1 (± 19,2)	0.583
FEV1, litres	2.7 (± 0,9)	3.3 (± 0,6)	0.92
FEV1, % predicted	94.4 (± 19,1)	97.1 (± 18.0)	0.720
DLCO, ml min kPa	6.2 (± 2,6)	7.4 (± 1,8)	0.234
DLCO, % predicted	73.9 (± 23,8)	75.3 (± 15,3)	0.881
Chest X-rays stage (%)			
Stage 0	15 (7.5)	0 (0)	
Stage 1	59 (29.7)	5 (50)	
Stage II	89 (44.7)	3 (30)	0.671
Stage III	30 (15.1)	2 (20)	
Stage IV	6 (3.0)	0 (0)	
ACE (U/L)			
At diagnosis	55.1 (± 36,7)	94.9 (± 43,9)	0.02
First relapse	86.0 (± 56,4)	-	N/A
Last follow up	50.7 (± 41,7)	83.3 (± 42,5)	0.04
Relapses	36 (18.9%)	0 (0%)	N/A
Organ involvement			
Mediastinum	159 (79.9)	8 (80)	0.994
Lungs	137 (68.8)	7 (70)	0.939
Skin	53 (26.6)	4 (40)	0.354
Lymph-nodes	34 (17.6)	3 (30)	0.321
Eyes	12 (6.0)	1 (10)	0.612
Spleen	12 (6.0)	1 (10)	0.612
Liver	17 (8.5)	0 (0)	0.335
Salivary glands	5 (2.5)	0 (0)	0.999
Nervous system	7 (3.5)	0 (0)	0.999
Hypercalcemia	1 (0.5)	0 (0)	0.999
Bones	2 (1.0)	1 (10)	0.137
ORL	1 (0.5)	0 (0)	0.999
Gastrointestinal	2 (1.0)	0 (0)	0.999
Lacrimal glands	1 (0.5)	1 (10)	0.094
Bone marrow	2 (1.0)	0 (0)	0.999
Heart	1 (0.5)	0 (0)	0.999
Testis	1 (0.5)	0 (0)	0.999

Continuous variables are expressed as mean±SD if normally distributed, as median (min, max) if non-normally distributed; categorical variables are expressed as absolute numbers and percentages
N/A = not applicable

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Checklist of items that should be included in reports of cohort studies

Title and abstract

a) Indicate the study's design a commonly used term in the title or the abstract: the study design is included in the title

b) Provide in the abstract an informative and balanced summary of what was done and what was found: abstract is not required for "research letters"

Introduction

Background/rationale: Explain the scientific background and rationale for the investigation being reported: see introduction of the manuscript (from row 1 to raw 7)

Objectives: State specific objectives, including any pre-specified hypotheses (from row 8 to row 9)

Methods

Study design: present key elements of study design early in the paper (see row 10)

Setting: describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (from row 11 to row 13)

Participants

(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (from row 14 to row 18)

(b) For matched studies, give matching criteria and number of exposed and unexposed (not applicable)

Variables. Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (from row 14 to row 18)

Data sources/measurement. For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (from row 14 to row 18)

Bias. Describe any efforts to address potential sources of bias: because of the limited number of words of a "research letter", we described sources of bias in the discussion

Study size. Explain how the study size was arrived at: this is a retrospective study, therefore we predefined a time interval considering all the patients with sarcoidosis, with and without lymphoma, referred to our Center for Rare Lung Diseases during the previously established period of time

Quantitative variables. Explain how quantitative variables were handled in the analyses. If applicable describe which groupings were chosen and why: 2 statisticians co-authored this "research letter". They therefore decided the most appropriate statistics to apply to this study

Statistical methods

(a) Describe all statistical methods, including those used to control for confounding

(b) Describe any methods used to examine subgroups and interactions

(c) Explain how missing data were addressed

(d) If applicable, explain how loss to follow-up was addressed

(e) Describe any sensitivity analyses

2 statisticians co-authored this “research letter”. They therefore decided the most appropriate statistics to apply to this study

Results

Participants

a) Report numbers of individuals at each stage of study - eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (from row 19 to row 21)

b) Give reasons for non-participation at each stage (because of the retrospective design of the study, we included all patients with a diagnosis of sarcoidosis with or without lymphoma)

c) Consider use of a flow diagram: a flow diagram was not included

Descriptive data

a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (from row 15 to row 18)

b) Indicate number of participants with missing data for each variable of interest (because of the retrospective design of the study, we included only patients with a diagnosis of sarcoidosis with or without lymphoma)

c) Summarise follow-up time (eg, average and total amount)

Outcome data. Report numbers of outcome events or summary measures over time (from row 29 to row 41)

Main results

a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg. 95% confidence interval). Make clear which confounders were adjusted for and why they were included (from row 19 to 42)

b) Report category boundaries when continuous variables were categorized: N/A

c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period: N/A

Other analyses. Report other analyses done - eg analyses of subgroups and interactions, and sensitivity analyses: N/A

Discussion

Key results. Summarise key results with reference to study objectives (from row 41 to row 48)

Limitations. Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (from row 64 to row 85)

Interpretation. Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (from row 68 to row 70)

Generalisability. Discuss the generalisability (external validity) of the study results (from row 68 to row 70)

Other information

Funding. Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based. (the present study has no funding sources).

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U.O. Clinica Pneumologica
Direttore: Prof Alberto Pesci

Modena, 24th December 2018

Dear Editor, Dear Prof. Martin Kolb
European Respiratory Journal

The association between sarcoidosis and lymphoma is well established. Indeed, patients with sarcoidosis are up to 11 times more likely to develop lymphoma as compared to the general population. However, the distinctive clinical features of patients with sarcoidosis and lymphoma, and the mechanism underlying this association are still unclear.

Including 209 patients, in this manuscript, that we would like to propose as "research letter", we retrospectively reviewed differences in sarcoidosis patients with and without lymphoma, showing clinical, radiological and serological differences, including lung function and chest X ray presentation. Furthermore, serum angiotensin-converting enzyme (ACE) levels resulted significantly higher in patients with sarcoidosis-lymphoma syndrome both at the time of the diagnosis and at the last follow-up measurement available, indicating that those patients with persistently elevated serum ACE levels should be carefully monitored over longer periods, despite the similar outcomes in term of mortality between the two groups.

To our knowledge, this is the first observation showing the existence of clinical, radiological and serological differences in sarcoidosis with or without lymphoma syndrome.

The material included in this manuscript is original, is not being considered for publication elsewhere, including publicly accessible websites or e-print servers, no part of the research presented has been funded by tobacco industry sources, and all authors have read the manuscript and approve its submission. Finally, the Authors do not have any competing financial interest.

We hope you will consider our manuscript for publication in the Journal.

Thank you very much indeed for your interest in our work.

Looking forward to hearing from you.

Yours sincerely,

Fabrizio Luppi (for the Authors)