ISSN 1124-0490 Volume 37 N. 3 - 2020
http://www.sarcoidosis.it

SARCOIDOSIS

VASCULITIS AND DIFFUSE LUNG DISEASES

OFFICIAL JOURNAL OF WASOG

Mattioli 1885 for: ITS – AIPO - SIMER

SARCOIDOSIS

VASCULITIS AND DIFFUSE LUNG DISEASES

FORMERLY "SARCOIDOSIS" (UP TO 1995) FOUNDED 1984 BY GIANFRANCO RIZZATO

PROPERTY AND COPYRIGHT

ITS – AIPO - Associazione Italiana Pneumologi Ospedalieri
Via Antonio da Recanate, 2
20124 Milano
Tel. +39 02 36590367
Fax +39 02 67382337
www.aiponet.it
aiposegreteria@aiporicerche.it

Società Italiana Di Pneumologia / Italian Respiratory Society (SIP/IRS)
Via San Gregorio, 12
20124 – Milano – Italy
Tel. +39 0249453331
Fax + 39 0287036090
mail segreteria@sipirs.it
web www.sipirs.it

PUBLISHER
Mattioli 1885 - Strada di Lodesana 649/sx, Loc. Vaio - 43036 Fidenza (Parma)
Tel. +39 0524 530383 - Fax +39 0524 82537 - www.mattiolihealth.com - edit@mattioli1885.com

Bibliographic Indices:
This journal is regularly listed in bibliographic services, including Current Contents/Clinical Medicine, the Science Citation Index, Sci Search, Research Alert and EMBASE/Excerpta Medica, PubMed/Medline, PubMed Central (Priority Journals)

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (3); e2020001 DOI: 10.36141/svdld.v37i3.9362

Methotrexate in sarcoidosis: hematologic and hepatic toxicity encountered in a large cohort over a six year period

Robert P. Baughman[1], Johanna P. Cremers[2], Martina Harmon[1], Elyse E. Lower[1], Marjolein Drent[2,3,4]

[1] University of Cincinnati Medical Center, Cincinnati, OH, USA; [2] ild care foundation research team, Ede, the Netherlands; [3] ILD Center of Excellence, Department of Respiratory Medicine, St. Antonius Hospital, Nieuwegein, The Netherlands; [4] Department of Pharmacology and Toxicology, FHML, University Maastricht, the Netherlands

Abstract. *Background:* Methotrexate (MTX) is a second line agent for treatment of sarcoidosis. Its long term safety and efficacy in sarcoidosis remains unclear. *Methods*: This was a retrospective review of patients seen at the University of Cincinnati Sarcoidosis Clinic over a six year period. For each visit, complete blood count, liver function testing, and dosing and outcome of MTX was noted. For efficacy, we compared the outcome of therapy of a matching subgroup of patients treated with either MTX or infliximab for one year and results scored as improved, stable, or worse based on response of the target organ. *Results:* Over six years, 1606 sarcoidosis patients were seen with a total of 13,576 clinical visits. During the study period, 607 patients (38% of total) were receiving MTX and had available blood work. Moderate elevation of alanine aminotransferase (ALT) (>3 times upper limit normal) was seen in nine (1.6%) patients. White blood count of <1500 cells per cu mm was seen in one patient. At six months, over half of the 44 patients initiated on infliximab and with at least six months of follow-up were better, while only 23% of the 44 of a matched subset of MTX treated patients were better (Chi square=10.566, p=0.0143). At the 12 month assessment, the infliximab treated patients were still more likely to be better than those treated with MTX (Chi square=10.033, p=0.0183). Only 23% of those treated with MTX were worse at twelve months. *Conclusion:* In our study, MTX therapy was associated with very few hepatic or hematologic complications. MTX was less likely than infliximab to improve clinical status. However, only 20% were worse after one year of MTX. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (3): e2020001)*

Key words: sarcoidosis, methtorexate, liver function tests, leukopenia, infliximab

Introduction

Methotrexate (MTX) has been used to treat chronic sarcoidosis for many years (1-3). It has been reported as effective in treating pulmonary,(2;4) ocular,(5) and neurologic disease.(6) In a double blind, placebo controlled trial, it was superior to placebo as a steroid sparing agent.(7) In two surveys, over 90 percent of sarcoidosis specialists considered MTX the drug of choice in patients who had developed intolerance to prednisone.(8;9)

However, a multi-national survey of sarcoidosis specialists revealed that 41% of responding physicians used MTX on five or less of their patients in the previous year, with 10% of the total responders not having used MTX in the past year.(8) For those not using MTX, fear of toxicity was the most commonly cited reason. In addition, some clinicians were unsure of the effectiveness of MTX in treating sarcoidosis.

Received: 2 February 2020
Accepted after revision: 24 June 2020
Correspondence: Robert P. Baughman MD,
200 Albert Sabin Way, Room 1001,
Cincinnati, OH 45267-0565
E-mail: bob.baughman@uc.edu

The major toxicities of MTX beside gastrointestinal side effects, mainly nausea, are leukopenia and hepatotoxicity (10). Guidelines have been developed to minimize these toxicities in rheumatoid arthritis(11), and similar guidelines have been proposed for sarcoidosis patients.(8) We were interested in the frequency of major hematologic and liver toxicity in our sarcoidosis patients.

There are several agents available to treat advanced sarcoidosis.(12) In addition to MTX, there are other immunosuppressants such as azathioprine,(4) leflunomide,(13;14) and mycophenolate mofetil (15). The tumor necrosis factor (TNF) inhibitors such as infliximab have been reported effective agents for advanced sarcoidosis.(16;17)

The aims of this study were to evaluate the real life safety of MTX in patients with advanced sarcoidosis and to compare the effectiveness of MTX treatment with infliximab.

Methods

We performed a retrospective review of patients seen at the University of Cincinnati Sarcoidosis Clinic over a six year period. Each clinic visit was recorded in a data base (ACCESS, Microsoft) with additional clinical and laboratory information obtained. Patients prescribed MTX alone or in combination with other agents were identified. We also recorded the age, gender, and self-reported race of all patients. Organ involvement was defined using standard criteria.(18) Other data collected included complete blood count, white blood count, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, MTX dosage, concurrent prednisone and/or cytotoxic drug use, and presence of known sarcoid liver disease (defined as positive liver biopsy or >3 times upper limit of normal (ULN) liver tests prior to treatment). The protocol has been approved by the University of Cincinnati Institutional Review Board.

Our standard practice is to institute treatment with MTX at an initial dose of 10 mg orally once a week unless the patient's baseline white blood cell count is <4000 cells/cu mm. In leukopenic patients the dose was reduced. Likewise, patients with an elevated serum creatinine would have does adjusted, and those patients with a serum creatinine >2.0 mg would not receive MTX. Complete blood counts along with liver and renal function are assessed every three months while on therapy. For patients whose ALT or AST rose to greater than three times the ULN, the MTX was discontinued. Patients who had MTX discontinued were treated with alternative agents, such as azathioprine. We did not rechallenge patients with MTX.

Toxicity

The study focused on two major side effects of MTX, leukopenia and hepatoxicity. Serial testing including complete blood count (CBCs) and liver function tests (LFTs) were scheduled to be performed every three months. All available testing was recorded at the time and subsequently available for review.

In patients who developed severe leukopenia, defined as a total WBC <1.5*10^3 cells per cu mm, MTX was discontinued. Abnormal liver function testing was defined as an alanine aminotransferase (ALT) >1.5x upper limit of normal (ULN), and an ALT >3x ULN required MTX discontinuation.

Response Assessment

Assessment was performed in those patients who instituted new treatment with a minimum of six months of follow up. To compare the efficacy of MTX to infliximab, a clinical response tool was developed. Known sarcoidosis patients with target organ involvement of lung, skin, eye, or liver were included, and patients were classified according to their response to therapy over the six months after initiation of therapy.

We identified 44 patients who initiated infliximab during the study period and had sufficient follow-up data at six months. We compared these patients to a matched group of MTX treated patients based on age, race, and organ involvement who had also begun therapy during the study period and there was at least six months of follow-up data. None of the MTX group were on infliximab at the time of the analysis of response to therapy. The infliximab patients were on MTX and/or other immunosuppressants including prednisone and azathioprine. However, all patients had progressive disease at the time of starting infliximab.

The clinical response tool captured patient data including drug therapy and dosage at the initial, 6-month, and 12-month interval, affected organs, FVC% predicted, and the evaluator's overall global assessment. For the global assessment, the physician's clinical assessment along with changes in FVC% or steroid-sparing drugs were considered and rated on a Likert scale of 1 to 5 (1=much worse, 2=worse, 3=same, 4=better, 5=much better).

The target organ was that manifestation which was identified as the reason for treatment. Response of the target organ to therapy was determined using predefined criteria for individual organ assessment as well as for the entire patient. Criteria for response are listed below:

Pulmonary

Improvement in the target organ was defined as an increase in at least one of the lung function test parameters by ≥10% of the predicted value or reduction of inflammation in chest imaging (based on official interpretation of chest imaging). Stable was defined as no clinically significant change (increase or reduction ≤10%) in lung function or chest imaging with reduction in steroid dosage. Patients were determined to be worse if there was ≥10% decline in lung function, worsening of chest imaging, or steroid dose could not be tapered. (7, 19, 32)

Ocular

Improvement was considered present when at least one of the inflammatory signs of eye show complete clearance or improvement in one without deterioration in others. Stabilized is defined as all inflammatory signs of eye remain unchanged, and deterioration as an increase in at least one inflammatory sign. Improvement, stabilization, or worsening of disease activity was assessed by the physician based upon increase or decrease in topical steroids such as eye drops, periocular injections given within the past two months and/or patient-reported changes in visual acuity. (7,19;20)

Hepatic

Appropriate laboratory test results were assessed by the physician, especially alkaline phosphatase and bilirubin, before and after therapy. Improvement was defined when elevated tests decreased by 50% of the upper limit of normal. Stabilization included levels which remained unchanged despite reduction in prednisone dosage. Worsened disease included an increase in abnormal LFTs or an increase in prednisone dosage. (14, 16)

Cutaneous

Change from baseline skin lesions were considered improved if lesions were reduced by greater than 50%, stable if there was no clinically significant change, and worse for increase ≥10%. Comparisons of lesions were reported by the same physician. (14, 32)

Assessment of target organ response was based on physician's comments in the patient record. We developed this assessment tool specifically for this study and it was applied to all patients. This assessment was performed by an independent reviewer (MH) who evaluated all patients. This reviewer was not involved in patient care.

Statistics

Results are reported as mean with standard deviations. Comparisons were made between groups using Student t test with a p value of <0.05 being considered significant. Multi regression analysis was performed by incorporating any feature in the model which had a p value of <0.10 on univariate analysis.

Results

Toxicity

During the time period, a total of 1606 sarcoidosis patients were seen with a total of 13,576 clinical visits. Figure 1 demonstrates the number of patients evaluable, including the number who received MTX at any time (869 [54% of total]). During the study period, 607 patients (38% of total) were receiving drugs and had available blood work. These 607 patients constituted the study group. The demographic characteristics and major organ for the group involvement are summarized in Table 1. Granulomatous liver involvement was confirmed by liver biopsy in 48 patients (7.9%); whereas, 11 patients had granulomas identified in their bone marrow.

Table 2 demonstrates the clinical features of those with abnormal liver function test abnormalities

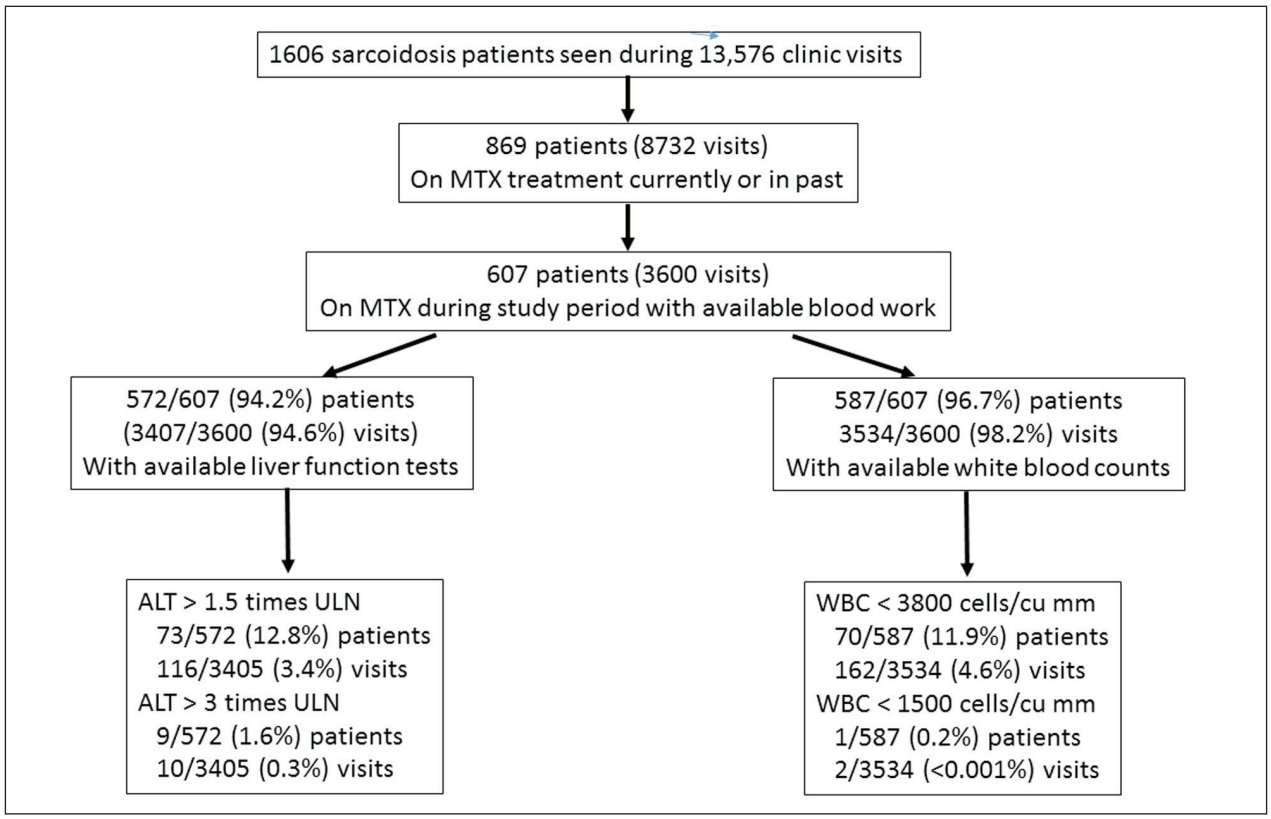

Fig. 1. A flow diagram of all visits seen during the study time period. MTX: methotrexate; ALT: alanine aminotransferase; ULN: upper limit normal; WBC: white blood count

Table 1. Clinical features of the studied methotrexate treated sarcoidosis patients

	Number	Percent of total
Total	607	
Female	446	73.5%
Caucasian *	345	56.8%
Organ		
Lung	491	80.9%
Eyes	264	43.5%
Skin	234	38.6%
CNS	96	15.8%
Liver	48	7.9%
Extra thoracic lymph nodes	67	11.0%
Spleen	30	4.9%
Cardiac	28	4.6%
Sinus	41	6.8%
Bone marrow †	11	1.8%

*Remaining patients are African American except one from India.
†Positive granulomas in bone marrow

(LFTA) versus the remainder of the treated patients. In multiple regression analysis only sex and hepatic sarcoidosis were independent predictors of LFTA. Elevated LFTs were reported within three months of MTX institution in more than half of patients with LFTA. However less 2% of patient had LFTA greater than 3 times upper limit normal and only 1 patient had an ALT or AST greater than 5 times upper limit normal. .

Table 3 depicts the clinical features of those patients with leukopenia versus the remainder of the MTX treated patients. In multiple regression analysis only race but not MTX dose was an independent predictor of leukopenia. None of the patients had infections associated with their leukopenia, including the one patient with a WBC of less than 1500 cells/cu mm.

All nine patients with ALT greater than three times the upper limit of normal had MTX withdrawn and follow-up LFT testing was normal. Patients with leukopenia had their dose of MTX adjusted and follow up testing was stable.

Table 2. Clinical features of the studied sarcoidosis patients with or without liver function abnormalities

	LTA (ALT >1.5 time ULN)	No LTA (ALT<1.5 times ULN)	P value
Total	73 (12.8%)	499 (87%)	
Age, years	48 ± 9.8 *	50 ± 10.8	>0.05
Female §	63 (86%)	324 (72%)	0.010
African American	33 (46%)	219 (44%)	>0.05
Serum creatinine >1.2 mg/dL	3 (4%)	22 (95%)	>0.05
Liver sarcoidosis §	13 (8%)	36 (8%)	0.008
Methotrexate dosage mg/week	9.9 ± 2.6	9.8 ± 3.1	>0.05
Concurrent prednisone	50 (68%)	260 (58%)	>0.05

Table 3. Clinical features of those sarcoidosis patients with or without leukopenia during methotrexate therapy

	Leukopenia (WBC<3800 cells/cu mm)	No leukopenia (WBC>3800 Cells/cu mm)	p-value
Total	70 (11.9%)	517 (88.1%)	
Age, years	50 ±9.5 *	50 ±10.7	>0.05
Female	47 (67%)	387 (75%)	>0.05
African American	39 (56%)	215 (42%)	0.027
Serum creatinine >1.2 mg/dL	4 (5%)	28 (6%)	>0.05
Hepatic sarcoidosis	5 (7%)	47 (9%)	>0.05
Methotrexate dosage mg/week	9.0 ±2.5	9.9 ±2.9	<0.0001
Concurrent prednisone	36 (51%)	307 (60%)	>0.05

Therapy Response

We identified 44 patients who initiated infliximab during the study period and had sufficient follow-up data at six months. We compared these patients to a matched group of MTX treated patients based on age, race, and organ involvement who has also begun therapy during the study period and in whom there was at least six months of follow-up data. The infliximab and MTX groups both con-

tained (28 females and 16 males). There was a similar proportion of lung, skin, eye, or liver involvement in the two groups.

Patient response to either therapy was calculated after six months and twelve months of treatment and summarized in Table 4. Only 10 (23%) of patients who started with MTX were worse by one year of therapy. No patient was much worse after either treatment and we did not include that response in our subsequent Chi square analysis. While there was an increase in the number of patients who were stable or better after 12 versus six months of MTX therapy, the differences in response were not significant (Chi square=3.294, p>0.05%). There was no significant difference in the response rate after 6 versus 12 monhts of infliximab.

Figure 2 summarizes the response rate for the MTX versus infliximab treated patients. Sarcoidosis patients treated with infliximab experienced a more favorable response to therapy compared to MTX treated patients at both six and 12 months. At six months, over half of the patients treated with infliximab were better or much better, while only 21% of the MTX treated patients were better and around a third of the MTX treated patients were worse. The response rate was significantly different between these two regimens (Chi square=11.804, p=0.0081). At the 12 month assessment, the infliximab treated patients were still more likely to be better than those treated with MTX. On the other hand, the percent of patients who worsened with MTX decreased from 36% at 6 months to 21% at 12 months. Again the rates of response significantly differed between MTX and infliximab (Chi square=11.141, p=0.011). The majority of patients in both groups were either stable or better at both the six and 12 months timepoints. Patients treated with infliximab had a more favorable response to therapy compared to MTX at both six and 12 months.

Discussion

We identified more than 600 sarcoidosis patients treated with MTX at our institution over a six year period. Leukopenia and elevated liver transaminases were identified in about ten percent of cases. Severe leukopenia was found in only one patient. Elevation of transaminases to greater than three times upper limit normal was seen in only nine patients. MTX was effective in treating the majority of these sarcoidosis patients. We did not encounter any other adverse events leading to discontinuation of MTX during this time period. This may in part to our dosage of methotrexate. The initial dose of MTX used at our center was 10 mg once a week. This dose was developed by our group as part of our initial reports on using methotrexate in sarcoidosis (2;21). This dose was used in the only double blind, placebo controlled trial of methotrexate for sarcoidosis (7).

MTX has been reported as a steroid sparing agent in sarcoidosis for many years (1;22). However, these early studies evaluated short courses of treatment and dosing based on experience in malignancy and rheumatoid arthritis. We started prospectively using MTX in sarcoidosis patients with two major

Table 4. Patient outcomes in infliximab versus methotrexate treatment

	1=Much Worse	2=Worse	3=Same	4=Better	5=Much Better	Chi	p-value
6 Months infliximab	0	7	13	19	5	11.804	0.008
6 months methotrexate	0	17	20	9	1		
12 months infliximab	0	5	14	15	5	11.141	0.011
12 months methotrexate	0	10	27	10	0		

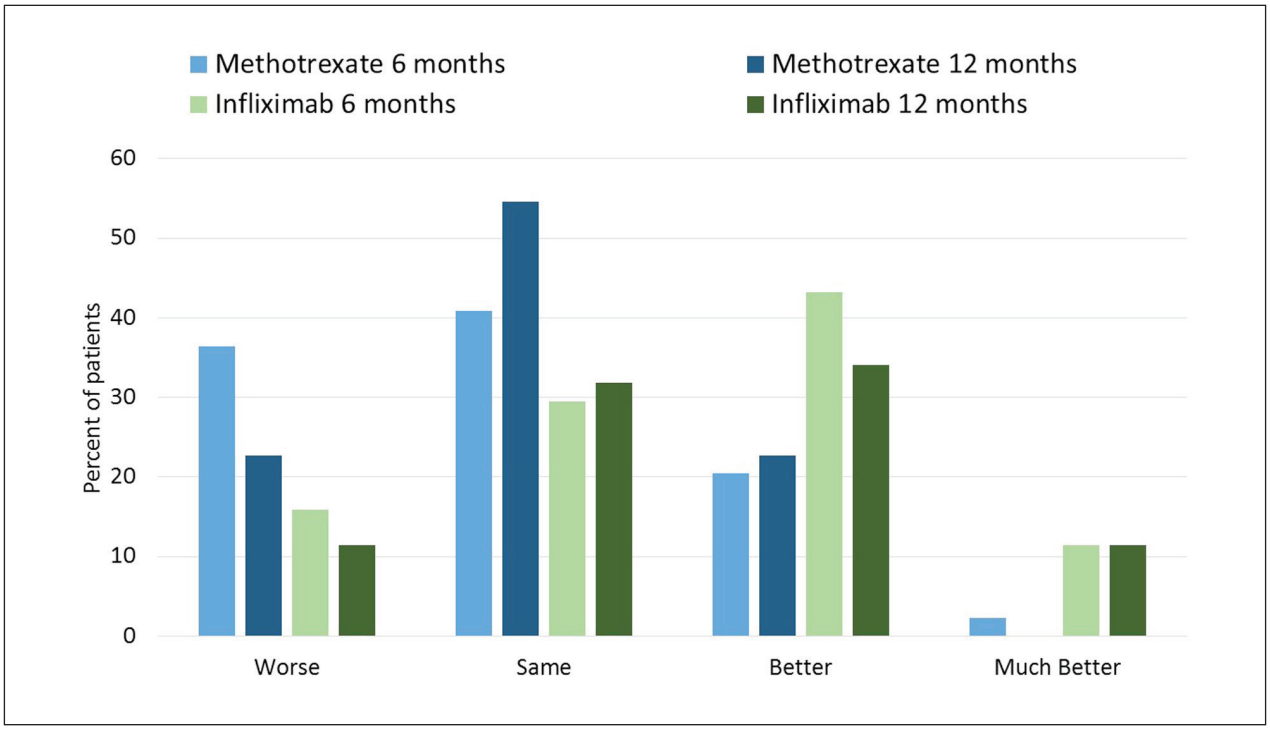

Fig. 2. Response rate for methotrexate at 6 months (light blue) and 12 months (dark blue) versus infliximab at 6 months (light green) and 12 months (dark gree). The response rate was significantly different between these two regimens (Chi square=11.804, p=0.0081). See text for details regarding level of response.

modifications from the early literature. We chose to use a lower dose, since we had demonstrated sarcoidosis patients often have hematologic abnormalities due to bone marrow suppression from the disease (23;24). We also noted that it could take six months or longer to achieve objective disease improvement with MTX treatment (2). Furthermore, we have reported that prolonged use of MTX is associated with good clinical response with limited toxicity (21). Others have demonstrated that MTX is effective in sarcoidosis and less toxic than other agents such as azathioprine (4;25).

The initial dose of MTX we used in this study was 10 mg once a week. This is consistent with our prior studies and within published guidelines for treating sarcoidosis (8) and reported by others (3). Others have used initially 10 mg, but would titrate up to 15 mg a week based on blood monitoring (4). It is unclear that higher doses are more effective and they may just be more toxic. The routine use of 10 mg a week or lower was based on observation of the low toxicity seen in our original series (2;21). This report found a similar very low rate of hepatic and hematologic toxicity. In rheumatoid arthritis, it has been found that higher doses of methotrexate are associated with a better clinical response but more toxicity (26). In a retrospective observational study of sarcoidosis patients, the dose of MTX varied based on treating physician preference. There was no difference in response rate between 10, 12.5, or 15 mg a week (3).

While some physicians feel MTX should be considered as first choice for steroid sparing in sarcoidosis (9;27), a poll of sarcoidosis experts revealed a significant proportion rarely if ever used MTX (8). This paradox seemed be due multiple factors including concern about drug toxicity, poor understanding of MTX efficacy as a steroid sparing agent, misperception of low dose steroid toxicity, and lack of experience with the agent. While there is little one can do about lack of experience except encourage use of drug, we felt that better defining the risk and effectiveness of MTX in sarcoidosis could enhance the usage of this steroid sparing agent.

Based on expert opinion, guidelines have been established regarding the frequency of performing CBC and LFTs (8). In those guidelines, a range frequency employed various centers was reported. Based on these reports the guideline say, "When starting MTX or increasing the dose, ALT with or without AST, creatinine and CBC should be monitored every 3–6 weeks until a stable dose is reached, and every 1–3 months thereafter; after stabilization the monitoring interval can be extended to every 6 months". This statement was not supported by any clinical studies, which was acknowledged in the guideline report (8). In the current study, we report the outcome of monitoring patients every three months as long as they are on therapy. Our results demonstrate that every three month monitoring was a safe method of evaluating patients when using the doses we prescribe.

MTX suppresses the bone marrow and at high doses can cause pancytopenia. In our practice, we use low doses of MTX to avoid such toxicity, and more important, in our experience this lower dose is effective. As seen in Figure 1, our protocol avoids significant leukopenia in the vast majority of patients. This study would suggest that such a guideline would identify the rare patient who develops leukopenia and that severe leukopenia may then be avoided. For patients with leukopenia, the MTX dosage was lower (Table 3). During the study period, the dose of MTX was adjusted based on WBC and MTX was not an independent predictor of leukopenia.

MTX can be hepatotoxic (28). Unlike leukopenia, the rate of liver damage from MTX does not seem to be dose dependent (29). It has been suggested that cumulative dose may be associated with increased risk of hepatotoxicity (30), however not all studies found such an association (10;31), including a study in sarcoidosis (32). This study was not able to detect an impact for cumulative dose, in part because of the small number of cases with significant liver function test abnormalities. We feel that continued liver function testing is warranted in these patients, even with prolonged therapy.

Liver biopsy is the most definitive way to identify MTX hepatotoxicity. We previously reported on the role of liver biopsy in detecting MTX hepatotoxicity (32). However, that procedure has been mostly replaced by liver function testing and recommendations based on the results of serial testing. In rheumatoid arthritis, it has been proposed that a serum transaminase of greater than three times the upper limit of normal be considered abnormal (33). This has been adapted for sarcoidosis patients (8). In sarcoidosis, one has to consider that liver involvement from the underlying disease may cause elevated transaminases (32). The current study rarely identified increased transaminases on serial testing. When it was encountered, our practice was to switch to azathioprine or mycophenolate, since these agents have reported lower rates of hepatotoxicity (12). Others have found that the changes in liver testing may reverse with reducing the dose of MTX. MTX was often used in conjunction with the anti-TNF agents infliximab and adalimumab. This has been recommended to reduce allergic reactions to infliximab and may increase effectiveness of the anti-TNF agents (34;35).

Several potential drugs, including MTX and infliximab, have been shown effective in patients who have worsening disease despite treatment with prednisone and cytotoxic agents (17;36). These studies focused on response of the lung, usually assessed by changes in forced vital capacity. Our study confirmed that infliximab was effective in the majority of patients able to take at least six months of therapy. In order to capture the benefit of therapy in various sarcoidosis phenotypes, we used a novel instrument which assessed individual organs affected and incorporated the physician global assessment. The use of physician global assessment and scoring individual organ response have been reported previously (17;37). While our system was not prospectively captured, it was easily adapted to information readily available in the patient's chart. The instrument was developed to evaluate response rates of different target organs. In our study, the number of patients with specific organ involvement such as brain or heart was insufficient to provide comparison of response rate.

We subsequently used this instrument to assess patient outcomes in infliximab treated patients versus a subset of the MTX treated patients. The MTX treated patients were matched to the infliximab treated patients to compare response rates. We chose this comparison because previous data had suggested infliximab was more potent that MTX in sarcoidosis. In line with another study, the onset of action and efficacy was less robust with MTX compared to infliximab (2). However, after one year, only 21% of

patients felt worse while receiving MTX. This is in line with other studies evaluating MTX response in sarcoidosis (3;21). However, decisions about treatment need to incorporate expected outcomes along with cost, drug availability, and possible long term toxicities which were not assessed in this study. For this comparison, we chose MTX treated patients who had not received infliximab or other third line treatments during the time of analysis. On the other hand, the infliximab patients had been receiving MTX, prednisone, and/or other antimetabolites at time of initiating infliximab. Infliximab is a third line agent that is usually added when patients have continued progression on first and second line immunosuppressants (27). The response rate to infliximab was the effect of addition of that drug to baseline treatment.

There are several limitations to this retrospective study. We focused on hematologic and hepatotoxicity. Other complications such as mucositis, nausea, and infection were not analyzed. While no patient discontinued drug for those reasons, minor dose modifications may have occurred as a result of these complications. Although there were no established criteria for changing drug therapy, only two health care providers (RPB and EEL) evaluated and prescribed treatment. Since sarcoidosis is a multi-organ disease, response in one organ may not mean a similar response in another organ. The study focused on the clinically important target organ for which the patient was undergoing therapy. This instrument was able to detect a significant difference in the two treatment modalities for the whole patient population. Given the relative small number of patients treated with infliximab, we did not perform analysis on specific manifestations of sarcoidosis. We also chose to compare MTX to infliximab. We did not analyze the rate of response of other anti-metabolites such as azathioprine. Infliximab treated patients had usually progressed despite treatment with MTX or similar second line agents (27). Therefore, the MTX patients were probably less severe. However the fact that patients had a better response rate to infliximab enforces the perception that anti-TNF agents are more potent in sarcoidosis. The dose of prednisone was not kept the same through the study. This the response to MTX and infliximab may have been skewed by concomitant glucocorticoid use. During the time of this study, many patients were withdrawn from infliximab because of insurance and/or infections. This is a less common problem now, but we still see that a quarter of patients on infliximab have drug discontinued for these reasons (38). We did not further analyze those patients who received less than one year of treatment and this may bias the response rate we reported with infliximab.

We conclude that MTX was a safe and effective treatment for sarcoidosis patients. The proposed monitoring of sarcoidosis patients treated with MTX (8) was effective in detecting liver and hematologic abnormalities.

References

1. Israel HL. The treatment of sarcoidosis. Postgrad Med J 1970; 46:537-540.
2. Lower EE, Baughman RP. The use of low dose methotrexate in refractory sarcoidosis. Am J Med Sci 1990; 299:153-157.
3. Fang C, Zhang Q, Wang N, Jung X, Xu Z. Effectiveness and tolerability of methotrexate in pulmonary sarcoidosis: a single center real-world study. Sarcoidosis Vasc Diffuse Lung Dis 2019; 36(3):217-227.
4. Vorselaars AD, Wuyts WA, Vorselaars VM, Zanen P, Deneer VH, Veltkamp M et al. Methotrexate versus azathioprine in second line therapy of sarcoidosis. Chest 2013; 144:805-812.
5. Baughman RP, Lower EE, Ingledue R, Kaufman AH. Management of ocular sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2012; 29:26-33.
6. Lower EE, Broderick JP, Brott TG, Baughman RP. Diagnosis and management of neurologic sarcoidosis. Arch Intern Med 1997; 157:1864-1868.
7. Baughman RP, Winget DB, Lower EE. Methotrexate is steroid sparing in acute sarcoidosis: results of a double blind, randomized trial. Sarcoidosis Vasc Diffuse Lung Dis 2000; 17:60-66.
8. Cremers JP, Drent M, Bast A, Shigemitsu H, Baughman RP, Valeyre D et al. Multinational evidence-based World Association of Sarcoidosis and Other Granulomatous Disorders recommendations for the use of methotrexate in sarcoidosis: integrating systematic literature research and expert opinion of sarcoidologists worldwide. Curr Opin Pulm Med 2013; 19:545-561.
9. Schutt AC, Bullington WM, Judson MA. Pharmacotherapy for pulmonary sarcoidosis: a Delphi consensus study. Respir Med 2010; 104(5):717-723.
10. Dubey L, Chatterjee S, Ghosh A. Hepatic and hematological adverse effects of long-term low-dose methotrexate therapy in rheumatoid arthritis: An observational study. Indian J Pharmacol 2016; 48(5):591-594.
11. Pavy S, Constantin A, Pham T, Gossec L, Maillefert JF, Cantagrel A et al. Methotrexate therapy for rheumatoid arthritis: clinical practice guidelines based on published evidence and expert opinion. Joint Bone Spine 2006; 73(4):388-395.
12. James WE, Baughman R. Treatment of sarcoidosis: grading the evidence. Expert Rev Clin Pharmacol 2018;1-11.
13. Sahoo DH, Bandyopadhyay D, Xu M, Pearson K, Parambil JG, Lazar CA et al. Effectiveness and safety of leflunomide for pulmonary and extrapulmonary sarcoidosis. Eur Respir J 2011; 38:1145-1150.
14. Baughman RP, Lower EE. Leflunomide for chronic sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2004; 21:43-48.
15. Hamzeh N, Voelker A, Forssen A, Gottschall EB, Rose C, Mroz P

et al. Efficacy of mycophenolate mofetil in sarcoidosis. Respir Med 2014; 108:1663-1669.
16. Jamilloux Y, Cohen-Aubart F, Chapelon-Abric C, Maucort-Boulch D, Marquet A, Perard L et al. Efficacy and safety of tumor necrosis factor antagonists in refractory sarcoidosis: A multicenter study of 132 patients. Semin Arthritis Rheum 2017; 47(2):288-294.
17. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, Du BR et al. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med 2006; 174(7):795-802.
18. Judson MA, Baughman RP, Teirstein AS, Terrin ML, Yeager HJr, the ACCESS Research group. Defining organ involvement in sarcoidosis: the ACCESS proposed instrument. Sarcoidosis Vasc Diffuse Lung Dis 1999; 16:75-86.
19. Erckens RJ, Mostard RL, Wijnen PA, Schouten JS, Drent M. Adalimumab successful in sarcoidosis patients with refractory chronic non-infectious uveitis. Graefes Arch Clin Exp Ophthalmol 2012; 250:713-720.
20. Baughman RP, Lower EE, Bradley DA, Raymond LA, Kaufman A. Etanercept for refractory ocular sarcoidosis: results of a double-blind randomized trial. Chest 2005; 128(2):1062-1067.
21. Lower EE, Baughman RP. Prolonged use of methotrexate for sarcoidosis. Arch Intern Med 1995; 155:846-851.
22. Lacher MJ. Spontaneous remission response to methotrexate in sarcoidosis. Ann Intern Med 1968; 69:1247-1248.
23. Lower EE, Smith JT, Martelo OJ, Baughman RP. The anemia of sarcoidosis. Sarcoidosis 1988; 5:51-55.
24. Browne PM, Sharma OP, Salkin D. Bone marrow sarcoidosis. JAMA 1978; 240:43-50.
25. Vucinic VM. What is the future of methotrexate in sarcoidosis? A study and review. Curr Opin Pulm Med 2002; 8(5):470-476.
26. Visser K, van der Heijde D. Optimal dosage and route of administration of methotrexate in rheumatoid arthritis: a systematic review of the literature. Ann Rheum Dis 2009; 68(7):1094-1099.
27. Rahaghi FF, Baughman RP, Saketkoo LA, Sweiss NJ, Barney JB, Birring SS et al. Delphi consensus recommendations for a treatment algorithm in pulmonary sarcoidosis. Eur Resp Rev 2020;in press.
28. Schnabel A, Gross WL. Low-dose methotrexate in rheumatic diseases--efficacy, side effects, and risk factors for side effects. Semin Arthritis Rheum 1994; 23(5):310-327.
29. Fathi NH, Mitros F, Hoffman J, Straniero N, Labreque D, Koehnke R et al. Longitudinal measurement of methotrexate liver concentrations does not correlate with liver damage, clinical efficacy, or toxicity during a 3.5 year double blind study in rheumatoid arthritis. J Rheumatol 2002; 29(10):2092-2098.
30. Kevat S, Ahern M, Hall P. Hepatotoxicity of methotrexate in rheumatic diseases. Med Toxicol Adverse Drug Exp 1988; 3(3):197-208.
31. Hashkes PJ, Balistreri WF, Bove KE, Ballard ET, Passo MH. The relationship of hepatotoxic risk factors and liver histology in methotrexate therapy for juvenile rheumatoid arthritis. J Pediatr 1999; 134(1):47-52.
32. Baughman RP, Koehler A, Bejarano PA, Lower EE, Weber FL, Jr. Role of liver function tests in detecting methotrexate-induced liver damage in sarcoidosis. Arch Intern Med 2003; 163(5):615-620.
33. Kremer JM, Alarcon GS, Lightfoot RW, Jr., Willkens RF, Furst DE, Williams et al. Methotrexate for rheumatoid arthritis. Suggested guidelines for monitoring liver toxicity. American College of Rheumatology. Arthritis Rheum 1994; 37(3):316-328.
34. Drent M, Cremers JP, Jansen TL, Baughman RP. Practical eminence and experience-based recommendations for use of TNF-alpha inhibitors in sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2014; 31(2):91-107.
35. Pouw MF, Krieckaert CL, Nurmohamed MT, van der Kleij D, Aarden L, Rispens T et al. Key findings towards optimising adalimumab treatment: the concentration-effect curve. Ann Rheum Dis 2015; 74(3):513-518.
36. Crommelin HA, Vorselaars AD, Van Moorsel CH, Korenromp IH, Deneer VH, Grutters JC. Anti-TNF therapeutics for the treatment of sarcoidosis. Immunotherapy 2014; 6(10):1127-1143.
37. Judson MA, Baughman RP, Costabel U, Flavin S, Lo KH, Kavuru MS et al. Efficacy of infliximab in extrapulmonary sarcoidosis: results from a randomised trial. Eur Respir J 2008; 31(6):1189-1196.
38. Lower EE, Sturdivant M, Grate L, Baughman RP. Use of third-line therapies in advanced sarcoidosis. Clin Exp Rheumatol 2019;14410.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (3); e2020002 DOI: 10.36141/svdld.v37i3.9261

Patterns of healthcare resource utilization in patients with sarcoidosis: a cross-sectional study

Nynke A. Kampstra[1], Paul B. van der Nat[2], Frouke T. van Beek[3], Jan C. Grutters[4], Douwe H. Biesma[5], Philip J. van der Wees[6]

[1] Department of Value-Based Healthcare, St. Antonius Hospital, Santeon-group. Radboud university medical center , Radboud Institute for Health Sciences, Scientific Center for Quality of Healthcare (IQ healthcare), Nijmegen, the Netherlands; [2] Department of Value-Based Healthcare, St. Antonius Hospital, Nieuwegein, the Netherlands. Radboud university medical center, Radboud Institute for Health Sciences, Scientific Center for Quality of Healthcare (IQ healthcare), Nijmegen, the Netherlands; [3] Interstitial Lung Diseases Center of Excellence, Department of Pulmonology, St. Antonius Hospital, Nieuwegein, the Netherlands; [4] Interstitial Lung Diseases Center of Excellence, Department of Pulmonology, St. Antonius Hospital, Nieuwegein, the Netherlands. Division of Heart and Lungs, University Medical Centre Utrecht, Utrecht, The Netherlands; [5] Department of Value-Based Healthcare, St. Antonius Hospital, Santeon-group Department of Internal Medicine, University Medical Centre Utrecht, The Netherlands, St. Antonius Hospital, Nieuwegein, the Netherlands; [6] Radboud university medical center, Radboud Institute for Health Sciences, Scientific Center for Quality of Healthcare (IQ healthcare), Department of Rehabilitation.

Abstract. *Background:* Limited data are available on healthcare resource use and costs in patients with sarcoidosis; *Objectives:* The primary aim of this study was to describe cost-drivers of the top 1% and top ≥1-5% high-cost patients with sarcoidosis. The secondary aim was to compare costs of patients with and without fatigue complaints and to compare comorbidities. *Methods:* We conducted a retrospective observational cross-sectional study in 200 patients diagnosed with sarcoidosis. Hospital administrative databases were used to extract healthcare utilization on the individual patient level. Healthcare costs were categorized into nine groups. *Results:* Average total health care costs for the top 1% (n=22), top ≥1%–5% (n=88) and bottom 95% beneficiaries (n=90) were € 108.296, €53.237 and €4.817, respectively. Mean treatment time in days for the top 1%, top ≥1-5% and the random sample of the bottom 95% was 1688 days (±225), 1412 days (±367) and 775 days (±659), respectively. Mean annual costs for the top 1%, top ≥1-5% and the random sample of the bottom 95% are €51.082, €27.840 and €8.692, respectively. We identified three cost-drivers in the top 5% high-cost patients: 1) expensive medication, 2) intensive care and 3) costs made at the respiratory unit. Patients with and without fatigue showed to have comparable mean costs. High-cost patients were more likely to have multiple organs involved due to sarcoidosis. *Conclusions:* We identified expensive medication as the main cost-driver in the top 5% high-cost patients with sarcoidosis. The study findings can help to tailor interventions for improving the quality of care and reducing overall costs. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (3): e2020002)*

Key words: sarcoidosis, costs, quality of care

Introduction

Sarcoidosis is a chronic granulomatous disease characterized by persistent body complaints in multiple organs and patients suffer from a broad range of nonspecific symptoms. (1–4) The inflammation level as well as organs affected are highly variable. In more than 90% of the cases, sarcoidosis affects the lungs. Health-related quality of life (HRQoL) and

Received: 7 February 2020
Accepted after revision: 17 August 2020
Correspondence: Nynke A. Kampstra
Koekoekslaan 1, 3435 CM Nieuwegein, The Netherlands
E-mail: n.kampstra@antoniusziekenhuis.nl
or nynkekampstra@gmail.com

health status is often reduced in patients with sarcoidosis. (5–8) Fatigue is an often reported symptom in patients with sarcoidosis. (2, 9, 10) In a sample of 1197 patients with sarcoidosis, 70% of the patients reported fatigue as a feature of their sarcoidosis. (10)

Detailed insights into the costs of patients with advanced sarcoidosis are however lacking. Efforts to further improve the quality of care could specifically target advanced sarcoidosis patients. Therefore, it would be useful to have a detailed picture of high-cost patients with sarcoidosis which can enable better evaluation of the current treatment choices.

The most complex patient group is that with the highest costs and needs, with a variety of complex medical conditions. About 5% of patients with various conditions account for 50% of the total health care spending. (11) Detailed insight into the disease specific costs regarding patients with sarcoidosis are getting more attention in the literature, although this is still limited. (12–14) Previous research has demonstrated that the cost distribution for patients with sarcoidosis is highly skewed. High-cost patients had more sarcoidosis related comorbidities compared to low-cost patients. (12–14) Previous research has shown commercial payers in the USA incurred a mean of $19,714 total annual healthcare costs per sarcoidosis patient. (12) It was furthermore reported that the main cost-drivers identified were outpatient visits (46% of the costs) and inpatient admissions (32% of the costs). Mean health care costs in a US-based patient group in the top quintile were $73,346. The authors furthermore concluded that this subgroup of most costly patients with sarcoidosis might be worthwhile to invest in concerning outcome improvement efforts. Another study, based on a U.S. national health care database, found that the median healthcare costs were $18,663 for patients with sarcoidosis per year. (14) The top 5% costliest patients with sarcoidosis exceeded $90,000 per year. However, little is known on the health care utilization, the patient characteristics and the relationship with fatigue complaints in high-cost patients with sarcoidosis. Furthermore, no other studies have tried to identify cost-drivers in this high-cost patient subgroup and identify their patient characteristics in a (Dutch) cohort. This can be valuable as it will enable to assess the effectiveness of current treatment choices. Therefore, the primary aim of this study was to describe cost-drivers of the top 1% and top ≥1-5% high-cost patients with sarcoidosis. The secondary aim was to compare costs of patients with and without fatigue complaints and to compare comorbidities.

Methods

Design and context

This study was a cross-sectional study using internal data of patients with sarcoidosis who visited the St. Antonius Hospital in the Netherlands. The study used administrative data provided by the business intelligence unit of the St. Antonius hospital. Patients were treated in the St. Antonius Hospital between January 1st 2011 and November 1st 2016. In total, 2251 patients with sarcoidosis were identified, from which 200 patients were used for the final analysis. When being enrolled in our cohort, there was at least of follow-up period of six months. The opening of the first diagnosis related group (DRG) due to their treatment for sarcoidosis was between the 11th of January 2011 and the 11th of May 2016.

We examined healthcare costs and identified the beneficiaries within the top 1% and the top ≥1%–5% of total costs. (16) The 2251 patients from our cohort were ranked in total costs after which the top 1% (0-0.99) and the ≥1-5% (1.0-4.99) most expensive patients were identified (n=110). From the remaining 2141 patients, a random sample of 90 patients was selected. Thus, from the total cohort, additional data was collected for 200 patients in total. Patients in the top 5% (so the top 1% and the top ≥1-5% together) of total health care costs were defined as high-cost patients.

Data collection

Baseline patient characteristics were examined for all patients with sarcoidosis (n=200). Patient characteristics included age, gender, BMI, Scadding stage, survival, fatigue, treatment time and the year of the diagnosis. Furthermore we collected information regarding the sarcoidosis related organ involvement (pulmonary, cardiac and neurologic). In addition, we collected data on whether or not patients suffered from pulmonary hypertension and obstructive sleep apnea (OSAS).

Mean treatment time was defined as the first date of receiving treatment minus the last date of receiving treatment at the St. Antonius hospital between January 1st 2011 and January 1st 2016. When the patient reported to experienced fatigue more than three times during the outpatient visits, he/she was seen as a patient with fatigue complaints in the analysis.

Healthcare utilization and cost calculation

Hospital administrative databases were extracted for all healthcare utilization as part of the diagnosis code for sarcoidosis patients. Next, this was grouped into six categories: 1) expensive medication (infliximab, adalimumab and rituximab), 2) intensive care/general ward nursing 3) respiratory medicine, 4) clinical chemistry, 5) radiology/ nuclear medicine, and 6) other.

Total costs per patient were calculated by summing the number of resources used multiplied by the costs per resource. We calculated costs for any given month in which care related to sarcoidosis was delivered in our hospital. Total annual costs were calculated by summing up the costs of all individual months the patient received care and subsequently correcting for the number of months. Months when no care was delivered were disregarded. Total costs per resource item were based on the national diagnosis treatment combination rates as defined in 2015 by the St. Antonius hospital. Costs of drugs were based on the lowest reported drug price according to the information of the Health Care Institute of the Netherlands valued in 2019. Total drug costs per patient were calculated using the amount of drugs (in mg) used multiplied with the price per mg in Euros.

Analyses

Descriptive statistics were used to present total costs. Costs were expressed as mean and interquartile range (IQR) due to skewness of the data. Overall mean costs (IQR) were presented for total health care costs made between January 2011 and January 2016. Treatment time and time between diagnosis/ first visit were expressed as mean (±SD). All categorical data were presented in *n* and percentage (%). All analyses were performed in SPSS (IBM SPSS Statistics version 24).

Results

For the 200 patients analysed, average total health care costs for the top 1%, top ≥1%–5% and bottom 95% beneficiaries were €108.296, €53.237 and €4.817, respectively. The mean annual cost are €51.082, €27.840 and €8.692, respectively. Mean treatment time in days for the top 1%, top ≥1-5% and the random sample was 1688 days (±225), 1412 days (±367) and 775 days (±659), respectively. Table 1 presents the demographics of the study population. In all groups, males were overrepresented, especially in the top 1%, where 68.2% of the patients were male. Mean age at diagnosis was comparable between the groups. In Table 2, the demographics are presented for the patients with less advanced sarcoidosis (without cardiac sarcoidosis, neurosarcoidosis or pulmonary hypertension). Here, top 1% beneficiaries were much older than the top ≥1-5% and the random sample of the bottom 95%. Furthermore, mortality was higher for high-cost patients, as 18% in the top 1% and 9.1% in the top ≥1%–5% died. In the random sample of the bottom 95%, 2.2% of the patients died.

Association of fatigue

In the top 1% and top ≥1%-5% 81.8% and 75% showed the experience fatigue complaints, respectively. In the random sample of the bottom 95%, 38.9% showed to have fatigue complaints.

In Table 3 the mean costs and patient characteristics are presented for patients with and without fatigue complaints for two groups: for the top 5% (the top 1% and top ≥1%-5% together) and for the random sample of the bottom 95%. Patients with and without fatigue showed to have comparable mean costs. Average total costs for the top 5% patients with fatigue were €65.512 and €60.165 without fatigue complaints. Average annual costs for the top 5% patients with fatigue were €33.039 and €30.311 without fatigue complaints. For the random sample of the bottom 95%, average total costs for patients with fatigue was €6.546 for patients with fatigue complaints and €3.716 for patients without fatigue complaints. In both the top 5% and random sample of the bottom 95%, patients with fatigue complaints were more likely to have pulmonary sarcoidosis, pulmonary hypertension, cardiac sarcoidosis, neurosarcoidosis and OSAS.

Table 1. Patient characteristics for three cost groups

Patient characteristics	Top 1%(n=22)	Top ≥1%-5%(n=88)	Sample of bottom 95%(n=90)
Male (n, %)	15 (68.2%)	52 (59.1%)	51 (56.7%)
Age at diagnosis (years ±SD)	44 ±12.3	43 ±11.0	45 ±12.6
Mean total costs (€, IQR)	€108.296 €25.497	€53.237 €39.384	€4.817 €4.466
Mean annual costs (€, IQR)	€51.082 €24.366	€27.840 €21.253	€8.692 €8.825
Treatment time (days ±SD)	1688 ±225	1412 ±367	775 ±659
Time between diagnosis/first visit (years ±SD)	8.8 ±6.2	7.3±6.7	6.1 ±9.1
BMI (±SD)	32.0 ±7.1	27.8 ±4.7	29.1 ±5.9
Fatigue (n, %)	18 (81.8%)	66 (75.0%)	35 (38.9%)
Deceased (n, %)	4 (18%)	8 (9.1%)	2 (2.2%)
Pul. sarc (n, %)	20 (90.9%)	81 (92.0%)	56 (62.2%)
Pul. Hypertension (n, %)	3 (13.6%)	9 (10.2%)	2 (1.7%)
Cardiac sarc (n, %)	2 (9.1%)	10 (11.3%)	5 (5.6%)
OSAS (n, %)	7 (31.8%)	9 (10.2%)	14 (15.6%)
Neurosarcoidosis (n, %)	6 (27.3%)	19 (21.6%)	12 (13.3%)
Scadding stage			
Scadding 0 (n, %)	4 (18.2%)	16 (18.2)	0 (0%)
Scadding I (n, %)	2 (9.1%)	13 (14.8%)	19 (21.1%)
Scadding II (n, %)	6 (27.3%)	18 (20.5%)	15 (16.7%)
Scadding III (n, %)	4 (18.0%)	13 (14.8%)	4 (3.0%)
Scadding IIII (n, %)	4 (18.0%)	28 (31.8%)	13 (14.4%)

IQR= Inter quartile range.

Comorbidities

In the top 1% and the top ≥1-5% there was more organ involvement of sarcoidosis compared to the random sample of the bottom 95%. Cardiac and pulmonary sarcoidosis and neurosarcoidosis were more likely in the top 1% and top ≥1-5%. OSAS was more present in the top 1% compared to the random sample of the bottom 95% (31.8% versus 15.6%). When patients with cardiac sarcoidosis, neurosarcoidosis or pulmonary hypertension were excluded, OSAS was still more present in the top 1% of the patients compared to both the top ≥1-5% and the random sample of the bottom 95% (33% versus 5% and 10%, respectively).

Cost-driver profile

The top 1% (n=22) spent a total of €3824 thousand. The top ≥1-5% patients (n=88) spent a total of €3,824,766. Figure 1A-C presents the share per category in the total costs. The top 3 cost-drivers identified in both the top 1% and top ≥1-5% high-cost patients were: 1) expensive medication, 2) intensive care/general ward nursing, and 3) costs made at the respiratory medicine department. Finally, the random sample of the bottom 95% spent a total of €442,019. Expenses made at the respiratory medicine department was the main cost-driver. Within the cost for expensive medication, infliximab accounted for 83% of the costs in the top 1%. In the

Table 2. Patient characteristics for three cost groups (without cardiac sarcoidosis, neurosarcoidosis and pulmonary hypertension)

Patient characteristics*	Top 1% (n=12)	Top ≥1%-5% (n=51)	Sample of bottom 95% (n=70)
Male (n, %)	8 (66.7%)	31 (60.8%)	39 (55.7%)
Age (years ±SD)	47 ±14.3	41± 11.0	44 ±12.1
Mean total costs (€, IQR)	€107.970	€50.861	€4.184
	€24.064	€45.551	€3.844
Mean annual costs (€, IQR)	€50.837	€25.322	€9.472
	€22.245	€17.413	€10.307
Treatment time (days ±SD)	1716 ±243	1411 ±352	700 ±650
Time between diagnosis/first visit (years ±SD)	7.9 ±7.8	7.3 ±5.7	5.9 ±9
BMI (±SD)	33.8 ±6.9	27.0 ±4.0	28.7 ±4.9
Fatigue (n, %)	10 (83.3%)	35 (68.6%)	21 (30%)
Deceased (n, %)	2 (16.7%)	5 (9.8%)	0 (0.0%)
Pul. sarc (n, %)	11 (91.7%)	47 (92.2%)	44 (62.9%)
OSAS (n, %)	4 (33.0%)	3 (5.0%)	7 (10.0%)
Scadding stage			
Scadding 0 (n, %)	2 (16.7%)	7 (13.7%)	5 (7.1%)
Scadding I (n, %)	0 (0.0%)	5 (9.8%)	14 (20.0%)
Scadding II (n, %)	3 (25.0%)	13 (25.5%)	15 (21.4%)
Scadding III (n, %)	3 (25.0%)	9 (17.6%)	4 (5.7%)
Scadding IIII (n, %)	4 (33.3%)	17 (33.3%)	9 (12.9%)
Unknown	0	0	23 (32.9%)

IQR= Inter quartile range. *without cardiac sarcoidosis, neurosarcoidosis and pulmonary hypertension.

top ≥1-5% this was 80% and in the random sample this was 98%. Adalimumab accounted for 16%, 19% and 0% of the total costs for expensive medication, respectively.

Discussion

In this study, we provide cost-related information and describe characteristics of 200 patients with sarcoidosis in a Dutch patient cohort using administrative data. The health care cost distribution for patients with sarcoidosis are highly skewed. The average healthcare costs of the top 1% patients with sarcoidosis were 22 times higher compared to that of the random sample of the bottom 95% (€108.296 vs. €4.817, respectively). The mean annual healthcare costs of the top 1% patients with sarcoidosis were 6 times higher compared to that of the random sample of the bottom 95% (€51.082 vs. €8.692, respectively). Treatment time of the most expensive 1% was 2.5 times longer compared to the random sample. The top 1% patients and top ≥1-5% patient showed to have more sarcoidosis related organ involvement compared to the random sample of the bottom 95%, indicating those are patients with more advanced sarcoidosis. Unexpectedly, patients with and without fatigue showed to have comparable mean costs and mean annual costs.

Although methodology and the data source used differ, our findings are consistent with other manuscripts studying the costs in patients with sarcoidosis. (12–14) Moreover, one study showed that patients in the top 5% in terms of costs, spent $93,201. (14) An-

Table 3. Patient characteristics for patients with and without fatigue

Patient characteristics	Top 0-5%		Random sample	
	With fatigue (n=84)	Without fatigue (n=26)	With fatigue (n=35)	Without fatigue (n=55)
Male (n, %)	49 (58.3%)	18 (69.2%)	15 (42.9%)	5 (9.1%)
Age (years ±SD)	53 (±11.1)	48 (±9.0)	51 (±12.5)	52 (±11.5)
Mean costs (€, IQR)	€65.512 (€44.486)	€60.165 (€54.307)	€6.546 (€6.080)	€3.716 (€2.552)
Mean annual costs (€, IQR)	€33.039 (€22.439)	€30.311 (€35.966)	€10.129 (€8.479)	€7.704 (€10.068)
Treatment time (days ±SD)	1484 (±347)	1410 (±401)	978 (±679)	634 (±613)
Time between diagnosis/first visit (years ±SD)	7.4 (±6.8)	8.3 (±5.7)	6.9 (±9.1)	5.6 (±9.0)
BMI (±SD)	28.7 (±5.3)	28.6 (±6.2)	29.7 (±6.8)	28.6 (±5.2)
Deceased (n, %)	10 (11.9%)	2 (7.7%)	1 (52.9%)	0 (0%)
Pulmonary sarcoidosis (n, %)	78 (92.9%)	23 (88.5%)	24 (68.6%)	32 (58.2%)
Pulmonary hypertension (n, %)	10 (11.9%)	2 (7.7%)	2 (5.7%)	1 (1.8%)
Cardiac sarcoidosis (n, %)	10 (11.9%)	2 (7.7%)	4 (11.4%)	6 (10.9%)
OSAS (n, %)	16 (19%)	0 (0%)	8 (22.9%)	0 (0%)
Neurosarcoidosis (n, %)	22 (26.2%)	3 (11.5%)	7 (20.0%)	5 (9.1%)
Scadding III (n, %)	13 (15.5%)	4 (15.4%)	2 (5.7%)	2 (3.6%)
Scadding IV (n, %)	23 (27.4%)	11 (42.3%)	7 (20.0%)	6 (10.9%)

IQR= Inter quartile range.

other study showed that the mean annual health care costs for patients in the top quintile were $73,346 (based on 2015 data). (13) This was 10 times greater compared to the mean annual health care costs for the remaining patients. Further, to our knowledge, this is the first study to use such data to characterize high-cost patients with sarcoidosis and identify key drivers that contribute to costs in this patient population.

This study has a number of limitations. First, we have studied a group of patients visiting our center for a period of 5 years. Thus, total costs for the full treatment time per patient are not presented in this study. Moreover, it would have been interesting to see total costs over a full treatment period per patient and next, to identify the most expensive treatment period looking at the patient cohort. Secondly, we were unable to include the societal costs. This includes costs due to work loss of patients not being able to work due to the severity of their disease course. Thirdly, this study was based on a population in the Netherlands. Therefore, this information cannot be generalized to other populations where cost of health care utilization can be very different. Also, some treatment options can be more expensive in other countries. It is known that performing a PET scan is more expensive in the USA compared to the Netherlands. As a consequence, the PET scan is performed less often in the USA. If we would leave out the PET scan cost, there would be no major changes in the cost distribution presented in Figure 1. Fourthly, we did not have a sufficient number of patients to perform an analysis of cost of the top 5% for the four major groups (neurosarcoidosis, cardiac sarcoidosis, pulmonary hypertension, and advanced sarcoidosis pulmonary). In a larger population it would have been very interesting to see whether total annual costs vary, depending upon underlying morbidity. Fifthly, we were unable to collect data when a patient was hospitalized outside our medical center.

Table 4. Patient characteristics for patients with and without fatigue (without cardiac sarcoidosis, neurosarcoidosis and pulmonary hypertension)

Patient characteristics	Top ≥1-5%		Random sample	
	With fatigue (n=45)	Without fatigue (n=18)	With fatigue (n=21)	Without fatigue (n=49)
Male (n, %)	27 (60%)	12 (66.7%	8 (38.1%%)	31 (63.3%)
Age (years ±SD)	52 (±12.1)	48 (±8.9)	47 (±11.9)	52 (±11.4)
Mean costs (€, IQR)	€65.044 (€46.618)	€53.476 (€48.151)	€6.103 (€3.361)	€3.361 (€5.632)
Mean annual costs (€, IQR)	€31.220 (€23.170)	€26.497 (€24.401)	€12.787 (€10.036)	€7.893 (€10.890)
Treatment time (days ±SD)	1502 (±326)	1387 (±414)	896 (±683)	609 (±621)
Time between diagnosis/first visit (years ±SD)	6.7 (±6.0)	9.1 (±6.1)	6.0 (±8.2)	5.9 (±9.4)
BMI (±SD)	28.9 (±5.3)	27.0 (±5.3)	28.5 (±4.6)	28.8 (±5.1)
Deceased (n, %)	5 (11.1%)	2 (11.1%)	0 (0%)	0 (0%)
Pulmonary sarcoidosis (n, %)	42 (93.3%)	16 (88.9%)	15 (71.4%)	29 (59.2%)
OSAS (n, %)	7 (15.6%)	0 (0%)	3 (14.3%)	4 (8.2%
Scadding III (n, %)	8 (17.8%)	4 (22.2%)	2 (9.5%)	2 (4.1%)
Scadding IV (n, %)	13 (28.9%)	8 (44.4%)	4 (19%)	5 (10.2%)

IQR= Inter quartile range. *without cardiac sarcoidosis, neurosarcoidosis and pulmonary hypertension

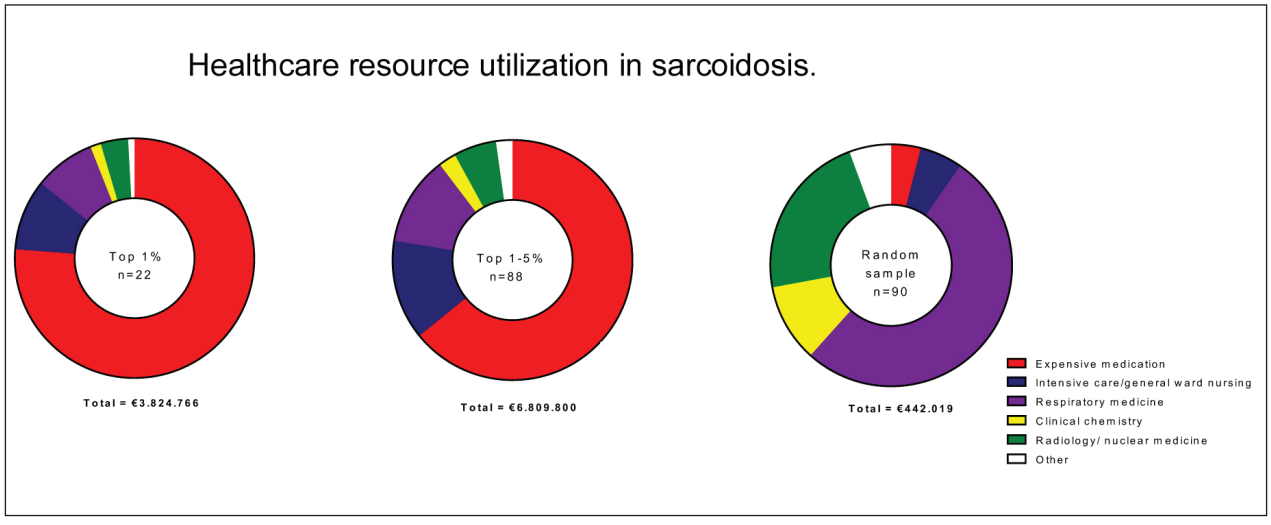

Fig. 1. Distribution of costs by resource categories for the top 1%, top ≥1-5% high-cost patients and the low-cost random sample.

However, we do not expect this to be a major (hidden) cost-driver. The Netherlands is a small country and planned admissions of tertiary patients are generally located in our center. A single emergency admission may sometimes take place regionally, but some of these patients will be transferred to us within a few days. Sixthly, we did not look into individual treatment schemes and were therefore unable to re-

port whether the patients were actively treated for e.g. pulmonary hypertension and how this affected their (medication) costs. Finally, a limitation with using annual costs is that we only used costs when care was delivered on a monthly basis. So when a patient would come back after six months for follow-up, the months in between visits were not part of our annual costs definition.

In conclusion, this study found that high-cost patients with sarcoidosis were patients with higher rates of comorbidities and had increased use of health care resources. Specifically, they were more often in need of expensive medication for their treatment. Both the management as well as physicians can specifically use this information to realize improvements in the quality of care and reducing overall costs for patients diagnosed with sarcoidosis, especially in referral centers of excellence. Efforts to further improve the quality of care and clinical outcomes for patients with sarcoidosis could specifically target the most expensive patients, which can potentially reduce the overall costs.

Support statement: This work was supported by The Netherlands Organisation for Health Research and Development (ZonMw) under project number 842001005. The funder had no role in the study design, data collection, analysis or decision of where to publish the manuscript.

References

1. Baughman RP, Lower EE, Gibson K. Pulmonary manifestations of sarcoidosis. Presse Med. Elsevier; 2012; 41: e289–e302.
2. Drent M, Lower EE, De Vries J. Sarcoidosis-associated fatigue. Eur. Respir; 2012; 40: 255–263.
3. Costabel U, Hunninghake GW, Committee SS. ATS/ERS/WASOG statement on sarcoidosis. Eur. Respir. J. Wiley Online Library; 1999; 14: 735–737.
4. Iannuzzi MC, Fontana JR. Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics. Jama American Medical Association; 2011; 305: 391–399.
5. De Vries J, Drent M. Quality of life and health status in sarcoidosis: a review. Semin. Respir. Crit. Care Med. New York: Thieme Medical Publishers, c1994-; 2007. p. 121–127.
6. Cox CE, Donohue JF, Brown CD, Kataria YP, Judson MA. Health-related quality of life of persons with sarcoidosis. CHEST J. American College of Chest Physicians; 2004; 125: 997–1004.
7. Michielsen HJ, Drent M, Peros-Golubicic T, De Vries J. Fatigue is associated with quality of life in sarcoidosis patients. CHEST J. American College of Chest Physicians; 2006; 130: 989–994.
8. de Kleijn WP, De Vries J, Lower EE, Elfferich MD, Baughman RP, Drent M. Fatigue in sarcoidosis: a systematic review. Curr. Opin. Pulm. Med. CoRPS, Department of Medical Psychology, Tilburg University, Tilburg, the Netherlands.; 2009; 15: 499–506.
9. Marcellis RG, Lenssen AF, Elfferich MD, De Vries J, Kassim S, Foerster K, Drent M. Exercise capacity, muscle strength and fatigue in sarcoidosis. Eur. Respir. J. Dept of Respiratory Medicine, ild Care Consultancy, Maastricht University Medical Centre, NC Maastricht, The Netherlands.; 2011; 38: 628–634.
10. Hinz A, Fleischer M, Brähler E, Wirtz H, Bosse-Henck A. Fatigue in patients with sarcoidosis, compared with the general population. Gen. Hosp. Psychiatry Elsevier; 2011; 33: 462–468.
11. Blumenthal D, Abrams MK. Tailoring complex care management for high-need, high-cost patients. Jama American Medical Association; 2016; 316: 1657–1658.
12. Rice JB, White A, Lopez A, Conway A, Wagh A, Nelson WW, Philbin M, Wan GJ. Economic burden of sarcoidosis in a commercially-insured population in the United States. J. Med. Econ. Taylor & Francis; 2017; 20: 1048–1055.
13. Rice JB, White A, Lopez A, Nelson WW. High-Cost Sarcoidosis Patients in the United States: Patient Characteristics and Patterns of Health Care Resource Utilization. J. Manag. care Spec. Pharm. Academy of Managed Care Pharmacy; 2017; 23: 1261–1269.
14. Baughman RP, Field S, Costabel U, Crystal RG, Culver DA, Drent M, Judson MA, Wolff G. Sarcoidosis in America. Analysis based on health care use. Ann. Am. Thorac. Soc. Am Thoracic Soc; 2016; 13: 1244–1252.
15. Kaplan RS, Porter ME. How to solve the cost crisis in health care. Harv. Bus. Rev. Harvard Business School, USA.; 2011; 89: 46–52, 54, 56-61 passim.
16. Wammes JJG, Tanke M, Jonkers W, Westert GP, Van der Wees P, Jeurissen PP. Characteristics and healthcare utilisation patterns of high-cost beneficiaries in the Netherlands: a cross-sectional claims database study. BMJ Open; 2017; 7: e017775-2017–017775.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (3); e2020004 DOI: 10.36141/svdld.v37i3.9273

Macrophage Migration Inhibitory Factor is not Associated with Sarcoidosis Susceptibility or Severity in Whites or Blacks

Camila D. Odio[1], Eward J. Miller[1], Maor Sauler[1], Lin Leng[1], MartaPiecychna[1], Wonder P. Drake[2], Richard Bucala[1]

[1]Yale School of Medicine, [2]Vanderbilt School of Medicine

Abstract. *Background:* Macrophage migration inhibitory factor (MIF) is an inflammatory cytokine, and increased *MIF* expression has been associated with the development and severity of multiple granulomatous, autoimmune diseases. However, *MIF* association studies have been discordant in sarcoidosis. *Objective:* To evaluate associations between macrophage migration inhibitory factor (*MIF*) promoter polymorphisms and sarcoidosis susceptibility and severity. *Methods:* Three hundred and fifty one patients with sarcoidosis were recruited through the Genomic Research in Alpha-1 Antitrypsin Deficiency and Sarcoidosis (GRADS) study. Genomic DNA was isolated from serum, and the *MIF* -173G/C SNP [*rs755622*] and *MIF* -794 $CATT_{5-8}$ microsatellite repeat [*rs5844572*] were genotyped. Allelic frequencies were compared between cases and healthy controls and associations between *MIF* alleles and sarcoidosis severity were assessed. *Results:* The frequencies of the high expression -173C SNP and the low expression -794 $CATT_5$ containing genotypes in white and black sarcoidosis patients were the same as those of healthy controls. High expression *MIF* alleles were not associated with sarcoidosis severity. Associations between *MIF* alleles and extrapulmonary sarcoidosis phenotypes were limited by small sample sizes. *Conclusions:* High expression *MIF* genotypes were not associated with the susceptibility to or severity of pulmonary sarcoidosis in a large North American cohort. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (3): e2020004)*

Key words: macrophage migration inhibitory factor, sarcoidosis, GRADS, susceptibility, severity

Introduction

Macrophage migration inhibitory factor (MIF) is an inflammatory cytokine that sustains macrophage activation and suppresses glucocorticoid signaling (1). *MIF* expression is associated with two commonly occurring promoter polymorphisms. The -794 CATT microsatellite has 5 to 8 repeats, and longer length results in increased gene expression due to enhanced binding of the transcription factor ICBP90 (2). The high expression allele, -794 $CATT_{7,}$ is in linkage disequilibrium with the -173C single nucleotide polymorphism (SNP)(1), and this SNP sometimes reveals associations because of reduced locus heterogeneity. Increased *MIF* expression has been associated with the development and severity of multiple granulomatous, autoimmune diseases including granulomatosis with polyangitis (3), hypersensitivity pneumonitis (4), and idiopathic pulmonary fibrosis (5).

Despite these reports, *MIF* association studies have been discordant in sarcoidosis. Amoli et al.

Received: 10 February 2020
Accepted after revision: 17 August 2020
Correspondence: Richard Bucala, M.D., Ph.D.
300 Cedar Street, Ste S411 - New Haven, CT 06519
Richard.bucala@yale.edu
Wonder Puryear Drake
A-2206 MCN - 1161 21st Ave South - Nashville, TN 37232
Wonder.drake@vanderbuilt.edu

identified a greater frequency of the -173C allele in patients with erythema nodosum (EN) due to sarcoidosis compared to those with EN due to other etiologies (6). A subsequent study reported that the *MIF* -173C allele is associated with Lofgren's syndrome but not with susceptibility to sarcoidosis (7). Plant et al. examined the functional -794 $CATT_{5\text{-}8}$ microsatellite and reported no association between the low expression $CATT_5$ allele and susceptibility to sarcoidosis, EN, or disease severity (8). These differing conclusions may be related to variable disease definitions, population stratification, or underpowered sample sizes.

The Genomic Research in Alpha-1 Antitrypsin Deficiency and Sarcoidosis (GRADS) study is an observational cohort study designed to examine the pathobiology of these two diseases. Patients with sarcoidosis were enrolled with the goal of identifying biomarkers and genotypes associated with the various sarcoidosis phenotypes. Patient data included detailed medical and exposure histories, blood samples, spirometry and radiology studies (9). We examined associations between sarcoidosis phenotypes and *MIF* promoter polymorphisms in these patients.

Methods

The GRADS study recruited 351 sarcoidosis patients from nine clinical centers across the United States, and 371 healthy controls were culled from a previously published registry (10). The relevant Institutional Review Boards approved this study, and all subjects gave signed informed consent. The easyDNA kit (Invitrogen) was used to isolate genomic DNA from serum samples and polymorphisms were identified as described previously (11). In short, the -173G/C SNP [*rs755622*] was analyzed with the established Taqman assay for allelic discrimination (Applied Biosystems, ABI), and the -794 $CATT_{5\text{–}8}$ microsatellite repeat [*rs5844572*] genotyping was performed by polymerase chain reaction with analysis by automated capillary electrophoresis (3730xl Genetic Analyzer, ABI).

Because *MIF* allelic frequencies vary by race (10), we stratified our cohort into self-identified white (including two Latinos) and black subjects. Asians and Native Americans were excluded due to small sample sizes. Race categories were confirmed by genetic admixture analysis in the control group. The -173G/C SNP conformed to Hardy-Weinberg equilibrium in both whites and blacks. Differences in genotype frequencies were assessed by logistic regression adjusting for age and sex. Pulmonary sarcoidosis severity was characterized by forced expiratory volume in one second (FEV1), forced vital capacity (FVC), diffusing lung capacity for carbon monoxide (DLCO) and Scadding stage. Differences in these measurements by genotype were assessed using a two-sample t-test for normally distributed samples and Mann-Whitney test for non-normally distributed two group comparisons. Chi-square tests for homogeneity were used to compare the proportions of white patients with each Scadding stage by genotype. Genotype frequencies in cardiac sarcoidosis patients versus healthy controls were compared using chi-square tests. Analyses were performed in RStudio v 1.2.1335 for Mac.

Results

After age and sex adjustment, there were no differences in the frequency of the low *MIF* expression -794 $CATT_5$ containing genotype between sarcoidosis subjects and healthy controls in whites (41% vs. 36% in controls, $p = 0.169$) or blacks (47% vs. 52% in controls, $p = 0.744$). The -173C containing genotype was not more common in the sarcoidosis patients versus controls regardless of race (whites: 31% vs 40% in controls, $p = 0.153$; blacks: 63% vs. 64% in controls, $p = 0.150$). All sarcoidosis patients had normal (> 80% predicted) FEV1 and FVC regardless of their -794 CATT and -173 SNP genotypes. However, while whites had normal DLCO, blacks had reduced DLCO. The severity of this impairment was not associated with genotype (black DLCO: -794 $CATT_5$ 71.4% predicted vs. -794 $CATT_{non\text{-}5}$ 66.6% predicted $p = 0.508$; -173G/G 66.6% predicted vs -173C 69.2% predicted, $p = 0.595$). The proportion of white patients with each Scadding stage did not vary by genotype (Table 1). While the sample sizes for black patients with each Scadding stage were too small for statistical analyses, the proportions of patients with each Scadding stage appeared similar regardless of genotype.

The potential role of MIF in extrapulmonary sarcoidosis phenotypes was examined. Sixteen pa-

Table 1. The number (%) of white (A) and black (B) subjects with each Scadding stage by genotype. Chi-square tests for homogeneity were used to compare the proportions of white subjects with each Scadding stage by genotype

A

Whites				
Scadding Stage n (%)	-794 $CATT_5$ (n = 104)	-794 $CATT_{non\text{-}5}$ (n = 151)	-173G/G (n = 176)	-173C (n = 78)
0	11 (10.6)	26 (17.6)	24 (13.7)	13 (17.1)
1	29 (27.9)	32 (21.6)	42 (24.0)	18 (23.7)
2	32 (30.8)	41 (27.7)	56 (32.0)	17 (22.4)
3	15 (14.4)	19 (12.8)	24 (13.7)	10 (13.2)
4	17 (16.3)	30 (20.3)	29 (16.6)	18 (23.7)
p		0.661		0.728

B

Blacks				
Scadding Stage n (%)	-794 $CATT_5$ (n = 37)	-794 $CATT_{non\text{-}5}$ (n = 42)	-173G/G (n = 29)	-173C (n = 49)
0	3 (8.1)	2 (4.8)	1 (3.4)	4 (8.2)
1	7 (18.9)	6 (14.3)	5 (17.2)	7 (14.3)
2	11 (29.7)	13 (31.0)	11 (37.9)	13 (26.5)
3	3 (8.1)	8 (19.0)	3 (10.3)	8 (16.3)
4	13 (35.1)	13 (31.0)	9 (31.0)	17 (34.7)

tients had sarcoidosis with neurologic involvement, 4 with CNS disease (2 black and 2 white) and 12 with peripheral nerve involvement (5 black and 7 white). Among the patients with neurologic sarcoid, the -794 $CATT_5$ containing genotype was common in both racial groups (6 of 9 white patients and 5 of 6 black patients with CATT genotyping available), while the -173C SNP was more common in blacks as compared to whites (83% vs 22%). Erythema nodosum occurred in 8 whites and 1 black patient, and Lofgren syndrome occurred in 5 whites and no black patients. Among the white patients, 6 of those with erythema nodosum and 3 of those with Lofgren's syndrome had the -794 $CATT_5$ containing genotype. In contrast, the high expression -173C SNP was present in 2 of those with erythema nodosum and 1 of those with Lofgren's syndrome. Cardiac sarcoidosis was reported in 53 white and 7 black subjects. There were no differences in the frequency of the -794 $CATT_5$ containing genotype between white cardiac sarcoidosis subjects and healthy controls (46% vs. 36% in controls, p = 0.231). The -173C SNP frequency was the same in white patients with this phenotype and healthy controls (-173C: 37% vs. 40% in healthy controls, p = 0.894).

Discussion

Because MIF is associated with other autoimmune (10) and granulomatous (4,10,12) diseases, some have hypothesized that variability in *MIF* expression may contribute to sarcoidosis susceptibility or severity (6–8). Examination of the GRADS co-

hort revealed no apparent relationship between either the -794 $CATT_5$ allele or the -173C SNP and sarcoidosis development, which is consistent with previous studies (7,8). However, two groups have reported associations between the *MIF* -173C allele and sarcoidosis-related erythema nodosum (6) and Lofgren's syndrome (7). These associations were not observed in our study, but our cohort was limited by small extrapulmonary sarcoidosis subgroups. In sum, this is the third study to examine *MIF* allelic frequencies in sarcoidosis and none have found associations between MIF and disease development. In contrast, previous work has reported increased frequency of the -173C SNP in patients with erythema nodosum and Lofgren's syndrome and further examination of MIF in these phenotypes is warranted. Our study was not powered to study these sarcoidosis subtypes.

Separately, it is well established that African Americans have more severe sarcoidosis presentations than Caucasian Americans (13), and blacks in our cohort had lower DLCO than whites, which is consistent with previous work (14). Because *MIF* allelic frequencies vary by race (10), we hypothesized that some of the increased sarcoidosis severity observed in blacks could be related to differences in *MIF* genotypes. However, our patients had the same *MIF* allelic frequencies as healthy controls regardless of race. Moreover, when stratified by disease severity, there were no differences in allelic frequencies between blacks.

Other studies have examined possible determinants of sarcoidosis severity and some may be associated with race including less physical activity and more mineral exposures in blacks compared to whites (13). Improving our understanding of the environmental and genetic factors that contribute to racial disparities in sarcoidosis will be critical to bolstering the health of the African American community. While MIF may play a role in the racial differences observed in other diseases given its strong population stratification (15,16), it does not appear central to pulmonary sarcoidosis pathogenesis.

Our findings are consistent with previous reports (7,8) suggesting that *MIF* expression is not associated with sarcoidosis susceptibility or severity of pulmonary disease regardless of race.

This research was supported by the following grants: K24 HL127301 and NIH AR049610

References

1. Bucala R. MIF, MIF alleles, and prospects for therapeutic intervention in autoimmunity. J Clin Immunol. 2013 Jan;33 Suppl 1:S72-78.
2. Yao J, Leng L, Sauler M, Fu W, Zheng J, Zhang Y, et al. Transcription factor ICBP90 regulates the MIF promoter and immune susceptibility locus. J Clin Invest. 2016 Feb;126(2):732–44.
3. Sreih AG, Ezzedine R, Leng L, Fan J, Yao J, Reid D, et al. Role of Macrophage Migration Inhibitory Factor in Granulomatosis With Polyangiitis. Arthritis & Rheumatology (Hoboken, NJ). 2018 Dec;70(12):2077–86.
4. Suga M, Yamasaki H, Nakagawa K, Kohrogi H, Ando M. Mechanisms accounting for granulomatous responses in hypersensitivity pneumonitis. Sarcoidosis Vasc Diffuse Lung Dis. 1997 Sep;14(2):131–8.
5. Olivieri C, Bargagli E, Inghilleri S, Campo I, Cintorino M, Rottoli P. Macrophage migration inhibitory factor in lung tissue of idiopathic pulmonary fibrosis patients. Exp Lung Res. 2016;42(5):263–6.
6. Amoli MM, Donn RP, Thomson W, Hajeer AH, Garcia-Porrua C, Lueiro M, et al. Macrophage migration inhibitory factor gene polymorphism is associated with sarcoidosis in biopsy proven erythema nodosum. J Rheumatol. 2002 Aug;29(8):1671–3.
7. Karakaya B, van Moorsel CHM, van der Helm-van Mil AHM, Huizinga TWJ, Ruven HJT, van der Vis JJ, et al. Macrophage migration inhibitory factor (MIF) -173 polymorphism is associated with clinical erythema nodosum in Löfgren's syndrome. Cytokine. 2014 Oct;69(2):272–6.
8. Plant BJ, Ghani S, O'Mahony MJ, Morgan L, O'Connor CM, Morgan K, et al. Sarcoidosis and MIF gene polymorphism: a case-control study in an Irish population. Eur Respir J. 2007 Feb;29(2):325–9.
9. Moller DR, Koth LL, Maier LA, Morris A, Drake W, Rossman M, et al. Rationale and Design of the Genomic Research in Alpha-1 Antitrypsin Deficiency and Sarcoidosis (GRADS) Study. Sarcoidosis Protocol. Ann Am Thorac Soc. 2015 Oct;12(10):1561–71.
10. Sreih A, Ezzeddine R, Leng L, LaChance A, Yu G, Mizue Y, et al. Dual effect of the macrophage migration inhibitory factor gene on the development and severity of human systemic lupus erythematosus. Arthritis Rheum. 2011 Dec;63(12):3942–51.
11. Wu S-P, Leng L, Feng Z, Liu N, Zhao H, McDonald C, et al. Macrophage migration inhibitory factor promoter polymorphisms and the clinical expression of scleroderma. Arthritis & Rheumatism. 2006;54(11):3661–9.
12. Das R, Koo M-S, Kim BH, Jacob ST, Subbian S, Yao J, et al. Macrophage migration inhibitory factor (MIF) is a critical mediator of the innate immune response to Mycobacterium tuberculosis. Proc Natl Acad Sci USA. 2013 Aug 6;110(32):E2997-3006.
13. Caruana LB, Redwine GD, Rohde RE, Russian CJ. A prospective study of patients diagnosed with sarcoidosis: Factors - environmental exposure, health assessment, and genetic outlooks. Sarcoidosis Vasculitis and Diffuse Lung Disease. 2019 Sep 16;36(3):228–42.
14. Zhou Y, Lower EE, Feng Y, Du S, Li H, Baughman RP. Clinical characteristics of sarcoidosis patients in the United States versus China. Sarcoidosis Vasculitis and Diffuse Lung Disease. 2017;34(3):209–16.
15. Zhong X, Leng L, Beitin A, Chen R, McDonald C, Hsiao B, et al. Simultaneous detection of microsatellite repeats and SNPs in the macrophage migration inhibitory factor (MIF) gene by thin-film biosensor chips and application to rural field studies. Nucleic Acids Res. 2005;33(13):e121.
16. Reid D, Shenoi S, Singh R, Wang M, Patel V, Das R, et al. Low expression Macrophage Migration Inhibitory Factor (MIF) alleles and tuberculosis in HIV infected South Africans. Cytokine: X. 2019 Mar 1;1(1):100004.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (3); e2020004 DOI: 10.36141/svdld.v37i3.9342

Cytokine gene polymorphisms in Pigeon Breeder's Disease expression

Cláudia Freitas [1,2], Bruno Lima[3], Natália Martins[1,2,4,7], Natália Melo[1], Patrícia Mota[1,2], Hélder Novais-Bastos[1,2,4], Helena Alves[5], Oksana Sokhatska[6], Luís Delgado[6,8], António Morais[1,2]

[1]Pulmonology Department, Centro Hospitalar e Universitário de São João, Porto, Portugal; [2]Department of Medicine, Faculty of Medicine, University of Porto, Portugal; [3]Oficina de Bioestatística, Ermesinde, Portugal; [4]Institute for Research and Innovation in Health (I3S), University of Porto, Portugal; [5]National Health Institute Doutor Ricardo Jorge, Porto, Portugal; [6]Basic and Clinical Immunology Unit, Department of Pathology, Faculty of Medicine, University of Porto, Portugal; [7]Laboratory of Neuropsychophysiology, Faculty of Psychology and Education Sciences, University of Porto, Portugal; [7]Center for Health Technology and Services Research (CINTESIS), Faculty of Medicine, University of Porto, Portugal

Abstract. *Background:* Exaggerated immunological response to repeated inhalation of organic or chemical dusts may lead to Hypersensitivity Pneumonitis among sensitized individuals. Only a few exposed individuals became ill and disease expression pattern is highly variable which suggest that genetic factors may play a role. *Aim:* To investigate interferon (IFN)-γ, tumour necrosis factor (TNF)-α, interleukin (IL)-6, transforming growth factor (TGF)-ß, and IL-10 gene polymorphisms in a cohort of pigeon breeder's disease (PBD) patients in comparison with exposed but healthy controls and the association with different patterns of disease. *Methods:* We evaluated 40 PBD patients and 70 exposed controls. IFN-γ, TNF-α, IL-6, TGF-ß, and IL-10 polymorphisms were determined by polymerase chain reaction–sequence specific primer amplification. *Results:* Polymorphism analysis of IFN-γ, TNF-α, IL-6, TGF-ß, and IL-10 genotypes and allele frequencies showed no differences between patients and controls. IFN-γ T/T genotype frequency was increased among patients with chronic presentation (RR=2.33, p=0.047) compared with those with acute/subacute presentation. Also, chronic presenting patients had an increased frequency of IFN-γ T allele (50% vs 22.5%, RR=1.76, p=0.011). No differences were found in TNF-α, IL-6, TGF-ß, and IL-10 genotypes neither allelic frequencies between both groups of patients. IL-6 C/C genotype was more frequent in patients who showed chronic evolution (RR=2.54, p=0.017), when comparing with patients with disease resolution. *Conclusion:* IFN-γ T/T and the IL-6 C/C genotypes seem to play a role in HP expression due to avian exposure, as their frequencies are increased in chronic presentations or in those with chronic evolution one year after the initial diagnosis, respectively. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (3): e2020004)*

Key words: Pigeon breeder's disease, hypersensitivity pneumonitis, cytokines, genetic polimorphisms

Introduction

Hypersensitivity pneumonitis (HP) is an immunologically mediated diffuse lung disease (DLD) caused by repeated inhalation of organic and/or chemical dusts by sensitized individuals (1–3). One of the most common forms of HP is pigeon breeder's disease which results from inhalation of avian proteins (1,2). The acute phase of the disease is characterized by specific antibody reaction to inhaled antigens and a putative local immune complexes deposition within the alveolar walls, triggering a neutrophil-mediated inflammatory response (4–6). Afterwards, subacute and chronic phases may devel-

Received: 26 February 2020
Accepted after revision: 9 September 2020
Correspondence: Cláudia Freitas
Pulmonology Department, Centro Hospitalar e Universitário de São João, Porto, Portugal
E-mail: claudiaasfreitas@gmail.com

op (7). These are proposed to be mediated by macrophages (8), cytotoxic (9–11) and type 1 T helper (Th1) (12) lymphocytes with consequent increasing of several cellular immunity-related cytokines, such as interferon-gamma (IFN-γ), tumour necrosis factor (TNF)-α, interleukin (IL)-2, along with other pro-inflammatory cytokines such as IL-6 and IL-8 (13,14). Thus, a Th1-driven delayed-type hypersensitivity may eventually result in granuloma formation and, later, pulmonary fibrosis (15–17). On the other hand, Th1 activity may be under the regulatory influence of other cytokines, such as transforming growth factor (TGF)-β and IL-10 (14,18,19).

Although HP diagnostic criteria of the disease have been well discussed and proposed based on clinical, radiologic and immunological features (20,21), there are still some unexplained features, namely the reason why only a small proportion of antigen exposed individuals develop the disease and individuals with similar exposure levels have different patterns of disease expression and/or evolution. These observations suggest that, similarly to other granulomatous DLD such as sarcoidosis, individual genetic factors may play a role in susceptibility and variability of HP expression (22–26). In this sense, cytokines, while essential for cellular communication and also having a relevant role in immune response modulation, may show individual variability in tissue expression and/or circulating levels (25). Several studies have shown a connection between some cytokine gene polymorphisms (TNF-α, IFN-γ, IL-6, TGF-ß, and IL-10) and the level of expression of those cytokines (25–32).

Regarding HP, a limited number of studies relating cytokine genetic polymorphisms with disease development have been performed, with controversial results (25,29,33–35). So, we investigated IFN-γ, TNF-α, IL-6, TGF-ß, and IL-10 gene polymorphisms among Portuguese patients with pigeon breeder's disease, comparatively with exposed but healthy controls. Furthermore, we compared the same genetic polymorphisms among pigeon breeder's disease patients with different patterns of disease expression and evolution.

Material and Methods

Patients' selection

HP patients (n=40) were prospectively and consecutively recruited from the Centro Hospitalar e Universitário de São João (CHUSJ), a University Hospital in Porto, Portugal. All patients had pigeon breeder's disease (PBD) and were native Portuguese, Caucasians and unrelated. The diagnosis PBD was established by a multidisciplinary team (MDT) (36,37) according to the following criteria (38): 1) avian exposure; 2) clinical and imagiological features of HP and 3) bronchoalveolar lavage (BAL) lymphocytes > 40%. These criteria were not totally met in eleven participants, in whom HP diagnosis required surgical lung biopsy. HP patients were followed-up in our center for 6.1±3.6 years. Disease presentation was classified in acute, subacute or chronic depending on clinical features. Acute presentation was defined as development of influenza-like symptoms such as chills, fever, sweating and myalgias commonly associated to respiratory symptoms such as dyspnea, cough, chest tightness and bibasilar crackles in a few hours after antigen exposure and lasting from hours to days. Subacute presentation consisted in a gradual onset of respiratory symptoms, such as cough and dyspnea, over several days to weeks, which may progress and lead to hospitalization. Chronic presentation was defined as insidious onset of progressive exertional dyspnea and dry cough, frequently concomitant fatigue and weight loss for several months (39,40). Since there is evidence that subacute presentation is difficult to individualize and some authors propose an alternative classification in two phenotypes (38,41–43), for subgroup analysis and concerning disease presentation, we evaluated two subgroups of HP patients: acute/subacute *versus* chronic. A consensual time period since diagnosis in which HP evolution is definitely considered to have a chronic evolution is not established (38); thus, in the present study, patients who evolved to chronicity presented persistence of respiratory symptoms and/or irreversible lung fibrosis after one year of the initial diagnosis. On the other hand, disease resolution was considered when respiratory and/or systemic symptoms disappeared and there was no clinical, functional or imagiological evidence of interstitial lung disease.

All patients underwent the same HP management protocol from our center. This protocol include antigen avoidance as the first step and, if there is worsening or even persistence of symptoms and/or imagiological findings, oral corticosteroids were initiated (usually prednisolone, 0.5 mg/kg), followed by progressive tapering according to clinical evolution (44). When disease is progressive despite corticosteroids or if required for long periods, aiming to a steroid-sparing effect, other immunosuppressants are considered, namely azathioprine (2 mg/kg, with a maximum dose of 150 mg/day) or mycophenolate mofetil (1500-2000 mg/day) (45,46).

Our control group consisted of 70 exposed, unrelated, and healthy bone marrow donors, all from the same region and ethnic background of the included patients. Exposure was determined by direct questionnaire. These controls had a regular medical follow-up in their general practitioner, without any clinical or imagiological suspicion of respiratory disease. Written informed consent was obtained of all participants and the study had approval from Ethics Committee of CHUSJ.

DNA extraction

Genomic DNA was isolated from anticoagulated venous blood samples by phenol precipitation followed by digestion with proteinase K using Puregene DNA isolation Kit (Gentra Systems, Minneapolis MN).

Cytokine genotyping

Cytokine genotypes were determined by polymerase chain reaction (PCR)–sequence specific primer amplification using the Cytokine Genotyping Tray (One Lambda, Inc., Canoga Park, CA). Single-nucleotide polymorphisms for five cytokines were analyzed (47,48); the positions of the polymorphic sites tested were as follow (47,48): –174 base pair (BP) promoter/enhancer region of IL-6 (31); –1082, –819, and –592 bp promoter region of IL-10 (49,50); –308 bp promoter/enhancer region of TNF-α (49,51); CA dinucleotide repeats in intron 1 of IFN-γ (49,52); and codons 10 and 25 of the signal sequence of TGF-β (49,53,54). All typing analyses described above included positive and negative controls. All PCR products were fractionated electrophoretically in 2% agarose-gel and visualized by ethidium bromide staining and ultraviolet light.

Statistical analysis

Genotype and allele frequencies were determined by direct counting. Goodness of fit of controls' allele frequencies to Hardy-Weinberg equilibrium were analyzed using a chi-square test. Differences between groups were evaluated through chi-square or Fisher exact tests, when appropriate. Odds ratios (OR) or Relative Risks (RR) and their respective 95% confidence intervals (95% CI) were also calculated. Lung functional and BALF parameters in patients' genotypes subgroups were compared through Kruskal-Wallis test. Two-sided P-values lower than 0.05 were considered statistically significant. All analysis was performed within R Studio, a computing environment for R programming language.

Results

The included patients were older than the exposed healthy controls (48.2±15.4 *versus* 32.9±7.9 years-old; p<0.001), although with a similar gender distribution (females 60% *versus* 65%, p=0.69) and without statistically significant differences on the mean of exposure time (12.4±11.9 *versus* 8.9±7.3 years, p=0.10). Demographic and clinical features of HP patients are shown in **Table 1**. Concerning disease classification, half of the patients had acute/subacute presentations while the other half had chronic forms. Resolution was documented in 52.6% and evolution to chronicity in 47.4% of patients. No significant differences on the treatment protocol (antigen avoidance with or without immunosuppression) were seen between subgroups (data not shown).

Allele distributions of IFN-γ (+874 T/A), TNF-α (-308 A/G), IL-6 (-174), TGF-ß (codon 10 T/C, codon 25 C/G) and IL-10 (-592 C/A,- 819 T/C, -1082 G/A), revealed no statistically significant deviations from Hardy-Weinberg equilibrium in the control group (data not shown).

Analysis of the studied gene polymorphisms encoding IFN-γ, TNF-α, IL-6, TGF-ß and IL-10 and their allelic frequencies showed no statistically significant differences between HP patients and control groups (**Supplementary Tables 1 and 2**).

Table 1. Clinical and demographics characteristics of HP patients

Characteristics	HP Patients N=40
Gender,	
Female	24 (60%)
Male	16 (40%)
Age (years),	48.2±15.4
Exposure time (years)	12.4±11.9
Evolution (N=38)	
Chronicity	18 (47.4%)
Resolution	20 (52.6%)
Presentation	
Acute	11 (27.5%)
Sub-acute	9 (22.5%)
Chronic	20 (50.0%)
Lung functional parameters	
FVC % of predicted	71 (16.6-122)
FEV1 % of predicted	69 (19.2-118)
TLC % of predicted	76 (42-121.5)
DLCO % of predicted	58 (23-134)
DLCO/VA % of predicted	66 (39-168.9)
PaO2 mmHg	69 (45.4-101)
BAL features	
Cells x10-5/ml	4.6 (2-24)
Lymphocytes %	61.3 (24.4-87.6)
Neutrophils %	3.8 (0-33)
Eosinophils %	1 (0-11)
Mastocytes %	0.2 (0-2)
CD4+ lymphocytes %	39.05 (7.3-85.6)
CD8+ lymphocytes %	46.35 (7.1-80.1)
CD4+/CD8+ ratio	0.85 (0.12-12.1)

HP characteristics were described as n (%), mean ± SD or median (min-max) as appropriated.
HP Hypersensitivity pneumonitis; FVC – forced vital capacity; FEV1 – forced expiratory volume in one second; TLC – total lung capacity; DLCO – diffusing capacity of the lung for carbon monoxide; DLCO/VA – diffusing capacity of the lung for carbon monoxide divided by alveolar volume; PaO2 – partial pressure of oxygen in arterial blood; BAL – bronchoalveolar lavage.

However, when comparing patients with acute/subacute with those with chronic presentations (**Table 2**), the IFN-γ T/T (high) genotype frequencies were significantly increased among the latter (RR=2.33, p=0.047). Conversely, although not statistically significant, patients with chronic presentation tended to have lower IFN-γ A/A (low) genotype frequencies (RR= 0.5, p=0.053). Regarding allelic frequencies analysis (**Table 2**), patients with chronic presentation had an increased frequency of IFN-γ T allele (50% vs 22.5%, RR=1.76, p=0.011), comparatively to those with acute/subacute presentations. No differences were found in TNF-α, IL-6, TGF-ß, and IL-10 encoding genes neither in allelic frequencies between both groups of patients (data not shown).

Comparing patients who evolved to chronicity with those with disease resolution (**Table 3**), the IL-6 C/C (low) genotype was more frequent in those with chronic evolution (RR= 2.54, p=0.017). With regards to allelic frequencies (**Table 3**), IL-6 C allele tended to be more frequent among patients who evolved to chronicity (41.7% vs 22.5%, RR= 1.55, p=0.07). No differences were found in the studied IFN-γ, TNF-α, TGF-ß, and IL-10 gene polymorphisms, neither in its allelic frequencies, between both groups (data not shown). Finally, lung functional parameters (namely forced vital capacity, forced expiratory volume in one second, total lung capacity, diffusing capacity of the lung for carbon monoxide, diffusing capacity of the lung, partial pressure of oxygen in arterial blood) and bronchoalveolar lavage (BAL) features (such as total and relative lymphocyte numbers, CD4+ and CD8+ lymphocytes) among patients' genotypes subgroups did not show any statistically significant differences (data not shown).

Discussion

In this study, we found no statistically significant differences in genotype or allelic frequencies of single-nucleotide polymorphisms in the promoter/enhancer region of cytokine genes (IFN-γ, TNF-α, IL-6, TGF-ß, and IL-10) in this Northern Portuguese cohort of patients with pigeon breeder's disease, comparatively with healthy exposed controls. However, further evaluating the different clinical patterns of disease, our results showed that patients with a chronic presentation have higher frequencies of IFN-γ T/T (high) genotype, comparatively with

Table 2. Comparison of IFN-γ genotypes and allele frequencies in patients with chronic (n=20) and with acute/subacute (n=20) presentations.

	Chronic	Acute/Subacute	RR	CI 95%	p value†
IFN-γ (+874)					
A/A (low)	5(25%)	11(55%)	0.5	0.23 ; 1.1	0.053
T/A (int)	10(50%)	9(45%)	1.11	0.6 ; 2.06	0.752
T/T (high)	5(25%)	0(0%)	2.33	1.59 ; 3.42	0.047‡
A allele	20(50%)	31(78%)	0.57	0.37 ; 0.87	0.011
T allele	20(50%)	9(23%)	1.76	1.16 ; 2.68	

RR Relative Risk; CI Confidence Interval; †Chi-square test; ‡ Fisher exact test

Table 3. Comparison of IL-6 genotypes and allele frequencies patients whose disease evolved to chronicity (n=18) and patients with disease resolution (n=20).

	Chronicity	Resolution	RR	CI 95%	p value†
IL6 (–174)					
C/C (low)	5(28%)	0(0%)	2.54	1.66 ; 3.88	0.017‡
G/C (high)	5(28%)	9(45%)	0.66	0.3 ; 1.46	0.272
G/G (high)	8(44%)	11(55%)	0.8	0.41 ; 1.58	0.516
C allele	15(42%)	9(23%)	1.55	0.98 ; 2.43	0.073
G allele	21(58%)	31(78%)	0.65	0.41 ; 1.02	

RR Relative Risk; CI Confidence Interval; †Chi-square test; ‡ Fisher exact test

those with acute/subacute presentations. Moreover, concerning disease evolution, the IL-6 C/C (low) genotype was more frequent in patients who evolved to chronicity than in those whose disease resolved. This supports the hypothesis that individual genetic factors may play a role in the variability of HP expression namely, in pigeon breeder´s disease, functional gene polymorphisms of the IFN-γ and IL-6 cytokines.

Pigeon breeder's disease is a common HP form caused by avian antigens inhalation. Patients exposed to proteins of bird droppings and feathers, usually in a domestic environment, develop acute, subacute or chronic illnesses, and some of them evolve to diffuse pulmonary fibrosis (39,40). The underlying mechanism consists of a complex immunological process, characterized by immune complexes formation, which trigger a neutrophilic recruitment and activation, and delayed hypersensitivity involving a Th1-cell mediated immune response (12,40). Since only a small proportion of exposed individuals develop the disease and both its presentation form and evolution varies, it has been hypothesized that genetic predisposition plays a role in disease expression.

IFN-γ is one of the key cytokines in T1 (Th1 and Tc1) lymphocyte responses and is classically associated with diseases characterized by granuloma formation (55). Specifically, in HP, T1 lymphocyte responses are deleterious, and it has been proposed that INF-γ is a relevant mediator in granuloma formation and progression to fibrosis. Experimental models have shown that IFN-γ knockout mice developed minimal inflammatory response and no granulomas when exposed to a standardized antigen preparation; furthermore, when IFN-γ was administered, these mice developed granulomatous lung inflammation (56). In a similar model, IFN-γ knockout mice had a decrease in the lung recruitment of

$CXCR3^+/CD4^+$ T cells and impaired granuloma formation, associated with the reduced levels of the IFN-γ-induced chemokines (the CXCR3 ligands: CXCL9, 10 and 11) (57). These CXCR3/CXCL10 interactions were also shown to be involved in HP in humans, namely the CXCL10 expression and secretion by alveolar macrophages in response to IFN-γ, resulting in the lung recruitment of T1 lymphocytes (58,59). Other experimental studies found that treating previously exposed mice with monoclonal antibody anti-IFN-γ associates with a decrease in lung inflammatory and fibrotic response (60) and also that the protective role of CD4+/CD25+ T regulatory cells in HP is achieved through IFN-γ production suppression (61).

Additionally, and in order to highlight IFN-γ role in HP, Nance and co-workers (62) reported that not only T-cells were able to produce IFN-γ, as innate immune cells are also an important source, and sufficient to granuloma formation. These findings were supported by a study in which IL-4-producing natural killer (NK) T-cells exerted a protective role in experimental HP development by suppressing IFN-γ-producing neutrophils (63).

In our study, although no differences were found in genotype distributions of IFN-γ (+874 T/A) polymorphism between HP patients and exposed controls, the IFN-γ T/T genotype and IFN-γ T allele frequency, which associate with higher IFN-γ expression levels (52), were higher among patients with chronic presentation. In fact, a more indolent clinical presentation might be explained by the higher IFN-γ expression levels, possibly related to an increased and persistent T1-mediated (delayed) inflammation, perpetuating granuloma formation and enabling subsequent evolution to chronicity.

We did not find association of the IL-6 (-174) gene polymorphism and pigeon breeder's disease, as the genotype distributions and allelic frequencies had no differences between HP patients and exposed controls. This was corroborated by data from another study in Japan, also including summer-type HP (34). However, the IL-6 -174C/C genotype, which has been associated with reduced IL-6 expression and serum levels (31), was more frequent among patients who evolved to chronicity, i.e. those with no resolution, one year after the initial diagnosis. On the other hand, studying a limited number of 8 HP cases, Vasakova et al. (25) showed that the IL-6 -174C/G genotype associated with higher ENA-78 BALF levels, an angiogenic CXC chemokine that may participate in tissue repair (64), suggesting a possible role of IL-6 polymorphisms on HP prognosis. Interestingly, Grutters et al (65), studying 248 sarcoidosis patients from two different European countries (each with their own controls), found the IL6 -174C allele frequency increased in Stage IV sarcoidosis, suggesting it might also have a role in the genetics underlying sarcoidosis severity or progression. Moreover, this polymorphism was also suggested by Jung et al. (66) to be related to vasculitis' susceptibility, especially for large and medium vessels. In a HP mice model, the administration of neutralizing anti-IL-6 antibody was associated with a more robust neutrophilic and an fibrotic response; conversely, when IL-6 was given, the inflammatory cell recruitment and fibrotic response diminished (67). In humans, a case control study also showed that pigeon breeder´s disease patients had significantly lower BALF IL-6 levels than their asymptomatic counterparts, supporting a mechanism through IL-6 production of downregulation the lung inflammatory response to antigen exposure (68) with impact in its resolution.

TNF-α is a pleiotropic cytokine, primarily produced by activated macrophages, with broad immune regulation functions that has been closely associated to the development of HP (69). The allele frequency of TNF-2 allele (A at position-308, associated with high TNF-α production) was reported to be higher in farmer's lung disease patients (28). Camarena et al. (35) also showed a probable relevance of $TNF\text{-}2^{-308}$ allele in HP susceptibility, as it was more frequently found in PBD patients than in control groups ($p<0.05$). However, the comparison of TNF-α concentrations, either in BAL or plasma, showed no differences between patients exhibiting $TNF\text{-}2^{-308}$ with those with other TNF-α polymorphisms. In our population, no differences were found in these polymorphisms among cases and exposed but healthy subjects. Similarly, Kondoh et al (34), in a study carried out in Japan, did not find any significant difference in TNF-α polymorphisms in HP patients.

IL-10 is an important anti-inflammatory cytokine that can inhibit distinct proinflammatory cytokines production, including TNF-α, IFN-γ and IL-1ß by Th1 cells (70). High- and intermediate-producers IL-10 genotypes were more frequent in sarcoidosis patients compared with healthy controls,

suggesting its role in other granulomatous lung diseases (71). In a murine model of farmer's lung, IL-10 knockout mice had a more severe granulomatous inflammatory response than normal mice (72). Another study demonstrated that patients with HP caused by diisocyanate showed decreased IL-10 mRNA expression levels in antigen-stimulated peripheral blood mononuclear cells, whereas cells from exposed but asymptomatic subjects responded with enhancement in IL-10 mRNA expression (73). TGF-ß is another cytokine that is consistently found to be upregulated in various experimental fibrotic diseases (18), and higher TGF-ß BAL levels have been reported in HP patients than in normal subjects (74). In our study, we did not find an increased susceptibility to HP related to the studied IL-10 and TGF-ß polymorphisms, findings also corroborated by Kondoh et al (34). Also, Yang et al (75) did not find differences in the same IL-10 genotypes and alleles between PBD patients, healthy exposed and unexposed controls.

The present study has a few limitations. Firstly, only a small number of patients were enrolled and the sample size limits the statistical power. Secondly, each cytokine gene polymorphism was analysed independently and, most probably, there are other factors influencing HP phenotype, namely other genetic factors such as HLA or chemokines (76,77), or exposure features such as antigen dose, its dispersion within the respiratory tract or systemic dissemination and the repetitive, chronic nature of the exposure (40). Although the diagnosis was established with a MDT approach, HP diagnostic criteria are still not validated across different centres. Regarding the healthy exposed controls, although they had no clinical or imagiological suspicion of respiratory disease (unrelated healthy bone marrow donors, with domestic avian exposure), they were not followed up or subjected to a more detailed evaluation (lung function, HRCT scan) in order to a definite exclusion of a subclinical HP. The study has also several strengths, such as the prospective design, enrolling consecutive cases in our reference centre with a well-defined exposure and diagnosed by the same DLD multidisciplinary team. Also, patients' follow-up was taken under the care of the same experienced team and long enough to determine the definitive disease evolution at least one year after exposure cessation. Finally, only patients with PBD, which represent a subgroup of HP, were studied. In fact, the relevance of the antigen type in HP phenotype and prognosis is still controversial. (78–81). Since we only included HP patients with the same type of avian exposure, we avoided the influence of a heterogeneous antigen exposure on our analysis. Our findings supporting the influence of cytokine gene polymorphisms in the different patterns of HP expression, may also suggest that a different genetic background is at least partially responsible for the natural history of progressive fibrosing DLD. In our understanding, different genetic polymorphisms may be clinically relevant for this pattern of disease progression, with the potential to identify patients with more accelerated lung function decline and health-related quality of life deterioration that may have treatment implications (82–85).

In conclusion, in PBD, genotype and allele frequencies of the studied INF-γ, IL-6, TNF-α, TGF-ß, and IL-10 polymorphisms were not significantly different from unrelated healthy exposed controls of the same ethnic origin. However, both the IFN-γ T/T (high) and the IL-6 C/C (low) genotypes may play a role in the genetic predisposition underlying a different disease expression, as their frequencies are significantly increased in HP patients due to avian exposure with chronic presentation or no disease resolution one year after the initial diagnosis, respectively. Future collaborative and more extensive genetic studies will be needed to corroborate these findings.

Acknowledgements

We acknowledge M. Beltrão, MSc for the contribution on the bronchoalveolar lavage analysis.

N. Martins would like to thank the Portuguese Foundation for Science and Technology (FCT-Portugal) for the Strategic project ref. UID/BIM/04293/2013 and "NORTE2020 - Northern Regional Operational Program" (NORTE-01-0145-FEDER-000012)and the Horizon 2020 Program (PTDC/PSI-GER/28076/2017).

References

1. Fink JN, Ortega HG, Reynolds HY, Cormier YF, Fan LL, Franks TJ, et al. Needs and opportunities for research in hypersensitivity pneumonitis. Am J Respir Crit Care Med. 2005;171(7):792–8.
2. Selman M, Pardo A, King TE. Hypersensitivity pneumonitis: Insights in diagnosis and pathobiology. Am J Respir Crit Care Med. 2012;186(4):314–24.

3. Nogueira R, Melo N, Novais e Bastos H, Martins N, Delgado L, Morais A, et al. Hypersensitivity pneumonitis: Antigen diversity and disease implications. Pulmonology [Internet]. 2018; Available from: https://doi.org/10.1016/j.pulmoe.2018.07.003
4. Pepys J, Riddell RW, Citron KM, Clayton YM. Precipitins against extracts of hay and moulds in the serum of patients with Farmer's Lung, Aspergillosis, Asthma, and Sarcoidosis. Thorax. 1968;17(4):366–74.
5. Dreisin RB. Lung diseases associated with immune complexes. Am Rev Respir Dis. 1981;123:748–52.
6. Winck J, Delgado L, Murta R, Lopez M, Marques J. Antigen characterization of major cork moulds in Suberosis (cork worker's pneumonitis) by immunoblotting. Allergy. 2004;59(7):739–45.
7. Morais A, Winck JC, Delgado L, Palmares MC, Fonseca J, e Sá JM, et al. Suberosis and bird fancier's disease: comparative study of radiological, functional and bronchoalveolar characteristics profile. Rev Port Pneumol [Internet]. 2004;10(1):63–75. Available from: http://dx.doi.org/10.1016/S0873-2159(15)30559-6
8. Delgado JL, Ramos JP, Winck JC, Rodrigues J, Fleming-Torrinha JA. Heterogeneity of alveolar macrophages in granulomatous lung diseases. Characterization by flow cytometry. Pulmonology. 1996;1:15–43.
9. Delgado JL, Ramos JP, Winck JC, Cuesta C, Fleming-Torrinha JA. Cytotoxic lymphocytes in hypersensibility pneumonitis. Clin Exp Allergy. 1993;23(1).
10. Sokhatska O, Padrão E, Sousa-Pinto B, Beltrão M, Mota P, Melo N, et al. NK and NKT cells in the diagnosis of diffuse lung diseases presenting with a lymphocytic alveolitis. BMC Pulm Med. 2019;19(1):39.
11. Couto M, Palmares C, Beltrão M, Neves S, Mota P, Morais A, et al. Integrin α E β 7 (CD103) expression in bronchoalveolar lymphocytes of patients with hypersensitivity pneumonitis. Int Arch Occup Environ Health. 2015;88(2):167–73.
12. Yamasaki H, Ando M, Brazer W, Center DM, Cruikshank WW. Polarized type 1 cytokine profile in bronchoalveolar lavage T cells of patients with hypersensitivity pneumonitis. J Immunol [Internet]. 1999;163(6):3516–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10477626
13. Schuyler M, Gott K, Cherne A. Mediators of hypersensitivity pneumonitis. J Lab Clin Med. 2000;136(1):29–38.
14. Woda BA. Hypersensitivity pneumonitis: An immunopathology review. Arch Pathol Lab Med. 2008;132(2):204–5.
15. Patel AM, Ryu JH, Reed CE. Hypersensitivity pneumonitis: Current concepts and future questions. J Allergy Clin Immunol. 2001;108(5):661–70.
16. Semenzato G, Adami F, Maschio N, Agostini C. Immune mechanisms in interstitial lung diseases. Allergy Eur J Allergy Clin Immunol. 2000;55(12):1103–20.
17. Barrios R, Fortoul TI, Lupi-Herrera E. Pigeon breeder's disease: immunofluorescence and ultrastructural observations. Lung. 1986;164(1):55–64.
18. Bartram U, Speer CPC. The role of transforming growth factor beta in lung development and disease. Chest [Internet]. 2004;125(2):754–65. Available from: http://dx.doi.org/10.1378/chest.125.2.754
19. Gudmundsson G, Bosch A, Davidson B, Berg D, Hunninghake G. Interleukin-10 modulates the severity of hypersensitivity pneumonitis in mice. Am J Respir Cell Mol Biol. 1998;19:812.
20. Selman M. Hypersensitivity pneumonitis. In: Schwarz MI KTJ, editor. Interstitial Lung Disease. 5th ed. Shelton, USA: People's Medical Publishing House; 2011. p. 597.
21. Giacomi F De, Andreano A, Faverio P, Biffi A, Ruvolo L, Sverzellati N, et al. Utility of precipitating antibody testing in the diagnostic evaluation of chronic hypersensitivity pneumonia. Sarcoidosis, Vasc Diffus lung Dis Off J WASOG [Internet]. 2017/04/28. 2017;34(2):149–55. Available from: https://pubmed.ncbi.nlm.nih.gov/32476836
22. Karakaya B, Schimmelpennink M, Kocourkova L, van der Vis J, Meek B, Grutters J, et al. Bronchoalveolar lavage characteristics correlate with HLA tag SNPs in patients with Löfgren's syndrome and other sarcoidosis. Clin Exp Immunol. 2019;196(2):249–58.
23. Morais A, Lima B, Alves H, Melo N, Mota P, Marques A, et al. Associations between sarcoidosis clinical course and ANXA11 rs1049550 C/T, BTNL2 rs2076530 G/A, and HLA class I and II alleles. Clin Respir J. 2018;12(2):532–7.
24. Morais A, Alves H, Lima B, Delgado L, Gonçalves R, Tafulo S. HLA class I and II and TNF-alpha gene polymorphisms in sarcoidosis patients. Pulmonology. 2008;14(6):727–46.
25. Vasakova M, Sterclova M, Kolesar L, Slavcev A, Pohunek P, Sulc J, et al. Cytokine gene polymorphisms and BALF cytokine levels in interstitial lung diseases. Respir Med. 2009;103(5):773–9.
26. Alhamad EH, Cal JG, Shakoor Z, Almogren A, AlBoukai AA. Cytokine gene polymorphisms and serum cytokine levels in patients with idiopathic pulmonary fibrosis. BMC Med Genet. 2013;14(66):1–13.
27. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor promoter on transcriptional activation. Proc Natl Acad Sci. 2002;94(7):3195–9.
28. Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. J Immunol [Internet]. 2001;166(6):3915–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11238636
29. Schaaf BM, Seitzer U, Pravica V, Aries SP, Zabel P. Tumor necrosis factor-α -308 promoter gene polymorphism and increased tumor necrosis factor serum bioactivity in farmer's lung patients. Am J Respir Crit Care Med. 2001;163(2):379–82.
30. Murako G, Gaede K, Zissel G, Schlaak M, Muller-Quernheim J. Analysis of gene polymorphisms in interleukin-10 and transforming growth factor-beta 1 in sarcoidosis. Sarcoidosis, Vasc Diffus Lung Dis Off J WASOG. 2001;18(2):165e9.
31. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. The Effect of Novel Polymorphisms in the Interleukin-6 ({IL}-6) Gene on {IL}-6 Transcription and {PlasmaJuvenile} Chronic Arthritis. J Clin Invest. 1998;102(7):1369–76.
32. Pravica V, Perrey C, Stevens A, Lee J-H, Hutchinson I V. A single nucleotide polymorphism in the first intron of the human IFNy gene. Hum Immunol. 2000;61:863–6.
33. Falfán-Valencia R, Camarena A, Pineda C, Montaño M, Juárez A, Buendía-Roldán I, et al. Genetic susceptibility to multicase hypersensitivity pneumonitis is associated with the TNF-238 GG genotype of the promoter region and HLA-DRB1*04 bearing HLA haplotypes. Respir Med. 108AD;1(211–217).
34. Kondoh K, Usui Y, Ohtani Y, Inase N, Miyake S, Yoshizawa Y. Proinflammatory and anti-inflammatory cytokine gene polymorphisms in hypersensitivity pneumonitis. J Med Dent Sci. 2006;53(1):75–83.
35. Camarena A, Juárez A, Mejía M, Estrada A, Carrillo G, Falfán R, et al. Major histocompatibility complex and tumor necrosis factor-alpha polymorphisms in pigeon breeder's disease. Am J Respir Crit Care Med. 2001;163(7):1528–33.
36. Walsh S, Wells A, Desai S, Poletti V, Piciucchi S, Dubini A, et al. Multicentre evaluation of multidisciplinary team meeting agreement on diagnosis in diffuse parenchymal lung disease: a case-cohort study. Lancet Respir Med. 2016;4(7):557–65.
37. Biglia C, Ghaye B, Reychler G, Koenig S, Yildiz H, Lacroix V, et al. Multidisciplinary management of interstitial lung diseases: A real-life study. Sarcoidosis Vasc Diffus lung Dis. 2020;36(3):108–15.
38. Vasakova M, Morell F, Walsh S, Leslie K, Raghu G. Hypersensitivity Pneumonitis: Perspectives in Diagnosis and Management. Am J Respir Crit Care Med [Internet]. 2017;196(6):680–9. Available from: http://www.atsjournals.org/doi/10.1164/rccm.201611-2201PP
39. Richerson HB, Bernstein IL, Fink JN, Hunninghake GW, Novey HS, Reed CE, et al. Guidelines for the clinical evaluation of hypersensitivity pneumonitis. Report of the Subcommittee on Hypersensitivity

Pneumonitis. J Allergy Clin Immunol. 1989;84(5 PART 2):839–44.
40. Reynolds HY. Hypersensitivity pneumonitis: correlation of cellular and immunologic changes with clinical phases of disease. Lung. 1988;166(4):189–208.
41. Hanak V, Golbin J, Ryu J. Causes and presenting features in 85 consecutive patients with hypersensitivity pneumonitis. Mayo Clin Proc. 2007;82:812–6.
42. Quirce S, Vandenplas O, Campo P, Cruz M, de Blay F, Koschel D, et al. Occupational hypersensitivity pneumonitis: an EAACI Position Paper. Allergy. 2016;71:765–79.
43. Sforza GGR, Marino A. Hypersensitivity pneumonitis: a complex lung disease. Clin Mol Allergy. 2017;15(6):1–8.
44. Kokkarinen JI, Tukiainen HO, Terho EO. Effect of corticosteroid treatment on the recovery of pulmonary function in farmer's lung. Am Rev Respir Dis [Internet]. 1992;145(1):3—5. Available from: https://doi.org/10.1164/ajrccm/145.1.3
45. Morisset J, Johannson KA, Vittinghoff E, Aravena C, Elicker BM, Jones KD, et al. Use of Mycophenolate Mofetil or Azathioprine for the Management of Chronic Hypersensitivity Pneumonitis. Chest [Internet]. 2016/11/03. 2017 Mar;151(3):619–25. Available from: https://pubmed.ncbi.nlm.nih.gov/27816444
46. Terras Alexandre A, Martins N, Raimundo S, Melo N, Catetano Mota P, Novais e Bastos H, et al. Impact of Azathioprine use in chronic hypersensitivity pneumonitis patients. Pulm Pharmacol Ther. 2020;60(December 2019):101878.
47. Budak F, Göral G, Heper Y, Yilmaz E, Aymak F, Baştürk B, et al. IL-10 and IL-6 gene polymorphisms as potential host susceptibility factors in Brucellosis. Cytokine. 2007;38(1):32–6.
48. Karaoglan I, Pehlivan S, Namiduru M, Pehlivan M, Kilinçarslan C, Balkan Y, et al. TNF-alpha, TGF-beta, IL-10, IL-6 and IFN-gamma gene polymorphisms as risk factors for brucellosis. New Microbiol. 2009;32(2):173–8.
49. Perrey C, Pravica V, Sinnott PJ, Hutchinson I V. Genotyping for polymorphisms in growth factor + 1 and tumour necrosis factora genes : a technical report. Transpl Immunol. 1998;3274(98):193–7.
50. Turner D, Williams D, Sankaran D, Lazarus M, Sinnott P, Hutchinson I. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet. 1997;24(1):1–8.
51. Wilson A, di Giovine F, Blakemore M, Duff G. Single-base polymorphism in the human tumour necrosis factor-a (TNF-a) gene detectable by Ncol restriction of the PCR product. Hum Mol Genet. 1992;1(353).
52. Pravica V, Asderakis A, Perrey C, Hajeer A, Sinnott P, Hutchinson I. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. Eur J Immunogenet. 1999;26(1):1–3.
53. Awad M, El-Gamel A, Hasleton P, Turner D, Sinnott P, Hutchinson I. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation. 1998;66(8):1014–20.
54. El-Gamel A, Awad M, Hasleton P, Yonan N, Hutchinson J, Campbell C, et al. Transforming growth factor-beta (TGF-beta1) genotype and lung allograft fibrosis. J Hear Lung Transplant. 1999;18(6):517–23.
55. Actor JK, Leonard CD, Watson VE, Wells A, Jagannath C, Hunter RLJ, et al. Cytokine mRNA expression and serum 290 cortisol evaluation during murine lung inflammation induced by Mycobacterium tuberculosis. Comb Chem High Throughput Screen. 2000;3:343–51.
56. Gudmundsson G, Hunninghake GW. Interferon-gamma is necessary for the expression of hypersensitivity pneumonitis. J Clin Invest. 1997;99(10):2386–90.
57. Nance S, Cross R, Fitzpatrick E. Chemokine production during hypersensitivity pneumonitis. Eur J Immunol. 2004;34(3):677–85.
58. Agostini C, Calabrese F, Poletti V, Marcer G, Facco M, Miorin M, et al. CXCR3/CXCL10 interactions in the development of hypersensitivity pneumonitis. Respir Res. 2005;22(6):20.
59. Limongi F, Fallahi P. Hypersensitivity pneumonitis and alpha-chemokines. Clin Ther. 2017;168(2):e140–5.
60. Denis M, Ghadirian E. Murine hypersensitivity pneumonitis: Bidirectional role of interferon-gamma. Clin Exp Allergy. 1992;22(8):783–92.
61. Park Y, Oh SJ, Chung DH. CD4+CD25+ regulatory T cells attenuate hypersensitivity pneumonitis by suppressing IFN-γ production by CD4+ and CD8+ T cells. J Leukoc Biol. 2009;86(6):1427–37.
62. Nance S, Cross R, Yi AK, Fitzpatrick EA. IFN-γ production by innate immune cells is sufficient for development of hypersensitivity pneumonitis. Eur J Immunol. 2005;35(6):1928–38.
63. Hwang SJ, Kim S, Park WS, Chung DH. IL-4-Secreting NKT Cells Prevent Hypersensitivity Pneumonitis by Suppressing IFN- -Producing Neutrophils. J Immunol. 2014;177(8):5258–68.
64. Antoniou K, Tzanakis N, Tzortzaki E, Malagari K, Koutsopoulos A, Alexandrakis M, et al. Different angiogenic CXC chemokine levels in bronchoalveolar lavage fluid after interferon gamma-1b therapy in idiopathic pulmonary fibrosis patients. Pulm Pharmacol Ther. 2008;21(6):840–4.
65. Grutters J, Sato H, Pantelidis P, Ruven H, McGrath D, Wells A, et al. Analysis of IL6 and IL1A gene polymorphisms in UK and Dutch patients with sarcoidosis. Sarcoidosis, Vasc Diffus Lung Dis Off J WASOG. 2003;20(1):20–7.
66. Jung J, Seok H, Choi S, Song G, Han Y. Association between rs1800795 polymorphisms in the interleukin-6 gene and vasculitis: A meta-analysis: IL-6 polymorphisms in vasculitis. Sarcoidosis, Vasc Diffus Lung Dis Off J WASOG. 2019;36(4):302–10.
67. Denis M. Interleukin-6 in mouse hypersensitivity pneumonitis: Changes in lung free cells following depletion of endogenous IL-6 or direct administration of IL-6. J Leukoc Biol. 1992;52(2):197–201.
68. Jones KP, Reynolds SP, Capper SJ, Kalinka S, Edwards JH, Davies BH. Measurement of interleukin-6 in bronchoalveolar lavage fluid by radioimmunoassay: differences between patients with interstitial lung disease and control subjects. Clin Exp Immunol. 1991;83(1):30–4.
69. Chen B, Tong Z, Nakamura S, Costabel U, Guzman J. Production of IL-12, IL-18 and TNF-alpha by alveolar macrophages in hypersensitivity pneumonitis. Sarcoidosis, Vasc Diffus Lung Dis Off J WASOG. 2004;21:199–203.
70. Fiorentino D, Bond M, Mosnmann T. Two Types of Mouse T Helper Cell. IV. Th2 Clones Secrete a Factor that Inhibits Cytokine Production by Thl Clones. J Exp Med [Internet]. 1989;170(December 1989):2081–95. Available from: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:TWO+TYPES+OF+MOUSE+T+HELPER+CELL#5
71. Vasakova M, Sterclova M, Kolesar L, Slavcev A, Skibova J, Striz I. Cytokine gene polymorphisms in sarcoidosis. Sarcoidosis, Vasc Diffus Lung Dis Off J WASOG. 2010;27(1):70–5.
72. Gudmundsson G, Bosch A, Davidson BLB, Berg DJD, Hunninghake GWG. Interleukin-10 modulates the severity of hypersensitivity pneumonitis in mice. Am J Respir Cell Mol Biol. 1998;19(5):812.
73. Sumi Y, Kyi M, Miyazaki Y, Ohtani Y, Miyake S, Yoshizawa Y. Cytokine mRNA expression in isocyanate-induced hypersensitivity pneumonitis. Respiration. 2003;70(3):284–91.
74. Wolff H, Teppo AM, Mutanen P, Sutinen S, Backman R, Sutinen S, et al. Studies of cytokine levels in bronchoalveolar fluid lavage from patients with interstitial lung diseases. Scand J Clin Lab Invest. 2003;63(1):27–36.
75. Yang X, Wu C, Wang W, Pang B. Correlation of IL-10 and IL-2 single gene polymorphisms with the susceptibility to pigeon breeder ' s lung in Chinese Uygur population. In 2009.
76. Oshima M, Maeda A, Ishioka S, Hiyama K, Yamakido M. Expression of C-C chemokines in bronchoalveolar lavage cells from patients with granulomatous lung diseases. Lung. 1999;177(4):229–40.
77. Selman M, Pardo A, Barrera L, Estrada A, Watson SR, Wilson K, et al. Gene expression profiles distinguish idiopathic pulmonary fibro-

sis from hypersensitivity pneumonitis. Am J Respir Crit Care Med. 2006;173(2):188–98.
78. Adams TN, Newton CA, Glazer CS. Role of Antigen Type in Survival in Chronic Hypersensitivity Pneumonitis. Lung. 2019;197(1):113–4.
79. Pérez E, Swigris J, Forssén A, Tourin O, Solomon J, Huie T, et al. Identifying an Inciting Antigen Is Associated With Improved Survival in Patients With Chronic Hypersensitivity Pneumonitis. Chest. 2013;144(5):1644–51.
80. Hanak V, Golbin JM, Hartman TE, Ryu JH. High-resolution CT findings of parenchymal fibrosis correlate with prognosis in hypersensitivity pneumonitis. Chest. 2008;134(1):133–8.
81. Vourlekis JS, Schwarz MI, Cherniack RM, Curran-Everett D, Cool CD, Tuder RM, et al. The effect of pulmonary fibrosis on survival in patients with hypersensitivity pneumonitis. Am J Med. 2004;116(10):662–8.
82. Kolb M, Vašáková M. The natural history of progressive fibrosing interstitial lung diseases. BMC Respir Res. 2019;20(57):1–8.
83. Shibata S, Furusawa H, Inase N. Pirfenidone in chronic hypersensitivity pneumonitis: a real-life experience. Sarcoidosis, Vasc Diffus lung Dis Off J WASOG [Internet]. 2018/04/28. 2018;35(2):139–42. Available from: https://pubmed.ncbi.nlm.nih.gov/32476893
84. Cinetto F, Agostini C. Pirfenidone treatment in a patient with IPF and possible initial hypersensitivity pulmonitis. Sarcoidosis, Vasc Diffus Lung Dis Off J WASOG. 2013;30(Suppl 1):40–3.
85. Horimasu Y, Ishikawa N, Iwamoto H, Ohshimo S, Hamada H, Hattori N, et al. Clinical and molecular features of rapidly progressive chronic hypersensitivity pneumonitis. Sarcoidosis, Vasc Diffus lung Dis Off J WASOG [Internet]. 2017/04/28. 2017;34(1):48–57. Available from: https://pubmed.ncbi.nlm.nih.gov/32476822

Supplementary table 1. Comparison of cytokine genotypes frequencies between HP patients and controls.

Genotype (expression)	HP patients (N = 40)	Exposed Controls (N = 70)	OR	CI 95%	*p* value†
TNF-α					
A/A (high)	2 (5%)	4 (5.7%)	0.87	0.15-4.98	1
G/A (high)	7 (17.5%)	22 (31.4%)	0.46	0.18-1.2	0.17
G/G (low)	31 (77.5%)	44 (62.9%)	2.04	0.84-4.95	0.17
TGF-β					
C/C C/C (low)	0 (0%)	1 (1.4%)	0	--	1
C/C G/C (low)	2 (5%)	2 (2.9%)	1.79	0.24-13.22	0.96
C/C G/G (int)	4 (10%)	3 (4.3%)	2.48	0.53-11.69	0.44
T/C G/C (int)	3 (7.5%)	4 (5.7%)	1.34	0.28-6.31	1
T/C G/G (high)	16 (40%)	33 (47.1%)	0.75	0.34-1.65	0.60
T/T G/G (high)	15 (37.5%)	27 (38.6%)	0.96	0.43-2.14	1
IFN- γ, n (%)					
A/A (low)	16 (40%)	26 (37.1%)	1.13	0.51-2.51	0.93
T/A (int)	19 (47.5%)	29 (41.4%)	1.28	0.59-2.8	0.68
T/T (high)	5 (12.5%)	15 (21.4%)	0.52	0.17-1.56	0.36
IL-6, n (%)					
C/C (low)	5 (12.5%)	9 (12.9%)	0.97	0.3-3.12	1
G/C (high)	15 (37.5%)	32 (45.7%)	0.71	0.32-1.57	0.52
G/G (high)	20 (50%)	29 (41.4%)	1.41	0.65-3.08	0.50
IL-10, n (%)					
ACC/ACC (low)	5 (12.5%)	9 (12.9%)	0.97	0.3-3.12	1
ACC/ATA (low)	5 (12.5%)	10 (14.3%)	0.86	0.27-2.72	1
ATA/ATA (low)	3 (7.5%)	6 (8.6%)	0.86	0.2-3.64	1
GCC/ACC (int)	10 (25%)	21 (30%)	0.78	0.32-1.88	0.73
GCC/ATA (int)	10 (25%)	18 (25.7%)	0.96	0.39-2.35	1
GCC/GCC (high)	7 (17.5%)	6 (8.6%)	2.26	0.7-7.27	0.28

HP Hypersensitivity pneumonitis; OR Odds Ratio; CI Confidence Interval; †Chi-square test.

Supplementary table 2. Comparison of cytokine alleles frequencies between HP patients (n=40) and controls (n=70).

Allele	HP patients	Exposed Controls	OR	CI 95%	*p* value†
TNF-α -308					
A	11 (13.8%)	30 (21.4%)	0.58	0.28-1.24	0.159
G	69 (86.2%)	110 (78.6%)	1.71	0.81-3.63	
TGF-β codon10					
C	31 (38.8%)	49 (35%)	1.17	0.67-2.07	0.578
T	49 (61.2%)	91 (65%)	0.85	0.48-1.5	
TGF-β codon25					
C	5 (6.2%)	8 (5.7%)	1.1	0.35-3.48	0.871
G	75 (93.8%)	132 (94.3%)	0.91	0.29-2.88	
IFN- γ +874					
A	51 (63.8%)	81 (57.9%)	1.28	0.73-2.26	0.391
T	29 (36.2%)	59 (42.1%)	0.78	0.44-1.37	
IL-6 -174					
C	25 (31.2%)	50 (35.7%)	0.82	0.46-1.47	0.502
G	55 (68.8%)	90 (64.3%)	1.22	0.68-2.2	
IL-10 -1082					
A	46 (57.5%)	89 (63.6%)	0.78	0.44-1.36	0.374
G	34 (42.5%)	51 (36.4%)	1.29	0.74-2.26	
IL-10 -819					
C	59 (73.8%)	100 (71.4%)	1.12	0.61-2.09	0.711
T	21 (26.2%)	40 (28.6%)	0.89	0.48-1.65	
IL-10 -592					
A	21 (26.2%)	40 (28.6%)	0.89	0.48-1.65	0.711
C	59 (73.8%)	100 (71.4%)	1.12	0.61-2.09	

HP Hypersensitivity pneumonitis; OR Odds Ratio; CI Confidence Interval; †Chi-square test.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (3); e2020005 DOI: 10.36141/svdld.v37i3.9926

Latent tuberculosis infection associates with cardiac involvement in patients with sarcoidosis

Els Beijer[1], Annelies Bakker[2], Raisa Kraaijvanger[1], Bob Meek[3], Marco Post[2], Jan Grutters[1,4], Marcel Veltkamp[1,4]

[1] Interstitial Lung Diseases Center of Excellence, Department of Pulmonology, St. Antonius Hospital, Nieuwegein, The Netherlands; [2] Department of Cardiology, St. Antonius Hospital, Nieuwegein, The Netherlands; [3] Department of Medical Microbiology and Immunology, St. Antonius Hospital, Nieuwegein, The Netherlands; [4] Department of Pulmonology, University Medical Center, Utrecht, The Netherlands

Abstract. *Background:* Sarcoidosis is a systemic disease characterized by formation of non-caseating granulomas. About 5% of patients have symptoms of cardiac sarcoidosis. Identification of cardiac involvement is important since it is a major cause of death. Mycobacterial antigens have been linked to sarcoidosis pathogenesis. Previous findings suggest that a latent tuberculosis infection (LTBI) might associate with development of cardiac involvement in patients with sarcoidosis. The aim of the present study was to further evaluate these findings in another cohort of cardiac sarcoidosis patients. *Methods:* Interferon release assays (IGRAs) or tuberculin skin tests (TST) were analysed in a cohort of cardiac sarcoidosis patients (n=103) and compared to non-cardiac sarcoidosis patients (n=153). *Results:* In the cohort of patients with cardiac sarcoidosis, 7 could be diagnosed with a LTBI (6.8%) compared to only one of the non-cardiac patients (0.7%), p = 0.008. *Conclusions:* To conclude, we were able to show an association between a LTBI and cardiac involvement in patients with sarcoidosis. Future research is however required to unravel the mechanism involved in this association. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (3): e2020005)*

Key words: sarcoidosis, cardiac sarcoidosis, mycobacteria, tuberculosis

List of abbreviations
CI: Confidence interval
CMR: Cardiac resonance imaging
CS: Cardiac sarcoidosis
FDG-PET: Fluorodeoxyglucose positron emission tomography
HRS: Heart Rhythm Society
IGRA: Interferon gamma release assay
LTBI: Latent tuberculosis infection
MEC-U: Medical research Ethics Committees United
MTB: *Mycobacterium tuberculosis*
Non-CS: Non-cardiac sarcoidosis
OR: Odds ratio
P. acnes: *Propionibacterium acnes*
RA: Rheumatoid arthritis
TST: Tuberculin skin test

Received: 28 May 2020
Accepted after revision: 14 September 2020
Correspondence: Marcel Veltkamp
Interstitial Lung Diseases Center of Excellence,
Department of Pulmonology, St. Antonius Hospital,
Koekoekslaan 1, 3435 CM Nieuwegein, The Netherlands
e-mail address: m.veltkamp@antoniusziekenhuis.nl

Introduction

Sarcoidosis is a systemic disease characterized by the formation of noncaseating granulomas. Although intrathoracic lymph nodes and the lungs are the most common affected organs, any organ can be involved in this disease (1). About 5% of sarcoidosis patients have symptoms of cardiac sarcoidosis (CS), although the prevalence of CS is 20-30% in autopsy and imaging studies (2). Identification of cardiac involvement is important since it is a major cause of death in sarcoidosis patients (3,4).

The aetiology of sarcoidosis is not clear do date. However, several antigens have been related to sarcoidosis, including inorganic agents (5,6), auto-antigens (7) and bacteria (8). The two most extensive studied bacteria in relation to sarcoidosis pathogenesis are *Propionibacterium acnes* (*P. acnes*) (9) and Mycobacteria (10). Studies regarding *P. acnes* and mycobacteria in relation to sarcoidosis mainly focussed on cellular immune responses to antigens of *P. acnes* and mycobacteria (11,12) as well as detecting DNA or antigens in biopsy material of sarcoidosis patients and controls (13,14). Trigger related phenotypes, however, have not been described previously. In recent work from our group (15) sarcoidosis patients were tested for an immunological response towards several antigens related to sarcoidosis pathogenesis. In a total cohort of 201 patients with sarcoidosis, a latent tuberculosis infection (LTBI) was found in 5 patients (2.5%). Interestingly, when defining trigger-related phenotypes it was found that three of these LTBI patients had cardiac involvement of their sarcoidosis. The aim of the present study was to further evaluate these unexpected results. We used another cohort of CS patients to investigate whether a LTBI associates with cardiac involvement in sarcoidosis.

Methods

Study subjects

A cohort of CS patients registered in our hospital was retrospectively studied and included as CS group (approved by the local institutional review board (Z.19.004) of the St Antonius Hospital. Due to the use of clinical data, the need for informed consent was waived). A diagnosis of CS was made after advanced imaging with cardiac resonance imaging (CMR) and fluorodeoxyglucose positron emission tomography (FDG-PET). The likelihood of CS was assessed in a multidisciplinary team consisting of a pulmonologist, cardiologist, radiologist, nuclear specialist and nurse, predominantly based on the diagnostic criteria from the Heart Rhythm Society (HRS) consensus statement (16). Definite and probable CS were the gold standard for the diagnosis of CS.

Biopsy proven sarcoidosis patients or Löfgren syndrome patients without cardiac involvement, seen for the first time at the ILD outpatient clinic of the St Antonius hospital (Nieuwegein, The Netherlands) from May 1st 2016 till December 2017, were also retrospectively studied and included in the non-cardiac sarcoidosis (non-CS) group (approved by the Medical research Ethics Committees United (MEC-U) (R14.023), all these patients signed informed consent). The diagnosis of sarcoidosis had been established according to the criteria of the American Thoracic Society/European Respiratory Society (17).

Diagnosis of LTBI

Following the diagnostic criteria for sarcoidosis to exclude other causes of granulomatous disease, an Interferon Gamma Release Assay (IGRA) or tuberculin skin test (TST is performed in all sarcoidosis patients in the St. Antonius Hospital. Medical records of patients were searched for data of IGRAs (TB ELISpot or QuantiFERON tests). When data of those tests were not available, medical records were searched for results of tuberculin skin tests (TST). If IGRAs or TSTs were not performed in our hospital, available data of referring hospitals were retrieved. If data of IGRAs or TSTs were not available, patients were excluded from the study. Results of IGRAs or TSTs were compared between the CS group and the non-CS group. A diagnosis of LTBI was made when a cellular immune response against antigens of *Mycobacterium tuberculosis* (MTB) was present without bacterial, radiological or clinical signs of an active tuberculosis infection according to the current guidelines (18,19).

Statistics

Data were analysed using IBM SPSS statistics version 24. An unpaired T-test was used to compare numerical data between the non-CS and CS group. Non-parametric tests were used for non-normally distributed data (Mann-Whitney U test). Categorical data were compared using the Chi-squared test. If expected cell frequencies were below 5, Fisher's exact test was used for categorical data up to two categories. Odds ratios were calculated using a binary logistic regression model.

Results

Retrospective data of IGRAs or TSTs was available from 153 non-CS patients and 103 CS patients, which were included in the study (table 1). Establishment of the diagnosis of cardiac sarcoidosis is shown in supplementary table1. The CS group was significantly older at time of the IGRA or TST and consisted of more men. No difference in ethnicity was observed between the two study groups and also the percentage of patients that originated from another country than the Netherlands did not significantly differ between the non-CS and CS group either (3.9% vs 7.8%, p = 0.189, supplementary table 2).

In total, 7 CS patients were diagnosed with LTBI (6.8%) compared to only one patient of the non-CS group (0.7%), p = 0.008 (figure 1). When looking at the two different assays, in the CS group 6 of 96 patients had a positive IGRA (6.3%) compared to 1 of 149 patients of the non-CS group (0.7%) (p = 0.016). A positive TST was found in 1 of 7 CS patients (14.3%) and in none of the 4 non-CS patients (p = 1.000). An increased OR of 11.08 (CI: 1.34; 91.49) was observed for CS and a LTBI and an increased odds ratio (OR) of 9.87 (CI: 1.17; 83.29) was observed for a positive IGRA and CS. Furthermore, when these ORs were adjusted for age, also significant increased ORs of respectively 10.17 (CI: 1.23; 84.50) and 9.06 (CI: 1.06; 77.13) were observed.

Besides cardiac involvement, we also compared other involved organs between the 8 sarcoidosis patients with a LTBI and the other sarcoidosis patients. Prevalence of other involved organs was not different

Table 2. Organ involvement of LTBI and no LTBI sarcoidosis patients

Involved organ:	No LTBI (n=248)	LTBI (n=8)	P-value*
Heart (%)	96 (38.7)	7 (87.5)	0.008
Lymph nodes (%)	237 (96.3)	8 (100)	1.000
Lungs (%)	185 (74.6)	7 (87.5)	0.684
Nervous system (%)	36 (14.5)	0 (0.0)	0.605
Skin (%)	32 (12.9)	0 (0.0)	0.601
Eyes (%)	30 (12.1)	1 (12.5)	1.000
Bone (%)	16 (6.5)	0 (0.0)	1.000
Liver (%)	13 (5.2)	1 (12.5)	0.366
Spleen (%)	18 (7.3)	0 (0.0)	1.000

Table 1. Demographics of study patients

	Non-CS (n=153)	CS (n=103)	P-value *
IGRA / TST	149 /4	96 / 7	0.124
Age (mean ± SD)	47.23±12.64	51.39±10.87	0.005
Sex (male / female)	80 / 73	77 / 26	<0.001
Ethnicity (white / non-white)	135 / 18	92 / 11	0.788
Organ involvement:			
Lymph nodes (%)	146 (96.1)	99 (97.1)	0.744
Lungs (%)	114 (74.5)	78 (75.7)	0.825
Nervous system (%)	28 (18.3)	8 (7.8)	0.017
Skin (%)	28 (18.3)	4 (3.9)	0.001
Eyes (%)	23 (15.0)	8 (7.8)	0.081
Bone (%)	8 (5.2)	8 (7.8)	0.411
Liver (%)	7 (4.6)	7 (6.8)	0.443
Spleen (%)	5 (3.3)	13 (12.6)	0.004

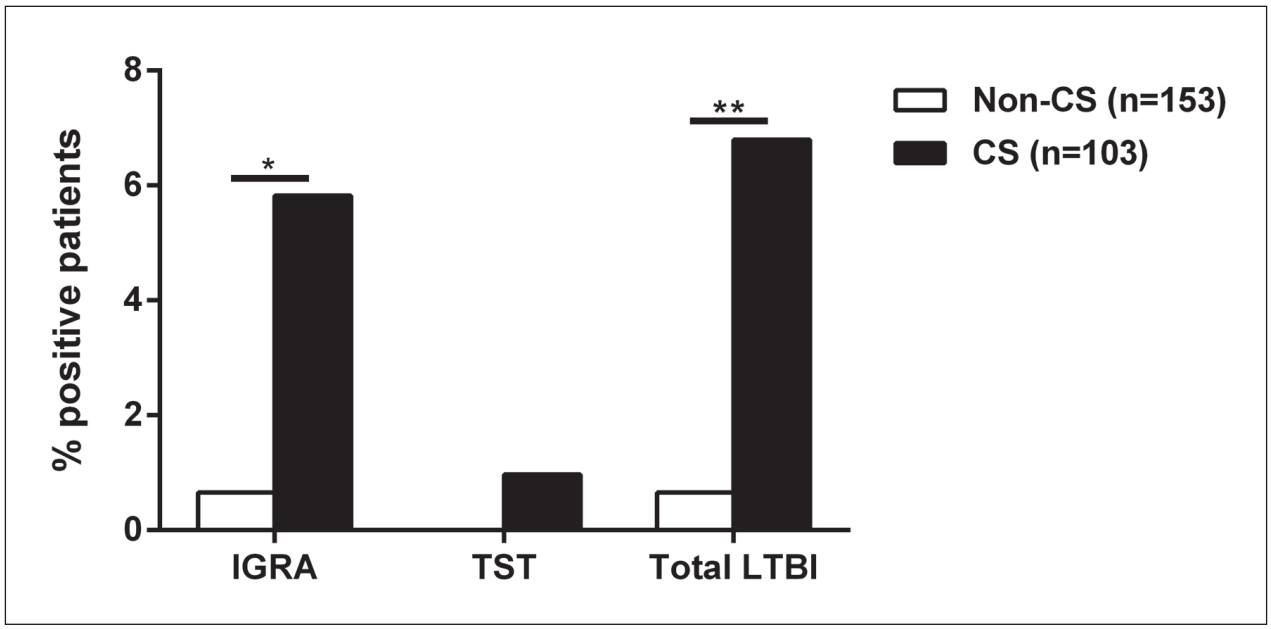

Fig. 1. Percentage of patients with a positive IGRA or TST, to determine a latent tuberculosis infection
Significant more CS patients had a positive IGRA test compared to the non-CS group (6 versus 1, respectively) (P = 0.016). In total, significant more CS patients were diagnosed with a LTBI compared to non-CS patients (7 versus 1, respectively) (P = 0.008).
IGRA included a QuantiFERON test or TB elispot.
Non-CS: Non-cardiac sarcoidosis, CS: Cardiac sarcoidosis, IGRA: Interferon gamma release assay, TST: tuberculin skin test, LTBI: latent tuberculosis infection.

between the patients with a LTBI and the remaining patients (table 2).

Discussion

In this study we were able to show that a latent tuberculosis infection is associated with cardiac involvement in patients already diagnosed with sarcoidosis, which is in line with our initial observation (15).

Although the finding that 6.8% of patients with CS were diagnosed with a LTBI seems low, this is an interesting observation since the Netherlands is a very low tuberculosis incidence country with a tuberculosis incidence of about 5 per 100.000 and a LTBI incidence of about 8 per 100.000 (20).

In previous papers, a possible link between LTBI and sarcoidosis has already been suggested (21). However, to the best of our knowledge, this is the first study to relate LTBI specifically to CS. It is known from literature that myocardial involvement in tuberculosis exists, but this is rare and has been occasionally described in case reports (22). Luk *et al*. a described a case of CS who underwent heart transplantation, developing recurrent CS in the graft following a mycobacteria tuberculosis infection (23). Since data of IGRAs or TSTs before heart transplantation were not presented, it is unclear whether this patient was suffering from a reactivation of a LTBI already present before heart transplantation, or a newly acquired tuberculosis infection. In a recent study in mice it was demonstrated that after intranasal infection, *Mycobacterium avium* was able to disseminate into cardiac tissue (24). Interestingly, infection with *Mycobacterium avium* was able to induce intracardial inflammatory gene expression and induce intracardiac tissue fibrosis. Although quite speculative, if other species of mycobacteria are also capable of inducing such damage to cardiac tissue, this could perhaps trigger a local granulomatous reaction in the heart we define as cardiac sarcoidosis. A possible explanation for our findings, may be molecular mimicry. Molecular mimicry has been described in T cell specific autoimmune diseases including multiple sclerosis and rheumatoid arthritis (RA) but also my-

ocarditis (25,26). Chodisetti *et al.* identified several T cell epitopes that are similar to peptides of mycobacterial antigens. Those epitopes may act as molecular mimics and result in an autoimmune response during an infection with *M. tuberculosis* (27). Cross-reactive antibodies with heart tissue were observed in rheumatic carditis patients (28). Further studies should clarify whether such cross reactive antibodies or shared epitopes are also present for mycobacteria and cardiac tissue.

If the association between LTBI and CS can be confirmed in further studies, this could be of interest for clinical management. For instance, it could be relevant to screen sarcoidosis patients with a LTBI for CS, even though symptoms may not be present (yet). Identification of cardiac involvement seems important since it is a major cause of death. Furthermore, in current clinical practice, if a patient is diagnosed with a LTBI they can either choose follow-up without medication or start LTBI treatment using Isoniazid/Rifampicin (29). One could speculate whether it would be beneficial for this group to start LTBI treatment instead of a wait-and-see policy directly after the diagnosis of LTBI is made.

A limitation of this retrospective study design is that the diagnosis of a LTBI was based on a positive result of different assay types (TST or IGRA). So, not all study patients had the same assay performed, although an IGRA was performed in over 95% of cases and results remained statistical significant when only LTBIs diagnosed by an IGRA were taken into account. A study regarding RA patients showed that results of IGRA tests did not correlate with use of corticosteroids, making these assays useful in RA patients (30), and likewise in sarcoidosis patients (31). Where results of IGRA are not affected by previous BCG vaccination, the TST is. However, no evidence for a previous BCG vaccination was found for the patient with a LTBI based on a positive TST. Moreover, there are several methods to search for an *Mycobacterium tuberculosis* (MTB) infection, which all have a different sensitivity and specificity. For instance, Masoud *et al.* showed that purified protein derivative antigens of MTB were present in tissue cells of 3 out of 10 sarcoidosis patients even when MTB DNA could not be detected (14). Based on our retrospective study design, we were not able to examine different methods to search for a MTB infection such as detection of mycobacterial antigens in tissue of patients. It would however be interesting to examine in future studies whether mycobacterial antigens are present in myocardial tissue of cardiac sarcoidosis patients diagnosed with a LTBI.

Another study limitation was that the non-CS and CS group were not similar regarding sex and age. Although tuberculosis is more common in the Netherlands among people with a higher age (32), the OR was still significantly increased after adjustment for age, suggesting that this difference between the groups have not induced a bias in our results. Although patients in the non-CS group did not have symptoms associated with CS, we cannot completely exclude the possibility that there might be some asymptomatic CS patients in this group, since a PET or MRI was not performed for the complete non-CS group.

To conclude, our data suggest that a latent tuberculosis infection associates with cardiac involvement in patients with sarcoidosis. Future research is required to unravel pathways involved in the association between a latent tuberculosis infection and cardiac involvement in sarcoidosis.

Funding: This study is part of the TopZorg Lung grant funded by ZonMw (nr 842002001).

References

1. Grunewald J, Grutters JC, Arkema E V., Saketkoo LA, Moller DR, Müller-Quernheim J. Sarcoidosis. Nat Rev Dis Prim. 2019 Dec 1;5(1).
2. Kouranos V, Tzelepis GE, Rapti A, Mavrogeni S, Aggeli K, Douskou M, et al. Complementary Role of CMR to Conventional Screening in the Diagnosis and Prognosis of Cardiac Sarcoidosis. JACC Cardiovasc Imaging. 2017 Dec 1;10(12):1437–47.
3. Judson MA. Screening sarcoidosis patients for cardiac sarcoidosis: What the data really show. Vol. 154, Respiratory Medicine. 2019. p. 155–7.
4. Bakker AL, Grutters JC, Keijsers RG, Post MC. Cardiac sarcoidosis: Challenges in clinical practice. Vol. 23, Current Opinion in Pulmonary Medicine. 2017. p. 468–75.
5. Beijer E, Meek B, Bossuyt X, Peters S, Vermeulen RCH, Kromhout H, et al. Immunoreactivity to metal and silica associates with sarcoidosis in Dutch patients. Respir Res [Internet]. 2020 Jun 8 [cited 2020 Jul 3];21(1):141. Available from: https://pubmed.ncbi.nlm.nih.gov/32513159/
6. Rafnsson V, Ingimarsson O, Hjalmarsson I, Gunnarsdottir H. Association between exposure to crystalline silica and risk of sarcoidosis . Occup Environ Med. 1998;55(10):657–60.
7. Kinloch AJ, Kaiser Y, Wolfgeher D, Ai J, Eklund A, Clark MR, et al. In situ humoral immunity to vimentin in HLA-DRB1*03+ patients with pulmonary sarcoidosis. Front Immunol [Internet]. 2018 Jul 9 [cited 2020 Aug 5];9(JUL). Available from: https://pubmed.ncbi.nlm.nih.gov/30038611/

8. Esteves T, Aparicio G, Garcia-Patos V. Is there any association between Sarcoidosis and infectious agents?: a systematic review and meta-analysis . BMC Pulm Med. 2016 Nov 28;16(1):165.
9. Zhou Y, Hu Y, Li H. Role of propionibacterium acnes in sarcoidosis: A meta-analysis. Vol. 30, Sarcoidosis Vasculitis and Diffuse Lung Diseases. Mattioli 1885 S.p.A.; 2013. p. 262–7.
10. Fang C, Huang H, Xu Z. Immunological Evidence for the Role of Mycobacteria in Sarcoidosis: A Meta-Analysis . PLoS One. 2016 Aug 1;11(8):e0154716.
11. Yorozu P, Furukawa A, Uchida K, Akashi T, Kakegawa T, Ogawa T, et al. Propionibacterium acnes catalase induces increased Th1 immune response in sarcoidosis patients. Respir Investig. 2015 Jul;53(4):161–9.
12. Oswald-Richter K, Sato H, Hajizadeh R, Shepherd BE, Sidney J, Sette A, et al. Mycobacterial ESAT-6 and katG are recognized by sarcoidosis CD4+ T cells when presented by the American sarcoidosis susceptibility allele, DRB1*1101. J Clin Immunol. 2010 Jan;30(1):157–66.
13. Negi M, Takemura T, Guzman J, Uchida K, Furukawa A, Suzuki Y, et al. Localization of propionibacterium acnes in granulomas supports a possible etiologic link between sarcoidosis and the bacterium. Mod Pathol [Internet]. 2012 Sep [cited 2020 Mar 20];25(9):1284–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22596102
14. Masoud S, Mihan P, Hamed M, Mehdi M, Mohamad RM. The presence of mycobacterial antigens in sarcoidosis associated granulomas. Sarcoidosis, Vasc Diffus lung Dis Off J WASOG [Internet]. 2017 [cited 2020 Aug 5];34(3):236–41. Available from: https://pubmed.ncbi.nlm.nih.gov/32476851/
15. Beijer E, Kraaijvanger R, Roodenburg C, Grutters JC, Meek B, Veltkamp M. Simultaneous testing of immunological sensitization to multiple antigens in sarcoidosis reveals an association with inorganic antigens specifically related to a fibrotic phenotype. Clin Exp Immunol. 2020 Sep 17;cei.13519
16. Birnie DH, Sauer WH, Bogun F, Cooper JM, Culver DA, Duvernoy CS, et al. HRS expert consensus statement on the diagnosis and management of arrhythmias associated with cardiac sarcoidosis. Hear Rhythm. 2014 Jul;11(7):1304–23.
17. Costabel U, Hunninghake GW. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Statement Committee. American Thoracic Society. European Respiratory Society. World Association for Sarcoidosis and Other Granulomatous Disorders. Eur Respir J. 1999;14(4):735–7.
18. Mack U, Migliori GB, Sester M, Rieder HL, Ehlers S, Goletti D, et al. LTBI: Latent tuberculosis infection or lasting immune responses to M. tuberculosis? A TBNET consensus statement. In: European Respiratory Journal [Internet]. Eur Respir J; 2009 [cited 2020 Aug 5]. p. 956–73. Available from: https://pubmed.ncbi.nlm.nih.gov/19407047/
19. Guidelines on the Management of Latent Tuberculosis Infection [Internet]. Guidelines on the Management of Latent Tuberculosis Infection. World Health Organization; 2015 [cited 2020 Aug 10]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25973515
20. Slump E, Erkens CGM, van Hunen MCJ, Schimmel HJ, van Soolingen D, de Vries G. Tuberculose in Nederland 2018- Surveillancerapport. In: Rijksinstituut voor Volksgezondheid en Millieu. 2019.
21. Oswald-Richter KA, Beachboard DC, Zhan X, Gaskill CF, Abraham S, Jenkins C, et al. Multiple mycobacterial antigens are targets of the adaptive immune response in pulmonary sarcoidosis. Respir Res. 2010 Nov 23;11:161.
22. Wallis PJW, Branfoot AC, Emerson PA. Sudden death due to myocardial tuberculosis. Thorax. 1984 Feb 1;39(2):155–6.
23. Luk A, Lee A, Ahn E, Soor GS, Ross HJ, Butany J. Cardiac sarcoidosis: Recurrent disease in a heart transplant patient following pulmonary tuberculosis infection. Can J Cardiol. 2010 Aug;26(7):e273–5.
24. Headley CA, Gerberick A, Mehta S, Wu Q, Yu L, Fadda P, et al. Nontuberculous mycobacterium M. avium infection predisposes aged mice to cardiac abnormalities and inflammation. Aging Cell. 2019 Jun;18(3):e12926.
25. Rojas M, Restrepo-Jiménez P, Monsalve DM, Pacheco Y, Acosta-Ampudia Y, Ramírez-Santana C, et al. Molecular mimicry and autoimmunity. J Autoimmun. 2018 Dec 1;95:100–23.
26. Bracamonte-Baran W, Čiháková D. Cardiac autoimmunity: Myocarditis. In: Advances in Experimental Medicine and Biology. 2017. p. 187–221.
27. Chodisetti SB, Rai PK, Gowthaman U, Pahari S, Agrewala JN. Potential T cell epitopes of Mycobacterium tuberculosis that can instigate molecular mimicry against host: implications in autoimmune pathogenesis. BMC Immunol. 2012 Mar 21;13:13.
28. Cunningham MW. Molecular Mimicry, Autoimmunity, and Infection: The Cross-Reactive Antigens of Group A Streptococci and their Sequelae. Microbiol Spectr. 2019 Jul 5;7(4).
29. Huaman MA, Sterling TR. Treatment of Latent Tuberculosis Infection—An Update. Vol. 40, Clinics in Chest Medicine. W.B. Saunders; 2019. p. 839–48.
30. Sargın G, entürk T, Ceylan E, Telli M, Çildağ S, Doğan H. Tst, Quantiferon-tb Gold test and t-spot.Tb test for detecting latent tuberculosis infection in patients with rheumatic disease prior to anti-tnf therapy. Tuberk Toraks. 2018 Jun 30;66(2):136–43.
31. Milman N, Søborg B, Svendsen CB, Andersen ÅB. Quantiferon test for tuberculosis screening in sarcoidosis patients. Scand J Infect Dis. 2011 Sep 27;43(9):728–35.
32. de Vries G, Riesmeijer R. Nationaal Plan Tuberculosebestrijding 2016-2020. Op weg naar eliminatie. [National Tuberculosis Control Plan 2016-2020. Moving towards Elimination]. 2016;1–53.

APPENDIX

Table 1 Establishment of the diagnosis of cardiac sarcoidosis

Total CS	103	
1.Histological diagnosis from myocardial tissue	3	
2a + 2b	**Histological diagnosis of extra-cardiac sarcoidosis (n=78)**	**Consensus diagnosis extra-cardiac sarcoidosis based on expert opinion (n=21)**
Steroid+/- immunosuppressant responsive cardiomyopathy or heart block	1	3
Unexplained reduced LVEF (<40%)	11	2
Unexplained sustained (spontaneous or induced) VT	9	4
Mobitz type II 2nd degree heart block or 3rd degree heart block	11	9
Patchy uptake on dedicated cardiac PET (in a pattern consistent with CS)	63	16
Late Gadolinium Enhancement on CMR (in a pattern consistent with CS)	67	15

The likelihood of cardiac sarcoidosis (CS) was assessed in a multidisciplinary team consisting of a pulmonologist, cardiologist, radiologist, nuclear specialist and nurse, predominantly based on the diagnostic criteria from the Heart Rhythm Society (HRS) consensus statement (17). Definite and probable CS were the gold standard for the diagnosis of CS

Table 2 Country of origin of study patients

Country of origin	Non-CS (n=153)	CS (n=103)
The Netherlands	146 (96.1)	95 (92.2)
Morocco	0	3 (2.9)
Curacao	0	2 (1.9)
Sri Lanka	2 (1.3)	0
Suriname	1 (0.7)	1 (1.0)
Colombia	1 (0.7)	0
Germany	0	1 (1.0)
Grenada	1 (0.7)	0
India	1 (0.7)	0
Syria	0	1 (1.0)
Unknown	1 (0.7)	0

The percentages of patients who originated from another country than the Netherlands did not significantly differ between the CS and non-CS group (p = 0.189).

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (3); e2020006 DOI: 10.36141/svdld.v37i3.9567

Fatal consequences of therapeutic thoracentesis in patients with systemic sclerosis

Tsvi Sirotkin[1], Aiman Natour[1], Ori Wand[2], Yair Levy[1]

[1]Department of Internal Medicine E, Meir Medical Center, Kfar Saba, Israel; [2]Department of Pulmonology, Meir Medical Center, Kfar Saba, Israel; Affiliated with the Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Abstract. Systemic sclerosis (SSc) is a systemic autoimmune disease, characterized by systemic fibrosis and involvement of visceral organs. Pulmonary complications are common and a leading cause of death. Pleural effusions, however, are rare. Thoracentesis is a common procedure, performed to reveal the cause of pleural effusion or to drain it and relieve dyspnea. Although generally considered a low-risk intervention, complications of thoracentesis can lead to increased morbidity and mortality. We describe three patients with SSc and symptomatic pleural effusion who required thoracentesis. All patients deteriorated shortly after the procedure and died. We assume that patients with SSc are at high-risk to develop complications after thoracentesis, most likely due to the low compliant lungs and the low elastance of the pleura. In this population, thoracentesis should be done with high caution, while measuring the pleural pressure – invasively, or with noninvasive surrogates. Further studies are required to determine mechanisms of the complication. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (3): e2020006)*

Key words: systemic sclerosis, pleural effusion, thoracentesis, pleural pressure monitoring, pulmonary hypertension

Introduction

Systemic sclerosis (SSc) is an autoimmune disease of unknown etiology, characterized by microvascular changes of intimal proliferation and dysregulated neoangiogenesis, systemic fibrosis, and involvement of visceral organs, including lungs, heart, gastrointestinal tract and kidneys (1). Pulmonary complications are common and are a leading cause of death (2-3). However, pleural effusions are rare, described in 7% of SSc patients, especially in the context of heart failure secondary to pulmonary hypertension (4-5). Pleural effusions in patients with SSc have been related to comorbidities, including concurrent infection or malignancy (6-7).

We present a case series of patients with SSc and symptomatic pleural effusion that required thoracentesis. All cases deteriorated shortly following the procedure and had fatal outcomes. Our goal is to raise awareness of thoracentesis as a potentially hazardous procedure among SSc patients.

Case series

We describe three patients with diffuse SSc who were under routine follow-up in our department. They developed pleural effusions that contributed to respiratory deterioration, leading to hospital admission. None of the patients had pericardial effusion.

Received: 19 April 2020
Accepted after revision: 9 July 2020
Correspondence: Tsvi Sirotkin MD, MHA1
1Department of Internal Medicine E,
Meir Medical Center, Kfar Saba, Israel
E-mail: tsvisirotkin@gmail.com

Patients' characteristics at admission are presented in Table 1. All three had undergone right-sided thoracentesis during hospitalization, while patient no. 2 also required pleurodesis for recurrent, intractable effusion. The patients died shortly after the pleural interventions.

Case 1

A 40-year-old male with SSc was admitted due to progressive dyspnea and anasarca. SSc was diagnosed 6 years earlier, when he presented with muscle weakness, Raynaud's phenomenon, sclerodactyly, and positive antinuclear and anti-Scl-70 antibodies. He developed pulmonary arterial hypertension (PAH), progressive interstitial lung disease (ILD), esophageal injury and right heart failure. Regular medications were carvedilol, furosemide, losartan, acetylsalicylic acid, mycophenolate mofetil, esomeprazole and macitentan. He required long-term, ambulatory oxygen therapy.

On admission, the patient was tachypneic, with signs of anasarca including ascites, limbs and abdominal wall edema. Bilateral pleural effusions, greater on the right, were identified on chest X-ray. Echocardiography showed a dilated, akinetic right ventricle due to severe pulmonary hypertension, with preserved left ventricular (LV) function. He was treated with intravenous furosemide and metolazone without significant improvement. A therapeutic thoracentesis was performed to improved dyspnea, with drainage of 1.2 liter from the right hemithorax. A few hours after thoracentesis he developed acute respiratory distress, with CO_2 accumulation and cardiovascular collapse necessitating intubation, mechanical ventilation and vasopressor therapy. After hemodynamic stabilization was achieved, he was treated with continuous intravenous infusion of epoprostanol. A right thoracic drain was inserted for continuous drainage over several days, but the patient continued to deteriorate, with multiorgan failure resulting in death. There were no signs of infection.

Table 1. Patients' characteristics

Characteristic	Patient No.		
	1	2	3
Sex	Male	Male	Female
Age at diagnosis, years	34	46	58
Duration of disease, years	6	13	3
Organs involved	Raynaud's Lungs (ILD+PAH) Skin (diffuse) Esophagus Myopathy	Raynaud's Lungs (PAH) Skin (diffuse) Esophagus Heart	Kidneys (SRC) Arthritis Skin (diffuse) Myositis Heart
Pulmonary hypertension	Yes	Yes	No
Pulmonary fibrosis	Yes	No	No
Pericardial effusion	No	No	No
Antibody status	ANA>1:640 fine speckled Anti-Scl-70	ANA>1:1000 fine speckled Anti-Scl-70	ANA>1:1280 Homogenous + nucleolar Anti-Scl-70
Pleural effusion characteristics, fluid/blood (ratio)			
Total protein (g/dL)	3.6/7.4 (0.48)	2.5/7 (0.35)	
Albumin (g/dL)		1.1/3 (0.36)	2.6/3.5 (0.74)
LDH (U/L)	336/532 (0.63)	227/369 (0.62)	313/407 (0.76)

ILD, interstitial lung disease; PAH, pulmonary arterial hypertension; SRC, scleroderma renal crisis; ANA, antinuclear antibodies; LDH, lactic dehydrogenase

Case 2

The patient was diagnosed with SSc at the age of 46, when he presented with Raynaud's phenomenon, which progressed to finger necrosis. He was treated with intravenous iloprost for several years and was positive for ANA and anti-Scl-70 antibodies. Over time, he developed PAH and esophageal injury, and complete AV block that required a pacemaker. He was maintained on iloprost, omeprazole, bosentan, nifedipine, and captopril.

Thirteen years after initial diagnosis, he was admitted with dyspnea related to bilateral pleural effusions, right larger than left, treated with repeated therapeutic thoracenteses of about 1 liter. Due to refractory right pleural effusion, he underwent right thoracoscopic pleurodesis under general anesthesia. A day after the procedure the patient deteriorated hemodynamically and developed respiratory distress. Echocardiography showed signs of severe pulmonary hypertension and a failing right ventricle, with preserved LV function. Chest imaging showed bilateral consolidations consistent with pulmonary edema, more pronounced at the right side. The patient refused endotracheal intubation and mechanical ventilation and continued to deteriorate until he succumbed.

Case 3

A 61-year-old female with SSc was admitted due to progressive dyspnea. SSc was diagnosed 3 years earlier, when she presented with myositis and arthritis that were treated with tocilizumab. She tested positive for ANA and anti Scl-70 antibodies. Two years later, she developed a scleroderma-related renal crisis, that was treated with high doses of captopril until hemodynamic stabilization was achieved. However, progressive renal failure ultimately required hemodialysis. Cardiac injury resulted in chronic left heart failure. An implantable cardioverter defibrillator (ICD) was installed for secondary prevention after an episode of ventricular tachycardia, a year prior to the current admission.

On admission, the known right pleural effusion was enlarged. At thoracentesis, 1 liter of fluid was drained. Immediately following the procedure, she became unresponsive with hemodynamic and respiratory collapse. Cardiopulmonary resuscitation was unsuccessful. ICD read did not show arrhythmia. A postmortem examination was declined by her family.

Discussion

We describe three patients with SSc and clinically significant pleural effusions requiring therapeutic thoracentesis. They had advanced heart failure secondary to SSc complications. One patient had PAH and ILD, the second had PAH, and the third had left heart failure, secondary to a hypertensive scleroderma renal crisis.

Pulmonary complications are a common visceral involvement in SSc and the leading cause of death (2-3). The two most significant forms are ILD and PAH (1). Other less common pulmonary manifestations are aspiration pneumonitis complicated by chronic gastroesophageal reflux, pulmonary hemorrhage, spontaneous pneumothorax and, uncommonly, pleural disease (8). Evidence of ILD can be found in up to 90% of patients with SSc at autopsy and in up to 85% with thin-section, high resolution computed tomography. Clinically significant ILD is less common, developing in 16–43%, and may remain asymptomatic until advanced (1, 9-10). Pulmonary hypertension develops in approximately 15% of patients with SSc, with several possible etiologies, including association with ILD, secondary to left heart disease, PAH, chronic thromboembolic disease, or multifactorial (11).

The prognosis of SSc-associated PAH is worse and treatment response poorer, than in idiopathic PAH. In many patients, it follows a downhill course with development of right heart failure. The median survival of SSc patients with untreated PAH is 1 year (2-3). In light of the poor prognosis of untreated PAH, all SSc patients should be screened for its presence at initial evaluation, and yearly echocardiographic screening for PAH is recommended.

Pleural effusions are a common presentation of heart failure. In connective tissue diseases (CTD), they may result from left heart failure or cor pulmonale mainly secondary to pulmonary hypertension, or may reflect direct pleural involvement by the disease as inflammatory serositis or pleural fibrosis. Previous studies reported higher rates of pleural effusions in patients with PAH associated with CTD, than in patients with idiopathic or familial PAH,

since some effusions might be due to the CTD itself (4), and reported a prevalence of 20-50% in patients with rheumatoid arthritis or systemic lupus erythematosus (6-7, 12). In SSc, however, pleural effusions are uncommon, and data regarding this association is surprisingly scarce. In a study which systematically evaluated patients with SSc for pleural and pericardial effusions, 4/58 had pleural effusion (7%) on chest X-ray (5). Another study compared X-ray findings in patients with "pure" SSc to patients with SSc-overlap syndromes. There were no pleural effusions in any of the 44 cases of "pure" SSc, while there were 3 cases of effusions is the 20 patients with SSc-overlap syndromes (15%) (13). In more contemporary studies using CT of the chest, pleural effusions were identified in 4/40 patients (10%) (10) ,5/28 patients (17.9%) (14), and 0/25 patients with SSc (15), but thoracentesis was not required in any case. Thus, the prevalence of pleural effusions in SSc is low, most effusions may be clinically insignificant, and some cases are probably related to comorbidities. Unlike pericardial effusions which are typically exudative reflecting active serositis (16-17), many cases of pleural effusions in SSc are transudates, and arise from heart failure and/or pulmonary hypertension, like in our subjects.

Thoracentesis is performed to reveal the cause of pleural effusion or to drain the effusion and relieve dyspnea. Although generally considered a low-risk intervention, complications, including pneumothorax, bleeding (puncture site bleeding, chest wall hematoma, hemothorax), and re-expansion pulmonary edema (REPE), can lead to increased morbidity, mortality, and healthcare costs (18). The use of ultrasonography to guide pleural procedures has been associated with fewer complications (19).

REPE is a rare complication of thoracentesis (0.01-0.5%) (20-21). It is characterized by the development of hypoxemia and new alveolar infiltrates, usually within several hours after pleural drainage. Symptoms consist of chest discomfort, persistent cough, dyspnea, and may progress to respiratory failure and hemodynamic instability. Management of these patients is supportive; diuresis, steroids, inotropic agents, and continuous positive airway pressure have all been suggested. It is believed that increased hydrostatic forces in the re-expanding lung, as well as direct injury to the alveolar-capillary barrier may contribute to REPE pathogenesis (22).

Older studies have shown that removal of large volumes of fluid are associated with the risk of REPE and cessation of fluid removal after drainage of 1-1.5 liters was advocated. However, current evidence suggests REPE is related to intrapleural pressure, rather than to the volume of fluid removed (20). In some centers, pleural pressure monitoring is available and experts recommend halting further fluid removal if end-expiratory pleural pressure drops below 20cm-H_2O. Pleural pressure can be directly measured during fluid removal with a manometer. Data suggest that the development of chest discomfort correlates with marked decreases in pleural pressure (23). In their recent study, Ault *et al.* used symptoms, including chest tightness, cough, and pain referred to the upper chest or neck, as a signal to halt fluid removal. This supports the efficacy of monitoring noninvasive, easily obtainable clinical surrogates for excessive negative pleural pressure (24).

Chowdhary *et al.* described REPE after thoracentesis in patients with left heart failure and suggested that the mechanism may be the rapid redistribution of fluid into the pulmonary extravascular space after pleural drainage, in the presence of a non-compliant LV. They postulated that significant fluid shift in the re-expanded lung (due to pulmonary edema) critically reduces the ventricular filling volume with poor LV reserve and leads to rapid cardiovascular collapse (25).

We believe that patients with SSc are prone to develop complications after thoracentesis, especially REPE, most likely due to non-compliant lungs and the low elastance of the pleura. Patients who have advanced heart failure are at high-risk for developing complications. Thoracentesis may also cause excessive vaso-vagal tone, resulting in bradycardia and hypotension that cannot be compensated for in patients with advanced cardiac failure.

Conclusions

Pleural effusion is not a rare complication in patients with SSc, especially in patients with advanced heart failure, and should be suspected in patients with respiratory deterioration. We assume that patients with SSc are at high-risk for developing complications post-thoracentesis, due to several possible mechanisms. In this population, therapeutic thora-

centesis should be performed very cautiously, while measuring pleural pressure, either invasively or with noninvasive surrogates. Prospective studies should be conducted to confirm this hypothesis.

References

1. Denton CP, Khanna D. Systemic Sclerosis. Lancet 2017; 390(10103): 1685-1699.
2. Tyndall AJ, Bannert B, Vonk M, et al. Causes and risk factors for death in systemic sclerosis: a study form the EULAR Scleroderma Trials and Research (EUSTAR) database. Ann Rheum Dis 2010;69(10):1809-1815.
3. Ioannidis JP, Vlachoyiannopoulos PG, Haidich AB, et al. Mortality in systemic sclerosis: an international meta-analysis. Am J Med 2005;118(1):2-10.
4. Luo YF, Robbins IM, Karatas M, et al. Frequency of pleural effusions in patients with pulmonary arterial hypertension associated with connective tissue diseases. Chest 2011;140(1):42-47.
5. Thompson AE, Pope JE. A study of the frequency of pericardial and pleural effusions in scleroderma. Br J Rheumatol. 1998;37(12):1320-1323.
6. Bouros D, Pneumatikos I, Tzouvelekis A. Pleural involvement in systemic autoimmune disorders. Respiration 2008;75(4):361-371.
7. Joseph J, Sahn SA. Connective tissue diseases and the pleura. Chest 1993;104(1):262-270.
8. Steen VD. The lung in systemic sclerosis. J Clin Rheumatol. 2005;11(1):40-46.
9. Kontur MR, Suresh P, Reddy VS. Systemic sclerosis with multiple pulmonary manifestations. J Clin Diagn Res. 2016;10(6):16-17.
10. Farrokh D, Abbasi B, Fallah-Rastegar Y, et al. The extrapulmonary manifestations of systemic sclerosis on chest high resolution computed tomography. Tanaffos 2015;14(3)193-200.
11. Fox BD, Shimony A, Langleben D, et al. High prevalence of occult left heart disease in scleroderma-pulmonary hypertension. Eur Respir J 2013;42(4):1083-1091.
12. Crestani B. The respiratory system in connective tissue disorders. Allergy 2005;60(6):715-734.
13. Taormina VJ, Miller WT, Gefter WB et al. Progressive systemic sclerosis subgroups: variable pulmonary features. Am J Roentgenol 1981;137:277-285.
14. Arakkal G, Chintagunta SR, Chandika V et al. Cardio-pulmonary involvement in systemic sclerosis: A study at a tertiary care center. Indian J Dermatol Venereol Leprol 2017;83(6):677-682.
15. Bonella F, Volpe A, Caramsaschi P, Nava C, Ferrari P, et al. Surfactant Protein D and KL-6 Serum Level in Systemic Sclerosis: Correlation with Lung and Systemic Sclerosis. Sarcoidosis Vasc Diffuse Lung Dis 2011; 28:27-33.
16. Kitchongcharoenying P, Foocharoen C, Mahakkanukrauh A et al. Pericardial fluid profiles of pericardial effusion in systemic sclerosis patients. Asian Pac J Allergy Immunol 2013;31(4):314-319.
17. Fernandez Morales A, Iniesta N, Fernandez-Codina A et al. Cardiac tamponade and severe pericardial effusion in systemic sclerosis: report of nine patients and review of the literature. Int J Rheum Dis 2017;20(10):1582-1592.
18. Cantey EP, Walter JM, Corbridge T, et al. Complications of thoracentesis: incidence, risk factors, and strategies for prevention. Curr Opin Pulm Med 2016;22(4):378-385.
19. Feller-Kopman D, Light R. Pleural disease. N Engl J Med 2018;378(8):740-751.
20. Feller-Kopman D, Berkowitz D, Boiselle P, et al. Large volume thoracentesis and the risk of reexpansion pulmonary edema. Ann Thorac Surg 2007;84(5):1656-1662.
21. Ault MJ, Rosen BT, Scher J, et al. Thoracentesis outcomes: a 12-year experience. Thorax 2015; 70(2):127-132.
22. Sherman SC. Reexpansion pulmonary edema: A case report and review of the current literature. J Emerg Med 2000;24(1):23-27.
23. Doelken P, Huggins JT, Pastis NJ, et al. Pleural manometry: technique and clinical implications. Chest 2004;126(6):1764-1769.
24. Feller-Kopman D, Walkey A, Berkowitz D, et al. The relationship of pleural pressure to symptom development during therapeutic thoracentesis. Chest 2006; 129(6):1556-1560.
25. Chowdhary M, Peng EW, Sarkar PK. The risk of fatal re-expansion pulmonary oedema in poor left ventricular reserve. Interact Cardiovasc Thorac Surg 2009;9(2):350-351.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (3); e2020007 DOI: 10.36141/svdld.v37i3.9346

Lung transplantation for Interstitial Lung disease, the experience of an outpatient clinic

Maria Jacob[1], *Carla Damas*[1]
[1] Pulmonology department, Centro Hospitalar Universitário de São João, Porto, Portugal

To the Editor,

Interstitial lung diseases (ILD) comprise a heterogeneous group of disorders that can lead to diffuse remodelling and structural damage to the healthy lung tissue and progressive loss of its function (1). Several ILDs are progressive, and their prognosis is often poor. Particularly, idiopathic pulmonary fibrosis (IPF) has a very poor outcome, resulting in progression to respiratory failure and death on an average of four years after the diagnosis (2). Meanwhile, other ILDs, such as idiopathic nonspecific interstitial pneumonia (NSIP), connective tissue disease-associated ILD (CTD-ILD), and chronic hypersensitivity pneumonitis (CHP), can also have a progressive course; however, their prognosis is usually more favourable than that of IPF. Although medical therapies have led to improvement in the prognosis of ILDs over the past years, they are rarely effective, and disease progression is inevitable. Therefore, lung transplant (LTx) remains as a viable treatment option (3).

LTx is a therapeutic option for selected patients with progressive and refractory ILDs that can potentially improve both the quality of life and life expectancy(4-5). According to the latest data from the International Society for Heart and Lung Transplantation (ISHLT), the second most common LTx indication is ILD (30%) and, idiopathic interstitial pneumonia (IIP), together with chronic obstructive pulmonary disease without Alpha-1-antitrypsin deficiency and cystic fibrosis, contributed the most to the growth in the number of transplants (3). The ISHLT has published specific referral and listing guidelines for ILDs (6) which include close monitoring for clinical and functional deterioration. Patients with advanced ILDs are prone to a fast decline or acute exacerbations that may require high-flow oxygen or mechanical ventilation. Although the latter could be a bridge to lung transplant, it lacks benefit in the majority of ILD patients (7). Awake extracorporeal membrane oxygenation (ECMO) as a bridge to lung transplant is gaining popularity, as improvements in technology have made it more feasible with less risk (8). Proper selection of patients is critical when deciding to implement awake ECMO support, especially in this category of patients.

The LTx outpatient clinic, at the Centro Hospitalar Universitário São João (Porto, Portugal), is a tertiary, non-transplant referring hospital. It is useful in achieving a systematic approach on the initial evaluation of LTx candidates, monitoring patients on waiting lists and also in the post-LTx follow-up. In this study, the authors aimed to show the clinical and demographic characteristics, at baseline, of ILD patients submitted to LTx. Moreover, we wanted to display the complications after LTx and survival.

This retrospective study included ILD patients evaluated for LTx at our outpatient clinic. Cases were included between 2006 and 2019. Categorical variables are presented as frequencies and percentages and

Received: 27 February 2020
Accepted after revision: 17 June 2020
Correspondence: Maria Jacob
Pulmonology Department,
Centro Hospitalar Universitário de São João
Alameda Prof. Hernâni Monteiro, 4200-319 Porto– Portugal
Email: maria.gsgj@gmail.com

were compared with the use of Fisher's test or the Chi-square test, as appropriated. Continuous variables are presented as mean and standard deviation, or median and interquartile range and were compared with the use of t-test or the Mann-Whitney test, as appropriated. Kaplan-Meier curve and the log-rank test were used to assess survival. All statistical analysis was performed using SPSS v.25.0 (IBM Corp., USA).

Overall, from the 213 patients in the LTx outpatient clinic, 72 (33.8%) have been evaluated for ILD. From those, 47 patients (65.3%) were referred for LTx and, 29 (56.9%) were transplanted. The majority were female (n=16, 55.2%), with a mean age of 48.4±11 years. Eighteen (72%) were non-smokers. The main indications for LTx were chronic hypersensitivity pneumonitis (n =13, 44.8%) and IIP (n=9, 31%; that included 7 patients with IPF and 2 with NSIP); followed by sarcoidosis (n=3, 10.8%), CTD-ILD (n=2, 6.9%; secondary to rheumatoid disease and to Sjogren's syndrome) and lymphangioleiomyomatosis (n=2, 6.9%). Unilateral LTx was performed in 24 (85.7%) patients and bilateral LTx in 4 cases (14.3%). Six months was the median while in waiting list (1 and 54 months, minimum and maximum respectively). There were two patients in which awake ECMO was used as a bridge to lung transplant: a 48 years-old women with CHP, admitted with acute exacerbation and submitted to a successful bilateral LTx (18 months in active list) and, a 46 years-old man with CHP, included in active list in the admission for an acute exacerbation, that died, while waiting for lung transplant, with acute left ventricular failure. Five (17.2%) patients died during the surgical procedure or shortly after that, and it was observed an association with males (p=0.011) and past smoking history (p=0.015), Table 1. Acute allograft rejection was diagnosed in 15 patients (51.7%), and in eleven cases happened in the first year post-transplant. In terms of late complications, the most commonly seen was chronic lung allograft dysfunction (CLAD, n=11, 44%), with a median time to rejection of 62.9 months (IQR: 72.5 months). Three patients with CLAD had, previously, acute allograft rejection. Other complications were infection (aspergillosis was diagnosed in 5 patients, CMV infection in 2 and pneumocystosis in 1) and malignancy (2 patients had squamous skin cancer). Two patients received re-transplantation. Ten patients died later on during follow-up. Survival at 1 and 5 years is 75% and 56%,

Table 1. Baseline characteristics of patients who underwent LTx – data are presented as n (%) or mean (±standard deviation). BMI: Body Mass Index; mMRC: modified Medical Research Council; FVC: forced vital capacity; DLCO: diffusing lung capacity for carbon monoxide; 6MWD: six-minute walk distance

	Total (n=29)	**Successful LTx (n=24)**	**Early death after LTx (n=5)**	**p-value**
Age, years	48.4 (±11)	48.2 (±11.3)	49.2 (10.3)	0.858
Male sex	13 (44.8)	8 (33.3%)	5 (100%)	**0.011**
BMI, kg/m2	26.5±7.1	25.8±4.8	29.9±15.6	0.638
Non-smoker	18 (62.1%)	18 (81.8%)	0	**0.015**
mMRC ≤2	21 (75%)	18 (78.3%)	3 (60%)	0.574
Oxygen requirement at rest	23 (82.1%)	20 (87%)	3 (60%)	0.207
Corticosteroid use	22 (78.6%)	17 (73.9%)	5 (100%)	0.553
Immunosuppressant use	16 (57.1%)	13 (56.5%)	3 (60%)	0.887
UIP pattern	24 (85.7%)	20 (87%)	4 (80%)	0.687
%FVC (%)	40.7±18.2	53±20.6	50.5±5.9	0.201
%DLco (%)	27.5±9.8	25.3±9.2	38.7±0.9	0.253
6MWD (m)	224.7±149	320±150.9	317.5±201.5	0.349
Median waiting time, months (IQR)	6 (7)	6 (9)	6 (8)	0.343
Unilateral LTx	24 (85.7%)	21 (87.5%)	3 (75%)	0.481

respectively. Median overall survival, after LTx, was 6.6 years, with a tendency to lower median survival in IIP (1.2 years) and CHP (4.2 years), though no statistical difference was observed between different categories (*Log Rank test*: p=0.759). The presence of acute allograft rejection is associated with a significantly lower median overall survival. CLAD seems to have better survival in the first two years after LTx, still with a tendency to worsen over time. It was diagnosed in a median 5.2 years after LTx, with a median overall survival of 7.3 years (Figure 1).

Of the non-LTx group, 18 (25%) patients were refused by the transplant centre, 7 (9.7%) were discharged (either due to transplant refusal or absen-

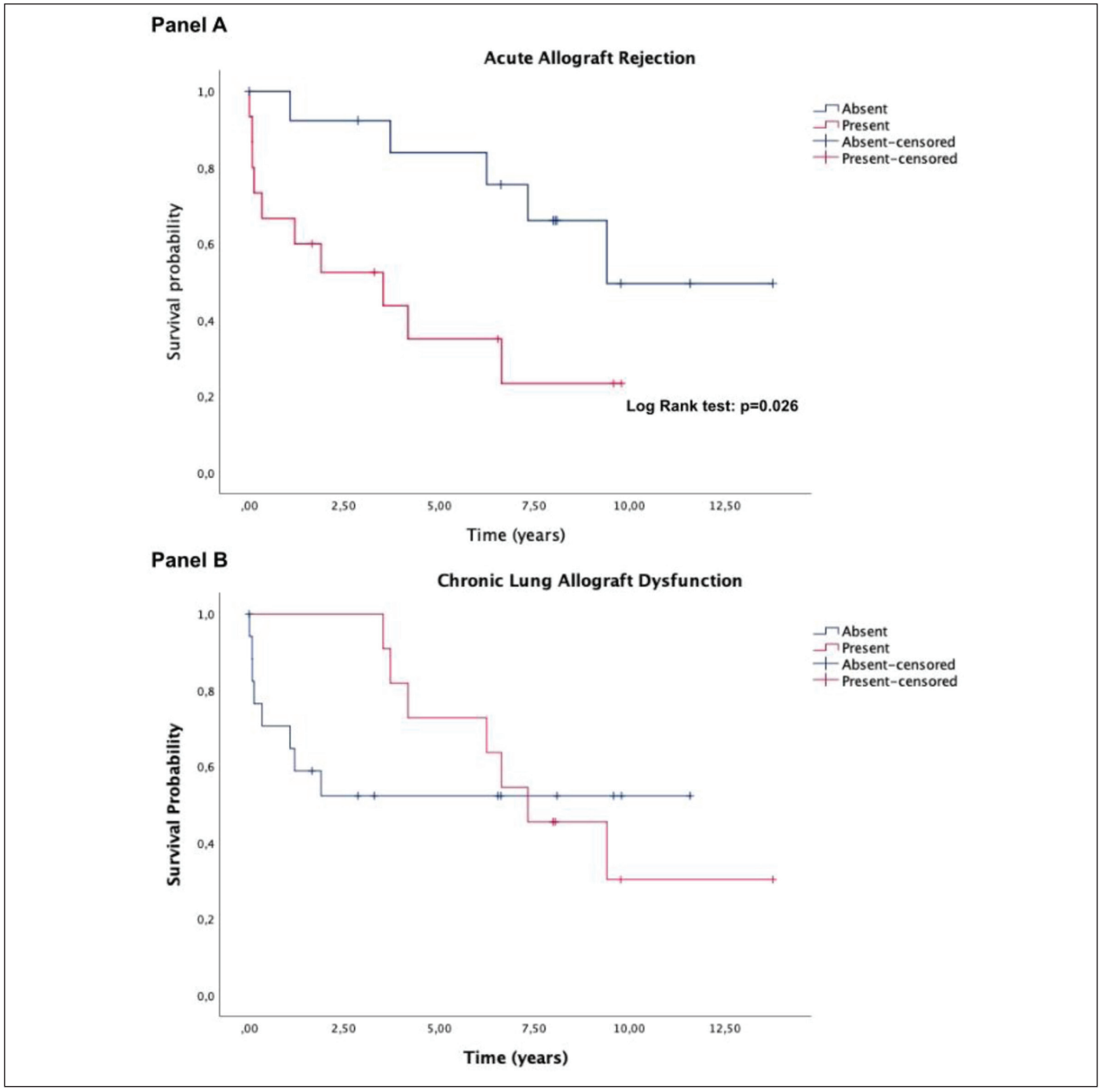

Fig. 1. Panel A - Acute allograft rejection is associated with a significantly lower median overall survival (3.5 vs. 9.4 years); Panel B - CLAD is associated with a median overall survival of 7.3 years (as in its absent, median overall survival was not reach yet). CLAD: chronic lung allograft dysfunction

teeism) and, 4 (5.6%) died while in active transplant list; the remaining are either actively on the LTx list (n=6), clinical surveillance (n=6) and in study (n=2).

Lung transplantation is challenging. The limited availability of lungs, the complexity of the medical intervention, that requires a dedicated recipient and medical team, represent just a few obstacles. Although lung transplantation in ILD has been steadily increasing in the past decades, the experience in the literature is still scarce. Our data demonstrate a higher LTx referral among patients with ILD than previously described in literature [to mention that the authors excluded patients with silicosis, previously described by Redondo, et al. (9)]. Also, the median overall survival shows a trend towards previous reports (4,6,10). More reports are needed about ILD disease and lung transplant, particularly to investigate which clinical, functional or disease specific characteristics are related with lung transplantation survival. These data support that lung transplantation remains an appropriate therapeutic option for selected ILD patients.

References

1. Travis WD, Costabel U, Hansell DM, King TE, Jr., Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. American journal of respiratory and critical care medicine. 2013;188(6):733-48.10.1164/rccm.201308-1483ST
2. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. American journal of respiratory and critical care medicine. 2011;183(6):788-824.10.1164/rccm.2009-040GL
3. Yusen RD, Edwards LB, Dipchand AI, Goldfarb SB, Kucheryavaya AY, Levvey BJ, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. J Heart Lung Transplant. 2016;35(10):1170-84.10.1016/j.healun.2016.09.001
4. Thabut G, Mal H, Castier Y, Groussard O, Brugière O, Marrash-Chahla R, et al. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. J Thorac Cardiovasc Surg. 2003;126(2):469-75.10.1016/s0022-5223(03)00600-7
5. Singer JP, Singer LG. Quality of life in lung transplantation. Semin Respir Crit Care Med. 2013;34(3):421-30.10.1055/s-0033-1348470
6. Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, et al. A consensus document for the selection of lung transplant candidates: 2014--an update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2015;34(1):1-15.10.1016/j.healun.2014.06.014
7. Faverio P, De Giacomi F, Sardella L, Fiorentino G, Carone M, Salerno F, et al. Management of acute respiratory failure in interstitial lung diseases: overview and clinical insights. BMC Pulm Med. 2018;18(1):70.10.1186/s12890-018-0643-3
8. Kearns SK, Hernandez OO. "Awake" Extracorporeal Membrane Oxygenation as a Bridge to Lung Transplant. AACN Adv Crit Care. 2016;27(3):293-300.10.4037/aacnacc2016792
9. Redondo MT, Vaz M, Damas C. End-stage silicosis and lung transplantation: A way forward. Rev Port Pneumol. 2014;20(6):341-.10.1016/j.rppneu.2014.09.004
10. De Oliveira NC, Osaki S, Maloney J, Cornwell RD, Meyer KC. Lung transplant for interstitial lung disease: outcomes for single versus bilateral lung transplantation. Interact Cardiovasc Thorac Surg. 2012;14(3):263-7.10.1093/icvts/ivr085

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2020; 37 (3); e2020008 DOI: 10.36141/svdld.v37i3.9939

Association of the calcitriol to calcifediol ratio with cardiac involvement in newly diagnosed sarcoidosis

Elias Gialafos[1,6], Lykourgos Kolilekas[2], Effrosyni Manali[3], Spyros Katsanos[5], Paschalis Steiropoulos[4], Elias Tsougos[6], Grigorios Stratakos[1], Mina Gaga[2], Nikos Koulouris[1], Spyros Papiris[3], Ioannis Ilias[7]

[1] 1st Respiratory Medicine Department, Athens Chest Hospital 'Sotiria' Medical School, National and Kapodistrian University of Athens, Greece; [2] 7th Respiratory Medicine Department, Athens Chest Hospital 'Sotiria', Athens, Greece; [3] 2nd Pulmonary Medicine Department, General University Hospital 'Attikon', Medical School, National and Kapodistrian University of Athens, Greece; [4] Department of Respiratory Medicine, University General Hospital, Medical School, Democritus University of Thrace, Greece; [5] 2nd Department of Cardiology, General University Hospital "Attikon", Medical School, National and Kapodistrian University of Athens, Greece; [6] 6th Department of Cardiology, Heart Failure and Preventive Cardiology Section, Ygeia Hospital, Athens, Greece; [7] Department of Diabetes, Endocrinology and Metabolism, Elena Venizelou Hospital, Athens, Greece

Vitamin D (VitD), a well-known regulator of calcium- and phosphate-metabolism has been shown to influence many non-skeletal conditions, including sarcoidosis and cardiovascular diseases; decreasing levels of vitamin are correlated with increased mortality[1]. In sarcoidosis (Sa), granuloma-derived interferon-gamma (among others) stimulates the production - and expresses to a high degree - one alpha hydroxylase, the enzyme that drives hydroxylation of 25(OH)D3 (calcifediol) to $1,25(OH)_2D3$ (calcitriol). The $1,25(OH)_2D3/25(OH)D3$ ratio (VDR) may reflect the efficiency of vitamin D hydroxylase activity [2]. A possible association between VitD metabolites and VDR with Sa severity has not been adequately evaluated. Cardiac involvement in Sa impairs prognosis, even with preserved left ventricular ejection fraction (EF). The aim of this study was to evaluate serum $1,25(OH)_2D3$, 25(OH)D3 and VDR vis-à-vis myocardial involvement in Sa.

In this study, we enrolled 87 newly diagnosed biopsy-proven Sa patients from our outpatient unit between March 2016 and September 2019. These were subjects who were referred for assessment of possible myocardial involvement according to Heart Rhythm Society (HRS) criteria[3]. The diagnosis of Sa was based on the presence of noncaseating granulomas on tissue biopsy specimens and compatible clinical and radiological findings based on the ATS/ERS/WASOG statement[4,5]. Inclusion criteria for this study were: patient age ≥18 years; no supplementation with calcium or vitD, absence of parathyroid dysfunction, kidney and/or liver failure. Exclusion criteria included known collagen vascular disease and cardiac dysfunction related to parathyroid disease, congenital heart disease, coronary artery disease, unrelated to sarcoidosis heart failure, valvular and pericardial disease. Also, patients with current treatment of arterial hypertension and diabetes mellitus were excluded.

All patients had a fasting morning blood collection for determination among others of inflammation markers (C-reactive protein [CRP] and fibrinogen) as well as Serum Angiotensin Converting Enzyme (SACE), Brain Natriuretic Peptide (BNP), Troponin, Parathyroid Hormone (PTH), serum calcium level, serum 25(OH)D3 and $1,25(OH)_2D3$ levels (the latter with Elecsys 25 (OH) D3 and DiaSorin Liaison 1,25 (OH)2 D3 chemilluminescence assays; Hoffman-La Roche AG, Basel, Switzerland and DiaSorin, Sallugia, VC, Italy, respectively) were

Received: 29 May 2020
Accepted after revision: 4 September 2020
Correspondence: Elias J. Giallafos, MD, PhD
Tel: 00306944399924
E-mail: gialaf@yahoo.com

used for the determination of the serum VitD metabolites levels respectively. Clinical parameters and prescribed therapies were recorded for each patient. The Body Mass Index (BMI) was calculated as the ratio of weight (Kg) per height in square (meter^2). Disease stage was assessed on chest X-ray according to the Scadding classification. All patients underwent pulmonary function testing (PFTs) and also baseline cardiac evaluation including cardiac Magnetic Resonance Imaging (c-CMR), in order to detect myocardial involvement according to HRS[4] consensus criteria. The EF and the E/E' ratio (mitral inflow E-wave divided by annular tissue e wave) were obtained from the cardiac echogram as systolic and diastolic function indices, respectively. The study protocol complied with the Declaration of Helsinki, was approved by the institutional ethics committee, and informed consent was obtained from all patients.

Table 1a. Demographic, Clinical and laboratory characteristics of all patients, without (Group A) and with Myocardial Sarcoidosis (Group B). With bold parameters with statistical significance

Parameters	All Patients (N=87)	Group A (n=66)	Group B (n=21)	p-Value
Demographic				
Sex (M)	42.53%	43,94%	38,09%	NS
Age (years)	49.51±11.5	49.67±11.65	49.04±11.49	NS
BMI(kg/m^2)	**27.73±5.17**	**27.09±5.14**	**30.06±4.63**	**0.013**
Hyperlipidemia (Yes)	22.9%	21.21%	28.6%	NS
Smoking (Yes)	20.69%	21.21%	19.05%	NS
Clinical				
Scadding's stage classification (0/1/2/3/4)	3/32/43/7/2	1/25/34/5/1	2/7/9/2/1	NS
Eye Involvement	4,59%	4,54%	4.76%	NS
Skin Involvement	13.79%	13.64%	14.29%	NS
Left Ventricular EF (%)	**62.73±3.85**	**63.26±3.54**	**60.9±4.36**	**0.009**
E/E'	7.47±2.17	7.44±2.18	7.58±2.21	NS
FEV1 (% of Predicted)	94.72±13.63	95.86±12.67	92.42±15.58	NS
FVC (% of Predicted)	97.31±14.36	98.00±12.21	96.94±18.41	NS
FEV1/FVC	80.99±7.26	81.37±7.39	79.83±6.84	NS
DLCO (% of Predicted)	84.08±17.21	84.53±17.27	82.68±17.41	NS
Laboratory				
Urea (mg/dL)	35.79±11.36	35.44±11.69	37.63±9.88	NS
Creatinine (mg/dL)	0.8±0.18	0.76±0.23	0.83±0.15	NS
CRP (mg/dL)	0.5±0.621	0.51±0.64	0.47±0.56	NS
Homocystein (μmol/L)	13.23±6.083	13.53±6.6	12.42±4.12	NS
Fibrinigen (mg/dL)	275.19±60.01	270.79±59.75	287.65±61.49	NS
25(OH)D3 (ng/mL)	**20.44±9.92**	**19.44±9.99**	**24.19±8.84**	**0.039**
1,25(OH) D3 (pg/mL)	24.85±4.57	24.61±4.53	25.9±4.64	NS
VitD Ratio	**1.5±0.77**	**1.6±0.84**	**1.16±0.33**	**0.0001**
PTH (pg/ml)	49.59±24.62	48.64±21.89	52.54±32.74	NS
Serum Calcium (mg/mL)	9.75±0.39	9.77±0.37	9.7±0.44	NS
SACE (U/L)	46.44±22.85	48.13±24.71	41.72±29.52	NS
Log BNP (pg/mL)	1.29±0.35	1.27±0.31	1.37±0.48	NS
Log Troponin (pg/mL)	0.26±0.47	0.24±0.43	0.33±0.57	NS

Table 1b. Stepwise backward logistic linear regression analysis

Variable	B (SE)	Significance	OR (95% CI)
Vitamin D ratio	-1.712 (0.738)	0.020	0.180 (0.042-0.767)
BMI	+0.249 (0.086)	0.003	1.283 (1.083-1.520)
EF	-0.196 (0.077)	0.015	0.821 (0.705-0.956)

SE: standard error; OR: odds ratio; 95% CI: 95% confidence interval

Statistical analyses were performed with SPSS (Version 20.0). Variables in the data set were expressed as mean ± standard deviation. If variables were not normally distributed median and interquartile range (IQR) were used. Dichotomous variables were expressed as frequency and percentage. Differences between continuous variables were tested for statistical significance using Student's t test or Mann-Whitney test. The Chi-squared test or Fisher's exact test were used to analyze categorical data. Further analysis for an association between possible variables (BMI, EF, VDR and 25(OH)D3) and myocardial involvement was done using stepwise backward logistic regression analysis; a two-sided P value <0.05 was considered as being statistically significant.

Table 1a presents baseline demographic, clinical, characteristics, and diagnostic findings in the 87 newly diagnosed Sa patients included in this study. According to the HRS consensus criteria, myocardial involvement was detected in 21 patients (Group B) while the rest formed (Group A). Group B had significantly higher BMI, lower EF, higher 25(OH) D3 and lower VDR (Table 1a). No significant differences were noted between the two groups regarding lung disease severity, other cardiac parameters and indices of inflammation. Logistic regression was performed to ascertain the effects of BMI, EF, VDR on the likelihood that subjects have cardiac involvement (disease stage and SACE levels were not associated with cardiac involvement). The logistic regression model was statistically significant (Chi square=21.257, p=0.0001). The obtained model correctly predicted 80.82% of cases. For each incremental increase in VDR or EF subjects were 5.55 or 1.22 times less likely to exhibit cardiac involvement, respectively, whereas for each incremental increase in BMI subjects were 0.78 times more likely to exhibit cardiac involvement (Table 1b).

Numerous clinical studies with different pathological conditions confirm an association between VitD abnormalities - especially deficiency - and increased morbidity and mortality. This assumption is based on the fact that active VitD metabolites can induce important biological effects at a molecular level in different organs. Mounting evidence suggests that VitD may influence the pathophysiology of heart failure through activation of VitD receptors in the cardiovascular system[6]. The latter interfere with the renin-angiotensin system (RAS), calcium handling, inflammatory status, and especially in cardiac fibrosis (mechanisms that active in myocardial Sa). Also, the complex and integrated regulatory pathways of VitD suggest that efficient regulation of vitD hydroxylation might be more crucial than the concentration of any D metabolite alone. Although several studies have previously reported an association between Sa and VitD, to our knowledge, this is the first one showing an association of myocardial involvement in Sa with a simple measure of VitD metabolites, thus correlating VDR with disease activity and/or possibly severit[7,8]. The exact mechanism of this association is unknown and speculative, however possible mechanisms can be mentioned. Either immunologic mechanisms through VitD metabolites to a sensitized myocardium or/ and the granuloma induced interferon-gamma and interleukin (IL) 2 production interferes with one alpha hydroxylase activity and production of active $1,25(OH)_2D3$, thus counter-regulating granuloma formation. Notably, we found that low VDR was a significant independent factor associated with the presence of cardiac involvement. Also, it is interesting to note the absence of association between pulmonary sarcoidosis with VitD metabolites, most probably due to the presence of mild pulmonary disease and the absence of important disease activity in the majority of patient[9,10]. This study was limited because we could not ascertain the duration of disease until diagnosis; furthermore it was limited by the number of patients studied and the non-inclusion of

newer markers of Sa activity such as of IL-2r or of chitotriosidase.

In conclusion, the role of Vit D metabolites and especially VDR may represent a promising simple informative tool for initially assessing cardiac involvement in Sa; further evaluation with follow-up studies in the future is ongoing.

References

1. Caristia, S.; Filigheddu, N.; Barone-Adesi, F.; Sarro, A.; Testa, T.; Magnani, C.; Aimaretti, G.; Faggiano, F.;Marzullo, P. Vitamin D as a Biomarker of Ill Health among the Over-50s: A Systematic Review of Cohort Studies. Nutrients 2019, 11, 2384.
2. Pasquali M, Tartaglione L, Rotondi S, Muci ML, Mandanici G, Farcomeni A, Marangella M, Mazzaferro S.: Calcitriol/calcifediol ratio: An indicator of vitamin D hydroxylation efficiency? BBA Clin. 2015 Jun; 3: 251–256
3. Birnie DH, Sauer WH, Bogun F, Cooper JM, Culver DA, Duvernoy CS, Judson MA, Kron J, Mehta D, Cosedis Nielsen J, Patel AR, Ohe T, Raatikainen P, Soejima K. HRS expert consensus statement on the diagnosis and management of arrhythmias associated with cardiac sarcoidosis. Heart Rhythm 2014; 11: 1305-23
4. Crouser ED, Maier LA, Wilson KC,. Bonham CA, Morgenthau AS, Patterson KC, Abston E, Bernstein RC, Blankstein R, Chen ES, Culver DA, Drake W, Drent M, Gerke AK, Ghobrial M, Govender P, Hamzeh N, James WE, Judson MA, Kellermeyer L, Knight S, Koth LL, Poletti V, Raman SV, Tukey MH,. Westney GE, and Baughman RP; on behalf of the American Thoracic Society Assembly on Clinical Problems. Diagnosis and Detection of Sarcoidosis. An Official American Thoracic Society Clinical Practice Guideline. Am J Respir Crit Care Med. 2020;201(8):e26-e51. doi:10.1164/rccm.202002-0251ST.
5. Hunninghake GW1, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, Eklund A, Kitaichi M, Lynch J, Rizzato G, Rose C, Selroos O, Semenzato G, Sharma OP.; American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 1999;16(2):149-173.
6. Nolte K, Herrmann-Lingen C, Platschek L, Holzendorf V, Pilz S, Tomaschitz A, Düngen HD, Angermann CE, Hasenfuß G, Pieske B, Wachter R, Edelmann F. Vitamin D deficiency in patients with diastolic dysfunction or heart failure with preserved ejection fraction. ESC Heart Failure 2019; 6: 262–270.
7. Niimi T, Tomita H, Sato S, Akita K, Maeda H, Kawaguchi H, Mori T, Sugiura Y, Yoshinouchi T, Ueda R. Vitamin D receptor gene polymorphism and calcium metabolism in sarcoidosis patients. Sarcoidosis Vasc Diffuse Lung Dis. 2000; 17:266–269. [PubMed: 11033842].
8. Rohmer J, Hadjadj J, Bouzerara A, Salah S, Paule R, Groh M, Blanche P, Mouthon L, Monnet D, Le Jeunne C, Guibourdenche J, Brézin A, Terrier B. Serum 1,25(OH)2 Vitamin D and 25(OH) Vitamin D Ratio for the Diagnosis of Sarcoidosis-Related Uveitis. Ocular Immunology & Inflammation, 2018; 00(00): 1–7
9. Kavathia D, Buckley JD, Rao D, Rybicki B, Burke R: Elevated 1,25-dihydroxyvitamin D levels are associated with protracted treatment in sarcoidosis. Respir Med 2010, 104:564–570.
10. Kamphuis LS, Bonte-Mineur F, van Laar JA, van Hagen PM, van Daele PL. Calcium and vitamin D in sarcoidosis: is supplementation safe?. J Bone Miner Res. 2014;29(11):2498-2503. doi:10.1002/jbmr.2262