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RESEARCH ARTICLE

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Peripheral blood natural killer cells in sarcoidosis are associated with early cardiac involvement

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Abstract

Aim: To evaluate the distribution of circulating immune cell subsets in peripheral blood of patients with sarcoidosis and investigate if there is an association with an underlying cardiac involvement.

Methods and results: Eighty-five newly diagnosed treatment-naïve patients with sarcoidosis (50 women) were included in the study. All patients underwent a thorough cardiac investigation, including cardiac magnetic resonance imaging (CMR). Of all patients, 19 (23.53%) had myocardial involvement, and the NK subpopulation in these patients in peripheral blood was significantly decreased compared to patients without (n = 63, $p = 0.001$ and $p = 0.003$ respectively). The absolute number of NKT cells (CD3+CD16/56^{+}) in patients with cardiac involvement was highly correlated with T2 map increased values in MRI ($r = -686$, $p = 0.041$) showing that low NKT cell count correlates with the inflammatory process of the heart. No difference in CD19, CD3, CD4, CD8 and CD3^{-}NK cell counts was found between groups. Lung severity was not found to correlate with the number of NK cells.

Conclusion: We found that low NK cell count in peripheral blood of patients with sarcoidosis is associated with cardiac involvement, and the number of NK-T

cells correlates with CMR findings indicative of myocardial inflammation. This finding might have a potential clinical application in detecting clinically silent cardiac involvement in sarcoidosis and may also suggest potential targets for therapeutic interventions.

KEYWORDS

cardiac magnetic resonance imaging, cardiac sarcoidosis, immunophenotype, NK cells, NK T cells

1 | BACKGROUND

Cardiac sarcoidosis (CS) is characterized by the presence of noncaseating granulomas in the heart. It often appears as a manifestation of systemic sarcoidosis, although isolated CS can occur in patients with no evidence of sarcoidosis in other organs.[1] Cardiac involvement is clinically manifested in approximately 5% of patients with systemic sarcoidosis, although the exact prevalence of CS is uncertain and is likely underestimated as many patients have nonspecific symptoms or subclinical disease. Based on autopsy and advanced imaging studies, an estimated 20%–25% of patients with systemic sarcoidosis have asymptomatic cardiac involvement (clinically silent disease).[1]

Cardiac magnetic resonance imaging (CMR) is considered a promising diagnostic modality for early detection of clinical or sub-clinical cardiac Sarcoidosis. Late gadolinium enhancement (LGE) in the myocardium, an important CMR technique for tissue characterization, has been recognized as an important prognostic factor that can lead to progressive heart failure or sudden death.[1] LGE could help quantify areas of focal myocardial fibrosis, which are substrates for ventricular arrhythmias. The presence of LGE in patients with magnetic resonance imaging (MRI) features of cardiac sarcoidosis could, therefore, aid in selecting those who could benefit from implantable cardiac defibrillator. Therefore, given the life-threatening nature of CS, it is pivotal to identify cardiac involvement on initial diagnostic evaluation through simple and easy to perform screening procedures to provide treatment when deemed necessary.[1]

Sarcoidosis is a multi-system disease of enhanced T-cell immunity at sites of granulomatous inflammation, where it expresses a highly polarized Th1 cytokine profile (Th1 polarized disorder), in contrast with common observed circulating lymphopenia.[2]

2 | AIM

We evaluated prospectively 85 consecutive patients newly diagnosed with sarcoidosis from two outpatient Sarcoidosis clinics, performing lymphocyte subset testing and cardiac magnetic resonance imaging (CMR), from 2019 to 2021. The goal of this study was to evaluate the distribution of circulating immune cell subsets in peripheral blood in a cohort of well-characterized treatment-naïve patients with sarcoidosis and investigate if there is an association with an underlying cardiac involvement as determined by CMR.

3 | METHODS

Eighty-five newly diagnosed treatment-naïve consecutive patients with sarcoidosis (50 women) were included in the study. Patient characteristics are shown in Table 1. Regarding comorbidities, in our cohort, three patients had also a diagnosis of diabetes mellitus. All patients underwent three Tesla CMR; T1 Native and T2 mapping were used to identify abnormalities with increased values because of the oedema associated with inflammation and granulomatous lesions. T1 Native and T2 are sensitive markers with ability to detect myocardial fibrosis, oedema and inflammation. Therefore, a combination of T1 Native (specific to abnormal myocardium) and T2 (discrimination between myocardial fibrosis and oedema) helps elucidate whether the cause of pathologic signal was either inflammatory (raised T2 map) or fibrotic (normal T2 map).[3] We also performed immunophenotyping of peripheral blood to evaluate CD19, CD3, CD4, CD8, NK T cell and NK cell counts.

Our study complies with the Declaration of Helsinki that the locally appointed ethics committee of Aeginition Hospital has approved the research protocol and that informed consent has been obtained from the subjects.

4 | RESULTS

In our study, 19 patients (23.53%) had myocardial involvement as detected by applying the Heart Rhythm Society (HRS) criteria (named as Group B) while the rest formed group A (the noncardiac group).[4] Fifty five of 85 patients

TABLE 1 Clinical/radiological and immunological parameters of the cohort

Parameter tested	Group A (Cardiac involvement −), $n = 20$	Group B (Cardiac involvement +), $n = 65$	All patients tested, $n = 85$	p-values (Group A vs. B)
General Characteristics				
M/F	28/37	10/10	38/47	NS
Age	50.02 ± 10.426	50.75 ± 12.328	50.51 ± 10.958	NS
Imaging				
MRI T2	47.9695 ± 4.91301	50.5356 ± 5.09008	49.109 ± 5.34982	NS
MRI T1	1254.4583 ± 42.42331	1249.625 ± 27.92816	1255.8182 ± 41.06356	NS
EF	63.24 ± 3.745	60.16 ± 5.890	62.55 ± 4.500	$p = 0.042$
Scadding stage				
0,1&2	58	19	77	NS
3,00	6	1	7	
4,00	1	0	1	
Serological parameters				
CRP	0.46 ± 0.616	0.55 ± 0.703	0.47 ± 0.625	NS
Fibrinogen	280.89±75.53	290.10±60.28	284.22±70.75	NS
SACE	47.27±31.62	48.42±41.47	47.26±33.41	NS
Troponin	2.83 ± 3.457	4.65 ± 5.581	3.2 ± 4.059	NS
BNP	23.34 ± 23.162	44.91 ± 71.860	28.9 ± 40.672	$p = 0.047$
WBC	6384.62 ± 2044.140	6465 ± 2791.580	6371.59 ± 2204.277	NS
Peripheral blood phenotype				
CD3 absolute number	1075.2769 ± 615.42329	1175.5789 ± 688.04896	1113.3793 ± 629.53682	NS
CD3%	65.9094 ± 10.50806	64.595 ± 9.69696	65.8875 ± 10.30065	NS
CD3CD4 absolute number	608.2031 ± 331.63214	736.9474 ± 469.59196	649.9419 ± 377.22867	NS
CD3CD4%	40.9809 ± 11.22818	42.4526 ± 11.46654	41.4166 ± 11.06044	NS
CD3CD8 absolute number	377.5172 ± 461.99166	361.9474 ± 258.17726	381.3733 ± 418.11239	NS
CD3CD8%	22.1638 ± 10.31822	20.1289 ± 7.40152	21.9801 ± 8.96561	NS
CD19 absolute number	207.2656 ± 144.54935	318.7895 ± 357.66179	230.3372 ± 212.94333	NS
CD19%	12.3183 ± 5.43675	15.1232 ± 6.23871	12.7418 ± 5.75385	NS
CD3-CD16+CD56+ absolute number	294.4531 ± 139.85377	305.5789 ± 153.68341	299.3488 ± 148.45738	NS
CD3-CD16+CD56+ %	20.1275 ± 9.64199	17.9416 ± 6.78882	19.5098 ± 9.06315	NS
CD3+CD16+CD56+ absolute number	103.254 ± 96.44286	56.3684 ± 41.55079	92.4941 ± 88.00103	$p = 0.003$
CD3+CD16+CD56+ %	6.4529 ± 5.20951	3.3042 ± 2.52290	5.7279 ± 4.89525	$p = 0.001$
Ratio CD4/CD8	2.2222 ± 1.04624	2.4826 ± 1.31392	2.2637 ± 1.10437	NS

Abbreviations: BNP, B-type natriuretic peptide; CRP, C-reactive protein; EF, ejection fraction; M/F, male/female ratio; MRI, magnetic resonance imaging; SACE, serum angiotensin-converting enzyme; WBC, white blood cells.

(65%) presented NK (CD3-CD16/56+) values lower than the normal range (5.9%–15.1% of total lymphocytes) of healthy individuals. Importantly, among various parameters tested, in patients with myocardial involvement (Group B), the NK subpopulation (either percentages and/or absolute numbers) in peripheral blood was significantly decreased compared to patients without ($n = 63$, $p = 0.001$ and $p = 0.003$ respectively) (Table 1). ROC curve analysis was performed to investigate the possible diagnostic utility of the absolute number of circulating CD3-positive NK cells (NK-T cells) in peripheral blood as assessed by immunophenotypic analysis. Peripheral blood NK-T cells showed an acceptable diagnostic performance; Analysis of the ROC curve was used to determine NK-T cells levels in peripheral blood as a diagnostic biomarker to differentiate sarcoidosis patients with cardiac involvement or not. The

AUC was 0.7089 (p value = 0.006), with optimal sensitivity and specificity values of 68,42 % and 66,67%, respectively, to the cut-off value of 3,385.

Moreover, in patients with myocardial involvement (Group B), the absolute number of NKT cells (CD3+CD16/56^{+}) was highly correlated with T2 map increased values in MRI ($r = -686$, $p = 0.041$, *data not shown*) showing possible interference of NKT cell with the inflammatory process of the heart. Moreover, NK-T cells (both absolute and percentages) positively correlated with T1 on MRI (Pearson Correlation = 0.475, $p = 0.005$ and Pearson Correlation = 0.463, 0 = 0.07 respectively) (Figure 1, CMR example of a sarcoidosis patient). We also compared the number of NK-T cells with various parameters clinical, radiological and serological in the cardiac group of sarcoidosis patients. NK-T cells exhibited only a trend towards positive correlation with B-type natriuretic peptide (BNP) (Pearson correlation = 0.445, $p = 0.073$, $n = 17$). No correlation was found regarding troponin levels or ejection fraction. No difference in CD19, CD3, CD4, CD8 and CD3^{-}NK cell counts was found between the two groups. Finally, lung severity according to Scadding classification was not found to correlate with the number of NK cells (Table 1).[5]

5 | DISCUSSION

We observed a significant decrease in the NK subpopulation in peripheral blood of patients with CS compared to those without. A previous report has shown significant lymphopenia, including CD4, CD8 and CD19 cells in patients with sarcoidosis with severe organ involvement compared to those with less severe manifestations.[2] However, this is the first study to examine lymphopenia considering specifically myocardial involvement. This finding, although myocardial biopsies were not performed, correlates with signs of acute inflammatory lesions as defined by T2 map in three Tesla MRI. CMR has emerged as a useful diagnostic surrogate, as endomyocardial biopsy despite being highly specific for diagnosing CS, has limited sensitivity due to disease's patchy/focal nature.[6]

The pathogenetic and regulatory role of NK cells in inflammatory heart disease remains still unclear. In general, NK cells provide a first-line defence against tumours and intracellular pathogens, and the effector mechanisms include cytotoxicity and secretion of inflammatory cytokines and chemokines.[7] The NK inflammatory response is mediated by tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ).[8] NKT cells represent a subset of T cells that share some of the receptors expressed on NK cells, and recent studies have shown that they are distinct cell populations from other T cells regarding their development, antigen recognition and function.[9] NKT cells seem to play a central role in the regulation of autoimmune responses.[10] It has been shown that the level of NKT cells is diminished early in the course of type I diabetes mellitus, both in humans and in NOD diabetic mice and especially in lpr/lpr mice, a model for studying lupus-like autoimmunity, disease was retarded by NKT.[10]

Recent reports have suggested a protective role of NK cells in sarcoidosis, supporting the hypothesis that granulomatous inflammation is favoured by NK deficiency.[11] NK cells can regulate the inflammatory cardiac environment by inhibiting viral replication, autoreactive T cells, innate lymphoid cell-derived Th2 cytokines, and activated cardiac fibroblasts and promoting eosinophil apoptosis and monocyte maturation.[11]

Previous studies identified in patients with sarcoidosis a subset of natural killer (NK) cells with immunoregulatory functions, the natural killer T cells (NKT), that express features of both T and natural killer (NK) cells (CD3+CD16/56+).[12] Impaired function of NKT cells may result in loss of the immunoregulation, which could play a critical role in the pathogenesis of sarcoidosis.[13]

NK cells have been shown to be dysregulated in many diseases characterized by cardiac inflammation, in which inflammatory cells infiltrate the myocardium leading to cardiac dysfunction, myocardial necrosis and fibrosis.[11] Clinical abnormalities in the numbers and functions of

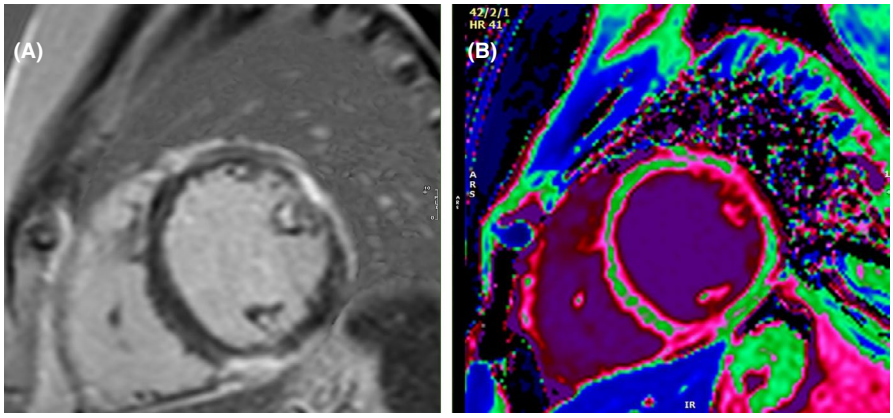

FIGURE 1 Native myocardial T1 maps in cardiac sarcoidosis. (A) Late gadolinium enhancement short-axis image, with areas of mainly subepicardial and midwall enhancement. (B) Corresponding T1 Native map short-axis image

NK cells are observed in myocarditis and inflammatory dilated cardiomyopathy. Specifically, lower percentages and total numbers of circulating NK cells with lower cytotoxicity potential and INFγ production ability have been found.[11] Frequency of NK cells has been found to be reduced in the bronchoalveolar lavage from patients with sarcoidosis compared to healthy controls and patients with other forms of interstitial lung diseases, such as idiopathic pulmonary fibrosis, chronic fibrotic hypersensitivity pneumonitis and nonspecific interstitial pneumonia.[13,14] Additionally, a study has shown that the natural killer T cells (NKT), a distinct subset of NK cells (CD56+ T-cells) with the capacity to produce large amounts of IFN-γ and TNF-a and participate actively in the T-helper (Th)-1-like inflammatory response, were found to accumulate in the lungs of patients with pulmonary sarcoidosis and may thereby enhance the inflammatory activity in the lungs.[13]

NK cells also participate in the formation of granulomas. Noncaseating epithelioid granulomas are the histologic hallmark of sarcoidosis. These are clusters containing macrophages, which will differentiate to epithelioid cells that subsequently fuse to form multinucleated giant cells, $CD4^+$ T helper cells, $CD8^+$ T cells, regulatory T cells, fibroblasts and B cells in the peripheral zone.[15] A viral infection of the heart or other antigens can elicit the activation of NK cells. By secreting cytokines, such as IFN-γ, NK cells can also contribute towards the establishment of an inflammatory, Th1-oriented response. Studies have shown that while some inflammation is required for repair during injury and is protective, prolonged inflammation leads to myocardial remodelling and apoptosis of cardiomyocytes. Moreover, persistence of an antigen in the heart leads to chronicity of the inflammation and a mature granuloma. Granulomas can heal, resulting in fibrosis, scarring and thinning of the ventricular walls with endocardial or pericardial involvement.[15] The observed lymphopenia in peripheral blood could be explained either by the deficiency in frequency and function of NK cells possibly caused by immunological abnormality, inherent defect or increased sensitivity to apoptosis, or by the translocation of NK cells to inflamed tissues due to the presence of chemo-attractants and cytokines and participation in immune responses.

CMR mapping offers a substantial value in detecting subclinical myocardial involvement in systemic sarcoidosis when other tests are normal. T2 mapping is more specific for active myocardial inflammation and oedema, whereas T1 mapping is sensitive (but lacks specificity) for myocardial fibrosis.[16] Our results from peripheral blood analysis implicate NK-T cells in active inflammatory processes in myocardium and homing to the inflamed tissue could be hypothesized (negative correlation among NK-T cells and T2 mapping). Histopathology evidence is needed to strengthen our results. Patients with lower NK-T cells displayed lower T1 mapping levels (positive correlation among NK-T cells and T1 mapping), a finding that could be interpreted by the short disease duration and the balance among scarring and active inflammation in the affected cardiac tissue.

In conclusion, our study found that low NK cell count in peripheral blood of patients with sarcoidosis is associated with cardiac involvement, and the number of NK-T cells correlates with CMR findings indicative of myocardial inflammation. This finding might have clinical application as a screening strategy to detect clinically silent cardiac involvement in sarcoidosis and may also suggest potential targets for therapeutic interventions.

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CONFLICTS OF INTEREST

No conflicts of interest.

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REFERENCES

1. Hulten E, Aslam S, Osborne M, Abbasi S, Bittencourt MS, Blankstein R. Cardiac sarcoidosis-state of the art review. *Cardiovasc Diagn Ther*. 2016;6:50-63.
2. Sweiss NJ, Salloum R, Gandhi S, et al. Significant CD4, CD8, and CD19 lymphopenia in peripheral blood of sarcoidosis patients correlates with severe disease manifestations. *PLoS One*. 2010;5:e9088.
3. Puntmann VO, Isted A, Hinojar R, Foote L, Carr-White G, Nagel E. T1 and T2 mapping in recognition of early cardiac involvement in systemic sarcoidosis. *Radiology*. 2017;285:63-72.
4. Crouser ED, Maier LA, Wilson KC, et al. Diagnosis and Detection of Sarcoidosis. An Official American Thoracic Society Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2020;201:e26-e51.
5. Scadding JG. Prognosis of intrathoracic sarcoidosis in England. A review of 136 cases after five years' observation. *Br Med J*. 1961;2:1165-1172.
6. Blankstein R, Waller AH. Evaluation of known or suspected cardiac sarcoidosis. *Circ Cardiovasc Imaging*. 2016;9:e000867.
7. Vivier E, Tomasello E, Baratin M, et al. Functions of natural killer cells. *Nat Immunol*. 2008;9:503-510.
8. Hua X, Song J. Immune cell diversity contributes to the pathogenesis of myocarditis. *Heart Fail Rev*. 2019;24:1019-1030.
9. Godfrey DI, Hammond KJ, Poulton LD, Smyth MJ, Baxter AG. NKT cells: facts, functions and fallacies. *Immunol Today*. 2000;21:573-583.
10. Rizzo C, La Barbera L, Lo Pizzo M, Ciccia F, Sireci G, Guggino G. Invariant NKT Cells and rheumatic disease: focus on primary sjogren syndrome. *Int J Mol Sci*. 2019;20:5435.

11. Ong S, Rose NR, Čiháková D. Natural killer cells in inflammatory heart disease. *Clin Immunol*. 2017;175:26-33.
12. Ho LP, Urban BC, Thickett DR, Davies RJ, McMichael AJ. Deficiency of a subset of T-cells with immunoregulatory properties in sarcoidosis. *Lancet*. 2005;365:1062-1072.
13. Katchar K, Söderström K, Wahlstrom J, Eklund A, Grunewald J. Characterisation of natural killer cells and CD56+ T-cells in sarcoidosis patients. *Eur Respir J*. 2005;26:77-85.
14. Bergantini L, Cameli P, d'Alessandro M, et al. NK and NKT-like cells in granulomatous and fibrotic lung diseases. *Clin Exp Med*. 2019;19:487-494.
15. Petersen HJ, Smith AM. The role of the innate immune system in granulomatous disorders. *Front Immunol*. 2013;4:120.
16. Greulich S, Kitterer D, Latus J, et al. Comprehensive cardiovascular magnetic resonance assessment in patients with sarcoidosis and preserved left ventricular ejection fraction. *Circ Cardiovasc Imaging*. 2016;9:e005022.

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