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Neutrophil gelatinase-associated lipocalin in patients with sarcoidosis

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ABSTRACT

Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein that is involved in the innate immune system and increased expression has been detected in diverse diseases. Sarcoidosis is a systemic granulomatous disorder and its clinical course and prognosis are changeable and highly divergent. This study aimed to examine the expression of NGAL in patients with sarcoidosis. In addition, we examined whether NGAL could serve as a marker of disease activity and prognosis.

Methods: Ninety-six sarcoidosis patients were studied. Serum samples collected at the time of diagnosis were examined for NGAL by cellular enzyme-linked immunosorbent assay. The level of NGAL was compared with clinical, radiological and laboratory data.

Results: Patients with sarcoidosis had significantly higher levels of NGAL (the median [interquartile range] was 35.1 ng/mL [23.5–60.8] in sarcoidosis patients *versus* 17.2 ng/mL [13.0–27.0] in the reference population, $p < .0001$). NGAL levels were not correlated with markers for disease activity. During the follow-up period, 26 patients (27.1%) deteriorated and received systemic corticosteroid therapy for organ dysfunction. In those patients, NGAL levels were significantly higher than in those who did not receive corticosteroid therapy (56.5 ng/mL [27.3–92.3] *versus* 34.3 ng/mL [23.0–53.0], $p = .0201$). Upon multivariate logistic regression analysis, elevated NGAL levels at diagnosis were associated with subsequent use of systemic corticosteroid therapy (hazard ratio, 1.20; 95% confidence interval, 1.09–1.31; p = .0004).

Conclusion: NGAL may be a useful marker to predict the disease course of sarcoidosis.

1. Introduction

Sarcoidosis is a systemic granulomatous disorder that commonly affects multiple organs [1–4]. Patients with sarcoidosis exhibit diverse and changeable clinical courses and prognosis [1–3]. Although the exact disease etiology is unknown, currently, sarcoidosis is understood to be the consequence of a chronic immune response associated with genetic predisposition and unidentified antigens [1–4]. Immunologically, it is assumed that specific environmental factors like transmissible infectious origins are antigens and the pathogen-associated molecular patterns of the antigen can trigger or amplify inflammation in sarcoidosis [1–4].

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa glycoprotein that is mainly released from activated neutrophil granules [5,6]. Although its precise and full functional role remains unclear, NGAL is regarded as a critical component of the innate immune system [6,7]. It has a high affinity for siderophores that bind to circulating and intracellular free iron [8] and limits bacterial growth by sequestrating the iron-laden siderophores [7]. To date, the expression of NGAL has been detected in diverse diseases [6,8–13]. In acute renal injury, the NGAL measurement is emerging as an early diagnosis and predictive tool in routine clinical practice [8,9]. In 402 patients with chronic obstructive pulmonary disease, plasma levels of NGAL were elevated and the number of exacerbations was associated with higher NGAL expression [11]. The levels of NGAL in patients with community-acquired pneumonia presenting to the emergency department increased proportionally with the severity of pneumonia. Based on its relationship with cell growth, differentiation and apoptosis [7], NGAL is also associated with tumor development in various types of cancer [8,14,15].

In this study, we aimed to examine the expression of NGAL in patients with sarcoidosis. In addition, we investigated the usefulness of NGAL as a marker of disease activity and prognosis.

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Table 1
Patient characteristics.

Characteristics	Sarcoidosis (n = 96)
Age, years (range)	55 (23–79)
Sex, male/female	38/58
Affected organs[a]	
Lungs	96
Eyes	49
Skin	15
Others	51
Duration of follow-up, years	7.7 (4.8–13.0)
Smoker	39 (40.6)
Blood % neutrophil, %	64.7 (59.5–70.9)
Serum ACE, IU/L	18.5 (14.1–23.1)
Pulmonary function tests	
FVC, % predicted	95.2 (84.4–107.4)
FEV_1/FVC ratio, %	79.4 (74.4–84.3)
Radiologic stage 0/I/II/III	4/44/36/12
Bronchoalveolar lavage	
Total cells, $\times 10^5$/ml	1.40 (0.86–2.50)
Lymphocytes, %	9.6 (6.2–19.4)
Neutrophils, %	0.4 (0–1.0)
CD4/CD8 ratio	4.8 (2.6–8.5)

Data are expressed as number (%) or median (interquartile range) unless otherwise indicated.
ACE, angiotensin converting enzyme; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s. Radiographic stages 0, I, II, and III, represent a normal appearance, bilateral hilar lymphadenopathy (BHL) alone, BHL and lung parenchymal involvement, and lung parenchymal involvement without BHL, respectively.
[a] Lungs include pulmonary hilar and mediastinal lymphadenopathy; others include muscle, liver, kidney, hypercalcemia, parotid gland, and extramediastinal lymphadenopathy.

2. Methods

2.1. Patient eligibility

A total of 96 consecutive patients who were diagnosed as having sarcoidosis and were observed for at least 2 years were included in this study (Table 1). They were ethnically homogeneous, Japanese patients. The diagnosis of sarcoidosis was based on the American Thoracic Society, the European Respiratory Society, and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) consensus statement [2] and the number of involved organs was counted according to the WASOG instrument [16]. This study was conducted in accordance with the amended Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Hamamatsu University School of Medicine (Hamamatsu, Japan, approval number: 15-165). The study was registered with the University Hospital Medical Information Network Clinical Trial Registry (UMIN ID 000028108).

2.2. Data collection

Clinical, radiological and laboratory data, including organs affected, pulmonary function, treatment, prognosis, bronchoalveolar lavage (BAL) and pathological information, were retrospectively obtained from medical records. Pulmonary function tests and laboratory parameters were assessed at the time of diagnosis.

2.3. ELISA for the detection of NGAL

Serum samples were collected at the time of diagnosis, when no patient was under systemic corticosteroid or immunosuppressive therapy, and stored at −30 °C until analysis. The enzyme-linked immunosorbent assay (ELISA) to detect serum NGAL was performed according to manufacturer's instructions (CircuLex Human NGAL kit; MBL, Nagoya, Japan). Forty-nine age- and gender-matched healthy subjects without any clinical, radiological, or serological evidence of infection, cardiovascular and renal disease, chronic obstructive pulmonary disease, tumor, sarcoidosis, or autoimmune disorders served as controls, which was approved by the ethical committee of Seirei Center for Health Promotion and Preventive Medicine (Hamamatsu, Japan, approval number; 26-05), and each subject directly provided informed consent.

2.4. Statistical analysis

The Wilcoxon signed rank test was used for continuous variables. Correlations between different parameters were undertaken using Spearman's rank correlation coefficient. Cox proportional hazard regression analysis was used to identify variables associated with the use of systemic corticosteroid therapy. p values of less than 0.05 were considered significant. Data are expressed as the median (interquartile range) unless indicated otherwise. All values were analyzed using JMP version 9.0.0 (SAS Institute Japan, Tokyo, Japan).

3. Results

3.1. Patient characteristics

Ninety-six patients with sarcoidosis were included in this study (Table 1). The median age at diagnosis was 55 years (range; 23–79 years) and 58 patients (60.4%) were female. Patients with sarcoid lesions in the lungs were divided into four groups based on the findings of chest radiography: four patients had a normal radiograph, 44 patients had bilateral hilar lymphadenopathy (BHL) alone, 36 patients had BHL and lung parenchymal involvement, and 12 patients had lung parenchymal involvement alone. The common extrapulmonary lesions were ocular and skin lesions, found in 51.0% and 15.6% of the patients, respectively. Of the 27 patients who had involvement of more than three organs, 85.2% had lung parenchymal lesions. About half of the patients had symptoms, three-fourths of which were ophthalmological manifestations. Serum angiotensin converting enzyme (ACE) levels were elevated above the upper normal range in 32 patients. In most patients, forced vital capacity (FVC), FVC% predicted, and forced expiratory volume in one second (FEV_1)/FVC ratio were within the normal range.

3.2. Expression of NGAL and correlation with findings in sarcoidosis

There were no significant correlation of NGAL levels with sex ($p = .11$), age ($r = 0.11$, $p = .31$), absolute neutrophil counts ($r = 0.15$, $p = .16$), hemoglobin ($r = 0.02$, $p = .84$) or mean corpuscular volume ($r = 0.002$, $p = .98$), estimate glomerular filtration rates ($r = -0.16$, $p = .83$), body mass index ($r = -0.03$, $p = .79$), or smoking pack-year histories ($r = -0.06$, $p = .57$). The median NGAL was 35.1 ng/mL (23.5–60.8) in sarcoidosis patients, which was significantly higher than in the reference population (17.2 ng/mL, [13.0–27.0], Fig. 1; $p < .0001$). The levels of NGAL did not differ among the groups defined by chest radiographic findings. When we divided patients into two groups according to the number of organs involved: those with two or fewer affected organs and those with more than three organs affected, there was no difference in NGAL levels (35.0 ng/mL *versus* 45.0 ng/mL, p = .13). NGAL levels were not correlated with markers for disease activity, such as serum ACE levels ($r = 0.10$, $p = .35$), the percentage of alveolar lymphocytes ($r = 0.14$, $p = .17$) and the CD4/CD8 ratio in BAL ($r = 0.02$, $p = .84$).

At the time of diagnosis, none of the patients had serious organ damage that needed systemic corticosteroid therapy. During the observation period (a median of 7.7 years), 26 patients deteriorated and received systemic corticosteroid therapy (median dose of 30 mg prednisolone per day, 0.5 mg/kg of body weight) for organ dysfunction; 16 patients for deterioration of pre-existing pulmonary disease with