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Clinical Significance of Elevated Serum Caspase-1 Levels in Patients With Ankylosing Spondylitis

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Dear Editor,

Ankylosing spondylitis (AS) is a chronic autoimmune disease that affects the axial skeleton and peripheral joints [1]. Although several etiologies for AS have been proposed, no clear cause has been identified to date [2]. Activated caspase-1 within the nucleotide-binding oligomerization domain-like receptor pyrin domain-containing-3 (NLRP3) inflammasome converts pro-interleukin (IL)-1β and pro-IL-18 into their biologically active forms, IL-1β and IL-18, respectively [3]. Given that AS is an inflammatory disease, it is hypothesized that inflammasomes activated during the immune response, such as the NLRP3 inflammasome, contribute to disease development and progression [4, 5]. Previously, we demonstrated that caspase-1 levels in the synovial fluid are higher in patients with spondyloarthritis than in patients with other types of arthritides [6]. In this study, we investigated whether serum caspase-1 level differentiates AS from other rheumatic diseases and examined the relationship between serum caspase-1 levels and AS disease activity.

Between June 2017 and August 2018, 126 study participants (20 AS, 23 gout, and 62 rheumatoid arthritis [RA] patients; 21 healthy controls) from Keimyung University Dongsan Hospital in Daegu were enrolled. The study was approved by the institutional review board (IRB; 2017-06-021) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. Blood samples were collected from each patient prior to initiating medical treatment. Patients met the 1984 New York criteria for AS [7], the 2010 RA classification criteria for RA [8], or had acute gout [9]. The control group consisted of healthy healthcare workers. We collected age, sex, disease duration, and blood chemistry data from all participants.

A second cohort of 22 AS patients was recruited from an outpatient rheumatology clinic at Hanyang University Hospital for Rheumatic Diseases, Seoul, Korea, and their serum caspase-1 levels were compared with indicators of AS disease activity. The study was approved by the IRB of Hanyang University Hospital (2008-09-001). Written informed consent was obtained from all patients. Blood samples were collected from AS patients for routine examination between September 2017 and March 2019.

We analyzed the relationship between serum caspase-1 level and serum erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), the Bath Ankylosing Spondylitis Disease Activity Index and Ankylosing Spondylitis Disease Activity Score [1]. Caspase-1, IL-1β, IL-18, and NLRP3 levels were measured using

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Table 1. Clinical and laboratory features of patients with ankylosing spondylitis, gout, and rheumatoid arthritis compared with healthy controls

Feature*	Ankylosing spondylitis (N=20)	Gout (N=23)	Rheumatoid arthritis (N=62)	Healthy controls (N=21)	$P^{\dagger}$
Age (yr)	35.5 (21.0)	56.0 (25.0)	61.5 (9.0)	41.0 (23.0)	**<0.001***
Sex (M/F)	18/2	20/3	24/38	1/20	
ESR (mm/hr)	20.0 (41.5)	43.5 (30.0) (N=10)	40.5 (56.0)	NA	0.316
CRP (mg/dL)	0.2 (1.6)	2.41 (8.14) (N=10)	0.32 (1.75)	NA	**0.020**
WBC ($\times 10^9$/L)	7.1 (2.6)	8.2 (6.0) (N=11)	7.6 (3.8)	NA	0.460
Caspase-1 (pg/mL)	201.1 (109.8)	137.3 (91.7)	144.6 (150.8)	71.5 (72.9)	**<0.001**
IL-1 (pg/mL)	2.39 (3.95)	4.1 (1.9)	2.1 (3.5)	6.1 (2.5)	**<0.001**
IL-18 (pg/mL)	1,585 (127)	1,520 (176)	1,572 (118)	1,695 (199)	**0.014**
NLRP3 (pg/mL)	0.2 (0.3)	0.5 (1.0)	0.6 (1.3)	0.0 (0.1)	**<0.001**
Caspase-1 >125 pg/mL‡	16 (80.0%)	15 (65.2%)	39 (62.9%)	3 (14.3%)	**<0.001**

*Continuous variables are shown as median (interquartile range); $^{\dagger}P$ values were determined using the Kruskal–Wallis test; bold numbers indicate statistical significance; ‡The frequency of high levels (≥125 pg/mL) of serum caspase-1 was significantly higher in ankylosing spondylitis than in the other groups as revealed by Fisher's exact test.
Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cells; IL, interleukin; NLRP3, nucleotide-binding oligomerization domain-like receptor pyrin domain-containing-3; NA, not available.

ELISA kits (Quantikine, R&D Systems, Minneapolis, MN, USA; Aviva Systems Biology, San Diego, CA, USA). Statistical analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA). Results are presented as median (interquartile range) or frequency (%). For continuous variables, the Mann–Whitney *U* test was used to compare the high CRP group with the normal CRP group and the Kruskal–Wallis test for between-group comparisons of AS, gout, RA, and healthy controls. For categorical variables, Fisher's exact test was used to compare the frequency of high levels (>125 pg/mL) of caspase-1 between groups. $P<0.05$ was considered statistically significant.

The summary statistics of the patients are presented in Table 1. The mean serum caspase-1 level was significantly higher in AS patients than in other groups ($P<0.001$). The frequency of caspase-1 level ≥125 pg/mL was also higher in the AS group than in the gout, RA, and healthy control groups. When we compared serum caspase-1 level according to high and normal CRP levels in the independent sample set, serum caspase-1 level was significantly higher in the high CRP groups than in the normal CRP group ($P=0.041$, Table 2).

Significantly higher caspase-1 levels were observed in spondyloarthritis than in other arthritic diseases, including gout, inflammatory arthritis, and osteoarthritis [6]. Concordantly, we found higher serum caspase-1 level in AS patients than in patients with other diseases and healthy controls, and patients with high CRP level had elevated serum caspase-1 levels. Caspase-1 activation is the hallmark of inflammasome activation [4], which is essential in inflammatory response induction in macrophages. A chronic inflammatory response is a major risk factor for autoimmune disease development. In AS patients, macrophages are abundantly present in not only synovial tissues but also sacroiliac joint and colonic mucosal tissues [6].

Table 2. Serologic and clinical features of patients with ankylosing spondylitis classified by CRP level in the independent sample set

Feature*	High CRP (≥0.8 mg/L) (N=12)	Normal CRP (<0.8 mg/L) (N=10)	$P^{\dagger}$
Age (yr)	29.5 (4.0)	30.0 (9.0)	0.205
Sex (M/F)	11/1	10/0	
Disease duration (month)	21.0 (85.5)	33.5 (86.0)	0.865
ESR (mm/hr)	42.5 (34.5)	4.5 (7.0)	**<0.001**
Caspase-1 (pg/mL)	86.7 (130.1)	32.1 (56.2)	**0.041**
BASDAI	5.1 (2.1)	1.9 (3.4)	**0.021**
ASDAS-CRP	3.8 (1.0)	1.8 (1.8)	**0.002**
Peripheral arthritis	4 (33%)	5 (50%)	

*Continuous variables are shown as median (interquartile range); $^{\dagger}P$ values were determined using the Kruskal–Wallis test, bold numbers indicate statistical significance.
Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; ASDAS, Ankylosing Spondylitis Disease Activity Score.

This study had some limitations. Because of the difficulty in obtaining a clinical sample from patients with high disease activity, the sample size was relatively small. Examining more di-

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rect evidence of the role of caspase-1 in AS is required. Further validation using larger sample sizes, alternative sample types (urine and bone), and animal models can help identify biomarkers for AS.

This study was the first to assess the association between serum caspase-1 level and AS. Serum caspase-1 levels were the highest in AS patients when compared with those in patients with other inflammatory arthropathies and healthy controls. Further, we observed high serum caspase-1 levels in AS patients that had high CRP levels. There is no clinically available biomarker for the early diagnosis or monitoring of AS. Our results suggest that serum caspase-1 is a helpful biomarker for AS and should be further investigated.

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AUTHOR CONTRIBUTIONS

Kim SH, Kim TH, Jun JB, and Son CN contributed to the study conception. Lee JH, Jeong HJ, Kim JM, Kim TH, and Son CN contributed to data curation. Kim SH, Jeong HJ, Kim TH, and Son CN performed the formal analysis. Son CN obtained funding for the project. Baek WK, Kim TH, Jun JB, and Son CN contributed to the methodology of the study. Kim SH, Jeong HJ, Kim JM, Baek WK, Kim TH, and Son CN administered the project. Kim SH, Kim TH, and Son CN wrote the draft of the manuscript. All authors reviewed and edited the manuscript.

CONFLICTS OF INTEREST

None declared.

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