Received: 21 March 2022 | Revised: 18 April 2022 | Accepted: 23 April 2022

DOI: 10.1002/jcla.24474

RESEARCH ARTICLE

WILEY

Association of serum hepatoma-derived growth factor levels with disease activity in rheumatoid arthritis: A pilot study

Sung Soo Ahn[1] | Hye Min Kim[2] | Younhee Park[3]

[1]Division of Rheumatology, Department of Internal Medicine, Yongin Severance Hospital, Yonsei University College of Medicine, Yongin, South Korea

[2]Department of Pathology, Yongin Severance Hospital, Yonsei University College of Medicine, Yongin, South Korea

[3]Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, South Korea

Correspondence
Younhee Park, Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seoul 03722, South Korea.
Email: younheep@yuhs.ac

Abstract

Background: Hepatoma-derived growth factor (HDGF) is reported to play an important role in tumorigenesis and cancer progression. However, growing evidence indicates its participation in immune system activation. This study analyzed the relationship among serum HDGF levels, disease activity, and laboratory markers in patients with rheumatoid arthritis (RA).

Methods: Blood samples from 165 patients with RA, 42 with osteoarthritis (OA), and 28 healthy controls, were used to evaluate the serum HDGF levels. Correlations of serum HDGF levels with age, 28-joint count disease activity score (DAS28), and laboratory findings were assessed by Pearson correlation and receiver operator characteristic (ROC) curve analyses to obtain HDGF optimal cutoffs according to the disease status. Immunohistochemical staining was performed on the knee synovial tissue samples from patients with RA and OA ($n = 10$ each) to investigate HDGF joint expression.

Results: Serum HDGF levels were significantly correlated with DAS28 erythrocyte sedimentation rate ($r = 0.412$, $p < 0.001$) and C-reactive protein values ($r = 0.376$, $p < 0.001$). The optimal cutoffs of serum HDGF levels from the ROC analysis were 5.79 and 5.14 for the differentiation of active/inactive disease and remission/non-remission, respectively. The ideal cutoff of serum HDGF levels to differentiate RA and OA was determined as 5.47. Serial serum HDGF level analyses in 21 patients with RA revealed that serum HDGF levels significantly decreased after improvement in disease activity ($p = 0.046$). HDGF expression was not observed in the synovial tissues of the patients with RA and OA.

Conclusion: Serum HDGF level could be a potential laboratory biomarker for the severity of RA.

KEYWORDS
alarmin, biomarker, disease activity, hepatoma-derived growth factor, rheumatoid arthritis

Sung Soo Ahn and Hye Min Kim contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2022 The Authors. *Journal of Clinical Laboratory Analysis* published by Wiley Periodicals LLC.

1 | INTRODUCTION

Hepatoma-derived growth factor (HDGF) is a heparin-binding 26-kDa glycoprotein, consisting of 240 amino acids, that was first purified from the supernatant of human hepatocellular carcinoma cell lines.[1] Early studies have revealed that HDGF functions as an essential growth factor that enhances cellular proliferation, invasion, and migration of cancer cells through receptor-mediated signaling pathways.[2] These effects were further illustrated by in vitro and in vivo experiments showing therapeutic effects after the inhibition and deletion of HDGF in various cancers.[3,4] HDGF is ubiquitously expressed in various tissues and is predominantly found in the nucleus.[5] The secretion of HDGF in the peripheral circulation is modulated via several non-classical pathways.[2] Previously, it was demonstrated that increased HDGF levels in the blood are associated with aggressive features and have prognostic implications in cancers, suggesting that it may be a treatment target and promising non-invasive biomarker.[6–8] Emerging evidence indicates that HDGF is an alarmin that is released following cell injury/death and plays an important role in the activation of the immune system by binding to its cognate receptor.[9] Nevertheless, compared to its prominent role in tumorigenesis and cancer progression, the precise role of HDGF in the immune system and immune-related diseases is not well characterized.

Rheumatoid arthritis (RA) is an autoimmune rheumatic disorder that usually involves the small joints and is typically associated with the development of chronic synovial tissue inflammation.[10] The underlying pathogenesis of RA is characterized by a complex interaction between dysregulated immune responses.[11] However, alarmins, initially considered to merely indicate cellular injury, have been reported to also influence innate and adaptive immunity to perpetuate the inflammatory cycle in autoimmune inflammatory arthritides.[12] Notably, among various alarmins, there are reports that have identified a significant correlation between 28-joint count disease activity score (DAS28) and high mobility group box 1, interleukin (IL)-33, and S100A8/9,[13–15] implying that alarmins may be utilized as biomarkers indicating the severity of disease in patients with RA. In general, persistently high disease activity has a major impact on the progression of joint destruction in RA,[16] and special attention has been paid to the discovery of putative markers. Accordingly, although the relevance of serum HDGF in RA is worthy of exploration, its clinical implications have not been reported in the literature. Hence, in this study, we analyzed the relationship between serum HDGF levels and indices of disease activity and laboratory markers to evaluate whether it could be a useful measure of inflammation in RA.

2 | MATERIAL AND METHODS

2.1 | Patients

We performed a pilot study using serum samples obtained from 165 patients with RA between March 2016 and February 2018, who were subjected to laboratory tests at the Division of Rheumatology, Severance Hospital. A blood test was performed at the time of providing routine patient care. The drawn blood was centrifuged immediately and the isolated sera were stored in a −70°C deep freezer for further analysis. Patients were classified as having RA according to the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria.[17,18] At the time of blood collection, the patients did not demonstrate any evidence of concurrent malignancy or infection.

For the comparison of serum HDGF levels, sera obtained from 42 patients with osteoarthritis (OA) and 28 healthy controls (HC) were used. Furthermore, sera were serially collected from 21 patients with RA, following a decrease in disease activity. The Severance Hospital's Institutional Review Board approved this study (4-2017-0761), and all relevant procedures were performed according to the ethical standards of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

2.2 | Clinical and laboratory findings and determination of serum HDGF and IL-6 level

Clinical and laboratory results were assessed retrospectively on the date of collection of patient sera. Demographic data including age, sex, and disease duration, as well as disease characteristics/activity of—seropositivity (defined according to rheumatoid factor and/or anti-cyclic citrullinated peptide), interstitial lung disease (ILD), abnormal renal function, DAS28 erythrocyte sedimentation rate (ESR), and DAS28 C-reactive protein (CRP)[19] were included. The investigated medications for the management of RA consisted of glucocorticoids and disease-modifying anti-rheumatic drugs (DMARDs) according to the treatment guidelines for RA.[20,21] Laboratory findings included white blood cell (WBC) and platelet counts, ESR, CRP, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase. Patients with DAS28 ESR ≥3.2 were regarded as having an active disease; the rest were classified as inactive.[22] On the other hand, the cutoffs—DAS28 ESR ≥2.6 and DAS28 ESR <2.6—were used to define non-remission and remission states.[23]

For the analyses of serum HDGF and IL-6 levels in the collected patient sera, enzyme-linked immunosorbent assay kits for HDGF (Cloud-Clone Corp, Katy, TX, USA) and IL-6 (R&D Systems) were used, and all experiments were performed following the manufacturer's instructions.

2.3 | Immunohistochemistry of HDGF in synovial tissues from patients with RA and OA

Tissue samples from patients with RA and OA ($n = 10$ each) who had undergone total knee replacement were used for immunohistochemistry using a commercially available antibody for HDGF (Abcam). Immunohistochemical (IHC) staining was performed on formalin-fixed, paraffin-embedded tissue sections after deparaffinization and rehydration in accordance with the manufacturer's protocol.

2.4 | Statistical analysis

Statistical analyses were performed using MedCalc statistical software version 20.011 (MedCalc Software), and a two-tailed *p*-value of <0.05 was considered significant. All continuous and categorical data are presented as median (interquartile range) and number (percentage), respectively. Differences in continuous data were evaluated using the Mann–Whitney *U* test or Kruskal–Wallis test as appropriate. The association between serum HDGF levels and continuous variables was evaluated using Pearson's correlation analysis. In addition, the optimal cutoff value of serum HDGF for identifying active disease was determined using the receiver operator characteristic (ROC) curve analysis. Serial changes in serum HDGF levels after treatment were assessed using the Wilcoxon signed-rank test.

TABLE 1 Baseline characteristics of the patients

	Values
Demographic data	
Age	57.0 (47.0–64.0)
Male sex	34 (20.6)
Disease duration, months	66.0 (12.5–159.3)
Disease characteristics/activity	
Seropositivity	153 (92.7)
Interstitial lung disease	12 (7.3)
Abnormal renal function	12 (7.3)
DAS28 ESR	3.2 (2.4–4.6)
DAS28 CRP	2.3 (1.4–3.8)
RA treatment	
Glucocorticoid	88 (53.3)
Any DMARDs	137 (83.0)
bDMARDs	86 (52.1)
Laboratory findings	
WBC count (/mm^3)	6820.0 (5262.5–8557.5)
Platelet count (×1000/mm^3)	266.0 (225.8–323.8)
ESR (mm/hr)	40.0 (18.0–65.0)
CRP (mg/L)	2.5 (0.6–12.7)
Alkaline phosphatase (IU/L)	66.0 (53.8–79.0)
AST (IU/L)	19.0 (15.8–25.0)
ALT (IU/L)	16.0 (11.0–24.0)
IL–6 (pg/mL)[a]	11.1 (3.5–27.6)
Serum HDGF (ng/mL)	4.6 (3.7–5.8)

Note: Data are expressed as median (interquartile ranges) and number (percentages).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; bDMARD, biologics disease-modifying anti-rheumatic drugs; CRP, C-reactive protein; DAS28, 28-joint count disease activity score; DMARD, disease-modifying anti-rheumatic drugs; ESR, erythrocyte sedimentation rate; HDGF, hepatoma-derived growth factor; IL, interleukin; WBC, white blood cell.

[a] Data are available for 158 patients.

3 | RESULTS

3.1 | Patient characteristics

The baseline patient characteristics are shown in Table 1. The median age of the patients was 57.0 years, 34 (20.6%) were male participants, and the median disease duration was 66.0 months. Concerning disease characteristics and activity, seropositivity and ILD were observed in 153 (92.7%) and 12 (7.3%) patients, respectively. The median DAS28 ESR and DAS28 CRP were 3.2 and 2.3, respectively. A total of 88 (53.3%) patients were on concomitant glucocorticoid treatment, and 137 (83.0%) and 86 (52.1%) patients were prescribed conventional synthetic DMARDs (76.2%) and biological DMARDs (bDMARDs), respectively. The median white blood cell count, ESR, CRP, IL-6, and serum HDGF were 6820.0/mm^3, 40.0 mm/h, 2.5 mg/L, 11.1 pg/ml, and 4.6 ng/ml, respectively.

3.2 | Relationship between serum HDGF with DAS28 values and laboratory findings

Serum HDGF was significantly correlated with DAS28 ESR (r = 0.412, 95% confidence interval [CI] 0.277, 0.532, p < 0.001)

TABLE 2 Correlation analysis of serum HDGF with age, DAS28, and laboratory findings

	Serum HDGF	
	Correlation coefficient (95% CI)[a]	*p*-value
Age	0.068 (−0.085, 0.219)	0.384
DAS28 ESR	0.412 (0.277, 0.532)	<0.001
DAS28 CRP	0.376 (0.237, 0.500)	<0.001
WBC count	0.238 (0.088, 0.377)	0.002
Platelet count	0.157 (0.004, 0.303)	0.044
ESR	0.368 (0.228, 0.493)	<0.001
CRP	0.166 (0.013, 0.311)	0.033
Alkaline phosphatase	0.073 (−0.081, 0.223)	0.352
AST	−0.253 (−0.391, −0.105)	0.001
ALT	−0.173 (−0.318, −0.021)	0.026
IL–6 (pg/mL)	−0.013 (−0.169, 0.143)	0.870

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; CRP, C-reactive protein; DAS28, 28-joint count disease activity score; ESR, erythrocyte sedimentation rate; HDGF, hepatoma-derived growth factor; IL, interleukin; WBC, white blood cell.

[a] The values represent correlation coefficient and 95% confidence interval.

and DAS28 CRP values ($r = 0.376$, 95% CI 0.237, 0.500, $p < 0.001$). Furthermore, positive correlations were observed between serum HDGF levels and WBC and platelet counts and ESR and CRP levels, whereas a negative correlation was observed between serum HDGF levels and aspartate aminotransferase and alanine aminotransferase (Table 2). Similarly, in a subgroup of patients with seropositive RA, serum HDGF had a significant impact on DAS28 ESR ($r = 0.441$, 95% CI 0.304–0.561, $p < 0.001$) and DAS28 CRP ($r = 0.408$, 95% CI 0.267, 0.532, $p < 0.001$) (Table 3). There was no difference in the level of serum HDGF according to the presence/absence of ILD and normal/abnormal renal function.

TABLE 3 Correlation of serum HDGF with age, DAS28, and laboratory findings in seropositive RA patients ($n = 153$)

	Serum HDGF	
	Correlation coefficient (95% CI)[a]	*p*-value
Age	0.070 (−0.090, 0.226)	0.389
DAS28 ESR	0.441 (0.304, −0.561)	<0.001
DAS28 CRP	0.408 (0.267, 0.532)	<0.001
WBC count	0.224 (0.068, 0.370)	0.005
Platelet count	0.142 (−0.017, 0.294)	0.080
ESR	0.373 (0.227, 0.502)	<0.001
CRP	0.160 (0.001, 0.311)	0.048
Alkaline phosphatase	0.126 (−0.034, 0.279)	0.121
AST	−0.270 (−0.411, −0.116)	<0.001
ALT	−0.185 (−0.334, −0.027)	0.022
IL−6 (pg/mL)	−0.007 (−0.169, 0.155)	0.930

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; CRP, C-reactive protein; DAS28, 28-joint count disease activity score; ESR, erythrocyte sedimentation rate; HDGF, hepatoma-derived growth factor; IL, interleukin; WBC, white blood cell.

[a]The values represent the correlation coefficient and 95% confidence interval.

3.3 | ROC curve analysis

We divided the patients into active/inactive disease and remission/non-remission groups and performed a ROC curve analysis to identify the optimal cutoffs to discriminate the disease activity status. The ROC curve analysis revealed that the cutoff value of serum HDGF were 5.79 and 5.14 showing differentiated active/inactive disease and remission/non-remission with the highest area under the ROC of 0.682 (95% CI 0.605, 0.752, $p < 0.001$) and 0.704 (95% CI 0.628, 0.772, $p < 0.001$) (Figure 1). However, the ideal cutoff of serum HDGF to differentiate RA and OA was determined as 5.47 (area under the ROC 0.608, 95% CI 0.538, 0.675, $p = 0.006$) (Figure 2).

3.4 | Serum HDGF level according to disease duration and treatment

A comparison of serum HDGF levels in patients with a disease duration of <3 and ≥3 months revealed that the levels were significantly higher in patients with a duration of <3 months ($p = 0.020$). Moreover, the levels were significantly lower in patients who were currently receiving DMARD and bDMARD treatments (all $p < 0.001$) (Figure 3).

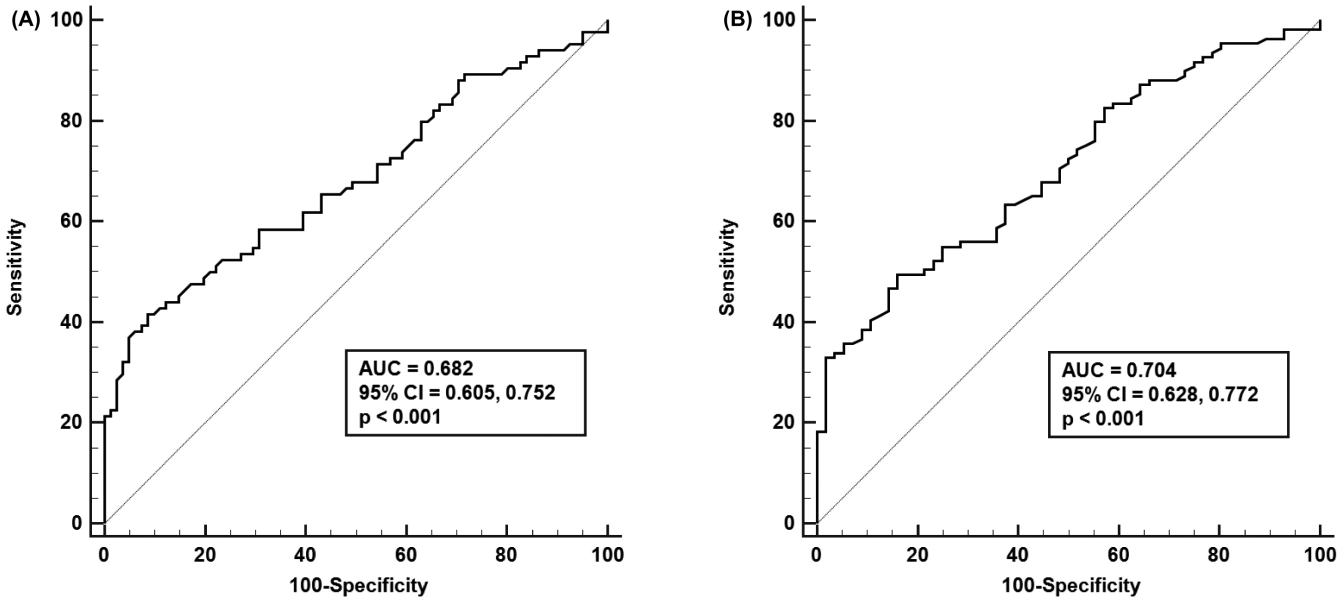

FIGURE 1 ROC curve analysis for discriminating disease activity status in RA. A ROC curve identified an optimal HDGF cutoff value of 5.79 in discriminating (A) active/inactive and 5.14 in (B) remission/non-remission. AUC, area under the curve; CI, confidence interval; HDGF, hepatoma-derived growth factor; RA, rheumatoid arthritis; ROC, receiver operator characteristic

3.5 | Comparison of serum HDGF levels in RA, OA, and HC and the changes in them after reduction of disease activity and IHC analysis

Serum HDGF levels were significantly higher in patients with active RA than in those with inactive RA, OA, and HC (all $p < 0.001$). Additionally, no difference was observed in the levels among patients with inactive RA, OA, and HC (Figure 4). To identify whether the alteration of serum HDGF levels is relevant to the disease severity, serial analyses of serum HDGF levels were performed in 21 patients, and the levels were found to significantly decrease after the improvement of disease activity ($p = 0.046$) (Figure 5). No HDGF expression was observed in the synovial tissues of patients with RA and OA based on an IHC analysis (Figure 6).

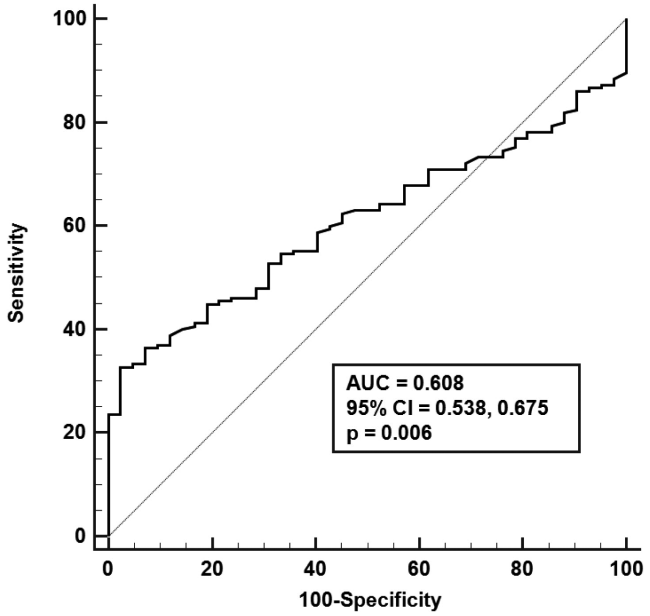

FIGURE 2 Optimal cutoff of serum HDGF in the differentiation of RA and OA. A serum HDGF cutoff level of 5.47 showed the highest predictive value in differentiating between RA and OA. AUC, area under the curve; CI, confidence interval; HDGF, hepatoma-derived growth factor; OA, osteoarthritis; RA, rheumatoid arthritis

4 | DISCUSSION

The present study evaluated the association between serum HDGF levels and disease activity in patients with RA, and several remarkable results were obtained. First, the serum HDGF levels were significantly correlated with DAS28, the most commonly used tool for assessing RA disease activity. Notably, these results were replicated in a subgroup analysis of patients with seropositive RA. Second, the serum HDGF levels were higher in patients with RA than in those with OA and HC, and an optimal cutoff for HDGF in the differentiation of RA severity and OA could be derived. Third, a significant decrease in serum HDGF levels was observed in patients with RA who showed an improvement in disease activity. Meanwhile, IHC analysis of synovial tissue revealed no remarkable HDGF expression in the RA and OA synovium. Accordingly, our results indicated that serum HDGF could be a potential laboratory biomarker in patients with RA. To the best of our knowledge, this is the first study to evaluate the clinical significance of serum HDGF levels in RA patients.

Several lines of evidence suggest that increased HDGF levels in circulation are associated with RA disease activity. HDGF is an

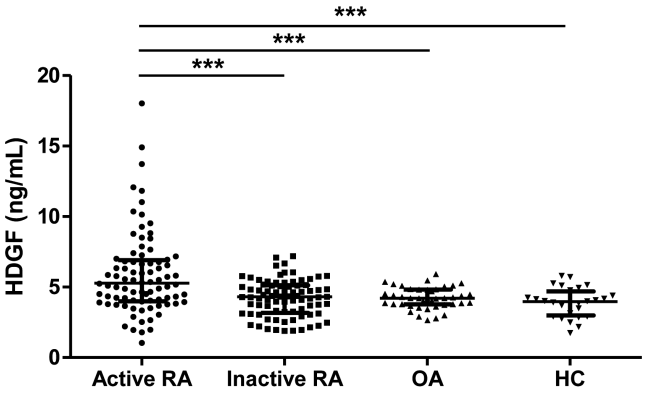

FIGURE 4 Serum HDGF level in patients with active and inactive RA, OA, and HC. Serum HDGF levels were compared between patients with active and inactive RA, patients with OA, and HC. ***$p < 0.001$. The error bars present median and interquartile ranges. HC, healthy control; HDGF, hepatoma-derived growth factor; OA, osteoarthritis; RA, rheumatoid arthritis

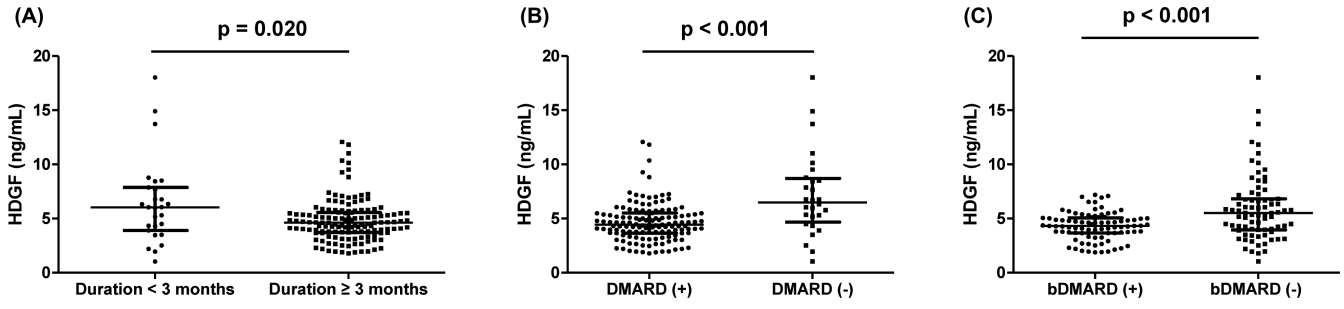

FIGURE 3 Comparison of serum HDGF level in patients with RA according to disease duration and DMARD treatment. Serum HDGF levels were compared in patients with RA based on (A) disease duration, (B) DMARD use, and (C) bDMARD use. The error bars present median and interquartile ranges. bDMARD, biologics disease-modifying anti-rheumatic drugs; DMARD, disease-modifying anti-rheumatic drugs; HDGF, hepatoma-derived growth factor; RA, rheumatoid arthritis

alarmin, which refers to a group of endogenous molecules that are linked to cellular and tissue injury.[24] Although initially identified as a damage-associated molecular pattern, it is being increasingly accepted that the overexpression of alarmins could have deleterious effects on RA by facilitating the production of tissue-degrading enzymes, inflammatory cytokines, chemokines, and reactive oxygen species.[12] Furthermore, alarmins can influence antigen processing and expedite the differentiation of naïve T cells into effector T cells, thereby sustaining chronic inflammation.[25] It has also been suggested that the binding of extracellular HDGF to its receptor activates the MAPK and PI3K signaling pathways, which are also considered to be critical pathways in RA pathogenesis.[26,27] Moreover, HDGF translocates to the nucleus, binds to DNA, and affects the expression of genes implicated in RA, such as tumor necrosis factor α (TNFα), IL-1, and IL-6.[28] Finally, HDGF has been reported to act as a growth factor and an angiogenic factor and also possesses an antiapoptotic effect.[2] The relationship between HDGF and RA activity could be proposed considering that synovial fibroblasts in RA, a major player in maintaining persistent inflammation in RA tissues,[29] are characterized by angiogenesis and defective apoptosis. However, different from the expression of HDGF in the sera, HDGF expression was not evident in RA and OA tissues in our study. We speculate that this discrepancy might be accounted for by the fact that findings present in RA synovium undergoing surgery more likely reflects the advanced stage of RA which is not proportional to the presence of active inflammation. It is obvious that the disparity of HDGF expression in the sera and tissue observed herein remains to be better investigated.

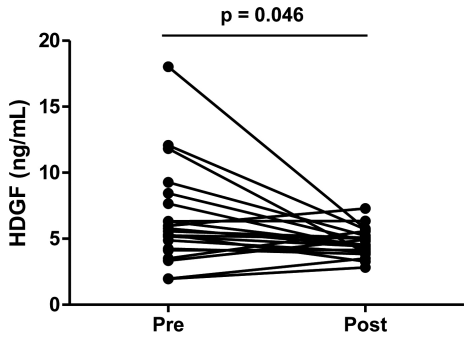

FIGURE 5 Changes in serum HDGF level after the decrease of disease activity. Serum HDGF levels decreased in patients with RA following the improvement of disease activity. HDGF, Hepatoma-derived growth factor

Correlation analysis demonstrated that serum HDGF levels were associated with disease activity indices namely DAS28 ESR and DAS28 CRP. This correlation was also found to be significant, with laboratory findings of WBC count, ESR, and CRP, a surrogate marker indicating inflammation in general. Notably, this relationship was also found to be consistent in patients with seropositive RA, an RA subtype considered to have a severe clinical presentation and poor prognosis.[30] Therefore, the assessment of serum HDGF in patients with RA appears to possess clinical value for

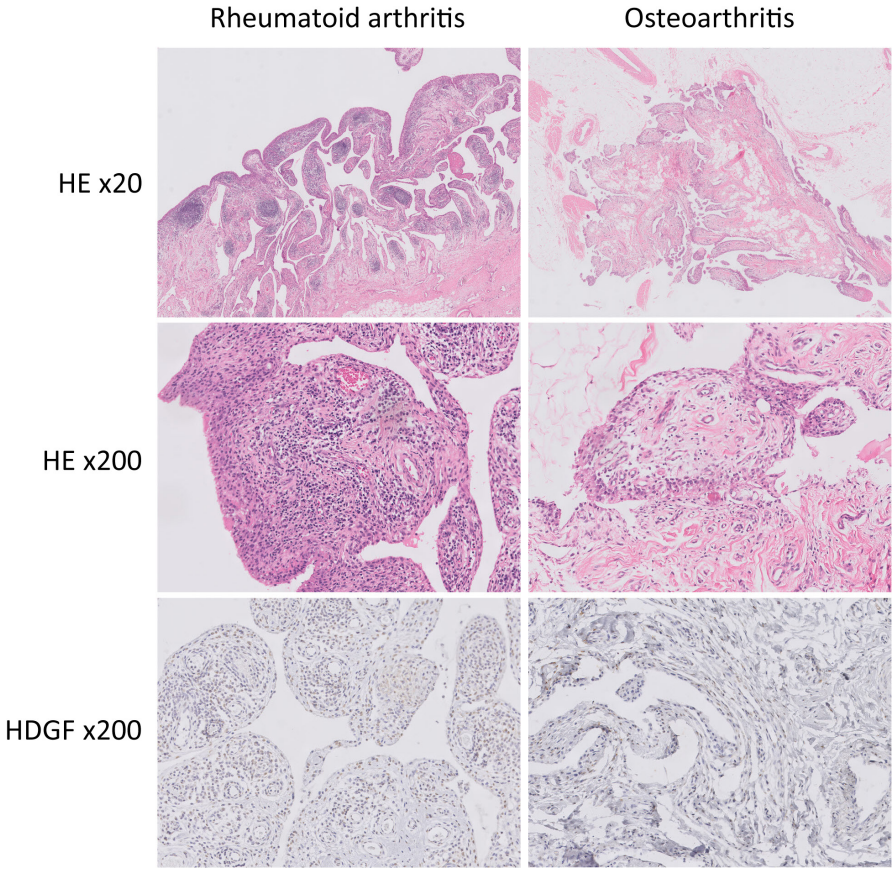

FIGURE 6 Immunohistochemical staining of HDGF in synovial tissues of patients with RA and OA. Hematoxylin and eosin staining showed villous synovial hyperplasia and hypertrophy with dense perivascular plasma cell, lymphocyte, and macrophage infiltration and germinal center formation in the RA knee, whereas the OA knee demonstrated myxoid degeneration with focal synovial hyperplasia with lymphoid follicles. In contrast, HDGF expression was not observed in the knee synovium of patients with RA or OA. HDGF, Hepatoma-derived growth factor; OA, osteoarthritis; RA, rheumatoid arthritis

the estimation of disease activity. This was further supported by the result that patients treated with DMARDs and bDMARDs had lower levels of serum HDGF, and serial testing confirmed a decrease in its level following a reduction in disease activity. Additionally, ROC curve analysis identified that a cutoff of serum HDGF of 5.79 and 5.14 could discriminate active/inactive and remission/non-remission in RA, and a cutoff >5.47 had the highest predictive value in differentiating between RA and OA. Notably, no association was found between serum HDGF and IL-6 levels in our patients. Given that IL-6 has been suggested to play a central role in RA pathogenesis, and a previous study has reported that HDGF increases the vascular expression of IL-6 in aortic smooth muscle cells from rats,[31,32] this result was not anticipated. This discrepancy could be partly explained by the diversity of disease-specific inflammatory environments and experimental settings. Alternatively, it could be possible that non-IL-6 dependent signaling exists with regard to HDGF and dysregulated immune response in RA or therapeutic effects of drugs/biologics toward IL-6 levels may have influenced the result.

This study had several limitations. First, this was a pilot study using serum samples that were collected during routine patient care, and patients' data collection was undergone retrospectively. Second, the number of treatment-naïve patients and those with seronegative RA were relatively small. Furthermore, the effect of medications on the treatment of RA could have affected the serum levels of HDGF, but analysis of these effects was not possible. Third, although a significant correlation between DAS28 and serum HDGF was present, the correlation coefficient was only moderate, and the AUROC curve value for discriminating RA disease activity and in patients with RA and OA was not decent. Fourth, the underlying mechanism linking the promotion of inflammation in RA and HDGF could not be evaluated in detail. Additional research is warranted in the future to reveal the precise role of serum HDGF in patients with RA.

In summary, we demonstrated that serum HDGF levels were correlated with disease activity in patients with RA and were significantly higher in those with active disease than in those with OA and the HCs. Serial testing of serum HDGF also revealed a decrease in its level after the improvement of RA disease activity, suggesting that it could be a potential laboratory biomarker reflecting the disease severity of RA.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PATIENT CONSENT STATEMENT

The requirement to obtain patient consent was waivered owing to the use of samples that were already available (leftover) during routine patient care and the retrospective nature of this study.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID

Sung Soo Ahn https://orcid.org/0000-0002-9002-9880
Younhee Park https://orcid.org/0000-0001-8458-1495

REFERENCES

1. Nakamura H, Izumoto Y, Kambe H, et al. Molecular cloning of complementary DNA for a novel human hepatoma-derived growth factor. Its homology with high mobility group-1 protein. *J Biol Chem*. 1994;269(40):25143-25149.
2. Enomoto H, Nakamura H, Nishikawa H, Nishiguchi S, Iijima H. Hepatoma-derived growth factor: an overview and its role as a potential therapeutic target molecule for digestive malignancies. *Int J Mol Sci*. 2020;21(12):4216.
3. Zhao J, Ma MZ, Ren H, et al. Anti-HDGF targets cancer and cancer stromal stem cells resistant to chemotherapy. *Clin Cancer Res*. 2013;19(13):3567-3576.
4. Liu C, Wang L, Jiang Q, et al. Hepatoma-derived growth factor and DDX5 promote carcinogenesis and progression of endometrial cancer by activating β-catenin. *Front Oncol*. 2019;9:211.
5. Izumoto Y, Kuroda T, Harada H, Kishimoto T, Nakamura H. Hepatoma-derived growth factor belongs to a gene family in mice showing significant homology in the amino terminus. *Biochem Biophys Res Commun*. 1997;238(1):26-32.
6. Koh HM, Hyun CL, Jang BG, Lee HJ. The relationship between hepatoma-derived growth factor and prognosis in non-small cell lung cancer: a systematic review and meta-analysis. *Medicine (Baltimore)*. 2020;99(51):e23837.
7. Zhang G, Liu Z, Chen Y, Zhang Y. High serum HDGF levels are predictive of bone metastasis and unfavorable prognosis in non-small cell lung cancer. *Tohoku J Exp Med*. 2017;242(2):101-108.
8. Liu XJ, Liu WL, Yang FM, Yang XQ, Lu XF. Hepatoma-derived growth factor predicts unfavorable prognosis of epithelial ovarian cancer. *Onco Targets Ther*. 2015;8:2101-2109.
9. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol*. 2007;81(1):1-5.
10. Orr C, Vieira-Sousa E, Boyle DL, et al. Synovial tissue research: a state-of-the-art review. *Nat Rev Rheumatol*. 2017;13(8):463-475.
11. Yap HY, Tee SZ, Wong MM, Chow SK, Peh SC, Teow SY. Pathogenic role of immune cells in rheumatoid arthritis: implications in clinical treatment and biomarker development. *Cells*. 2018;7(10):161.
12. Lavric M, Miranda-García MA, Holzinger D, Foell D, Wittkowski H. Alarmins firing arthritis: helpful diagnostic tools and promising therapeutic targets. *Joint Bone Spine*. 2017;84(4):401-410.
13. Goldstein RS, Bruchfeld A, Yang L, et al. Cholinergic anti-inflammatory pathway activity and high mobility group box-1 (HMGB1) serum levels in patients with rheumatoid arthritis. *Mol Med*. 2007;13(3-4):210-215.
14. Matsuyama Y, Okazaki H, Tamemoto H, et al. Increased levels of interleukin 33 in sera and synovial fluid from patients with active rheumatoid arthritis. *J Rheumatol*. 2010;37(1):18-25.
15. Kang KY, Woo JW, Park SH. S100A8/A9 as a biomarker for synovial inflammation and joint damage in patients with rheumatoid arthritis. *Korean J Intern Med*. 2014;29(1):12-19.
16. Edwards CJ, Kiely P, Arthanari S, et al. Predicting disease progression and poor outcomes in patients with moderately active rheumatoid arthritis: a systematic review. *Rheumatol Adv Pract*. 2019;3(1):rkz002.
17. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum*. 2010;62(9):2569-2581.
18. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis*. 2010;69(9):1580-1588.

19. Inoue E, Yamanaka H, Hara M, Tomatsu T, Kamatani N. Comparison of Disease Activity Score (DAS)28- erythrocyte sedimentation rate and DAS28- C-reactive protein threshold values. *Ann Rheum Dis*. 2007;66(3):407-409.
20. Fraenkel L, Bathon JM, England BR, et al. 2021 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Rheumatol*. 2021;73(7):1108-1123.
21. Lau CS, Chia F, Dans L, et al. 2018 update of the APLAR recommendations for treatment of rheumatoid arthritis. *Int J Rheum Dis*. 2019;22(3):357-375.
22. McWilliams DF, Kiely PDW, Young A, Joharatnam N, Wilson D, Walsh DA. Interpretation of DAS28 and its components in the assessment of inflammatory and non-inflammatory aspects of rheumatoid arthritis. *BMC Rheumatol*. 2018;2:8.
23. Fransen J, Creemers MC, Van Riel PL. Remission in rheumatoid arthritis: agreement of the disease activity score (DAS28) with the ARA preliminary remission criteria. *Rheumatology (Oxford)*. 2004;43(10):1252-1255.
24. Rider P, Voronov E, Dinarello CA, Apte RN, Cohen I. Alarmins: feel the stress. *J Immunol*. 2017;198(4):1395-1402.
25. Alvarez F, Al-Aubodah TA, Yang YH, Piccirillo CA. Mechanisms of T(REG) cell adaptation to inflammation. *J Leukoc Biol*. 2020;108(2):559-571.
26. Malemud CJ. Intracellular signaling pathways in rheumatoid arthritis. *J Clin Cell Immunol*. 2013;4:160.
27. Enomoto H, Nakamura H, Liu W, Nishiguchi S. Hepatoma-derived growth factor: its possible involvement in the progression of hepatocellular carcinoma. *Int J Mol Sci*. 2015;16(6):14086-14097.
28. Chung CL, Wu CH, Huang YH, et al. Blocking hepatoma-derived growth factor attenuates vasospasm and neuron cell apoptosis in rats subjected to subarachnoid hemorrhage. *Transl Stroke Res*. 2022;13(2):300-310.
29. Akhavani MA, Madden L, Buysschaert I, Sivakumar B, Kang N, Paleolog EM. Hypoxia upregulates angiogenesis and synovial cell migration in rheumatoid arthritis. *Arthritis Res Ther*. 2009;11(3):R64.
30. Albrecht K, Zink A. Poor prognostic factors guiding treatment decisions in rheumatoid arthritis patients: a review of data from randomized clinical trials and cohort studies. *Arthritis Res Ther*. 2017;19(1):68.
31. Kao YH, Chen PH, Sun CK, et al. Cyclic mechanical stretch upregulates hepatoma-derived growth factor expression in cultured rat aortic smooth muscle cells. *Biosci Rep*. 2018;38(2). doi:10.1042/BSR20171398
32. Hashizume M, Mihara M. The roles of interleukin-6 in the pathogenesis of rheumatoid arthritis. *Arthritis*. 2011;2011:765624.

How to cite this article: Ahn SS, Kim HM, Park Y. Association of serum hepatoma-derived growth factor levels with disease activity in rheumatoid arthritis: A pilot study. *J Clin Lab Anal*. 2022;36:e24474. doi:10.1002/jcla.24474