Pre-Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
48(11) 1–10
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/0300060520962954
journals.sagepub.com/home/imr
SAGE

Elevated serum granzyme B levels are associated with disease activity and joint damage in patients with rheumatoid arthritis

Junjie Qiao[1,#], Meng Zhou[2,#], Zheng Li[3], Jie Ren[3], Guanghan Gao[3], Jumei Zhen[1], Guanglei Cao[3,*] and Lixiang Ding[1,*]

Abstract

Objectives: Little is known about the roles of granzyme B in rheumatoid arthritis (RA). We aimed to evaluate the serum level of granzyme B in patients with RA and determine relationships with clinical features and joint destruction of RA.

Methods: We enrolled 100 patients with RA, 50 patients with osteoarthritis (OA), and 50 healthy controls (HC). Granzyme B serum concentrations were measured by ELISA; we then analyzed associations between granzyme B levels, clinical features, and joint destruction by calculating Sharp scores and disease activity as measured by Disease Activity Score-28 based on erythrocyte sedimentation rate (DAS28-ESR) in patients with RA.

Results: Compared with HC and patients with OA, serum granzyme B levels in patients with RA were remarkably elevated. Serum granzyme B levels did not differ between patients with OA and HC. Granzyme B levels correlated with ESR, rheumatoid factor, swollen joint counts, joint erosion scores, total Sharp scores, and DAS28-ESR. Moreover, patients with RA with high disease activity had higher granzyme B levels.

[1]Department of Orthopedics, Beijing Shijitan Hospital, Capital Medical University, Beijing, China
[2]Department of Orthopedics, Beijing Jishuitan Hospital, Fourth Medical College of Peking University, Beijing, China
[3]Department of Orthopedics, Xuanwu Hospital, Capital Medical University, Beijing, China

[#]These authors contributed equally to this work.

[*]These authors contributed equally to this work.

Corresponding authors:
Lixiang Ding and Guanglei Cao, Department of Orthopedics, Beijing Shijitan Hospital, Capital Medical University, No. 10 Tieyi Road, Yangfangdian, Haidian District, Beijing 100038, China; Department of Orthopedics, Xuanwu Hospital, Capital Medical University, No. 45, Changchun Street, Xicheng District, Beijing 100053, China.
Email: Dinglixiang2019@163.com; guanglei_cao@163.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Conclusions: Serum granzyme B levels were elevated significantly in patients with RA and correlated positively with disease activity and joint destruction. Serum granzyme B may have potential applications in laboratory evaluation of patients with RA.

Keywords
Rheumatoid arthritis, granzyme B, disease activity, joint destruction, Sharp score, DAS28

Date received: 21 March 2020; accepted: 4 September 2020

Introduction

Rheumatoid arthritis (RA) is a chronic immune-mediated disease characterized by degradation of articular cartilage and bone; it affects 0.2% to 1.0% of the population worldwide.[1] With progressive destruction of bone, patients with RA can develop obvious joint deformities and may suffer severe work disability. These outcomes have a considerable impact on patient quality of life and economic burden, both of which have received attention.[2] Previous studies have demonstrated that cytotoxic T cells and natural killer (NK) cells are significantly increased in rheumatoid synovial tissue,[3,4] and these cells contain a family of homologous serine proteinases called granzymes.[5]

Granzymes are important members of the serine protease family and have a wide range of functions.[6] In particular, granzyme B has been shown to serve a vital role in apoptosis of target cells through the granzyme–perforin apoptotic pathway.[7] Several studies have indicated the presence of granzyme-positive cells in synovial tissue from patients with RA, and expression of granzyme B was shown to be higher in rheumatoid synovial tissue than in osteoarthritic synovial tissue.[8,9] Recent work revealed a potential role of granzymes in increasing degradation of extracellular matrix (ECM), which might accelerate cartilage and bone destruction in RA.[10] Colombo et al.[11] found that granzyme B serum levels of 18 patients with RA were correlated with disease activity measured by Disease Activity Score 28-joint count–C-reactive protein (DAS28-CRP), and levels of granzyme B were significantly decreased after treatment. However, the significance of granzyme B, and especially its role in RA joint damage, for patients with RA remains largely unclear. Therefore, in this study, we aimed to investigate the potential functions of granzyme B in RA. We compared serum levels of granzyme B in patients with RA and osteoarthritis (OA) and in healthy individuals. We also analyzed correlations between serum granzyme B levels and clinical characteristics, disease activity, and joint damage of patients with RA.

Materials and Methods

Ethical approval

All participants gave written informed consent, and the study was approved by the ethical committee of Xuanwu Hospital.

Patients

Patients with RA ($n = 100$) fulfilling the 1987 American College of Rheumatology (ACR) revised criteria[12] and 2010

ACR/European League Against Rheumatism (EULAR) classification criteria[13] were recruited in our hospital. Patients with OA (n = 50) fulfilling the 1995 ACR criteria[14] and matched by sex and age were enrolled as disease controls in our hospital. All the patients with OA were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), which includes 24 items of pain (0–20), stiffness (0–8), and physical function (0–68), with a total score of 96.[15] The average WOMAC score of patients with OA in this study was 67.1 ± 7.9. Healthy individuals (n = 50) were recruited from the local community.

Clinical data

Clinical data included age, sex, duration of disease, and counts of tender and swollen joints. The following laboratory indices were recorded: white blood cell (WBC) count, red blood cells (RBC) count, hemoglobin (Hb), platelet (PLT) count, immunoglobulin (Ig)G, IgA, IgM, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), and anti-cyclic citrullinated peptide antibody (anti-CCP antibody). Disease activity of patients with RA was scored using the 28-joint Disease Activity Score (DAS28) calculated using the Nijmegen formula based on ESR.[16] A DAS28-ESR >5.1 is considered indicative of high disease activity in accordance with the EULAR recommendations.[17]

Joint damage assessment

All patients with RA had X-rays of the hands and wrists, and joint damage was assessed by the van der Heijde modified Sharp score.[18] Total Sharp scores were calculated on the basis of 17 areas evaluated for joint erosions and 18 areas evaluated for joint space narrowing. The assessments were completed independently by two experienced radiologists who were blinded to the patients' data.

ELISA detection of serum granzyme B levels

Serum samples were obtained from all patients with RA and OA, and HC; samples were coded and stored at −80°C until analysis. Serum granzyme B levels were detected by using DuoSet sandwich ELISA kits (catalog number DY2906-05) and DuoSet Ancillary Reagent Kit 2 (catalog number DY008) from R&D Systems (Minneapolis, MN, USA). In accordance with the manufacturer's instructions, detection was performed as follows. Briefly, plates were coated with capture antibody against human granzyme B overnight at room temperature (RT). Then, plates were washed three times in 0.05% Tween 20 in PBS and blocked with 1% bovine serum albumin in PBS for 1.5 hours at RT. The plates were washed as above. Standards and samples were added and plates incubated for 2 hours at RT. The plates were washed as above. Next, biotinylated anti-human granzyme B antibody and horseradish peroxidase (HRP)-conjugated streptavidin were used to detect the reaction. Tetramethylbenzidine was added as the substrate solution, and the reaction was stopped with 2 N H_2SO_4. The optical density was immediately measured using an automatic ELISA reader at 450 nm with a correction wavelength of 570 nm. The concentration was calculated from the standard curve.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 for Windows (IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation or median [interquartile range (IQR)]. The group

differences were assessed by one-way ANOVA test with Dunnett's T3 post hoc test (as appropriate), two-tailed independent samples Student's *t*-test, or Mann–Whitney rank-sum test. The correlations between two variables were analyzed by Spearman's correlation coefficient. The regression analysis was analyzed by logistic regression. A P-value < 0.05 was considered statistically significant.

Results

Clinical and demographic features of patients with RA

Table 1 summarizes the clinical and demographic data of participants enrolled in this study. The ratio of female to male patients with RA was approximately 3.76:1 (79 women, 21 men), and the mean age was 57.82 ± 12.42 years. The median (IQR) disease duration of patients with RA was 20 months (14–26.7 months). The mean DAS28-ESR score was 5.08 ± 1.31. All patients with RA completed X-ray examinations and joint damage assessment; the median (IQR) joint erosion scores, joint space narrowing scores, and total Sharp scores were 26.5 (8–56.75), 43.5 (27.25–60.75), and 66.5 (40–121.5), respectively.

Elevated serum granzyme B levels in patients with RA

Compared with patients with OA (22.99 ± 9.63 pg/mL) and HC (21.60 ± 8.58 pg/mL), serum granzyme B levels were significantly higher in patients with RA (55.34 ± 22.75 pg/mL) ($F = 84.27$, $P < 0.001$; RA vs. HC: $P < 0.001$; RA vs. OA: $P < 0.001$; OA vs. HC: $P = 0.847$) (Figure 1a). However, there was no difference in granzyme B levels between female and male patients with RA ($Z = -0.072$) (Figure 1b), and no correlation between granzyme B levels and duration of RA or age of patients with RA (duration: $r = 0.033$; age: $r = -0.007$) (Figure 1c, d).

Table 1. Demographic and clinical characteristics of participants.

Characteristics	Patients with RA (n=100)	Patients with OA (n=50)	Healthy controls (n=50)	*P*-value
Age (years)	57.82 ± 12.43	55.93 ± 8.60	56.18 ± 9.42	>0.05
Sex (F/M)	79/21	39/11	41/9	>0.05
Duration (months)	20 (14–26.7)	–	–	–
Swollen joint counts (0–28)	5 (2–12)	–	–	–
Tender joint count (0–28)	6 (2–13)	–	–	–
ESR (mm/h)	51.85 ± 32.85	–	–	–
CRP (mg/L)	18.29 (8.61–35.43)	–	–	–
RF (IU/mL)	261 (80.23–637)	–	–	–
Anti-CCP antibody (U/mL)	146.96(39.16–187.92)	–	–	–
DAS28-ESR	5.08 ± 1.31	–	–	–
Joint erosion scores	26.5 (8–56.75)	–	–	–
Joint narrow scores	43.5 (27.25–60.75)	–	–	–
Total Sharp scores	66.5 (40–121.5)	–	–	–

Values are mean ± standard deviation or median (interquartile range).

RA, rheumatoid arthritis; OA, osteoarthritis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; anti-CCP antibody, anti-cyclic citrullinated peptide antibody; DAS28-ESR, Disease Activity Score 28-joint count based on ESR.

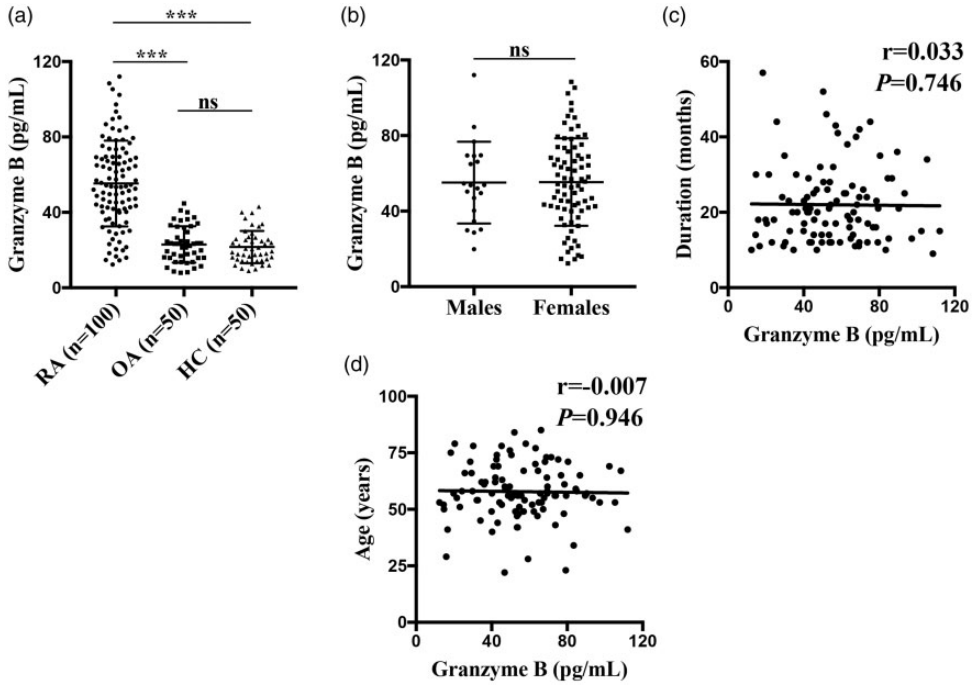

Figure 1. Comparison of serum granzyme B levels in patients with RA and OA, and HC. Concentrations of granzyme B were substantially increased in patients with RA compared with patients with OA and HC, but there was no difference between patients with OA and HC (a). No difference was found between female and male patients with RA (b). No correlation was found between granzyme B levels and duration of disease (c) or age (d) of patients with RA. Error bars indicate SD; ***$P < 0.001$, ns, nonsignificant
RA, rheumatoid arthritis; OA, osteoarthritis; HC, healthy controls.

Granzyme B levels and clinical characteristics in patients with RA

Next, we analyzed correlations between granzyme B levels and clinical features of patients with RA. Serum concentration of granzyme B was positively correlated with ESR ($r = 0.228$, $P = 0.023$), RF ($r = 0.329$, $P = 0.001$), and swollen joint counts ($r = 0.231$, $P = 0.021$) (Figure 2a-c). However, granzyme B level was not correlated with WBC count ($r = 0.003$), RBC count ($r = -0.196$), Hb ($r = -0.041$), PLT count ($r = 0.125$), IgM ($r = 0.195$), IgA ($r = 0.021$), IgG ($r = -0.142$), CRP ($r = -0.006$), anti-CCP antibody ($r = -0.059$), or counts of tender joints ($r = 0.156$) (data not shown). We then conducted multivariate logistic regression and the results showed that there was no independent risk factor for serum granzyme B level (odds ratio = 1.665; $P = 0.006$).

Granzyme B levels and joint damage in patients with RA

We found that granzyme B levels were associated with bone damage in patients with RA. Granzyme B levels were positively correlated with total Sharp scores ($r = 0.222$, $P = 0.026$) and joint erosion scores ($r = 0.202$, $P = 0.044$); no correlation was found between granzyme B levels and joint space narrowing scores ($r = 0.189$) (Figure 3a-c).

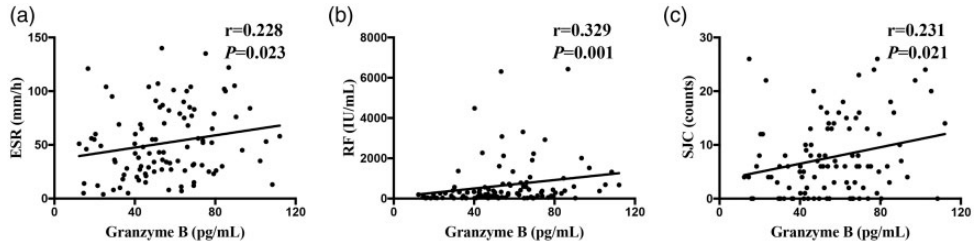

Figure 2. Associations between the levels of granzyme B and clinical characteristics of patients with RA. Serum granzyme B levels were positively correlated with ESR (a), RF (b), and SJC (c)
RA, rheumatoid arthritis; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; SJC, swollen joint counts.

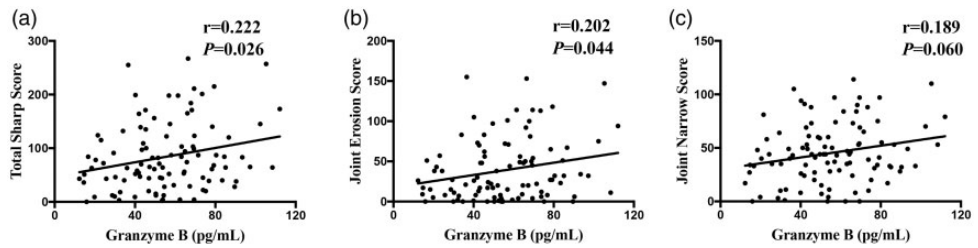

Figure 3. Correlations between granzyme B levels and joint damage of patients with RA. Serum granzyme B levels was positively correlated with total Sharp score (a) and joint erosion score (b), but not with joint space narrowing score (c).
RA, rheumatoid arthritis.

Granzyme B levels and disease activity score in patients with RA

In accordance with the recommendations from EULAR, the DAS28 based on ESR was evaluated as described. We found that serum levels of granzyme B were positively correlated with DAS28-ESR ($r = 0.322$, $P = 0.001$) (Figure 4a). Furthermore, patients with RA were classified into two groups based on their DAS28-ESR: a DAS28-ESR score ≤ 5.1 defined the low/moderate activity group (51 patients) and a DAS28-ESR score >5.1 defined the high activity group (49 patients). We then compared serum granzyme B levels among the two groups. Granzyme B levels were significantly higher in the high activity group (59.84 ± 23.12 pg/mL) than in the low/moderate activity group (51.02 ± 21.74 pg/mL) ($Z = -2.100$, $P = 0.036$) (Figure 4b).

Discussion

Rheumatoid arthritis is a chronic and progressive inflammatory disease affecting the synovial tissue in multiple joints, and it is characterized by a number of clinical symptoms.[19] Granzymes are a family of serine proteases derived from the granules of cytotoxic lymphocytes, and granzyme B is thought to be responsible for the apoptosis of target cells.[20] It has been demonstrated that the synovial tissue of RA is infiltrated by cytotoxic cells, which could then lead to a specific increase in granzyme B.[21]

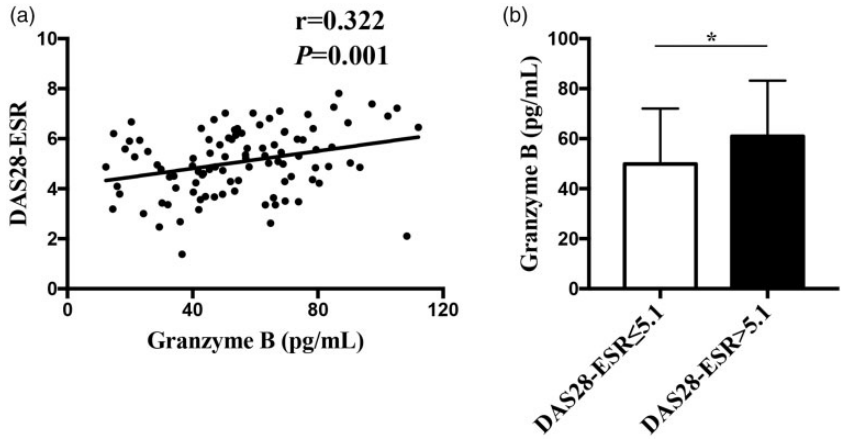

Figure 4. Correlation analysis of serum granzyme B levels with patients with RA disease activity. Serum granzyme B concentrations were positively correlated with DAS28-ESR in patients with RA (a). A significant difference in granzyme B level was found between the low/moderate disease activity group (DAS28-ESR $\leq$5.1) and the high disease activity group (DAS28-ESR $>$5.1) (b). Error bars indicate SD; *$P < 0.05$
RA, rheumatoid arthritis; DAS28-ESR, Disease Activity Score 28-joint count based on erythrocyte sedimentation rate.

The first findings of our study showed a significant increase in serum granzyme B concentrations in patients with established RA compared with patients with OA and healthy controls. The marked elevation of serum granzyme B associated with RA indicated that granzyme B expression is somehow related to the inflammatory activation of RA, consistent with previous studies.[22,23] Patients with autoimmune diseases have a high tendency to develop autoantibodies against nuclear components.[24] Defective clearance of apoptotic cells is believed to be one of several causes of RA, and apoptotic cells are present in synovial tissues and serum.[25] However, the origin of the increase in serum granzyme is not fully known. Previous studies have reported that the level of granzyme B was higher in synovial tissue than in corresponding serum samples, which indicated that the increase of granzyme B in serum may originate from the synovial tissues of the inflamed joint.[26] Following release from cytotoxic cells in synovial tissue, granzymes migrate into the circulatory system and reach a high concentration in serum. Soluble granzyme B is found extracellularly in normal plasma and is elevated in a number of diseases, including viral and bacterial infections and autoimmune diseases; therefore, granzyme B might be produced and secreted by non-immune cells.[5,27]

Although the finding that patients with RA have higher granzyme B concentrations than patients with OA has been demonstrated in some studies,[9,26] correlations between serum levels of granzyme B and clinical features in RA have not been fully elucidated. Goldbach-Mansky et al.[28] revealed a weak correlation between granzyme B and RF in patients with RA, whereas no obvious correlations were found between granzyme B concentrations and other clinical features. However, Tak et al.[26] reported highly significant correlations between serum granzyme B levels and serum levels of RF. Additionally, weakly positive correlations were found between serum granzyme B level and CRP level

and swollen joint counts.[26] Xu et al.[10] reported that the frequency of granzyme B-producing B cells was negatively correlated with disease activity in patients with RA. Our study also found that elevated granzyme B levels in the sera of patients with RA were correlated positively with RF and counts of swollen joints. Moreover, in the current study, we showed positive correlations between serum granzyme B levels and ESR and DAS28-ESR disease activity score. These discrepancies in reported results may be because Xu et al.[10] focused on granzyme B produced by B cells, whereas our study detected total serum granzyme B, which is produced by multiple cell types, including T cells, NK cells, B cells, and others. Although no evidence has yet shown a causal role for granzyme B in disease activity of RA, our results suggested that granzyme B may be an indicator by which to monitor disease activity.

The extracellular activity of granzyme B is an emerging focus of autoimmune disease research.[29] A study by Colombo et al. revealed that granzyme-positive cells were present at the cartilage-pannus junction, where damaged synovium invades cartilage and bone. The authors hypothesized that secreted granzymes may act as mediators of extracellular proteolysis and have potent ECM remodeling activity.[11] Therefore, in this study, we assessed the correlation between granzyme B levels and indices of bone destruction. As expected, levels of granzyme B were strongly and positively correlated with joint destruction scores in patients with RA, which indicated that granzyme B level might be a surrogate marker to predict the severity of joint damage.

There are several possible explanations for the relationship between granzyme B and joint destruction. First, granzymes are important mediators of apoptosis and might mediate cartilage destruction by inducing apoptosis in chondrocytes.[30] Second, granzyme B has enzymatic activity to cleave ECM proteins in the cartilage matrix,[31,32] and granzyme-mediated ECM degradation may facilitate the movement of cytotoxic lymphocytes and other leukocytes in vivo or the cleavage products may attract various immune cells, contributing to local inflammation.[33,34] Finally, extracellular granzyme B can cleave and activate several important proinflammatory cytokines, including interleukin (IL-6), IL-8, and tumor necrosis factor-α.[35] However, in vivo evidence for the contribution of granzyme B to the destructive process in RA is lacking and should be explored in further studies.

In the present study, we systematically measured serum levels of granzyme B in patients with RA and correlated these measures with clinical features and joint destruction. Our findings suggest that measurement of granzyme B may be of use in laboratory evaluations of patients with RA. Further functional studies are needed to fully elucidate the role of granzyme B in development of RA.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

The present study was supported by the National Natural Science Foundation of China (No. 81541135), Capital's Funds for Health Improvement and Research (No. 2018-2-2012), Capital Clinical Application Research and Achievement Promotion Fund (Z151100004015068) and Beijing Traditional Chinese Medical Science and Technology Development Fund (2018-A29).

ORCID iDs

Junjie Qiao https://orcid.org/0000-0001-6426-2024

Guanghan Gao https://orcid.org/0000-0002-6662-5463

References

1. Smolen JS, Aletaha D and McInnes IB. Rheumatoid arthritis. *Lancet* 2016; 388: 2023–2038.
2. Ostrowska M, Maslinski W, Prochorec-Sobieszek M, et al. Cartilage and bone damage in rheumatoid arthritis. *Reumatologia* 2018; 56: 111–120.
3. Conigliaro P, Scrivo R, Valesini G, et al. Emerging role for NK cells in the pathogenesis of inflammatory arthropathies. *Autoimmun Rev* 2011; 10: 577–581.
4. Aggarwal A, Sharma A and Bhatnagar A. Bi (o)communications among peripheral blood fractions: a focus on NK and NKT cell biology in rheumatoid arthritis. *Autoimmunity* 2013; 46: 238–250.
5. Turner CT, Lim D and Granville DJ. Granzyme B in skin inflammation and disease. *Matrix Biol* 2017.
6. Voskoboinik I, Whisstock JC and Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. *Nat Rev Immunol* 2015; 15: 388–400.
7. Joeckel LT and Bird PI. Are all granzymes cytotoxic in vivo? *Biol Chem* 2014; 395: 181–202.
8. Darrah E, Kim A, Zhang X, et al. Proteolysis by granzyme B enhances presentation of autoantigenic peptidylarginine deiminase 4 epitopes in rheumatoid arthritis. *J Proteome Res* 2017; 16: 355–365.
9. Kummer JA, Tak PP, Brinkman BM, et al. Expression of granzymes A and B in synovial tissue from patients with rheumatoid arthritis and osteoarthritis. *Clin Immunol Immunopathol* 1994; 73: 88–95.
10. Xu L, Liu X, Liu H, et al. Impairment of granzyme B-producing regulatory B cells correlates with exacerbated rheumatoid arthritis. *Front Immunol* 2017; 8: 768.
11. Colombo E, Scarsi M, Piantoni S, et al. Serum levels of granzyme B decrease in patients with rheumatoid arthritis responding to abatacept. *Clin Exp Rheumatol* 2016; 34: 37–41.
12. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315–324.
13. 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: 2569–2581.
14. Hochberg MC, Altman RD, Brandt KD, et al. Guidelines for the medical management of osteoarthritis. Part II. Osteoarthritis of the knee. *Arthritis Rheum* 1995; 38: 1541–1546.
15. Gandek B. Measurement properties of the Western Ontario and McMaster Universities Osteoarthritis Index: a systematic review. *Arthritis Care Res (Hoboken)* 2015; 67: 216–229.
16. Prevoo ML, Van't Hof MA, Kuper HH, et al. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 44–48.
17. Fransen J and Van Riel PL. The Disease Activity Score and the EULAR response criteria. *Rheum Dis Clin North Am* 2009; 35: 745–757, vii-viii.
18. Van Der Heijde DM, Van Riel PL, Nuver-Zwart IH, et al. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. *Lancet* 1989; 1: 1036–1038.
19. Guo Q, Wang Y, Xu D, et al. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Res* 2018; 6: 15.
20. Perl M, Denk S, Kalbitz M, et al. Granzyme B: a new crossroad of complement and apoptosis. *Adv Exp Med Biol* 2012; 946: 135–146.
21. Kurschus FC and Jenne DE. Delivery and therapeutic potential of human granzyme B. *Immunol Rev* 2010; 235: 159–171.
22. Knevel R, Krabben A, Wilson AG, et al. A genetic variant in granzyme B is associated with progression of joint destruction in

rheumatoid arthritis. *Arthritis Rheum* 2013; 65: 582–589.
23. Smeets TJ, Kraan MC, Galjaard S, et al. Analysis of the cell infiltrate and expression of matrix metalloproteinases and granzyme B in paired synovial biopsy specimens from the cartilage-pannus junction in patients with RA. *Ann Rheum Dis* 2001; 60: 561–565.
24. Fujii T. Direct and indirect pathogenic roles of autoantibodies in systemic autoimmune diseases. *Allergol Int* 2014; 63: 515–522.
25. Darrah E and Rosen A. Granzyme B cleavage of autoantigens in autoimmunity. *Cell Death Differ* 2010; 17: 624–632.
26. Tak PP, Spaeny-Dekking L, Kraan MC, et al. The levels of soluble granzyme A and B are elevated in plasma and synovial fluid of patients with rheumatoid arthritis (RA). *Clin Exp Immunol* 1999; 116: 366–370.
27. Horiuchi K, Saito S, Sasaki R, et al. Expression of granzyme B in human articular chondrocytes. *J Rheumatol* 2003; 30: 1799–1810.
28. Goldbach-Mansky R, Suson S, Wesley R, et al. Raised granzyme B levels are associated with erosions in patients with early rheumatoid factor positive rheumatoid arthritis. *Ann Rheum Dis* 2005; 64: 715–721.
29. Afonina IS, Tynan GA, Logue SE, et al. Granzyme B-dependent proteolysis acts as a switch to enhance the proinflammatory activity of IL-1alpha. *Mol Cell* 2011; 44: 265–278.
30. Saito S, Murakoshi K, Kotake S, et al. Granzyme B induces apoptosis of chondrocytes with natural killer cell-like cytotoxicity in rheumatoid arthritis. *J Rheumatol* 2008; 35: 1932–1943.
31. Buzza MS and Bird PI. Extracellular granzymes: current perspectives. *Biol Chem* 2006; 387: 827–837.
32. Buzza MS, Zamurs L, Sun J, et al. Extracellular matrix remodeling by human granzyme B via cleavage of vitronectin, fibronectin, and laminin. *J Biol Chem* 2005; 280: 23549–23558.
33. Boivin WA, Cooper DM, Hiebert PR, et al. Intracellular versus extracellular granzyme B in immunity and disease: challenging the dogma. *Lab Invest* 2009; 89: 1195–1220.
34. Susanto O, Trapani JA and Brasacchio D. Controversies in granzyme biology. *Tissue Antigens* 2012; 80: 477–487.
35. Wensink AC, Hack CE and Bovenschen N. Granzymes regulate proinflammatory cytokine responses. *J Immunol* 2015; 194: 491–497.