

Glucose-6-phosphate isomerase is associated with disease activity and declines in response to infliximab treatment in rheumatoid arthritis

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Abstract

Background: Rheumatoid arthritis (RA), a systemic autoimmune disease characterized by synovial inflammation, can cause cartilage and bone damage as well as disability. The aim of this study was to explore whether serum glucose-6-phosphate isomerase (GPI) is correlated with disease activity and the value of GPI in the evaluation of infliximab treatment in patients with RA.

Methods: Sixty-two patients with RA who had an inadequate response to methotrexate (MTX) were enrolled in Peking University People's Hospital from July 1, 2016 to July 31, 2018. Infliximab (3 mg/kg, intravenous at weeks 0, 2, and 6 and then every 8 weeks) was administered to patients with stable background MTX therapy. Serum samples were obtained at baseline and week 18. Serum GPI levels were determined using enzyme-linked immunosorbent assay. The associations between serum GPI levels and clinical features were analyzed.

Results: Serum GPI was positively correlated with Disease Activity Score in 28 joints (DAS28), swollen joint count, tender joint count and C-reactive protein level ($P < 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.033$, respectively). The change of DAS28 in GPI-positive patients was greater than that in GPI-negative patients ($P < 0.001$). Compared with those for patients receiving MTX monotherapy at baseline, the GPI levels were significantly declined when MTX was combined with infliximab ($P < 0.001$).

Conclusion: Serum GPI is related to disease activity and clinical response to infliximab treatment.

Keywords: Glucose-6-phosphate isomerase; Rheumatoid arthritis; Treatment

Introduction

Rheumatoid arthritis (RA), a systemic autoimmune disease characterized by synovial inflammation, can cause cartilage and bone damage as well as disability.^[1] RA pathogenesis is the result of complex interactions between genetic and environmental factors and activation of the immune system.^[2] Autoantibodies are the hallmark of this disease, but their pathogenic role remains unclear.

Tumor necrosis factor (TNF) inhibitors have become widely used biological agents for RA due to their clinical efficacy.^[3] However, a portion of patients do not respond to currently available treatments or lose their response to these treatments over time. There is an unmet need to identify predictors for therapeutic response to TNF inhibitors.

Glucose-6-phosphate isomerase (GPI), also known as phosphoglucose isomerase and phosphohexose isomerase, plays a crucial role in glycolysis and gluconeogenesis.^[4] GPI can be secreted by lectin-stimulated T cells^[5] and also acts as a cytokine in extracellular processes in addition to its enzymatic activity. Immunization with GPI can induce arthritis in genetically unaltered mice in a B cell-dependent manner.^[6] A previous study showed that GPI stimulated the proliferation and inhibited the apoptosis of fibroblast-like synoviocytes in RA, with the increased secretion of inflammatory cytokines.^[7] The concentration of GPI was shown to be elevated in the sera of RA patients, suggesting that GPI is involved in the pathogenesis of this disease.^[8]

In this study, we measured serum GPI levels in patients with RA and evaluated the relationships between GPI and clinical characteristics, disease activity and the response to

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TNF inhibitor treatment, especially in patients with an inadequate response to methotrexate (MTX).

Methods

Ethical approval

This study was approved by the Ethics Committee of Peking University People's Hospital (No. FWA00001384). All participants signed an informed consent form.

Study population

Sixty-two patients were consecutively enrolled in Peking University People's Hospital from July 1, 2016 to July 31, 2018. Eligible subjects fulfilled the RA classification criteria established by American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) in 2010.^[9] Patients who had an inadequate response to MTX (≥ 10 mg weekly, orally at least 3 months) received infliximab (3 mg/kg, intravenous at weeks 0, 2, and 6 and then every 8 weeks) plus background MTX treatment.

Clinical assessment

Clinical symptoms (swollen joint count [SJC] and tender joint count [TJC], and routine laboratory tests) were assessed at baseline and week 18. The erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, rheumatoid factor (RF) level, and anti-cyclic citrullinated peptide antibody (anti-CCP) level were tested.

Disease activity was assessed by calculating the Disease Activity Score 28-joint count with ESR (DAS28-ESR) as follows: $DAS28 = 0.56 \times \sqrt{(TJC)+0.28 \times \sqrt{(SJC)+0.70 \times \ln(ESR)} + 0.014 \times (\text{patient global assessment using visual analog scales, } 0-100 \text{ mm})$. Patients were categorized into groups with remission ($DAS28 \leq 2.6$) and low ($2.6 < DAS28 \leq 3.2$), moderate ($3.2 < DAS28 \leq 5.1$), and high ($DAS28 > 5.1$) disease activity.^[10] Patients were classified as having no, a moderate, or a good response to treatment according to the EULAR criteria.^[11]

Measurement of bone destruction

All available radiographs were graded using the modified Sharp/van der Heijde score (SHS), erosion (ERO) score and joint space narrowing (JSN) score.^[12]

Enzyme-linked immunosorbent assay to determine serum GPI

Serum samples were obtained at baseline and week 18. Serum levels of GPI were measured with a human GPI detection kit (Shanghai Beijia Biochemical Sciences Co., Ltd., Shanghai, China) according to the manufacturer's instructions. A concentration greater than 0.2 mg/L was considered a positive result.

Statistical analysis

Continuous data are presented as the mean \pm standard deviation (SD) or median and interquartile range (IQR). The Chi-square test was used to compare qualitative values

Table 1: Baseline characteristics of patients with RA who had an inadequate response to methotrexate.

Characteristics	All participants (N=62)
Female sex, n (%)	44 (71.0)
Age (years)	64 (49–75)
Smoker, n (%)	14 (22.6)
Duration of RA (months)	9.00 (3.75–15.25)
GPI positive, n (%)	49 (79.3)
RF positive, n (%)	44 (71.0)
Anti-CCP antibody positive, n (%)	49 (79.0)
CRP (mg/L)	41.9 (17.8–49.2)
ESR (mm/H)	75.0 (53.8–91.3)
TJC (0–28)	8.00 (2.00–20.25)
SJC (0–28)	4.50 (1.00–15.25)

Data are presented as median (interquartile range) unless stated otherwise. CCP: Cyclic citrullinated peptide; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; GPI: Glucose-6-phosphate isomerase; RA: Rheumatoid arthritis; RF: Rheumatoid factor; SJC: Swollen joint count; TJC: Tender joint count.

between groups. Student's *t* test or the Mann-Whitney rank-sum test was used for comparisons of quantitative values, depending on the distribution of data. Spearman correlation analysis was used to analyze the correlations between two variables. The Wilcoxon matched pairs signed-rank test was performed to analyze paired samples. Differences with a *P* value < 0.05 were considered to be statistically significant. Statistical analysis was performed with SPSS (version 20.0, IBM, NY, USA) or GraphPad Prism (version 7.0, GraphPad software).

Results

Baseline characteristics of patients with RA

Sixty-two patients were enrolled in this study. The average age of the patients was 61.9 ± 15.3 years, and there were 44 (71.0%) females among the enrolled patients. Among the patients, 79.0% (49/62) were positive for GPI (≥ 0.2 mg/L). The demographics and clinical characteristics of the patients are shown in Table 1.

Associations between serum GPI and clinical features in RA patients

Patients with high disease activity ($DAS28 > 5.1$) presented significantly higher levels of GPI than the other patients ($DAS28 \leq 5.1$) ($P = 0.035$). As shown in Figure 1, the GPI concentration was positively correlated with the DAS28 ($r = 0.6840$, $P < 0.001$). Among RA patients, serum GPI was positively correlated with SJC and TJC ($r = 0.4248$, $P = 0.001$, and $r = 0.6701$, $P < 0.001$, respectively, Figure 2, A and B). Serum GPI was also related to higher CRP levels ($r = 0.2706$, $P = 0.033$, Figure 2C). GPI concentration was not associated with ESR, the levels of immunoglobulin, anti-CCP or RF. These results are shown in Figure 2 D–I. Serum GPI levels were not correlated with the age of the RA patients or duration of RA.

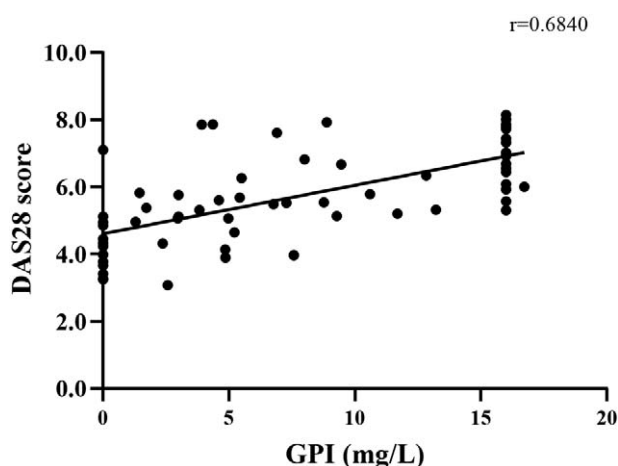


Figure 1: Serum GPI concentration in 62 RA patients is correlated with disease activity. A positive correlation was presented between GPI levels and DAS28 ($P < 0.001$). DAS28: Disease Activity Score 28-joint count; GPI: Glucose-6-phosphate isomerase; RA: Rheumatoid arthritis.

In addition, the association of GPI with radiographic joint destruction was analyzed. No statistically significant correlation between the GPI concentration and SHS, ERO score or JSN score was found [Figure 3].

We subsequently compared the characteristics of GPI-positive and GPI-negative patients. GPI-positive patients had a higher TJC and SJC ($P < 0.001$ and $P = 0.009$, respectively). There were significantly more smokers among GPI-negative patients than among GPI-positive patients ($P = 0.022$). These data are shown in Table 2.

GPI predicts the therapeutic response to infliximab treatment

After 18 weeks of infliximab treatment, disease activity assessed by the DAS28 was found to have a therapeutic benefit ($P < 0.001$, Figure 4A). The change of DAS28 was significantly greater in GPI-positive patients than in GPI-negative patients ($P < 0.001$, Figure 4B). There was no difference in the proportions of GPI-positive and

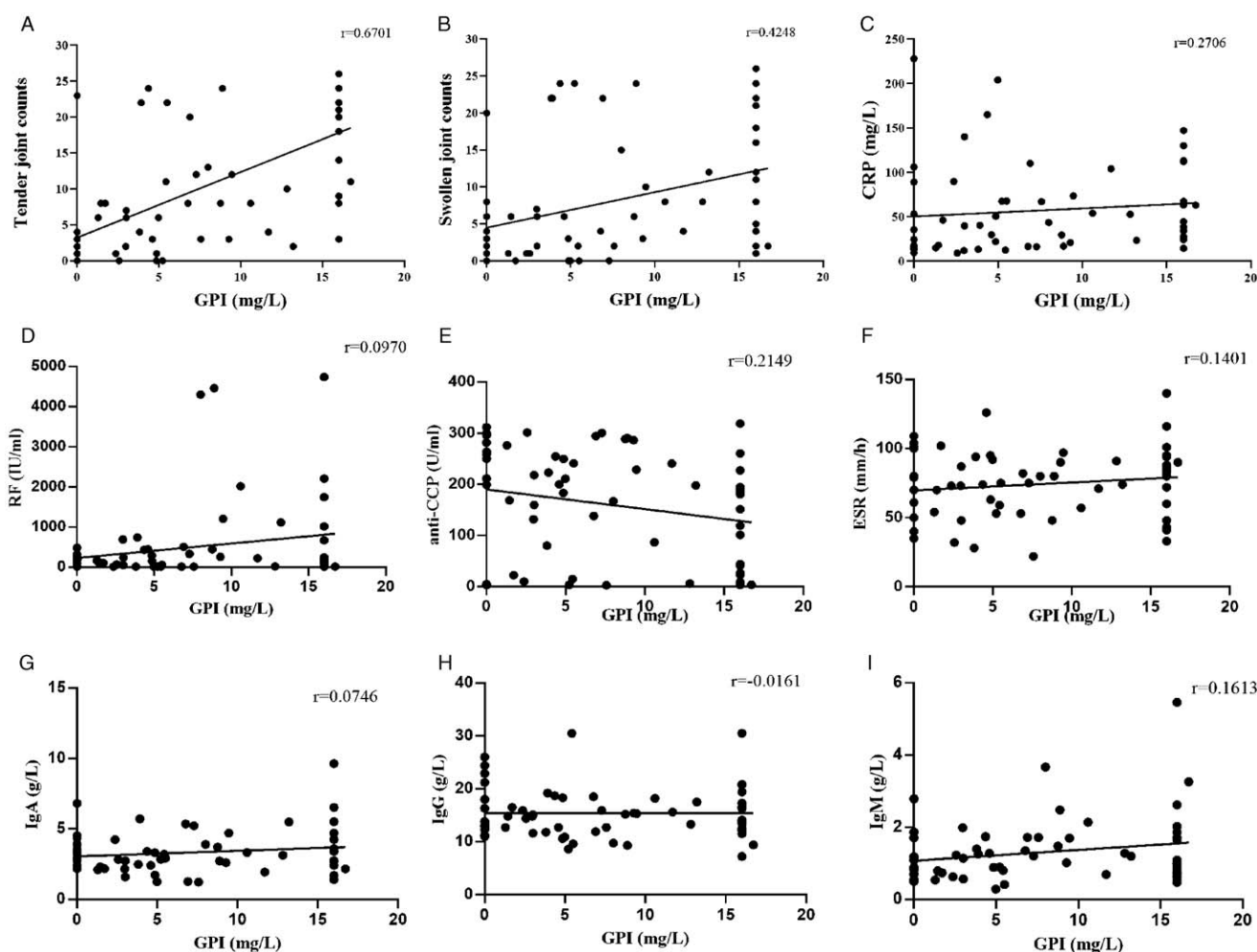


Figure 2: The GPI levels are correlated with clinical features in 62 RA patients. A positive correlation was observed between GPI levels and tender joint counts ($P < 0.001$, A), swollen joint counts ($P < 0.001$, B), and C-reactive protein ($P = 0.033$, C). No correlation was observed between GPI levels with rheumatoid factor ($P = 0.453$, D), cyclic citrullinated peptide antibody ($P = 0.094$, E), erythrocyte sedimentation rate ($P = 0.277$, F), immunoglobulin A ($P = 0.564$, G), immunoglobulin G ($P = 0.901$, H), and immunoglobulin M ($P = 0.211$, I). CCP: Cyclic citrullinated peptide; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; GPI: Glucose-6-phosphate isomerase; RA: Rheumatoid arthritis; RF: Rheumatoid factor.

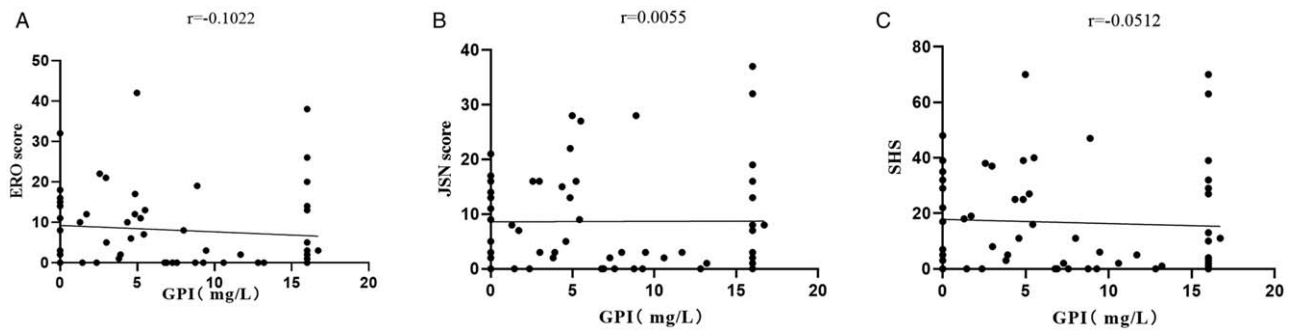


Figure 3: The correlation between serum glucose-6-phosphate isomerase (GPI) levels and measurement of joint destruction. (A) Erosion (ERO) score ($P = 0.429$). (B) Joint space narrowing (JSN) score ($P = 0.966$). (C) Modified Sharp/van der Heijde score (SHS, $P = 0.693$).

Table 2: Comparison between GPI-positive and GPI-negative patients with RA.

Characteristics	GPI-positive (n = 49)	GPI-negative (n = 13)	χ^2/Z	P
Female sex, n (%)	37 (75.5)	7 (53.8)	2.340*	0.126
Smoker, n (%)	8 (16.3)	6 (46.2)	5.229*	0.022
Duration of RA, months	10 (4.0–16.5)	6 (2.5–12.0)	-0.453†	0.651
RF, n (%)	35 (71.4)	9 (69.2)	0.240*	1.000
Anti-CCP, n (%)	40 (81.6)	9 (69.2)	0.954*	0.444
CRP (mg/L)	44.4 (22.5–70.6)	24.4 (13.6–71.0)	-1.332†	0.183
ESR (mm/H)	75.0 (55.5–90.5)	70.0 (45.0–100.5)	-0.303†	0.762
TJC (0–28)	10.0 (4.0–21.5)	0 (0–2.5)	-3.991†	<0.001
SJC (0–28)	6.0 (2.0–17.0)	1.0 (0–5.0)	-2.604†	0.009

Data are presented as median (interquartile range) unless stated otherwise. * χ^2 value. † Z value. CCP: Cyclic citrullinated peptide; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; RA: Rheumatoid arthritis; RF: Rheumatoid factor; SJC: Swollen joint count; TJC: Tender joint count.

GPI-negative patients that achieved a good EULAR response. The levels of ESR and CRP were decreased along with disease activity ($P < 0.001$). Additionally, GPI levels declined with infliximab treatment in patients who had an insufficient response to MTX ($P < 0.001$, Figure 4C). A higher GPI level predicted a greater improvement in disease activity [Figure 4D].

Discussion

This study evaluated the value of serum GPI levels in patients with RA. The results demonstrated that GPI levels were associated with disease activity and decreased with therapeutic response to infliximab treatment in patients who had an inadequate response to MTX.

RA is a chronic inflammatory disease involving the infiltration of immune cells, synovial inflammation and joint damage.^[13] Current guidelines recommend MTX as a first-line disease-modifying anti-rheumatic drug (DMARD) in RA, especially in early rheumatoid arthritis. However, some patients have an insufficient response to MTX monotherapy. Infliximab has been added to obtain a better therapeutic effect. Although patients achieve low disease activity or remission with currently available therapies, subclinical inflammation can result in radiographic joint damage.^[14] Therefore, it is necessary to find more

biomarkers to evaluate disease activity and predict treatment response.

A previous study showed that GPI was detected in RA patients and could be introduced as a diagnostic marker.^[15] In this study, the GPI concentration was found to be correlated with the clinical features CRP level, SJC, and TJC. Assessment of radiological damage was performed at baseline. The GPI concentration was not correlated with SHS, ERO score or JSN score. In contrast, the GPI concentration was associated with disease activity score. The GPI concentration was significantly decreased with infliximab treatment in patients with continued stable background MTX treatment. These results revealed that GPI can be an indicator to evaluate disease activity, which is consistent with a previous study.^[16] Furthermore, the improvement of clinical outcomes was significantly greater in GPI-positive patients than in GPI-negative patients, which suggested that GPI-positive patients are more likely to respond well to the treatment. Taken together, these results suggest GPI as a prognostic marker to assess the efficacy of infliximab.

Smoking has been reported as an important risk factor for RA. Previous data showed that smokers were more often found among anti-CCP-positive patients.^[17,18] Interestingly, a higher GPI-positive rate was found in nonsmokers in this study, which needs to be further

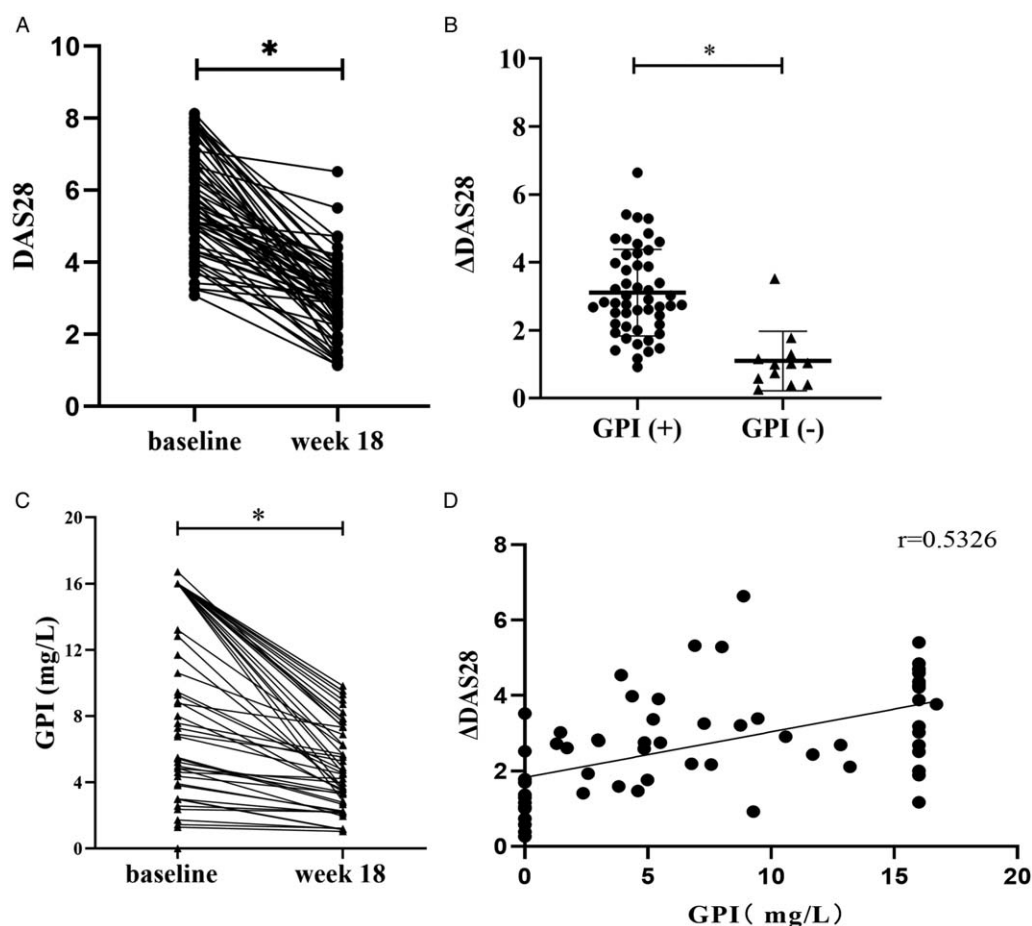


Figure 4: GPI predicts therapeutic response to infliximab treatment. (A) The decrease of DAS28 with infliximab treatment in patients who had an inadequate response to methotrexate. (B) The decrease of DAS28 score in the GPI-positive group was significantly higher than that in the GPI-negative group. (C) GPI levels declined with infliximab treatment. (D) The change of DAS28 score was positively correlated with GPI levels ($P < 0.001$). * $P < 0.05$ by Wilcoxon matched-pairs signed rank test and Mann-Whitney rank-sum test. DAS28: Disease Activity Score in 28 joints; GPI: Glucose-6-phosphate isomerase.

evaluated in a large-scale cohort. Furthermore, the mechanisms by which GPI is involved in RA etiology need to be further investigated.

Our study has several limitations. First, the sample size was small. The clinical characteristics of the patients in the study, such as disease duration, were heterogeneous. Another limitation is that radiographic progression could not be evaluated due to the short follow-up period. Prospective follow-up studies with a large sample size are required to confirm our findings and to further evaluate the role of GPI as a prognostic marker.

In conclusion, this study demonstrated that serum GPI is a marker to assess disease activity that might predict response to infliximab treatment.

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Conflicts of interest

None.

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