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LETTER TO THE EDITOR

WILEY

A pilot assessment of xanthine oxidase activity in plasma from patients with hematological malignancies using a highly sensitive assay

Xanthine oxidoreductase (XOR) is the key enzyme in the production of uric acid. XOR is abundantly expressed as xanthine dehydrogenase (XDH) mainly in the liver and intestine. XDH is physiologically converted to xanthine oxidase (XO) by proteases on the vasculature in the blood. Noticeably, when liver is suffered from hypoxia/inflammation, XDH is released into circulation and is subsequently converted into XO.[1] Elevated plasma XO level has been reported in familial hypercholesterolaemia and cardiovascular diseases.[2,3]

Regarding hematological malignancies, high value of XO activity in plasma has been reported in patients with non-Hodgkin lymphoma and acute lymphocytic leukemia.[4] Allogeneic hematopoietic stem cell transplantation (allo-HSCT) effectively eradicates a line of hematological malignancies, but graft-versus-host disease (GVHD) remains lethal in some cases. However, there seems no validated predictive biomarker for acute GVHD.[5] Of note, susceptible organs of GVHD such as the liver and intestine are the major production sites of XDH, a biological precursor of XO. In this context, to explore the clinical implication of plasma XO activity in patients with hematological malignancies, we performed an assessment of plasma XO activity in the clinical course of patients with hematological malignancies.

The present study was approved by the institutional ethical committee (approved number: 992, Institutional Ethical Committee of University of the Ryukyus on 2 September 2016). Written informed consent was obtained from all participants.

The present study was conducted in accordance with the Helsinki Declaration.

Plasma XO activity was determined using a fluorometric assay measuring the conversion of pterin to isoxanthopterin with a considerably high sensitivity.[6] (The details of measurement of XO activity in plasma are shown in Data S1.)

Thirty-five patients with hematological malignancies who received treatment at Ryukyu University Hospital during the period from October 2016 to August 2017 were enrolled in this study. Among the patients studied, in-depth assessments were performed for 10 patients whose plasma XO activity and clinical parameters were measured consecutively at least five times during the study period. Profile of patients is summarized in Table S1. To examine the possible difference in the value of plasma XO activity between patients with hematological malignancies and healthy subjects, we enrolled five healthy volunteers. Patients who routinely use XO inhibitors were carefully excluded. Alternatively, plasma XO activity was measured after the discontinuation of XO inhibitors.

Statistical analysis was performed using a standard software package (JMP version 14; SAS Institute Inc, Cary, North Carolina). All data were examined for normality by Shapiro-Wilk test, and logarithmic transformation of variables was performed, where applicable. Continuous variables were compared with the Wilcoxon rank sum test. The correlation between variables was calculated using Pearson correlation coefficient or Spearman rank correlation coefficient. *P* values less than.05 were considered statistically significant.

Among enrolled subjects, XO activities in plasma were assessed in five patients with hematological malignancies before receiving any chemotherapies. There were no apparent differences in plasma XO activities between five patients with hematological malignancies and five healthy volunteers (Table S2). Figure 1 (A) summarizes the value of plasma XO activity and clinical manifestations in four patients who underwent allo-HSCT (cases 1-4). All four cases developed grade 1 or 2 acute GVHD with skin symptoms such as erythema and diarrhea. In cases 1 and 2, there was no increase in plasma XO activity during the clinical course. Notably, in cases 1 and 2, no apparent changes were observed in liver transaminases (AST and ALT). On the other hand, in cases 3 and 4, the values of both plasma XO activity and liver transaminase (AST and ALT) were tightly associated and concomitantly elevated. Figure 1 (B) summarizes plasma XO activity in six patients who received chemotherapy (cases 5-10). No appreciable elevations in plasma XO activity, AST and ALT, were observed in cases 5, 6, and 9. On the other hand, in case 7, 8, and 10, the values of both plasma XO activity and liver transaminase (AST and ALT) were tightly associated and concomitantly elevated. Figure 2 (A) shows correlations between plasma XO activity and a line of clinical parameters in all of blood samples (n = 83) from 10 patients analyzed in the present study. For a series of clinical parameters correlated with the value of plasma XO activity shown in Figure 2 (A), analysis using interquartile range (IQR) was also carried out to further confirm whether such a correlation was similarly observed within intraindividual variations. IQR reflects physiological stability as well as pathological fluctuation in each case. Figure 2 (B) clearly shows a significantly positive correlation between plasma XO activity and liver transaminase even in the IQR analyses.

The major findings in the present study are as follows. In patients with hematological malignancies, the value of plasma XO activity was tightly associated with that of serum level of liver transaminases (AST

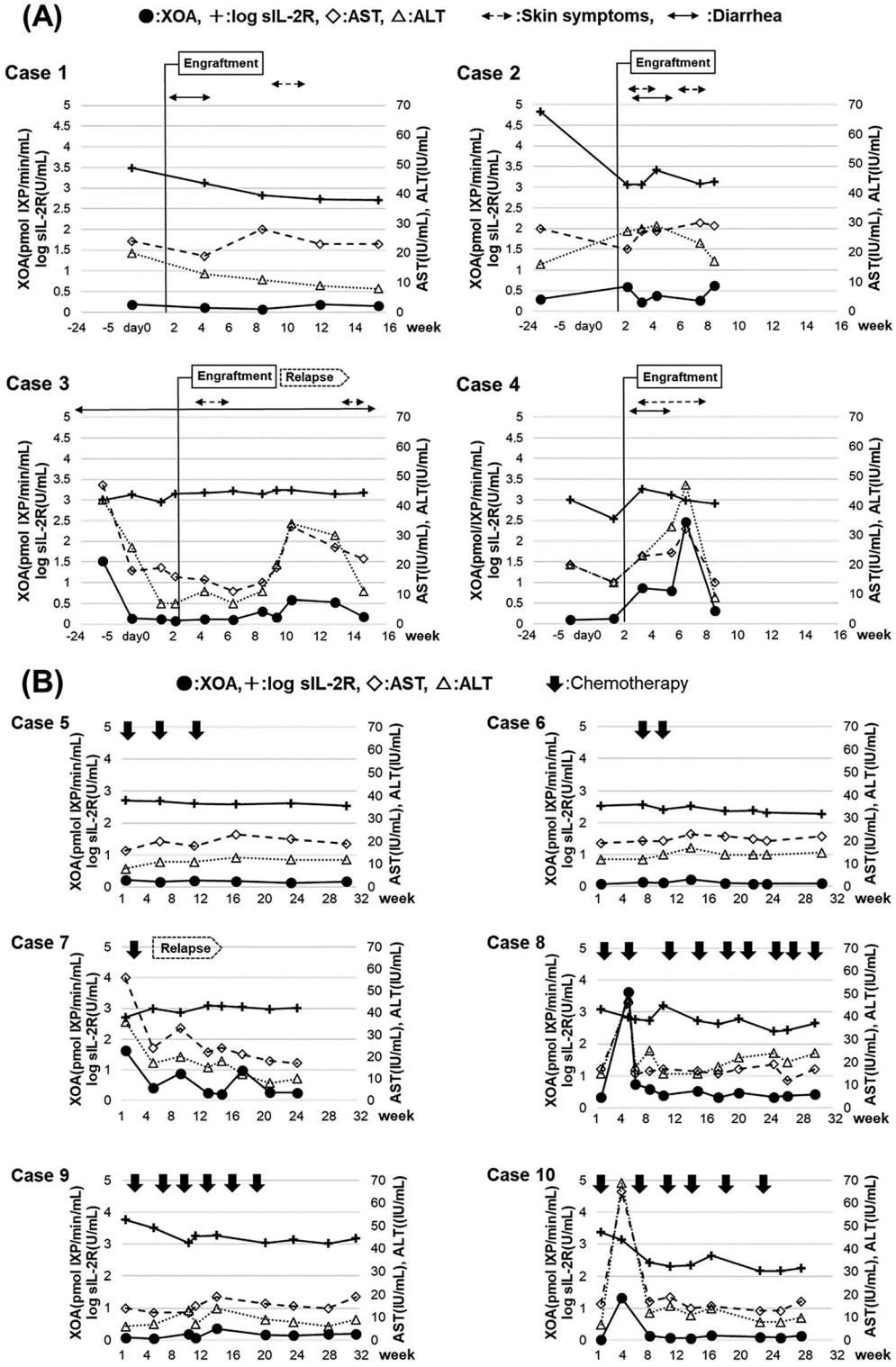

FIGURE 1 (A), Serial measurements of clinical parameters in four cases with allogeneic hematopoietic stem cell transplantation. After the engraftment, all patients showed skin symptoms and intestinal symptoms as graft-versus-host diseases (GVHD). In patients without apparent changes in serum levels of liver transaminase (AST and ALT), there was no significant elevation in plasma XO activity (case 1 and case 2). On the other hand, in case 3 and case 4, plasma XO activity and serum levels of liver transaminase (AST and ALT) were concomitantly elevated. Black circles, diamonds, triangles, and crosses in the figure indicate xanthine oxidase activity (XOA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and soluble form of interleukin-2 receptor (sIL-2R), respectively. The arrow line indicates the period of symptoms. The broken line indicates skin symptoms, and the solid line indicates diarrhea. (B), Serial measurements of clinical parameters in six cases treated with chemotherapy. In patients without apparent elevation in serum levels of liver transaminase (AST and ALT) (case 5, case 6, and case 9), there was no significant elevation in plasma XO activity. On the other hand, plasma XO activity value and serum levels of liver transaminase (AST and ALT) were concomitantly elevated in case 7, case 8, and case 10. Black circles, diamonds, triangles, and crosses in the figure indicate XOA, AST, ALT, and sIL-2R, respectively. Arrows indicate chemotherapies

and ALT) in the course of allo-HSCT and chemotherapies, suggesting that the value of plasma XO activity may reflect liver damage because of chemotherapies and related clinical interventions. Plasma XO activities in humans are considerably low as compared with those in rodents.[7] Therefore, the sensitivity of the measurement is critical to precisely evaluate the value of XO activity. However, the measurement methods of previous reports have shown difficulties in the detectable range.[8,9] To overcome this issue, in the present study, we measured XO activities using considerably high sensitive fluorometric assay, thereby precisely estimating the plasma XO activities in all samples studied. Our recent study demonstrated that the value of plasma XO activity is not affected by the time point, diet, or exercise.[10] The IQR analyses in the present study further confirmed that plasma XO activity was stable in each individual as far as levels of liver

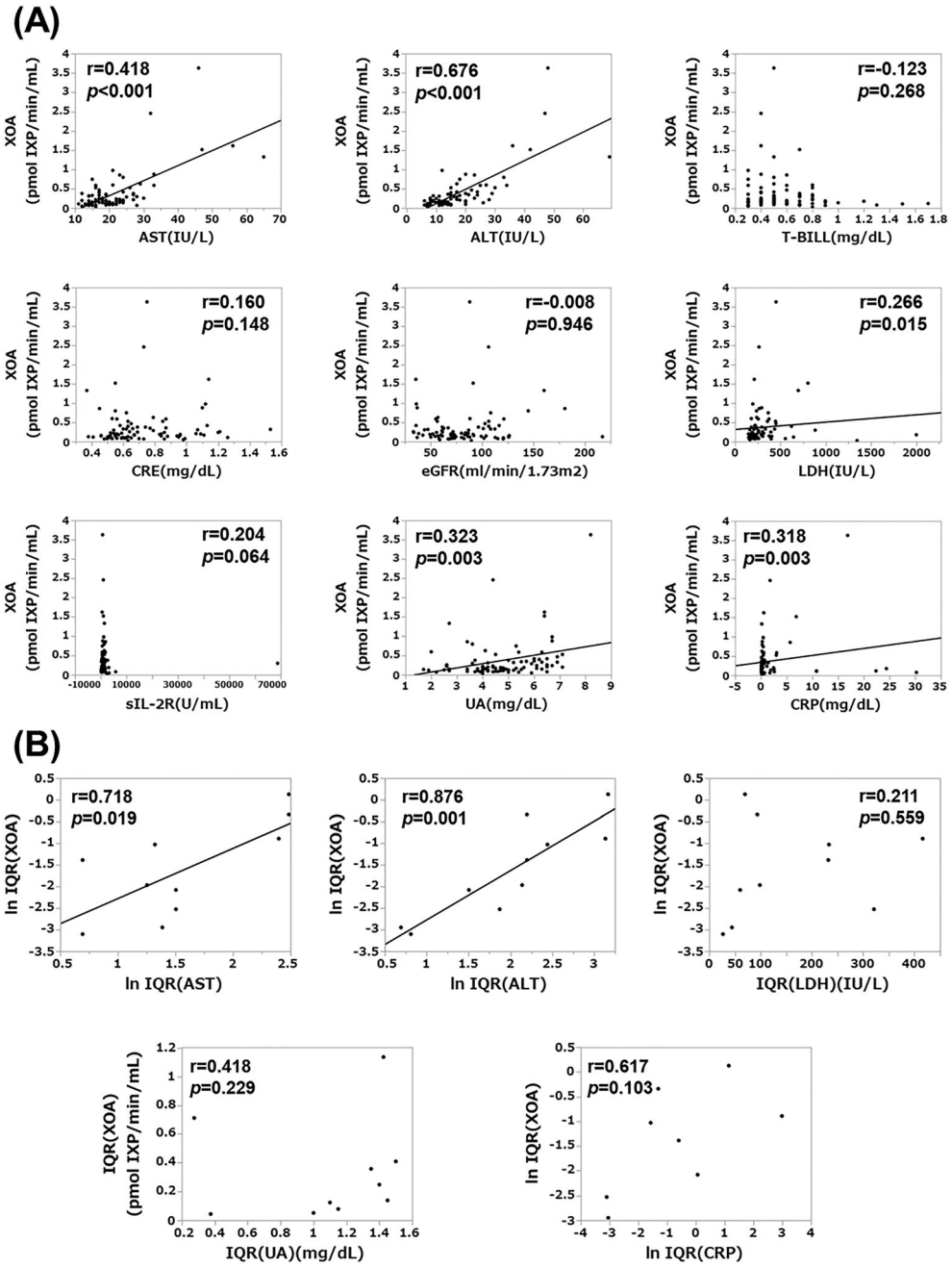

FIGURE 2 (A), Correlation between the level of plasma XO activity and a line of clinical parameters. Data were analyzed by Spearman rank correlation coefficient. *P* values of less than 0.05 were considered as statistically significant. (B), Correlation between the level of plasma XO activity and a line of clinical parameters via the interquartile range (IQR) analyses. The correlation between variables was calculated using Pearson correlation coefficient for data showing normal distribution or log-normal distribution. Spearman rank correlation coefficient was employed for data that did not show either normal distribution or log-normal distribution. *P* values of less than 0.05 were considered as statistically significant. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRE, creatinine; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin-2 receptor; T-BIL, total bilirubin; UA, uric acid; XOA, xanthine oxidase activity

transaminases were constant. Taken together, it is therefore reasonable to speculate that the value of plasma XO activity would be unique in each individual, potentially providing a novel avenue to estimate the responsiveness to therapies or conditions of diseases in patients with hematological malignancies. Because the number of patients studied was extremely limited, further extensive studies are required to strengthen our findings.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

N. Hokama and S. Sunagawa designed the present study, collected the data, and performed statistical analysis. T. Shirakura designed the study and reviewed the manuscript. S. Morishima, S. Nakachi, and Y. Nishi recruited patients, conducted laboratory tests, and reviewed the manuscript. T. Shirakura, C. Matsui, N. Hase, Y. Murayama, and M. Tamura performed assays for xanthine oxidase activity. S. Okamoto, M. Shimabukuro, and K. Nakamura reviewed the manuscript and provided a series of invaluable advices. H. Masuzaki designed the present study and wrote the manuscript. All authors contributed to the entire process of manuscript preparation and approved the final version. We thank I. Nomura, C. Horiguchi, and T. Ikematsu for technical assistance. We are also grateful to M. Hirata, I. Asato, H. Kaneshiro, T. Uema, and C. Noguchi for secretarial assistance.

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