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Pharmacokinetics and Disposition

Associations between plasma hydroxylated metabolite of itraconazole and serum creatinine in patients with a hematopoietic or immune-related disorder

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Abstract

Purpose: Serum markers of renal function have not been characterized in patients treated with itraconazole (ITZ). This study aimed to evaluate the associations between plasma ITZ and its hydroxylated metabolite (OH-ITZ) concentrations and serum markers of renal function in hematopoietic or immune-related disorder patients.

Methods: This study enrolled 40 hematopoietic or immune-related disorder patients receiving oral ITZ solution. Plasma concentrations of ITZ and OH-ITZ at 12 hours after dosing were determined at steady state. Their relationships with serum levels of creatinine and cystatin C, and their estimated glomerular filtration rate (eGFR) were evaluated.

Results: The free plasma concentration of ITZ had no correlation with serum creatinine and serum creatinine-based estimated glomerular filtration rate (eGFR-cre). The free plasma concentration of OH-ITZ was positively and negatively correlated with serum creatinine and eGFR-cre, respectively. The free plasma concentrations of ITZ and OH-ITZ had no association with serum cystatin C and serum cystatin C-based eGFR. Serum creatinine was higher by 16% after than before starting ITZ treatment, while eGFR-cre was lower by 9.3%. The serum creatinine ratio after/before ITZ treatment was positively correlated with the free plasma concentration of OH-ITZ. The patients co-treated with trimethoprim-sulfamethoxazole had higher serum creatinine. Concomitant glucocorticoid administration did not significantly alter serum cystatin C.

Conclusions: Hematopoietic or immune-related disorder patients treated with oral ITZ had a

higher level of serum creatinine. Although serum creatinine potentially increases in conjunction with the free plasma concentration of OH-ITZ, concomitant ITZ administration has a slight impact on the eGFR-cre level in clinical settings.

Key words:

itraconazole; metabolite; pharmacokinetics; creatinine; renal function; cystatin C

Abbreviations:

ITZ, itraconazole; CYP, cytochrome P450; OH-ITZ, hydroxyitraconazole; OATP, organic anion transporting polypeptide; MATE, multidrug and toxic compound extrusion; IC_{50}, half maximal inhibitory concentration; OCT2, organic cation transporter 2; IQR, interquartile range; eGFR, estimated glomerular filtration rate; eGFR-cre, serum creatinine-based eGFR; eGFR-cys, serum cystatin C-based eGFR; JSN, Japanese Society of Nephrology; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; TMP-SMX, trimethoprim-sulfamethoxazole

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Introduction

Itraconazole (ITZ), an azole-type antifungal drug with potent activity against *Aspergillus* and *Candida* spp., is commonly used for the prophylaxis and treatment of systemic and superficial fungal infections in clinical settings [1,2]. ITZ is predominantly converted to cytochrome P450 (CYP) 3A4-mediated metabolites, including hydroxyitraconazole (OH-ITZ) as a major metabolite in the liver [3]. Both ITZ and OH-ITZ are excreted only slightly from the kidney [4]. OH-ITZ has a similar pharmacokinetic profile to ITZ in humans and shows antifungal activity comparable to that of ITZ [5,6]. ITZ and OH-ITZ both inhibit CYP3A4 activity [7], although little data has been published on their differences in the inhibitory efficacy of CYP3A4 in human beings.

ITZ is highly bound to human plasma protein and its free fraction rate is 2–4% for ITZ [8–10]. In contrast, the plasma protein binding of OH-ITZ has not been fully evaluated in humans. An earlier report showed that the plasma free fraction rate of OH-ITZ was much higher than that of ITZ in patients with pulmonary aspergillosis [11]. Most hematopoietic or immune-related disorder patients who receive ITZ over a long period tend to have hypoalbuminemia owing to chronic and cancer-related inflammation. However, the free plasma concentrations of ITZ and OH-ITZ have not been characterized in hypoalbuminemia populations. Determination of the free plasma concentrations of ITZ and OH-ITZ may be helpful for the quantitative evaluation of antifungal efficacy in clinical settings.

Both ITZ and OH-ITZ inhibit P-glycoprotein activity *in vitro* and in humans [12,13].

Recent reports have showed that ITZ and OH-ITZ inhibit the activity of several drug transporting proteins including organic anion transporting polypeptide (OATP) 1B, multidrug and toxic compound extrusion (MATE) 1, and bile salt export pump [14,15]. At therapeutic doses, the plasma concentrations of ITZ and OH-ITZ potentially alter the activity of these drug transporting proteins based on their half maximal inhibitory concentration (IC_{50}). The IC_{50} for these drug transporting proteins are different for ITZ and OH-ITZ *in vitro* [14]. Few clinical reports have fully evaluated the inhibitory efficacy of ITZ and OH-ITZ on these drug transporting proteins [13].

Creatinine is a product converted from phosphocreatine in muscle and the majority is filtrated by the glomeruli. Serum creatinine is commonly used for an estimation of renal function in routine medical care. A part of the serum creatinine is excreted into the urine by drug transporting proteins including organic anion transporter 2, organic cation transporter 2 (OCT2), and MATEs [16]. Earlier reports showed a concomitant tyrosine kinase inhibitor or pyrimethamine administration raised serum creatinine through the inhibitions of MATEs [17,18]. Inhibition of tubular creatinine secretion does not commonly affect the glomerular function. However, these reports alerted the underestimation of renal function in MATEs inhibitor-treated patients. In contrast, serum cystatin C as a practical alternative for renal function has not been specifically recognized by the above drug transporting proteins [19] and is available for estimation of kidney function in special cases [20].

In hematopoietic or immune-related disorder patients treated with ITZ, dose of renal-

excretion drugs including trimethoprim-sulfamethoxazole (TMP-SMX), amphotericin B, ganciclovir, and acyclovir in supportive therapy need to be adjusted based on renal function. To date, the utilization of serum renal function markers remains to be characterized in ITZ-treated patients. This study aimed to evaluate the associations between plasma concentrations of ITZ and OH-ITZ and serum markers of renal function in patients with a hematopoietic or immune-related disorder.

Materials and methods

Patients and study schedule

This study enrolled 40 Japanese adult hematopoietic or immune-related disorder patients treated with oral ITZ solution (Janssen Pharmaceutical K.K. or Pfizer Japan Inc., Tokyo, Japan) for prophylaxis of fungal infections at Hamamatsu University Hospital (Hamamatsu, Japan). They received 200 mg of ITZ once daily before bedtime for at least 2 weeks. The exclusion criteria of the study were as follows: (1) patients who were being co-treated with an azole-type antifungal drug other than ITZ; (2) patients who were concomitantly receiving oral or intravenous cyclosporine; (3) patients who were being co-treated with a potent CYP3A4 inducer or inhibitor [21]; (4) patients who were concomitantly receiving metformin, cimetidine, or a tyrosine kinase inhibitor; (5) patients who had a severe bacterial infection; (6) patients with serum total bilirubin > 2.0 mg/dL or serum creatinine > 1.5 mg/dL before starting ITZ treatment; and (7) patients with poor adherence based on interviews and medical records. On the 14th day

after starting the ITZ treatment or later, blood specimens were collected at 12 hours after ITZ administration with the blood samples for the routine clinical laboratory tests. This study was registered with the University Hospital Medical Information Network (UMIN-CTR 000036201).

Determination of plasma total ITZ and OH-ITZ

Total ITZ and OH-ITZ in human plasma were determined by a previous method [22]. The calibration curves for total ITZ and OH-ITZ in human plasma were linear over the concentration ranges of 15–1500 ng/mL. The lower limits of quantification for total ITZ and OH-ITZ in plasma were 15 ng/mL. The intra- and inter-day accuracies for total ITZ and OH-ITZ were 94.1–101.8% and 97.6–101.7%, while their imprecisions were 0.8–4.0% and 0.6–6.5%, respectively.

Sample preparation of plasma free ITZ and OH-ITZ

Before ultrafiltration, 225 μL of acetonitrile was added into a weighted filtrate cup (Centrifree, a 30 kDa molecular weight cut-off, 4104, Merck Millipore Ltd, Billerica, MA, USA) to prevent the non-specific binding of ITZ and OH-ITZ to polyethylene surface. Plasma specimens were added into the sample reservoir of a Centrifree, centrifuged at 2000 × *g* at 37°C for 30 minutes using a fixed-angle rotor (AT-508C, Kubota Corporation co., ltd., Tokyo), and then the filtrate cup was weighted again. The ultrafiltrated plasma in filtrate cup was transferred to a low

adsorption microtube coated with 2-methacryloyloxyethyl phosphorylcholine polymer (Sarstedt K.K., Tokyo) containing 100 μL of ITZ-*d9* solution (500 ng/mL) as an internal standard, and then acetonitrile was added in order to adjust the total volume to 750 μL. After mixing, 700 μL of the supernatant after centrifugation at 18,000 × *g* was evaporated to dryness. The residues were reconstituted with 65 μL of mobile phase, and the supernatant was injected into the liquid chromatography system.

Determination of plasma free ITZ and OH-ITZ

Analytes in plasma were determined using a NexeraX2 liquid chromatography system coupled to an LCMS-8050 triple quadruple mass spectrometer (Shimadzu Corporation, Kyoto, Japan). Analytes were isocratically separated using a 3-μm particle C18 column (TSKgel ODS-100V, 75 mm length × 2.0 mm inner diameter, Tosoh, Tokyo) warmed at 40°C. The total run time was 10 minutes with a flow rate of 0.2 mL/min. The mobile phase consisted of acetonitrile and 5 mM ammonium acetate (pH 6.0) (57:43, v/v). Samples were introduced to the interface through a turbo ion spray with the temperature set at 305°C. The positive ion transitions were monitored using a dwell time of 200 milliseconds for each analyte: ITZ, *m/z*, 706.05/393.05; OH-ITZ, 721.15/408.15; and ITZ-*d9*, 714.25/401.15. Collision-induced-dissociation gas, drying gas, nebulizer gas, and heating gas were optimized at 17 kPa, 8.8 L/min, 2.4 L/min, and 9.1 L/min, respectively. Collision energies for ITZ, OH-ITZ, and ITZ-*d9* were –38, –37, and –36 volts, respectively. The calibration curves of free ITZ and OH-ITZ in plasma were linear over the

concentration ranges of 0.5–50 ng/mL ($r > 0.999$) and 1.5–150 ng/mL ($r > 0.999$), respectively. The lower limits of quantification for free ITZ and OH-ITZ in plasma were 0.5 and 1.5 ng/mL, respectively. The intra- and inter-day accuracies and imprecisions in human plasma were 95.7–101.7% and 1.8–7.0% for free ITZ, and 94.9–100.7% and 2.2–6.0% for free OH-ITZ, respectively. The plasma free fraction rate (%) was calculated using the equation: (free / total concentration) × 100.

Measurement of serum renal function markers

Serum creatinine was determined by an enzymatic method (Cygnas Auto-CRE, Shino-test Corporation, Tokyo) and its measurable range was 0.02–100 mg/dL. Serum cystatin C was measured using a latex coagulating nephelometry method (Nordia Cystatin C, Sekisui Medical Co., Ltd, Tokyo). The measurable range of serum cystatin C was 0.1–10 mg/L. The serum creatinine-based estimated glomerular filtration rate (eGFR) (eGFR-cre, mL/min/1.73 m^2) was calculated using the following Japanese Society of Nephrology (JSN) equation: 194 × (serum creatinine, mg/dL) $^{-1.094}$ × (age, years) $^{-0.287}$ × 0.739 (if female) [23]. Serum cystatin C-based eGFR (eGFR-cys, mL/min/1.73 m^2) was calculated using the following equation: (104 × (serum cystatin C, mg/L) $^{-1.019}$ × 0.996 $^{-(\text{age, years})}$ × 0.929 (if female)) – 8 [24]. Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [25] was also used to support data using Japanese eGFR equation. The serum creatinine ratio before and after starting ITZ treatment (after/before) was evaluated. Serum cystatin C was not evaluated before starting ITZ treatment.

Statistical analysis

All statistics were calculated using IBM SPSS Statistics software ver. 25 (IBM Japan Ltd., Tokyo). The associations between the total plasma concentrations of ITZ and OH-ITZ, between the total and free plasma concentrations, and between the plasma free fraction rates and the serum albumin were evaluated using Spearman's rank correlation coefficient test. The correlations between the plasma concentrations of ITZ and OH-ITZ, and serum markers of renal function, eGFR, or serum creatinine ratio were also calculated using Spearman's rank correlation coefficient test. The values of serum creatinine and eGFR before and after starting ITZ treatment were compared using the Wilcoxon signed rank test. The data before starting ITZ treatment means the data before at least two weeks of ITZ treatment. The associations between concomitant drug administration and serum marker levels of renal function were compared by the Mann-Whitney *U* test. Multiple regression analyses were performed to confirm the relative contribution of plasma ITZ and OH-ITZ to the changes in serum creatinine and its ratio, and eGFR-cre. A $P < 0.05$ was considered to indicate statistical significance.

Results

Patient characteristics

Table 1 shows the patient characteristics in this study population. This study population included patients with a median age of 60 years. The enrolled patients had low serum albumin

levels (median, 3.7 g/dL; and interquartile range (IQR), 3.4–4.0 g/dL). TMP-SMX doses of 1 g and 0.5 g daily were co-administered to 30 and 4 patients, respectively. Eleven patients received oral prednisolone (median, 30 mg daily) and 1 patient received oral hydrocortisone (10 mg daily). Primary diseases included acute myeloblastic leukemia (n = 12), non-Hodgkin lymphoma (n = 9), myelodysplastic syndrome (n = 7), acute lymphatic leukemia (n = 4), immune thrombocytopenia (n = 2), polyangiitis (n = 2), and others (n = 4).

Characterization of plasma ITZ and OH-ITZ

The total plasma concentration of ITZ was strongly correlated with that of OH-ITZ (r_s = 0.920, P < 0.001) and with free plasma concentration of ITZ (r_s = 0.562, P < 0.001) (Figure S1). In plasma OH-ITZ, the total concentration was not correlated with the free concentration (r_s = 0.261, P = 0.103). The median plasma free fraction rates of ITZ and OH-ITZ were 0.86% (IQR, 0.48–1.38%) and 2.23% (0.50–5.29%), respectively. No significant correlations were observed between the plasma free fraction rates of ITZ (r_s = 0.243, P = 0.132) and OH-ITZ (r_s = 0.236, P = 0.143) and serum albumin level.

Relationships with serum markers of renal function

Figure 1 shows the comprehensive correlation analysis between plasma concentrations of ITZ and OH-ITZ and serum markers of renal function in the patients. The total and free plasma concentrations of ITZ had no correlation with the serum creatinine level (r_s = 0.313, P = 0.052

and r_s = 0.307, P = 0.054, respectively). In contrast, the free plasma concentration of OH-ITZ (r_s = 0.405, P = 0.009), but not total plasma concentration of OH-ITZ (r_s = 0.311, P = 0.051), was positively correlated with the serum creatinine level. Multiple regression analysis confirmed that the plasma free OH-ITZ, but not total and free ITZ, and total OH-ITZ, was associated with serum creatinine (standardized partial regression coefficient, β = 0.499, P = 0.008). The total and free plasma concentration of ITZ had no correlations with the serum cystatin C level (r_s = 0.172, P = 0.289 and r_s = 0.238, P = 0.139, respectively). Additionally, the total and free plasma concentrations of OH-ITZ were not also correlated with the serum cystatin C level (r_s = 0.139, P = 0.392 and r_s = 0.179, P = 0.268, respectively).

Relationships with eGFR

Figure 2 shows the correlations of the free plasma concentrations of ITZ and OH-ITZ with eGFR in the patients. The free plasma concentration of ITZ was not correlated with eGFR-cre (r_s = –0.235, P = 0.145). The free plasma concentration of OH-ITZ was negatively correlated with eGFR-cre (r_s = –0.321, P = 0.044). Parametric multiple regression analysis with 2 outliers did not demonstrate the significant association between the plasma free OH-ITZ and eGFR-cre (β = –0.265, P = 0.099). The free plasma concentrations of ITZ and OH-ITZ had no correlation with eGFR-cys (r_s = –0.229, P = 0.155 and r_s = –0.118, P = 0.469, respectively). The free plasma concentrations of ITZ and OH-ITZ were negatively correlated with eGFR-cre using CKD-EPI equation (r_s = –0.382, P = 0.015 and r_s = –0.404, P = 0.010, respectively) (Figure

S2).

Renal function and its serum marker before and after starting ITZ treatment

Figure 3 shows the changes in serum creatinine and eGFR-cre after starting ITZ treatment. The serum creatinine level was higher after than before starting ITZ treatment ($P = 0.055$), while the eGFR-cre was lower ($P = 0.007$). The average change values in serum creatinine and eGFR-cre were 0.091 mg/mL and –9.8 mL/min/1.73 m^2, respectively, and their average change rates from baseline were 16% and –9.3%, respectively. Even after starting ITZ treatment, the eGFR-cre was higher than the eGFR-cys ($P < 0.001$). The eGFR-cys after starting ITZ treatment was lower than the eGFR-cre before starting ITZ treatment ($P < 0.001$). Using CKD-EPI equation, the eGFR-cre was lower after than before starting ITZ treatment ($P = 0.036$) and was higher than the eGFR-cys after starting ITZ treatment ($P < 0.001$) (Figure S3).

Relationships with the serum creatinine ratio

Figure 4 shows the correlations between the free plasma concentrations of ITZ and OH-ITZ and serum creatinine ratio before and after starting ITZ treatment in this study population. No correlation was observed between the free plasma concentration of ITZ and serum creatinine ratio ($r_s = 0.010$, $P = 0.950$). The free plasma concentration of OH-ITZ was positively correlated with the serum creatinine ratio ($r_s = 0.443$, $P = 0.004$). Multiple regression analysis confirmed that the plasma free OH-ITZ, but not free ITZ, was associated with serum creatinine ($\beta = 0.626$,

$P = 0.001$).

Relationships with concomitant drugs

The serum creatinine level was higher in the TMP-SMX co-treatment group than in the non-treatment group ($P = 0.021$) (Figure 5). In contrast, there was no difference in the serum cystatin C level between the TMP-SMX co-treatment and non-treatment groups ($P = 0.127$). No difference was observed in the serum creatinine level between the glucocorticoid co-treatment and non-treatment groups ($P = 0.469$). Concomitant glucocorticoid administration did not significantly increase the serum cystatin C level ($P = 0.075$).

Discussion

This study investigated the associations between plasma ITZ and OH-ITZ and serum markers of renal function in patients with a hematopoietic or immune-related disorder. To the best of our knowledge, this is the first report that has assessed the kinetic alterations of serum creatinine and serum creatinine-based estimation of renal function based on plasma ITZ and its metabolite in hematopoietic or immune-related disorder patients.

The present study needed to characterize the plasma ITZ and OH-ITZ in hematopoietic or immune-related disorder patients. The enrolled patients did not receive a potent CYP3A4 inducer or inhibitor according to the exclusion criteria. In the study population, the IQR of the plasma free fraction rate of ITZ and OH-ITZ were 0.48–1.38% and 0.50–5.29%, respectively.

In an earlier report, the ranges of the plasma free fraction rate of ITZ and OH-ITZ in pulmonary aspergillosis patients were 0.012–0.099% and 0.20–0.57%, respectively [11]. The patients with slightly low serum albumin levels had a larger variation in the plasma free fraction rates of OH-ITZ than ITZ in the present study. However, serum albumin in this population did not explain their plasma free fraction rates. The correlation between the total and free plasma concentrations in OH-ITZ was much weaker than that in ITZ. These data indicate that OH-ITZ weakly binds to the plasma proteins compared with ITZ and plasma free OH-ITZ is more variable than the plasma free ITZ. Additionally, the plasma free OH-ITZ had no correlation with serum C-reactive protein (data not shown). Thus, the plasma free OH-ITZ is not simply predictable using plasma total OH-ITZ and serum albumin in patients with a hematopoietic or immune-related disorder.

In the study population, the associations of plasma ITZ and OH-ITZ with serum markers of renal function were comprehensively examined to detect the statistically significant relationships. Only free OH-ITZ as active form in plasma had an association with serum creatinine in this study. Since both ITZ and OH-ITZ are only slightly eliminated by the kidneys [4], mild to moderate renal dysfunction has a negligible impact on plasma exposure to ITZ and OH-ITZ. In contrast, serum cystatin C as an alternative marker for renal function had no associations with plasma ITZ and OH-ITZ. These results suggest that plasma free OH-ITZ does not similarly alter the kinetics of two serum markers of renal function.

The subsequent analysis assessed the clinical impact of plasma free OH-ITZ on serum

markers-based eGFR calculations in ITZ-treated patients. The patients with higher plasma free OH-ITZ had the lower eGFR-cre, but not eGFR-cys. These data imply that plasma free OH-ITZ does not alter the renal function itself. Our previous study also reported that plasma OH-ITZ but not ITZ was inversely associated with eGFR-cre in another population [26]. Additionally, our data demonstrated that the serum creatinine was higher by 16% after starting ITZ treatment and its elevation was positively associated with plasma free OH-ITZ. There is a gender difference in creatinine production in humans [27]. Although the serum creatinine was higher in men than in women in the present study (data not shown), the serum creatinine ratio was positively associated with free OH-ITZ. These supplementary data confirmed that the serum creatinine increases in conjunction with the free plasma concentration of OH-ITZ in this study population. Since the eGFR estimation using serum creatinine is commonly employed in clinical settings, the slight underestimation of renal function may be observed in ITZ-treated patients. In the present study, the eGFR-cre had a slightly weaker association with plasma free OH-ITZ compared to serum creatinine. One possible explanation for the results is that eGFR-cre is estimated with the additional factors including age and body weight [23].

In patients with a hematopoietic or immune-related disorder, eGFR-cre was higher than eGFR-cys even after starting ITZ treatment. The unexpected findings may be caused by the increases of tubular secretion of creatinine in hypoalbuminemia [28]. In the study population, the patients tended to have low serum albumin levels. Hematopoietic or immune-related disorder patients have hypoalbuminemia due to chronic and cancer-related inflammation

[29,30]. Inflammatory condition or hypoalbuminemia abnormally increases the eGFR [28,31]. The present study patients potentially possess the promotion effect caused by hypoalbuminemia for tubular secretion of creatinine. This population included two patients with eGFR of close to 200 mL/min/1.73 m^2. They had serum creatinine of less than 0.5 mg/dL and its value in addition to inflammation may prompt inaccurate estimation of glomerular filtration rate [31]. Since serum albumin before and after ITZ treatments did not change in this study, our study demonstrated that the eGFR-cre slightly lowers after starting ITZ treatment.

This study abundantly included the patients with normal renal function or mild renal dysfunction. Serum creatinine can more accurately estimate renal function in patients with moderate to severe renal dysfunction [32]. Additionally, hypoalbuminemia also increases the eGFR [28]. The discrepancy between eGFR-cre and eGFR-cys potentially occurs in patients with normal renal function or mild renal dysfunction rather than moderate to severe renal dysfunction [33]. Our study population with the lower serum albumin may not clearly observe the decrease of eGFR-cre after starting ITZ treatment.

We needed to clarify the possible mechanisms that plasma free OH-ITZ increases the serum creatinine from the viewpoint of creatinine secretion by the proximal tubules. Serum creatinine is freely filtered by the glomeruli and additionally secreted by the proximal tubules in small amounts [34]. The observed creatinine clearance overestimates the actual glomerular filtration rate by 10–20% [35]. Creatinine secretion by the proximal tubules into urine is mediated by drug transporting proteins including MATEs and OCT2 in humans [16]. MATE1

is mainly located on the brush border side of proximal tubule cells in kidney [36] and inhibited by OH-ITZ but not ITZ based on their IC_{50} *in vitro* [14]. Twenty of 40 patients probably had a value higher than the IC_{50} of OH-ITZ until 12 hours after dosing of ITZ in our study. The present study patients had much lower plasma ITZ and OH-ITZ compared to the IC_{50} for OCT2 *in vitro* [14]. These data support the theory that serum creatinine was increased through the MATE1 inhibition by OH-ITZ in ITZ-treated patients. Earlier clinical studies also showed that MATE1 inhibitors such as cimetidine and abemaciclib raised serum creatinine through the inhibition of MATE1 [37,38].

This study needed to assess the substantive impact of concomitant TMP-SMX on our findings. Low-dose TMP-SMX co-treatment elevated the serum creatinine level in this study. At its preventive dose, trimethoprim administration inhibits MATEs and OCT2, resulting in elevation of serum creatinine [39]. Additionally, the plasma free OH-ITZ increased serum creatinine regardless of the presence or absence of TMP-SMX co-treatment in the present study. Concomitant trimethoprim probably raises serum creatinine by the inhibition of both MATEs and OCT2. Based on *in vitro* data [14], plasma free OH-ITZ may increase serum creatinine through the mediation of MATE1 rather than OCT2 in TMP-SMX co-treated patients.

This study needs to evaluate the associations between concomitant glucocorticoid and the serum markers of renal function. Glucocorticoid co-treatment did not alter the kinetics of serum creatinine and cystatin C in this study. Cystatin C is not secreted by the renal tubular cells, while concomitant glucocorticoid and cyclosporine treatments affect serum cystatin C

[40]. Although the present study did not enroll patients co-treated with cyclosporine according to the exclusion criteria, 12 patients received oral prednisolone (median, 30 mg daily) or hydrocortisone (10 mg daily). High-dose glucocorticoids have been reported to increase serum cystatin C in kidney transplant recipients, while low-dose glucocorticoids did not [41]. The glucocorticoid dose in our study population may be less than the threshold value that increase serum cystatin C.

This study has several limitations which need to be pointed out. First, this study evaluated serum markers of renal function using a single point at 12 hours after ITZ dosing. The relationships between the plasma exposure of ITZ and OH-ITZ and serum markers of renal function were not examined in this study. The plasma concentrations of ITZ and OH-ITZ in the elimination phase were well correlated with the plasma exposure of ITZ and OH-ITZ [42]. Analyses including the plasma concentration profiles of ITZ and OH-ITZ in addition to concomitant drugs would improve the estimation of renal function in hematopoietic or immune-related disorder patients. Second, the eGFR in the present study was calculated using the eGFR equation created by the JSN. Our findings of the present study were confirmed by the eGFR using the CKD-EPI equation [25] as well as the Japanese eGFR equation. The use of eGFR estimation used in the present study is suitable for Japanese population. Additionally, this study could not evaluate serum cystatin C before starting ITZ treatment. Changes in serum cystatin C and eGFR-cys before and after starting ITZ treatment may clarify the usefulness of serum cystatin C monitoring in ITZ-treated patients. Third, the present study could not evaluate renal

function using global gold standard methods such as the iohexol, inulin, ^{51}Cr-EDTA, or ^{99m}Tc-DTPA assays due to the observation study. The methods are not routinely performed because of invasiveness, time-consuming procedure, and medical cost. Intervention studies using the standard methods should confirm our findings. Fourth, this study did not determine plasma keto-itraconazole, which inhibits MATE1 [14]. A previous study demonstrated that the plasma keto-itraconazole concentration at steady state ranged from 1.1 to 5.4 ng/mL in ITZ-treated patients [22]. In clinical settings, the plasma keto-itraconazole may not strongly inhibit MATE1 based on its IC_{50} of 792 ng/mL [14].

For this population, serum creatinine potentially increases with the free plasma concentration of OH-ITZ. However, the serum creatinine increased by only 16% after starting ITZ treatment. The present study observed the ITZ- and concomitant drug-derived variations in serum creatinine in patients with a hematopoietic or immune-related disorder. The routine estimation of renal function using serum creatinine may not be suitable for patients receiving ITZ and/or TMP-SMX co-treatment. Moreover, hematopoietic or immune-related disorder patients tend to have low serum albumin and its condition leads to the increased eGFR-cre [28]. In contrast, the evaluation of renal function using serum cystatin C may be suitable in ITZ and/or TMP-SMX treated hematopoietic or immune-related disorder patients.

Conclusions

Hematopoietic or immune-related disorder patients treated with oral ITZ had a higher level of

serum creatinine. Although serum creatinine potentially increases in conjunction with the free plasma concentration of OH-ITZ, concomitant ITZ administration has a slight impact on the eGFR-cre level in clinical settings.

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Compliance with ethical standards:

This study was performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethics Committee of Hamamatsu University School of Medicine (approval number, 16-289) before starting the study. Each patient received information about the scientific aim of the study, and each provided written informed consent.

Consent for publication:

Not applicable.

Availability of data and materials:

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

Author's contributions:

YI and TN planned and designed this study. Acquisition of data was carried out by YI, YM, and TO. YI, TN, and JK contributed to the analysis and interpretation of data. All authors contributed to drafting and revision of the manuscript for important intellectual content and provided final approval for publication.

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Figure legends

Figure 1. Comprehensive correlation analysis between the plasma concentrations of itraconazole (ITZ) and its hydroxylated metabolite (OH-ITZ) and serum markers of renal function in patients with a hematopoietic or immune-related disorder.

Plasma (a) total ITZ, (b) free ITZ, (c) total OH-ITZ, (d) free OH-ITZ versus serum creatinine; and (e) total ITZ (f) free ITZ, (g) total OH-ITZ, and (h) free OH-ITZ versus serum cystatin C. The correlations were evaluated using Spearman's rank correlation coefficient test.

Figure 2. Correlations between the free plasma concentrations of itraconazole (ITZ) and its hydroxylated metabolite (OH-ITZ) and estimated glomerular filtration rate (eGFR) in patients

with a hematopoietic or immune-related disorder.

Free plasma concentrations of (a) ITZ and (b) OH-ITZ versus serum creatinine-based eGFR (eGFR-cre); and free plasma concentrations of (c) ITZ and (d) OH-ITZ versus serum cystatin C-based eGFR (eGFR-cys).

The correlations were calculated using Spearman's rank correlation coefficient test and a regression line (b) with a slope was added.

Figure 3. Changes in serum creatinine and estimated glomerular filtration rate (eGFR) in patients with a hematopoietic or immune-related disorder.

The data before starting ITZ treatment means the data before at least two weeks of ITZ treatment.

(a) Serum creatinine and (b) serum creatinine-based eGFR (eGFR-cre) before and after starting itraconazole (ITZ) treatment and (c) eGFR-cre and serum cystatin C-based eGFR (eGFR-cys) after starting ITZ treatment.

The differences were evaluated using Wilcoxon signed rank test.

Figure 4. Correlations between the free plasma concentrations of itraconazole (ITZ) and its hydroxylated metabolite (OH-ITZ) and serum creatinine ratio before and after starting ITZ treatment (after/before) in patients with a hematopoietic or immune-related disorder.

The data before starting ITZ treatment means the data before at least two weeks of ITZ

treatment.

Free plasma concentrations of (a) ITZ and (b) OH-ITZ.

The correlations were evaluated using Spearman's rank correlation coefficient test and a regression line (b) with a slope was added.

Figure 5. Relationships between concomitant drug administration and serum markers of renal function in itraconazole-treated patients with a hematopoietic or immune-related disorder.

(a) Serum creatinine and (b) serum cystatin C in trimethoprim-sulfamethoxazole (TMP-SMX) co-treated and non-treated patients; and (c) serum creatinine and (d) serum cystatin C in glucocorticoid co-treated and non-treated patients.

The differences were compared using the Mann-Whitney *U* test.

Supplementary Figure legend

Figure S1. Correlations between the total plasma ITZ and OH-ITZ (a) and between the total and free plasma concentrations of ITZ (b) and OH-ITZ (c) in patients with a hematopoietic or immune-related disorder.

The correlations were evaluated using Spearman's rank correlation coefficient test.

Figure S2. Correlations between the plasma free ITZ and OH-ITZ and estimated glomerular filtration rate (eGFR) using Chronic Kidney Disease Epidemiology Collaboration equation in

patients with a hematopoietic or immune-related disorder.

Free plasma concentrations of (a) ITZ and (b) OH-ITZ versus serum creatinine-based eGFR (eGFR-cre); and free plasma concentrations of (c) ITZ and (d) OH-ITZ versus serum cystatin C-based eGFR (eGFR-cys). The correlations were evaluated using Spearman's rank correlation coefficient test and a regression line (a, b) with a slope was added.

Figure S3. Changes in estimated glomerular filtration rate (eGFR) using Chronic Kidney Disease Epidemiology Collaboration equation in patients with a hematopoietic or immune-related disorder.

(a) Serum creatinine-based eGFR (eGFR-cre) before and after starting ITZ treatment and (b) eGFR-cre and serum cystatin C-based eGFR (eGFR-cys) after starting ITZ treatment.

The differences were evaluated using Wilcoxon signed rank test.

Table 1. Patient characteristics

(A) Physical data

Gender, male/female, n	23/17
Age, years	60 (52–67)
Body weight, kg	50.9 (46.8–58.2)
Body height, m	1.51 (1.44–1.65)

Data are expressed as median and interquartile range in parentheses.

(B) Clinical laboratory data

Itraconazole treatment	Before	After	*P*-value
Serum total protein, g/dL	6.1 (5.7–6.7)	6.0 (5.5–6.5)	0.134
Serum albumin, g/dL	3.7 (3.4–4.0)	3.7 (3.4–4.0)	0.188
Serum creatinine, mg/dL	0.67 (0.46–0.86)	0.69 (0.51–0.84)	0.055
Serum creatinine-based eGFR using JSN equation, mL/min/1.73 m^2	80 (64–110)	74 (58–103)	0.007
Serum creatinine-based eGFR using CKD-EPI equation, mL/min/1.73 m^2	107 (93–127)	105 (89–118)	0.036
Serum cystatin C, mg/L	−	1.17 (0.98–1.57)	−
Serum cystatin C-based eGFR, mL/min/1.73 m^2	−	59 (43–70)	−
Blood urea nitrogen, mg/dL	14.8 (9.4–21.5)	12.7 (10.7–20.4)	0.317
C-reactive protein, mg/dL	0.23 (0.14–0.39)	0.17 (0.08–0.74)	0.909
Total bilirubin, mg/dL	0.5 (0.4–0.7)	0.6 (0.4–0.8)	0.047
Aspartate aminotransferase, IU/L	21 (19–33)	21 (16–25)	0.258
Alanine aminotransferase, IU/L	26 (18–49)	24 (15–42)	0.505
γ-Glutamyl transpeptidase, U/L	41 (24–73)	51 (27–74)	0.536

eGFR, estimated glomerular filtration rate; JSN, Japanese Society of Nephrology, and CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration

Serum creatinine-based eGFR using JSN equation [23]: $194 \times (\text{serum creatinine})^{-1.094} \times (\text{age})^{-0.287} \times 0.739$ (if female). Serum creatinine-based eGFR using CKD-EPI equation [25]: Male

(serum creatinine < 0.9 mg/dL :141× (serum creatinine/0.9) $^{-0.411}$ × 0.993 ^{age}, ≥ 0.9 mg/dL :141× (serum creatinine/0.9) $^{-1.209}$ × 0.993 ^{age}) and female (serum creatinine < 0.7 mg/dL :144× (serum creatinine/0.7) $^{-0.329}$ × 0.993 ^{age}, ≥ 0.7 mg/dL :144× (serum creatinine/0.7) $^{-1.209}$ × 0.993 ^{age}). Serum cystatin C-based eGFR [24]: (104 × (serum cystatin C) $^{-1.019}$ × 0.996 $^{-(age)}$ × 0.929 (if female)) – 8

Data are expressed as median and interquartile range in parentheses.

Figure 1

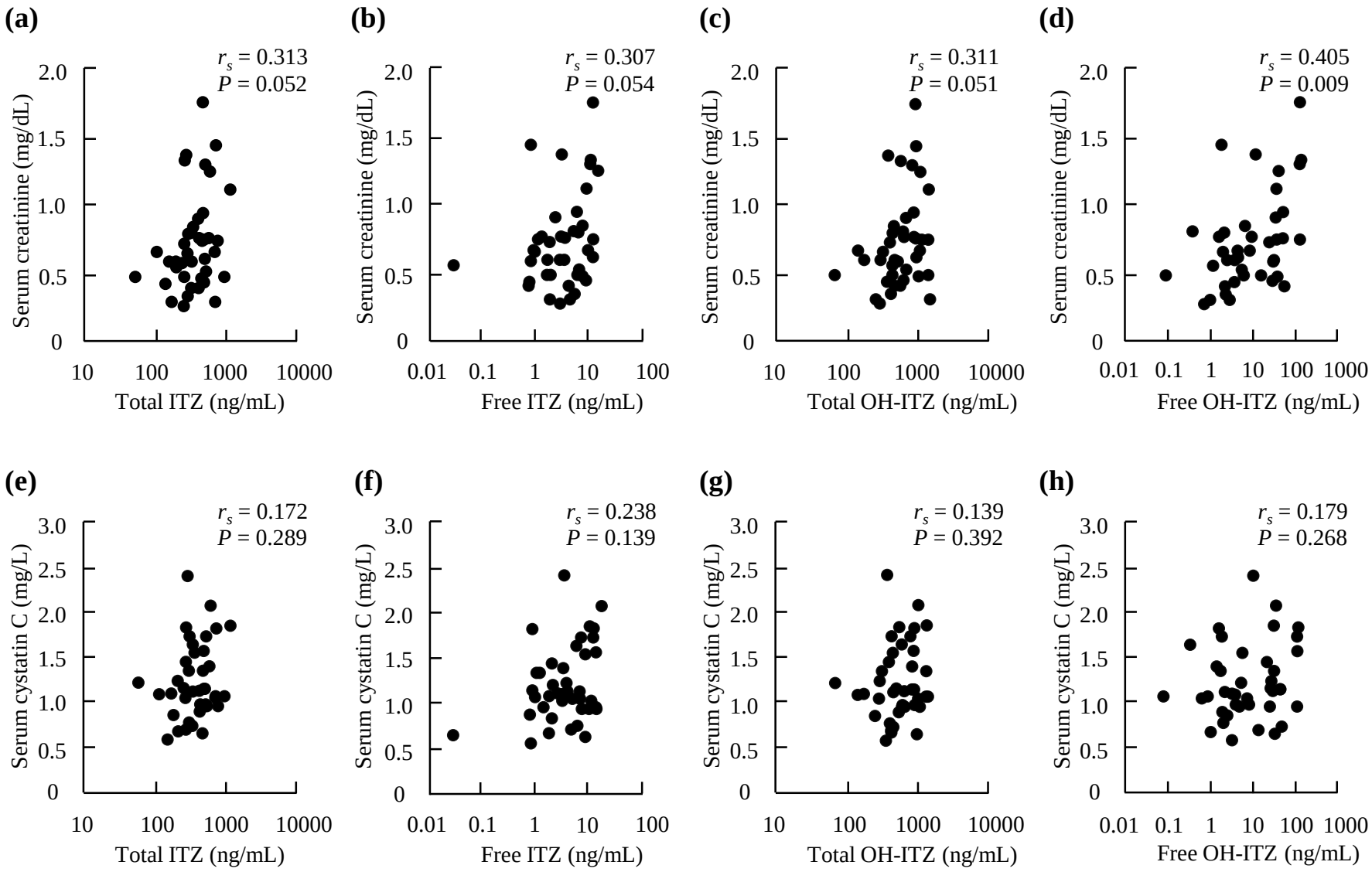
(a)
r_s = 0.313
P = 0.052
Serum creatinine (mg/dL)
Total ITZ (ng/mL)
(b)
r_s = 0.307
P = 0.054
Serum creatinine (mg/dL)
Free ITZ (ng/mL)
(c)
r_s = 0.311
P = 0.051
Serum creatinine (mg/dL)
Total OH-ITZ (ng/mL)
(d)
r_s = 0.405
P = 0.009
Serum creatinine (mg/dL)
Free OH-ITZ (ng/mL)
(e)
r_s = 0.172
P = 0.289
Serum cystatin C (mg/L)
Total ITZ (ng/mL)
(f)
r_s = 0.238
P = 0.139
Serum cystatin C (mg/L)
Free ITZ (ng/mL)
(g)
r_s = 0.139
P = 0.392
Serum cystatin C (mg/L)
Total OH-ITZ (ng/mL)
(h)
r_s = 0.179
P = 0.268
Serum cystatin C (mg/L)
Free OH-ITZ (ng/mL)

Figure 2

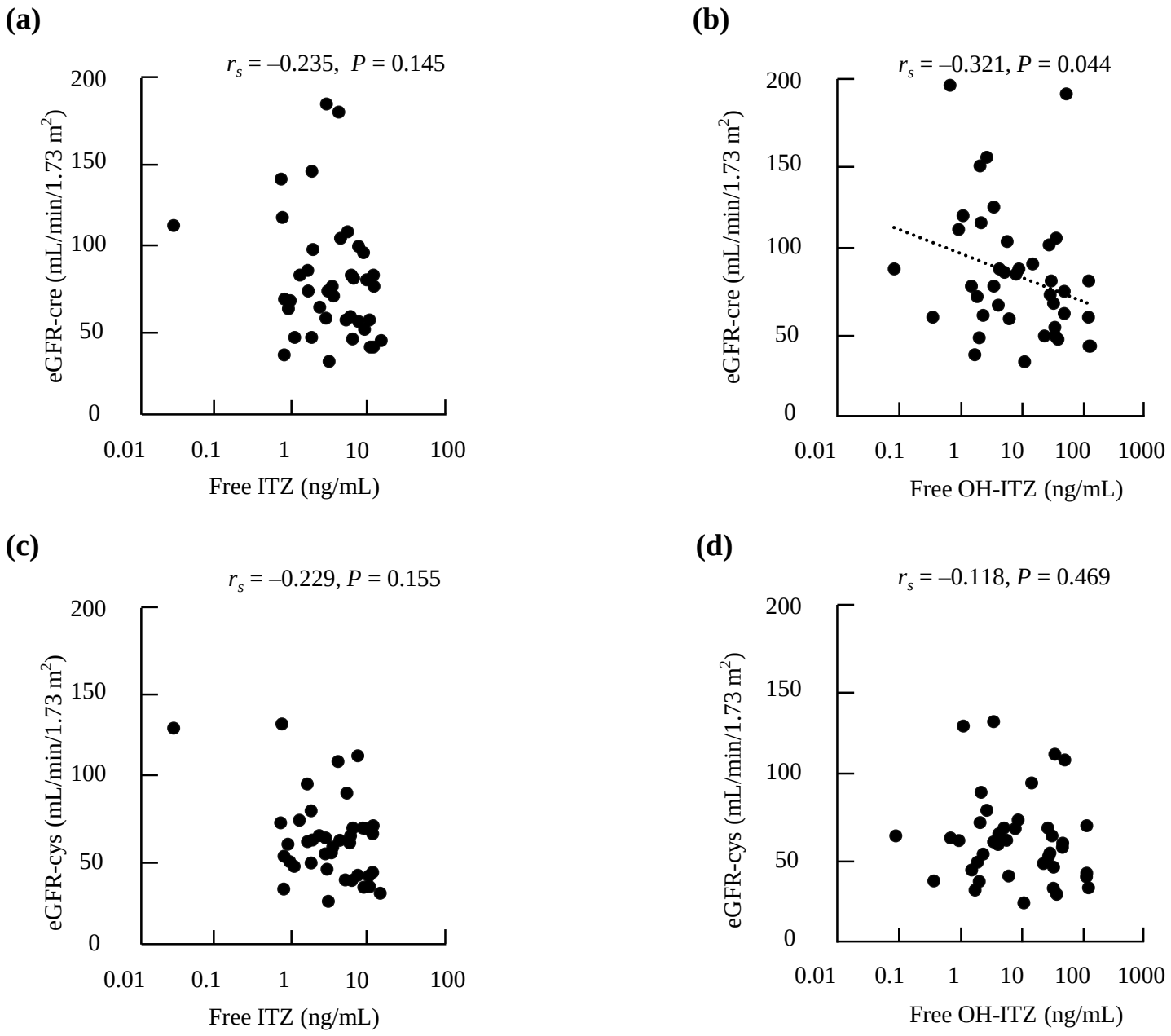
(a)
r_s = –0.235, P = 0.145
eGFR-cre (mL/min/1.73 m^2)
200
150
100
50
0
0.01
0.1
1
10
100
Free ITZ (ng/mL)
(b)
r_s = –0.321, P = 0.044
eGFR-cre (mL/min/1.73 m^2)
200
150
100
50
0
0.01
0.1
1
10
100
1000
Free OH-ITZ (ng/mL)
(c)
r_s = –0.229, P = 0.155
eGFR-cys (mL/min/1.73 m^2)
200
150
100
50
0
0.01
0.1
1
10
100
Free ITZ (ng/mL)
(d)
r_s = –0.118, P = 0.469
eGFR-cys (mL/min/1.73 m^2)
200
150
100
50
0
0.01
0.1
1
10
100
1000
Free OH-ITZ (ng/mL)

Figure 3

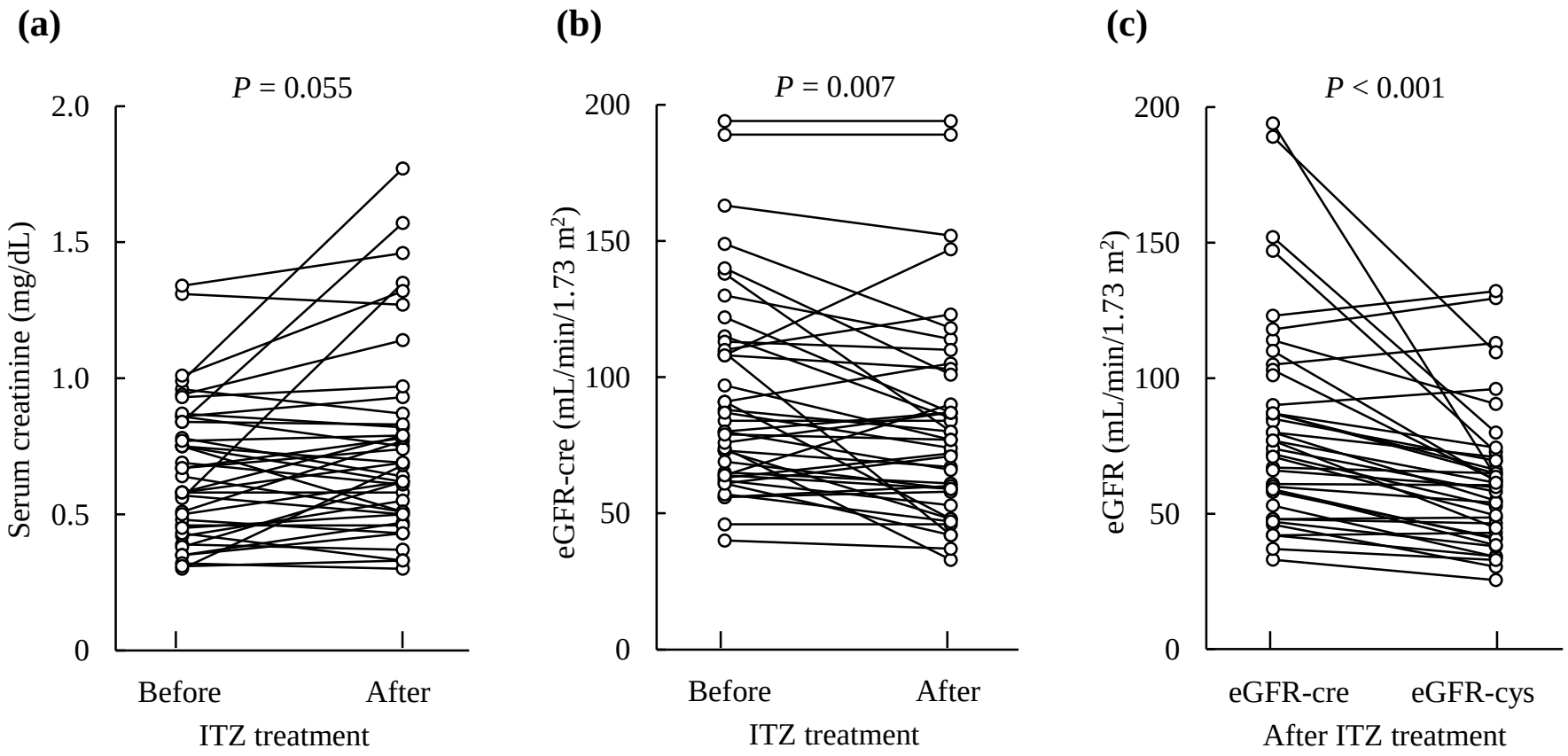
(a)
P = 0.055
Serum creatinine (mg/dL)
2.0
1.5
1.0
0.5
0
Before
After
ITZ treatment
(b)
P = 0.007
eGFR-cre (mL/min/1.73 m2)
200
150
100
50
0
Before
After
ITZ treatment
(c)
P < 0.001
eGFR (mL/min/1.73 m2)
200
150
100
50
0
eGFR-cre
eGFR-cys
After ITZ treatment

Figure 4

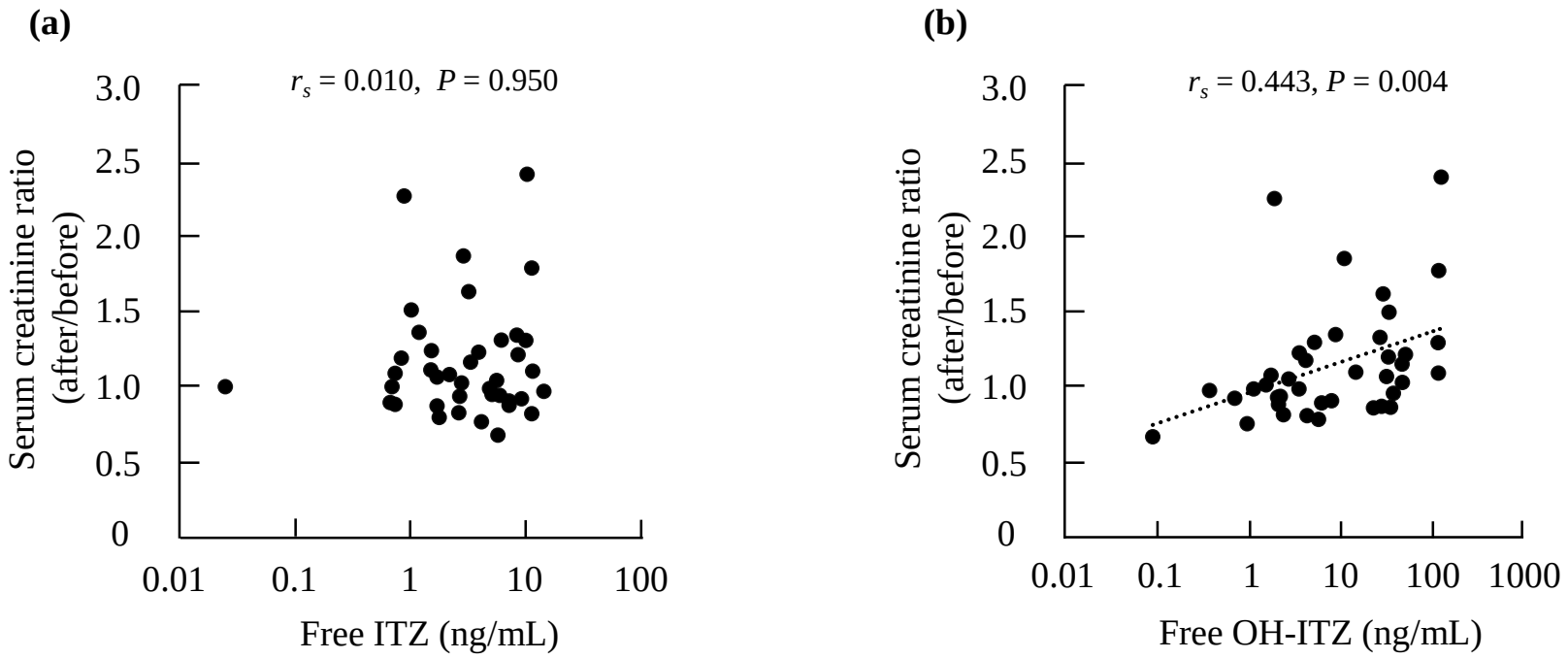
(a)
r_s = 0.010, P = 0.950
Serum creatinine ratio (after/before)
3.0
2.5
2.0
1.5
1.0
0.5
0
0.01
0.1
1
10
100
Free ITZ (ng/mL)
(b)
r_s = 0.443, P = 0.004
Serum creatinine ratio (after/before)
3.0
2.5
2.0
1.5
1.0
0.5
0
0.01
0.1
1
10
100
1000
Free OH-ITZ (ng/mL)

Figure 5

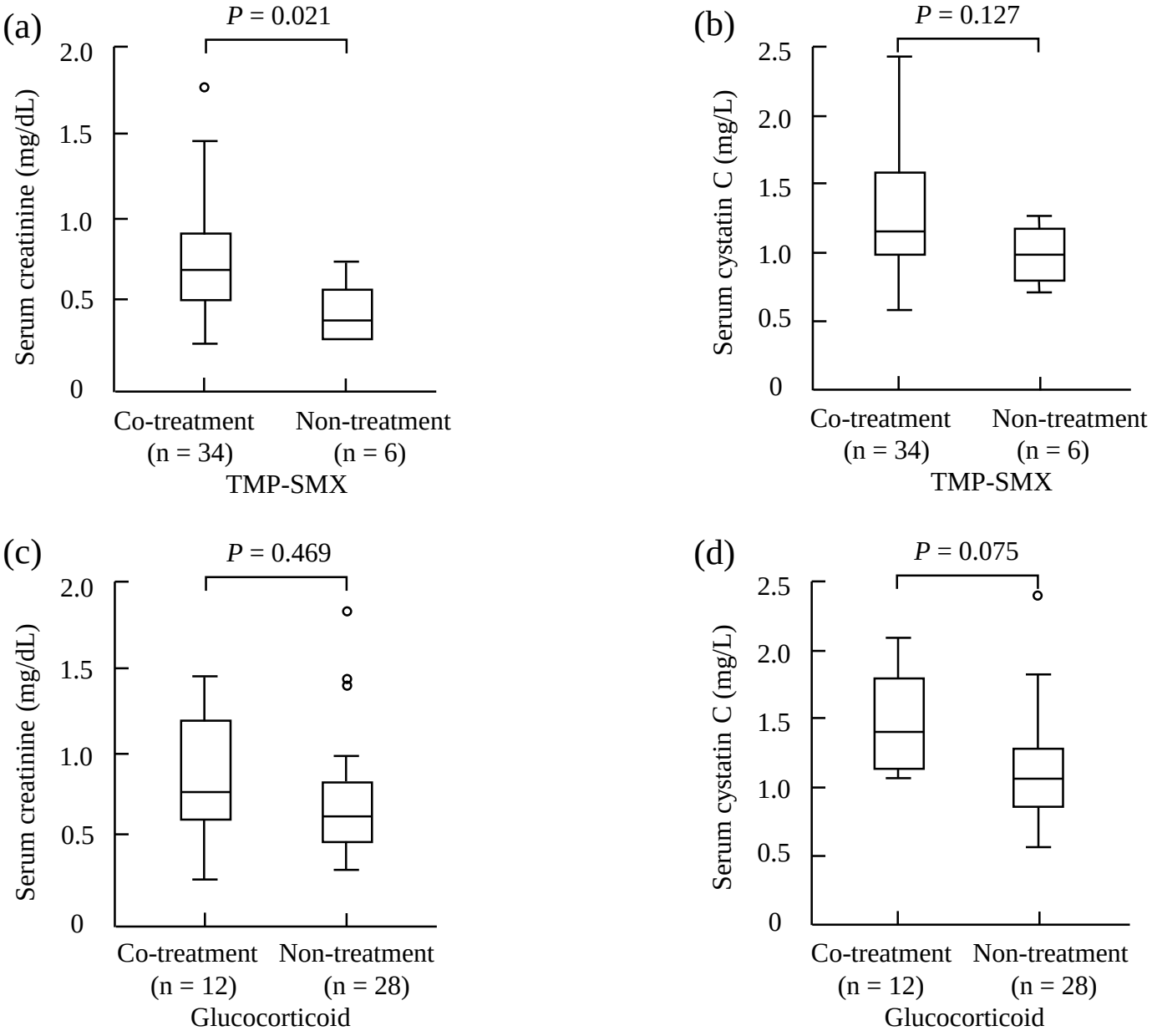
(a)
P = 0.021
Serum creatinine (mg/dL)
2.0
1.5
1.0
0.5
0
Co-treatment (n = 34)
Non-treatment (n = 6)
TMP-SMX
(b)
P = 0.127
Serum cystatin C (mg/L)
2.5
2.0
1.5
1.0
0.5
0
Co-treatment (n = 34)
Non-treatment (n = 6)
TMP-SMX
(c)
P = 0.469
Serum creatinine (mg/dL)
2.0
1.5
1.0
0.5
0
Co-treatment (n = 12)
Non-treatment (n = 28)
Glucocorticoid
(d)
P = 0.075
Serum cystatin C (mg/L)
2.5
2.0
1.5
1.0
0.5
0
Co-treatment (n = 12)
Non-treatment (n = 28)
Glucocorticoid

Figure S1

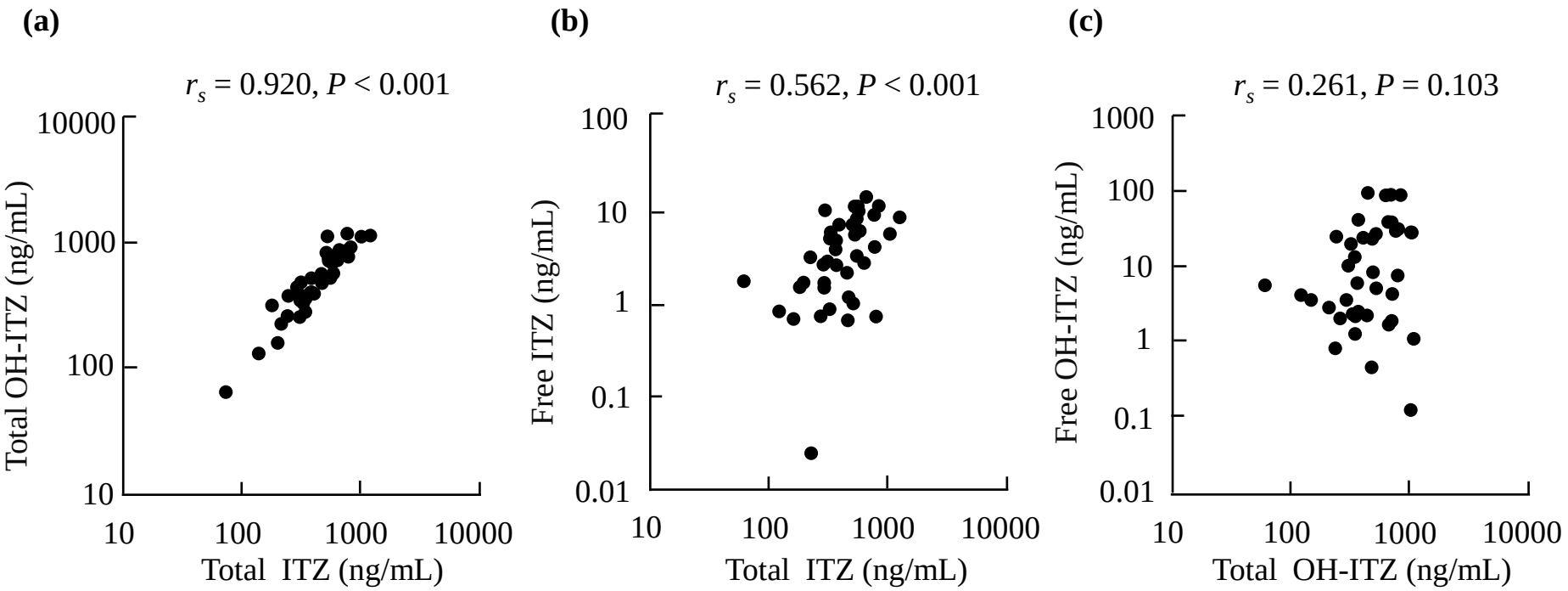

Figure S1. Correlations between the total plasma concentrations of ITZ and OH-ITZ **(a)** and between the plasma total and free concentrations of ITZ **(b)** and OH-ITZ **(c)** in patients with a hematopoietic or immune-related disorder.
The correlations were evaluated using Spearman's rank correlation coefficient test.

Figure S2

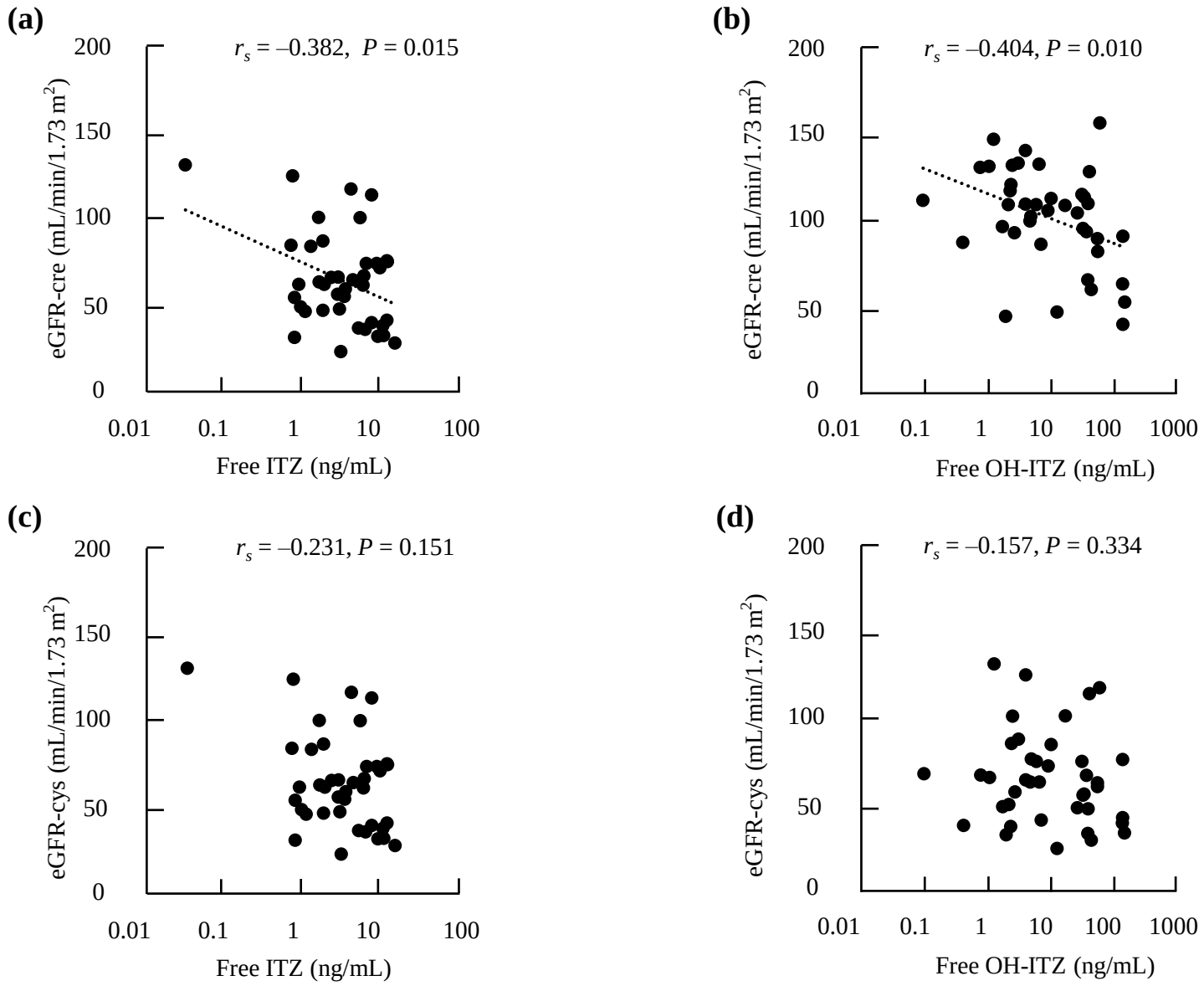

Figure S2. Correlations between the plasma free ITZ and OH-ITZ and estimated glomerular filtration rate (eGFR) using Chronic Kidney Disease Epidemiology Collaboration equation in patients with a hematopoietic or immune-related disorder.
Free plasma concentrations of **(a)** ITZ and **(b)** OH-ITZ versus serum creatinine-based eGFR (eGFR-cre); and free plasma concentrations of **(c)** ITZ and **(d)** OH-ITZ versus serum cystatin C-based eGFR (eGFR-cys). The correlations were evaluated using Spearman's rank correlation coefficient test and a regression line (a, b) with a slope was added.

Figure S3

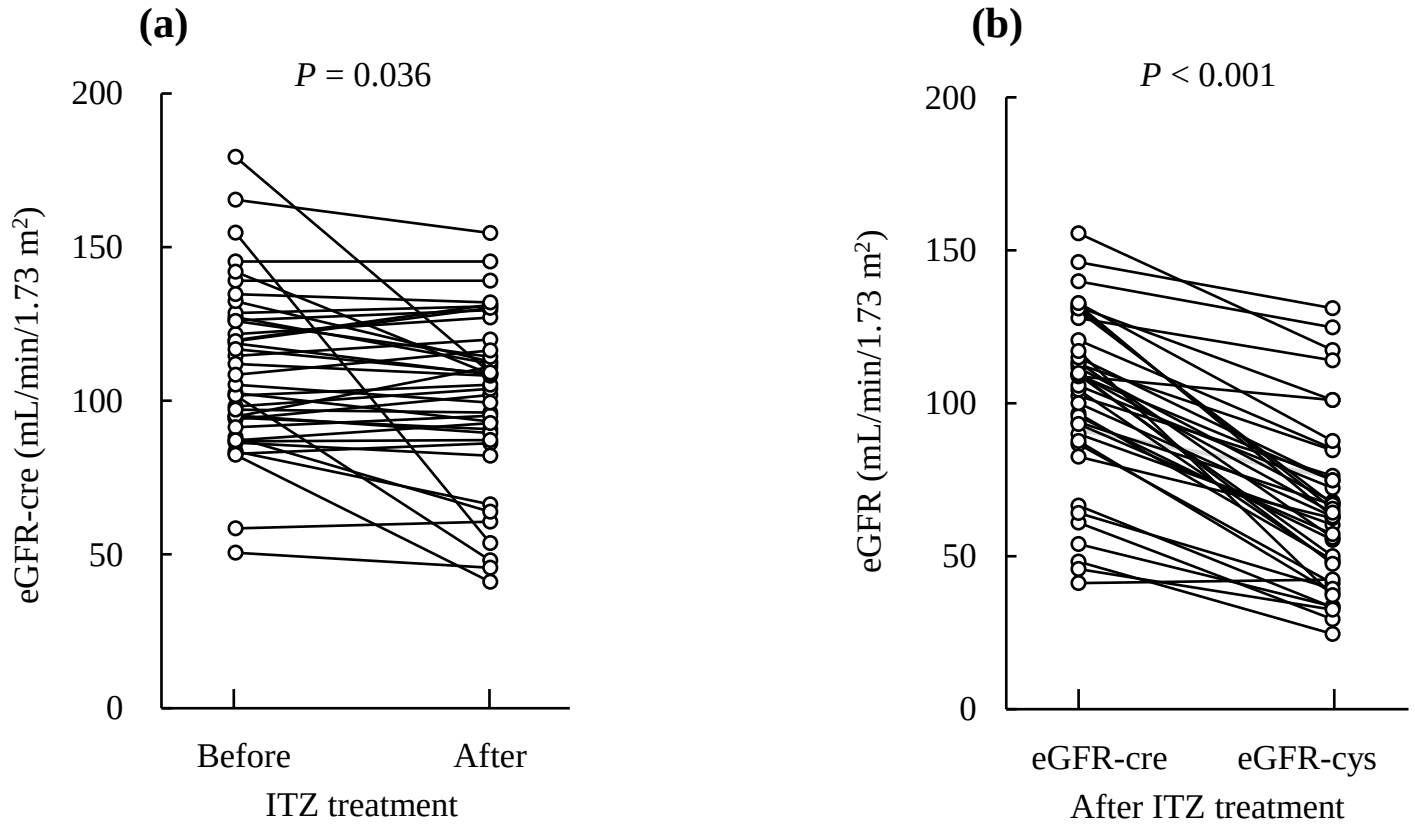

Figure S3. Changes in estimated glomerular filtration rate (eGFR) using Chronic Kidney Disease Epidemiology Collaboration equation in patients with a hematopoietic or immune-related disorder.
(a) Serum creatinine-based eGFR (eGFR-cre) before and after starting ITZ treatment and **(b)** eGFR-cre and serum cystatin C-based eGFR (eGFR-cys) after starting ITZ treatment.
The differences were evaluated using Wilcoxon signed rank test.