Nephrology Dialysis Transplantation (2023) 38: 644–654
https://doi.org/10.1093/ndt/gfac166
Advance Access publication date 2 May 2022

ORIGINAL ARTICLE

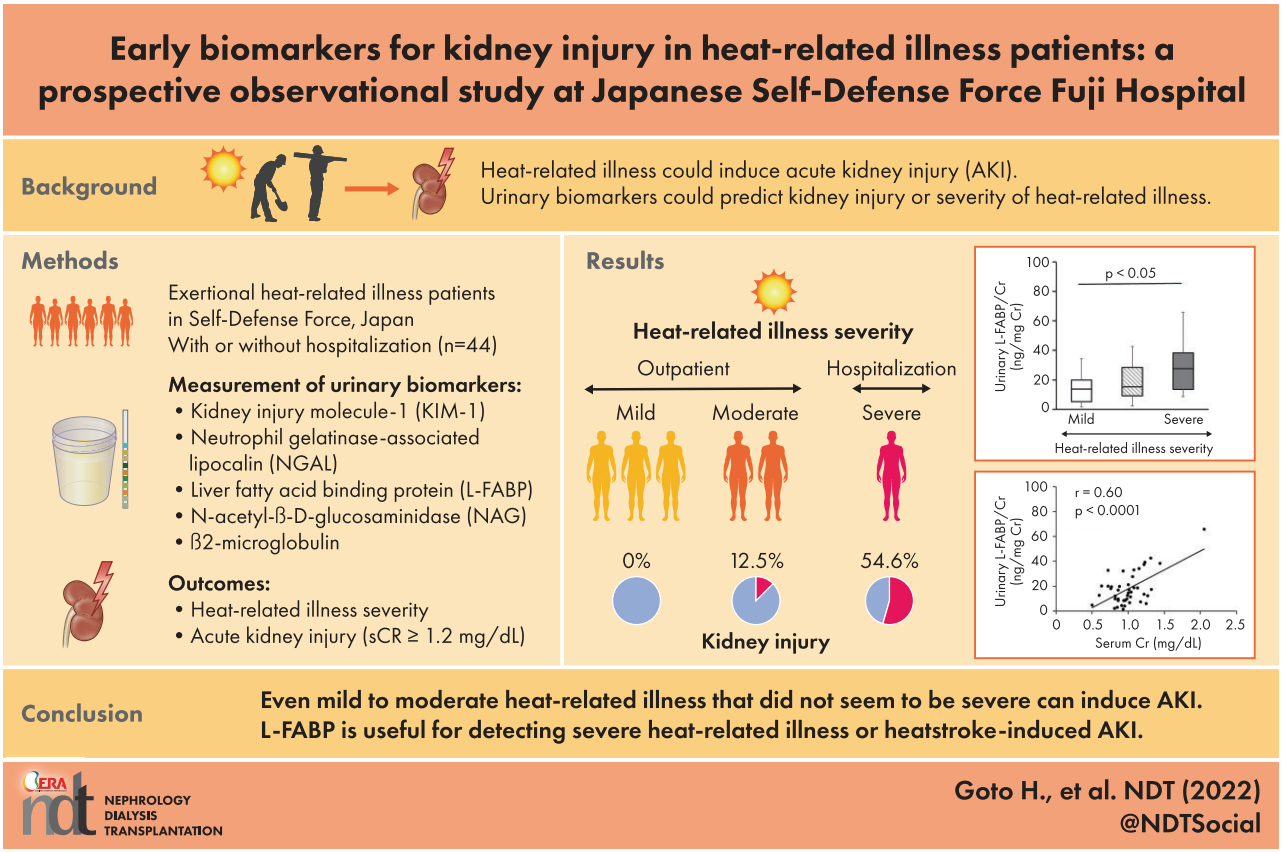
Early biomarkers for kidney injury in heat-related illness patients: a prospective observational study at Japanese Self-Defense Force Fuji Hospital

Hiroyasu Goto[1], Shinichi Shoda[2], Hiroyuki Nakashima[3], Midori Noguchi[1], Toshihiko Imakiire[1], Naoki Ohshima[1], Manabu Kinoshita[3], Soichi Tomimatsu[2] and Hiroo Kumagai[1]

[1]Department of Nephrology and Endocrinology, National Defense Medical College, Tokorozawa, Saitama, Japan, [2]Self-Defense Force Fuji Hospital, Subashiri, Shizuoka, Japan and [3]Department of Immunology and Microbiology, National Defense Medical College, Tokorozawa, Saitama, Japan

Correspondence to: Hiroyasu Goto; E-mail: hgoto@ndmc.ac.jp

GRAPHICAL ABSTRACT



KEY LEARNING POINTS

What is already known about this subject?

- Heat-related illness can induce acute kidney injury (AKI) by damaging kidney tubular cells.
- Although 30–40% of exertional heatstroke patients develop AKI, most studies have focused on patients hospitalized for heatstroke and it is not well known whether AKI also occurs in patients with mild to moderate heat-related illness.
- Urinary liver fatty acid–binding protein (L-FABP) and kidney injury molecule-1 (KIM-1) are well known to be useful for the early detection of AKI, but the relationship between these biomarkers and the severity of heat-related illness or AKI due to heatstroke remains unclear.

What this study adds?

- Twelve percent of moderate heat-related illness patients who did not require hospitalization had AKI.
- The urinary L-FABP levels were higher in severe heatstroke patients than the levels in mild heat-related illness patients and positively correlated with the serum creatinine levels.
- The urinary KIM-1 levels showed a marked correlation with serum cystatin C (sCysC).

What impact this may have on practice or policy?

- It should be noted that even mild to moderate heat-related illness can cause kidney damage.
- L-FABP is useful for monitoring athletes and laborers in hot environments to identify individuals who need to be treated immediately to prevent severe kidney injury.
- Although KIM-1 was not associated with the severity of heat-related illness, it had the potential to detect heatstroke-induced AKI in terms of sCysC.

ABSTRACT

Background. Since heatstroke-induced acute kidney injury (AKI) can progress to chronic kidney disease, it would be useful to detect heatstroke-induced AKI and severe heat-related illness in the early phase. We studied the epidemiology of heat-related illness among patients in the Japanese Ground Self-Defense Force and evaluated the relationship between heat-related illness severity and early urinary biomarkers for AKI.

Methods. We enrolled patients who were diagnosed with heat-related illness at the Self-Defense Force Fuji Hospital from 1 May to 30 September 2020. We compared the urinary kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), liver fatty acid–binding protein (L-FABP), N-acetyl-β-D-glucosaminidase (NAG) and β_2-microglobulin levels according to the severity of heat-related illness as defined by positive scores for the Japanese Association of Acute Medicine Heatstroke Working Group (JAAM-HS-WG) criteria (0, mild; 1, moderate; $\geq$2, severe).

Results. Of the 44 patients, kidney injury, defined as serum creatinine (sCr) $\geq$1.2 mg/dL, was seen in 9 (20.5%) patients. Urinary NAG, NGAL and L-FABP levels were significantly higher in the $\geq$2 JAAM-HS-WG criteria group than in the 0 group. Furthermore, urinary L-FABP levels were positively correlated with sCr levels. In contrast, the urinary KIM-1 levels showed the best correlation with serum cystatin C (sCysC) among these biomarkers.

Conclusions. We conclude even mild to moderate heatstroke could lead to AKI. Urinary L-FABP is useful for detecting heatstroke-induced AKI and patients with severe heat-related illness requiring immediate treatment. Urinary KIM-1 may detect heatstroke-induced AKI in terms of sCysC, although it was not related to the severity of heat-related illness.

Keywords: acute kidney injury, cystatin C, heatstroke, heat-related illness, KIM-1, L-FABP

INTRODUCTION

Global warming is progressing. The mean annual air temperature at the Earth's surface has increased by roughly 0.8°C since the 19th century and could warm by another 4°C by 2100 if the current trend continues [1]. Thus heat-related illness becomes an increasingly serious concern around the world. In Japan, the number of patients with heat-related illness has increased in recent years [2].

Heatstroke, which is the most hazardous, life-threatening heat-related illness, can be categorized into two types—classic (passive) and exertional—depending on its cause. Exertional heatstroke is associated with physical exercise and occurs when excessive production of metabolic heat overwhelms physiological heat loss. Since exertional heatstroke strikes young, healthy and highly active people, such as athletes, firefighters and military personnel, including the members of the Ground Self-Defense Force (GSDF), the mortality rate is lower than that of classic heatstroke. However, the risks of acute kidney injury (AKI) are higher with exertional heatstroke than with classic heatstroke [3].

Furthermore, recent studies have revealed that heatstroke-induced AKI is not transient and can actually result in progression to CKD [4, 5]. This is a serious health problem for athletes and laborers working in hot environments, as well as military personnel. It would thus be beneficial to be able to detect severe heat-related illness and heatstroke-induced AKI at an early phase and determine which patients need immediate treatment.

Recently, several urinary biomarkers have attracted attention for their usefulness in the early diagnosis of AKI [6–17].

Table 1. JAAM heat-related illness classification in 2015[a] [22]

Grade	Symptom	Treatment
I	Dizziness, faintness, slight yawing Heavy sweating, muscle cramps Impaired consciousness is not observed	First aid and observation
II	Headache, vomiting, fatigue, sinking feeling Decreased concentration and judgement	Should be taken to a medical institution
III	Includes at least one of following: Central nervous system manifestation (JCS ≥2 (impaired orientation, GCS E4V4M6), cerebellar symptoms, convulsive seizures) Hepatic/renal dysfunction (hepatic or renal impairment requiring inpatient hospital care) Coagulation disorder	Inpatient hospital care

[a]In this criteria, heat-related illness was classified into three grades according to patient symptoms and signs.
JCS, Japan Coma Scale.

Of these, neutrophil gelatinase–associated lipocalin (NGAL) and liver fatty acid–binding protein (L-FABP) have been approved for use in diagnostic tests for AKI in Japan. Kidney injury molecule-1 (KIM-1) is another AKI biomarker that was recently approved by the US Food and Drug Administration [18]. All three of these urinary biomarkers have been studied thoroughly among AKI patients subjected to cardiac surgery and admitted to intensive care units [6–9, 18, 19]. However, while some studies have reported an elevation in these urinary biomarkers when exercising in hot environments [10–14, 20, 21], the relationship between the severity of heat-related illness and these urinary biomarkers remains unclear.

In Japan, the severity of heat-related illness is usually assessed by the Japanese Association of Acute Medicine (JAAM) criteria published in 2015, with results classified into three grades: I, II and III [22] (Table 1). However, these criteria are intended to facilitate making decisions in the prehospital rescue setting and do not provide a scientifically objective definition of organ failure. The JAAM Heatstroke Working Group (JAAM-HS-WG) developed criteria for classifying the severity of heatstroke based on the Japanese Heat Stroke Registry. For the simplified classification, the JAAM-HS-WG criteria consist of the following four items: central nervous system disorder, kidney damage, liver damage and coagulation disorder in patients exposed to high-temperature environments [22]. The JAAM-HS-WG criteria positive score is the sum of individual positive items. We used the positive score to assess the severity of illness, since this value was reported in a recent study [23] to correlate with mortality due to heat-related illness.

In the present study we examined the relationship between the severity of heat-related illness and urinary biomarkers using urine samples from patients with heat-related illness at an outpatient clinic of the Japanese GSDF. Furthermore, we investigated which urinary biomarker was most useful for detecting severe heat-related illness and heatstroke-induced AKI.

MATERIALS AND METHODS

Study setting and design

This study is a prospective observational study of exertional heat-related illness patients who visited the Self-Defense Force (SDF) Fuji Hospital, Shizuoka Prefecture, Japan, between 1 May and 30 September 2020. The SDF Fuji Hospital is located near the Fuji maneuver area and many patients, including those who suffer heat-related illness during training, visit the hospital.

The wet bulb globe temperature (WBGT) was obtained from the website of the Japanese Ministry of the Environment (https://www.wbgt.env.go.jp). All patients diagnosed with heat-related illness by a physician were enrolled in this study.

This study was approved by the Research Ethics Committee of the National Defense Medical College, Japan (approval 4141) and all participants gave informed consent.

JAAM criteria for heat-related illness

The JAAM published their criteria for heat-related illness in 2015 (Table 1). Under these criteria, heat-related illness was defined as the presence of symptoms caused by a hot environment for which other obvious causes have been excluded. Heat-related illnesses are classified into three grades according to their severity: I, II and III (Fig. 1A) [22].

JAAM-HS-WG criteria items

The JAAM also established criteria for heatstroke (JAAM-HS-WG criteria) [22]. Heatstroke was determined when patients met at least one of the following criteria: Glasgow Coma Scale (GCS) score ≤14, serum creatinine (sCr) level ≥1.2 mg/dL, total bilirubin level ≥1.2 mg/dL and JAAM disseminated intravascular coagulation (DIC) score ≥4 [24].

The sum of the number of positive items for each patient was used to classify the severity of heatstroke as the JAAM-HS-WG criteria positive score (Fig. 1B).

Definition of heatstroke-induced AKI

Because the baseline sCr was unknown, we defined heatstroke-induced AKI as sCr ≥1.2 mg/dL, according to the JAAM-HS-WG criteria [22].

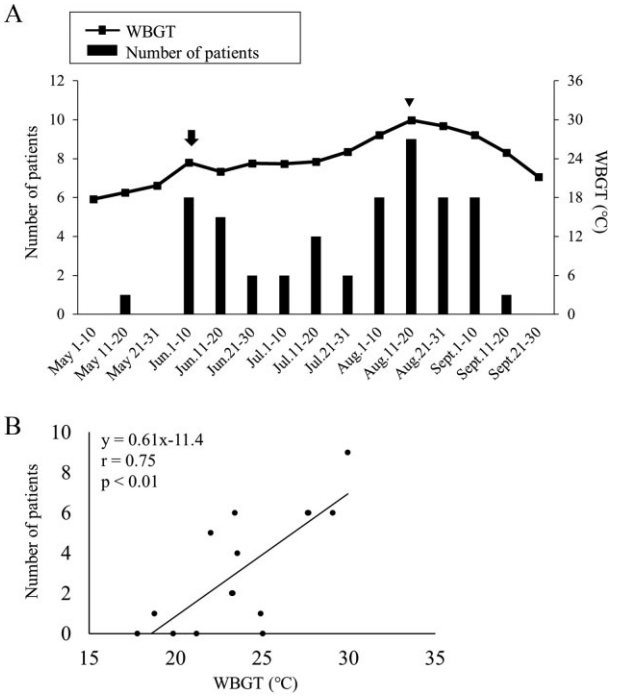


FIGURE 1: Time series of WBGT and the number of patients with heat-related illness every 10 days. (**A**) The number of patients (bar graph) indicates the total number of heatstroke patients encountered over a 10-day period. The WBGT (line graph) is the average of the maximum WBGT for each day during the 10-day period. From 1 to 10 June 2020, there was a sharp increase in the number of patients as well as in the WBGT (arrow). Both the number of patients and WBGT peaked from 11 to 20 August 2020 (arrowhead). (**B**) The total patient numbers during the 10-day periods correlated with the average of the maximum WBGT (Pearson's $r = 0.75$, $P < .01$). Data were analysed using Pearson's correlation coefficient.

Data collection

Serum and urine samples were collected from patients with heat-related illness and analysed at the SDF Fuji Hospital Laboratory. The laboratory data and medical history were collected from the medical records. The estimated glomerular filtration rate (eGFR) was calculated based on the Japanese equation of the eGFR for sCr [25] and serum cystatin C (scysC) [26]. Patients with heat-related illness were also asked to answer a questionnaire regarding the amount of water they had consumed that day.

The evaluation of exercise habits

We asked patients about their exercise habits using a questionnaire and calculated the metabolic equivalent of tasks (METs) per week for each patient using a conversion formula [27].

Measurement of urinary biomarkers

After collection, urine samples were centrifuged at 3500 *g* for 10 min at 4°C and then the supernatant was drawn off and stored at −80°C. Urinary KIM-1, NGAL and L-FABP were measured at the National Defense Medical College using the following commercial enzyme-linked immunosorbent assay (ELISA) kits: KIM-1 (R&D Systems, Minneapolis, MN, USA), NGAL (R&D Systems) and L-FABP (CMIC, Tokyo, Japan). The intra- and interassay coefficients of variation were 3.9–4.4% and 6.1–7.8% for KIM-1; 3.1–4.4% and 5.6–7.9% for NGAL and ≤15% and ≤10% for L-FABP [28]. Detection limits were 0.009 ng/mL for KIM-1, 0.012 ng/mL for NGAL and 0.3 ng/mL for L-FABP. Urinary N-acetyl-β-D-glucosaminidase (NAG) and β_2-microglobulin (β_2 m) levels were measured via an enzymatic method (SRL, Tokyo, Japan).

Statistical analyses

Variables are expressed as the median and interquartile range (IQR) for continuous data and as the number and percentage of patients for categorical data. Differences among the three groups were assessed by a one-way analysis of variance (ANOVA) with Tukey's post hoc test. Differences between two groups were assessed by the Mann–Whitney *U*-test. A correlation analysis was conducted using Pearson's correlation coefficient. A paired *t*-test was used to compare sCr at arrival and during follow-up. All statistical analyses were performed using the JMP software program (version 15; SAS Institute, Cary, NC, USA). *P*-values <.05 were considered statistically significant.

RESULTS

Relationship between the maximum WBGT and number of patients with heat-related illness

A total of 50 patients were included in this study. Figure 2 shows the WBGT and number of patients with heat-related illness every 10 days. There was a sharp increase in the WBGT from 1 to 10 June 2020, which was coincident with a marked increase in the number of patients with heat-related illness. Both the number of patients with heat-related illness and the WBGT peaked from 11 to 20 August 2020 (Fig. 2A). There was a significant positive correlation between the WBGT and patient number (Pearson's $r = 0.75$, $P < .01$; Fig. 2B).

Patients' characteristics

Because serum total bilirubin data were lacking in 2 patients and urine samples were not collected in 4 patients, we ultimately analysed 44 patients. The number of Grade I, II and III patients with heat-related illness based on the 2015 JAAM criteria [22] was 9 (20.5%), 24 (54.5%) and 11 (25.0%), respectively (Fig. 1A). The characteristics of patients with heat-related illness classified as severity Grade I, II and III are shown in Table 2. All but one patient, who had diabetes and hypertension, were healthy with no comorbidities. There were significant differences in the GCS ($P < .01$), blood urea nitrogen (BUN; $P < .01$), sCr ($P < .05$) and sCysC ($P < .05$) values among the three groups, whereas neither body temperature (BT) nor hematocrit was significantly different. The number of patients with central nervous system disorder and kidney injury increased with the grade of heat-related illness (Table 3).

The presence of kidney injury, defined as sCr ≥1.2 mg/dL, was noted in three patients (20.5%) of Grade II and in six patients (54.6%) of Grade III. We confirmed that these

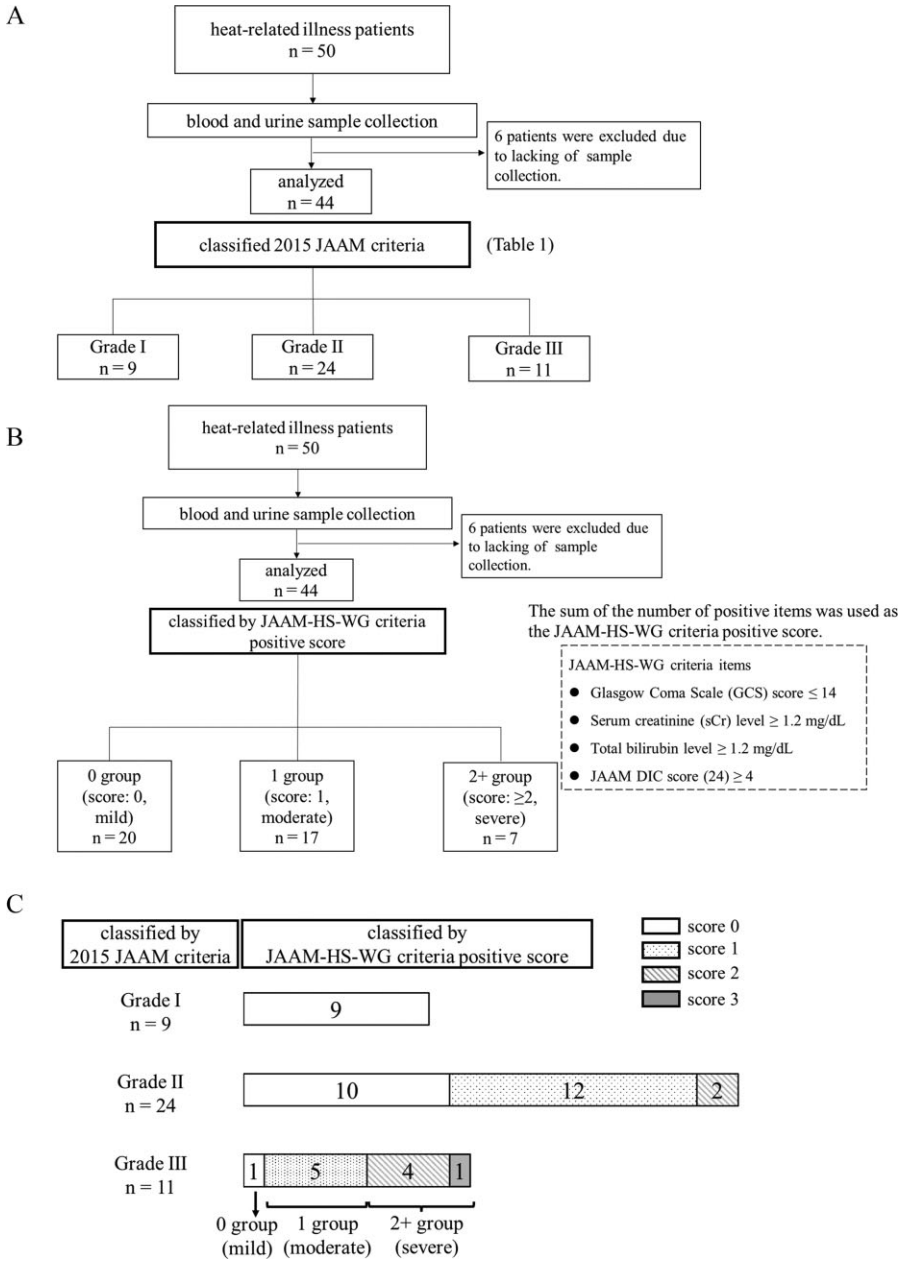


FIGURE 2: The flowchart of patient selection in this study. Following the exclusion of 6 patients, 44 patients were classified by the (**A**) 2015 JAAM criteria and (**B**) JAAM-HS-WG criteria positive score. (**C**) comparison of patient numbers by severity of heat-related illness classified by the 2015 JAAM criteria and the JAAM-HS-WG criteria positive score. All patients with Grade I classified by the 2015 JAAM criteria were classified into the 0 group by the JAAM-HS-WG criteria positive score. In contrast, there was only one patient classified into the 0 group who was Grade III. The numbers of patients by severity of heat-related illness classified by the JAAM-HS-WG criteria positive score are shown in the bar graph.

patients with kidney injury had markedly decreased sCr levels (≥0.3 mg/dL) during the follow-up period (day 1–7; Supplementary Fig. S1).

The positive items in the JAAM-HS-WG criteria were summed as the JAAM-HS-WG criteria positive score. The number of patients with a JAAM-HS-WG criteria positive score of 0, 1, 2 and 3 was 20 (45.5%), 17 (38.6%), 6 (13.6%) and 1, respectively. No patients met all four criteria. As shown in Fig. 1C, all patients with Grade I exhibited a score of 0 according to the JAAM-HS-WG criteria. In contrast, in Grade II patients, 10 had score of 0, 12 had a score of 1 and 2 had a score of 2. In Grade III patients, 1 had a score of 0, 5 had a score of 1, 4 had a score of 2 and 1 had a score of 3.

Elevation of urinary NGAL and L-FABP levels in severe heatstroke patients

To assess the relationship between urinary biomarkers and the severity of heat-related illness, we measured five urinary biomarkers (KIM-1, NGAL, L-FABP, NAG and β_2 m) and

Table 2. Patient characteristics

Variable	Grade I	Grade II	Grade III	*P*-value
Patients, *n*	9	24	11	
Age (years)	21 (19–27)	25 (20–34)	27 (20–39)	.37
Male, *n* (%)	7 (77.8)	23 (95.8)	10 (90.9)	.14
BMI (kg/m^2)	22.4 (20.5–25.4)	22.8 (22.1–24.9)	24.9 (22.9–25.3)	.38
GCS at arrival, *n* (%)	15 (15–15)	15 (14–15)	14 (10–15)	<.01
BT at arrival (°C)	36.4 (36.2–36.8)	36.7 (36.3–37.1)	36.4 (35.9–36.8)	.64
HR at arrival (bpm)	69 (61–77)	72 (56–86)	77 (66–95)	.18
sBP at arrival (mmHg)	112 (103–126)	115 (109–130)	124 (110–128)	.42
Serum T.bil (mg/dL)	0.82 (0.69–1.07)	1.04 (0.1–1.54)	1.04 (0.93–1.39)	.23
Serum AST (U/L)	25 (19–31)	23 (19–29)	31 (24–36)	.80
Serum ALT (U/L)	29 (17–42)	21 (17–32)	26 (16–45)	.66
Serum CK (U/L)	259 (139–409)	193 (130–590)	313 (208–616)	.74
BUN (mg/dL)	12.5 (10.2–14.8)	15.1 (12.6–16.9)	19.8 (13.1–28.6)	<.01
Serum Cr (mg/dL)	0.86 (0.72–1.00)	0.97 (0.86–1.07)	1.22 (1.00–1.32)	<.05
eGFR (Cr) (mL/min/1.73 m^2)	89.6 (76.0–102.3)	80.9 (68.7–90.7)	65.3 (50.0–78.2)	<.05
Serum CysC (mg/L)	0.80 (0.75–0.91)	0.85 (0.80–0.91)	0.99 (0.81–1.20)	<.05
eGFR (CysC) (mL/min/1.73 m^2)	104.8 (97.0–145.3)	102.6 (93.0–112.1)	85.9 (72.7–103.0)	<.01
Serum CRP (mg/dL)	0.1 (0.0–0.4)	0.1 (0.1–0.25)	0.1 (0.1–0.4)	.88
Hematocrit (%)	46.5 (43.4–49.1)	46.7 (43.1–50.3)	46.4 (42.6–50.1)	.28
White blood cells ($\times 10^3/\mu$L)	6.7 (5.4–12.0)	9.9 (6.3–11.8)	10.1 (8.5–12.3)	.43
Platelet ($\times 10^4/\mu$L)	24.1 (22.3–26.6)	22.7 (19.5–28.2)	23.4 (18.7–27.1)	.61
PT-INR	0.97 (0.93–1.01)	0.97 (0.93–1.00)	0.94 (0.92–1.00)	.78
FDP (μg/mL)	0.9 (0.2–2.3)	1.1 (0.3–1.5)	1.2 (0.3–1.8)	.97
Baseline medications				
Oral hypoglycemic agent	0 (0.0)	0 (0.0)	1 (8.3)	.46
Antihytertensive agent	0 (0.0)	0 (0.0)	1 (8.3)	.46
Water consumption (L)	1.0 (0.6–1.3)	1.0 (0.6–1.5)	1.0 (0.5–2.0)	.67
Exercise habits (METs/week)	17.5 (0–30.3)	17.4 (10.2–28.4)	13.1 (6.5–26.0)	.41
JAAM-HS-WG criteria positive score	0 (0–0)	1 (0–1)	1 (1–2)	<.0001

Values are presented as median (IQR) unless stated otherwise. Continuous variables were analysed using one-way ANOVA and categorical variables were analysed using Fisher's exact test.

BMI, body mass index; HR, heart rate; sBP, systolic blood pressure; T.bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; CRP, C-reactive protein; PT-INR, prothrombin time-international normalized ratio; FDP, fibrin/fibrinogen degradation products.

Table 3. The JAAM-HS-WG criteria and heat-related illness grade

JAAM-HS-WG criteria item	Grade I, *n* (%)	Grade II, *n* (%)	Grade III, *n* (%)	*P*-value
GCS ≤14	0 (0)	6 (25.0)	6 (54.6)	<.05
T.bil ≥1.2 mg/dL	0 (0)	7 (29.2)	4 (36.4)	.13
sCr ≥1.2 mg/dL	0 (0)	3 (12.5)	6 (54.6)	<.01
JAAM DIC score	0 (0)	0 (0)	0 (0)	1.00

The number of patients who met each of the JAAM-HS-WG criteria was expressed by the severity of heat-related illness. The sum of the JAAM-HS-WG criteria positive items is used as the JAAM-HS-WG criteria positive score in Fig. 3. Data were analysed using Fisher's exact test.

T.bil, total bilirubin; DIC, disseminated intravascular coagulation.

compared them among patients with JAAM-HS-WG criteria positive scores of 0, 1 and ≥2 (2+), which mean mild, moderate and severe, respectively (Fig. 1B). The urinary NGAL/Cr, L-FABP/Cr and NAG/Cr were significantly higher in the ≥2 group than those in the 0 group ($P < .05$ for NGAL/Cr, L-FABP/Cr and NAG/Cr; Fig. 3B–D).

Elevation of urinary L-FABP levels in kidney injury patients

Among the five urinary biomarkers, only the urinary L-FABP/Cr was significantly higher in patients who had kidney injury than in those who did not have it ($P < .05$; Fig. 4C). Furthermore, there was a significant correlation between the urinary L-FABP/Cr and sCr values (Pearson's $r = 0.60$, $P < .0001$; Fig. 5C). Although heatstroke-induced AKI is considered to correlate with dehydration, there was no correlation between the hematocrit and urinary L-FABP or sCr values (Supplementary Fig. S2). No statistically significant correlations were found between sCr and KIM-1/Cr (Fig. 5A) or NGAL/Cr (Fig. 5B), but there was a significant positive correlation between the urinary NGAL and serum total bilirubin levels (Pearson's $r = 0.37$, $P = .01$; Supplementary Fig. S3).

We also analysed the relationships between the sCysC and urinary biomarker levels. Interestingly, there were significant correlations between the sCysC and urinary KIM-1/Cr and L-FABP/Cr ($r = 0.66$, $P < .0001$ for KIM-1; $r = 0.49$, $P < .001$ for L-FABP; Fig. 5F–J).

DISCUSSION

In the present study we evaluated the relationship between the JAAM-HS-WG criteria positive score and five urinary biomarkers. Our study demonstrated that the urinary NAG/Cr,

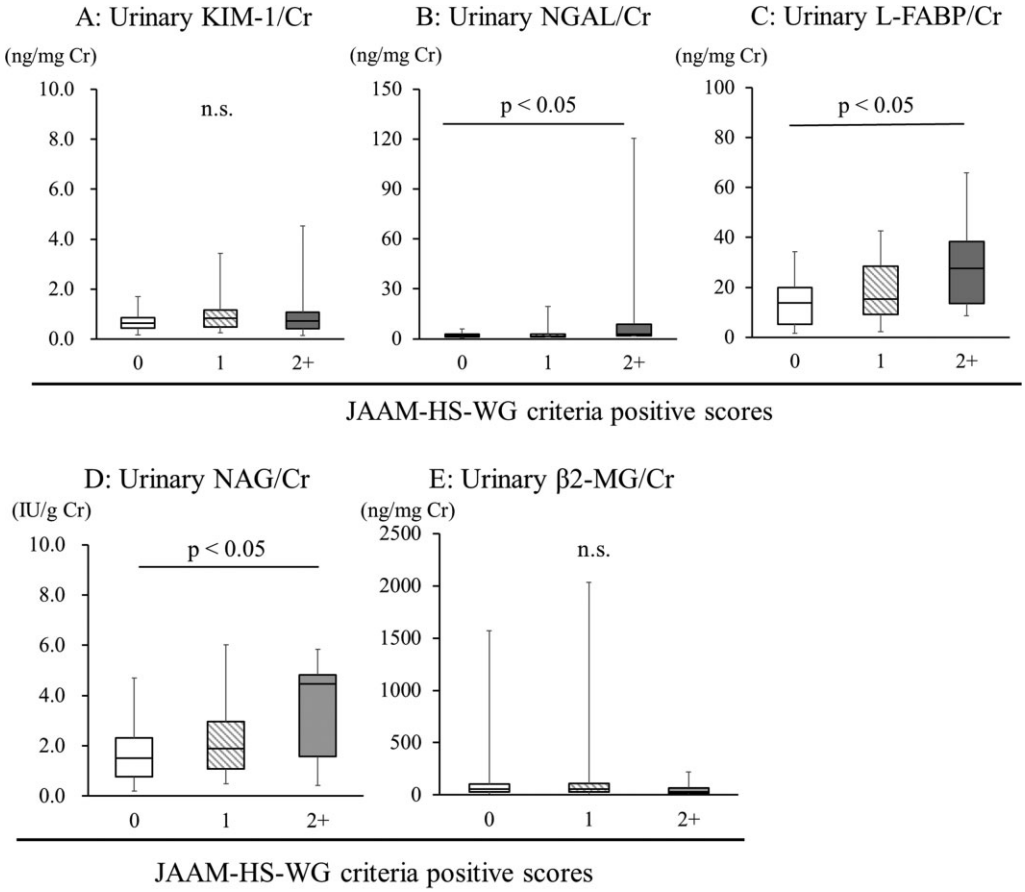


FIGURE 3: Relationship between urinary biomarkers and the JAAM-HS-WG criteria positive score. The JAAM-HS-WG criteria positive score on the *x*-axis was calculated as the sum of the items positive for JAAM-HS-WG criteria, shown in Table 3. Urinary biomarker levels in patients with positive scores of 0, 1 and ≥2 are shown. Data are presented as the median and IQR. Data were analysed using a one-way ANOVA with Tukey's post hoc test.

NGAL/Cr and L-FABP/Cr values were increased in patients with severe heat-related illness. Furthermore, the urinary L-FABP level was significantly and positively correlated with the sCr value.

In this study, urinary L-FABP was the only biomarker whose level was higher in heat-related illness patients with kidney injury than in those without it. L-FABP is a part of the FABP family, which is involved in fatty acid transport [18, 29]. In steady state, L-FABP is expressed predominantly in the proximal tubules, which uses fatty acids as a major source of energy metabolism [30]. When AKI occurs, L-FABP transits from the cytoplasm to the tubular lumen of the proximal tubule cells, which results in an increase in urinary L-FABP. Earlier studies reported the correlation between urinary L-FABP and microcirculatory disorder [29], and in addition, heat-related illness is considered to induce dehydration. Nevertheless, we noted no significant correlation between the hematocrit and L-FABP values in the current study. Several experimental studies have shown that heatstroke can induce not only prerenal AKI, but also heat stress–induced mitochondrial dysfunction, which suppressed adenosine triphosphate (ATP) production, resulting in the exacerbation of kidney damage in a mouse heatstroke model [31, 32]. Therefore our results imply that in heatstroke patients, heat stress–injured mitochondria in tubular cells increased the L-FABP expression in the tubular lumen to restore ATP production, resulting in an increase in urinary L-FABP values. In clinical settings, patients should receive urgent treatment when their urinary L-FABP levels are elevated, as it suggests mitochondrial dysfunction in tubular cells, which can induce further tubular damage. A simple measurement kit, such as the L-FABP dipstick, may be useful in the prehospital setting to make scientifically objective decisions concerning immediate treatment and prevent further kidney damage. Further studies focused on the relationship between mitochondrial dysfunction and L-FABP expression in tubular cells are needed.

We conducted a cohort study of AKI in healthy, highly active, young exertional heatstroke patients in the GSDF. Heatstroke-induced AKI occurred in ∼20% of exertional heatstroke patients, which is less than previously reported (30–40%) [33, 34]. The reason for this may be the low severity of the heat-related illness in this study. Patients in our study showed mostly mild to moderate heat-related illness of Grade I or II according to the 2015 JAAM criteria. However, this is a strong point of our study. The SDF Fuji Hospital is located near the Fuji maneuver area and it is easy for members of the GSDF to visit the hospital when they suffer any issues. Furthermore, we enrolled all the patients who visited the SDF

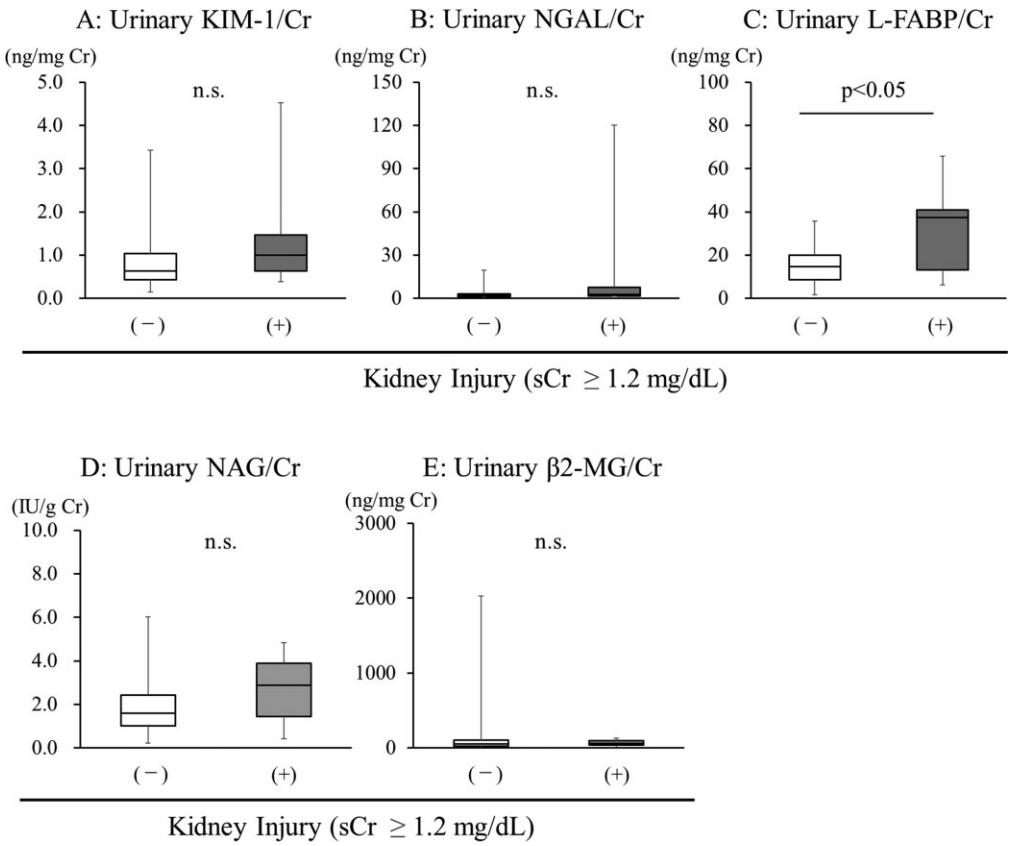


FIGURE 4: Relationship between urinary biomarkers and presence of kidney injury (sCr ≥1.2 mg/dL). Urinary biomarker levels are shown in patients without kidney injury (–) and with kidney injury (+). Data are presented as the median and IQR. Data were analysed using the Mann-Whitney *U*-test.

Fuji Hospital and were diagnosed with heat-related illnesses, regardless of whether or not the patients were thereafter hospitalized. In contrast, previous studies have focused on patients hospitalized due to exertional heatstroke [33, 34]. Therefore the low incidence of AKI in the patients with heat-related illness in our study reflects what actually happens in the field. Furthermore, it should be emphasized that even moderate heatstroke can induce kidney injury. Indeed, in our study, 3 of 25 (12%) Grade II patients, who did not seem to need hospitalization, had kidney injury.

KIM-1 is a type I membrane glycoprotein that contains both a novel six-cysteine immunoglobulin-like domain and a mucin domain in its extracellular portion [35]. A meta-analysis revealed that the area under the curve of KIM-1 for an AKI diagnosis was 0.86 (95% confidence interval 0.83–0.89) [11]. Although we evaluated urinary KIM-1 levels in patients with heat-related illness, we noted no marked difference in values between patients with mild and severe heat-related illness. Furthermore, urinary KIM-1 levels were not increased in patients with kidney injury compared with those without kidney injury. The levels of sCysC, another index of the kidney function, were significantly correlated with the urinary KIM-1/Cr and L-FABP/Cr. In particular, urinary KIM-1/Cr showed the best correlation with sCysC. It may be better to use sCysC instead of sCr as an index of renal function in a study of young, healthy people, such as our study, as some studies have indicated that sCysC is a better GFR marker than sCr, particularly for individuals with a small to moderate decrease in GFR [36]. Furthermore, some studies have suggested that the sCysC level is useful for detecting AKI, although the cut-off value remains controversial [37, 38]. Therefore our results suggest that urinary KIM-1 may be a candidate biomarker for detecting AKI in heat-related illness patients.

NGAL is a 5-kD lipocalin superfamily protein that is up-regulated in the proximal tubules, thick ascending limb and collecting ducts of mice after AKI [39–42]. Some studies have described the elevation of urinary NGAL when exercising or working in a hot environment [10–14], and we observed a significant difference in urinary NGAL/Cr levels between mild and severe heat-related illness patients, although there was no marked difference based on the presence of kidney injury. Liver injury also reportedly increases the serum and urinary NGAL levels [43], and we observed a positive correlation between the urinary NGAL and total bilirubin levels. Therefore the elevation of urinary NGAL/Cr in the patients with severe heat-related illness in our study may have been due, at least in part, to liver injury.

Several limitations associated with the present study warrant mention. First, since it was a single-center study, the sample size was small. Second, most of the patients in our study were male, and we did not assess the impact of gender differences on the study results. One study reported that the urinary NGAL level was lower in men than in women [14]. Third, urinary L-FABP levels in our study were not as

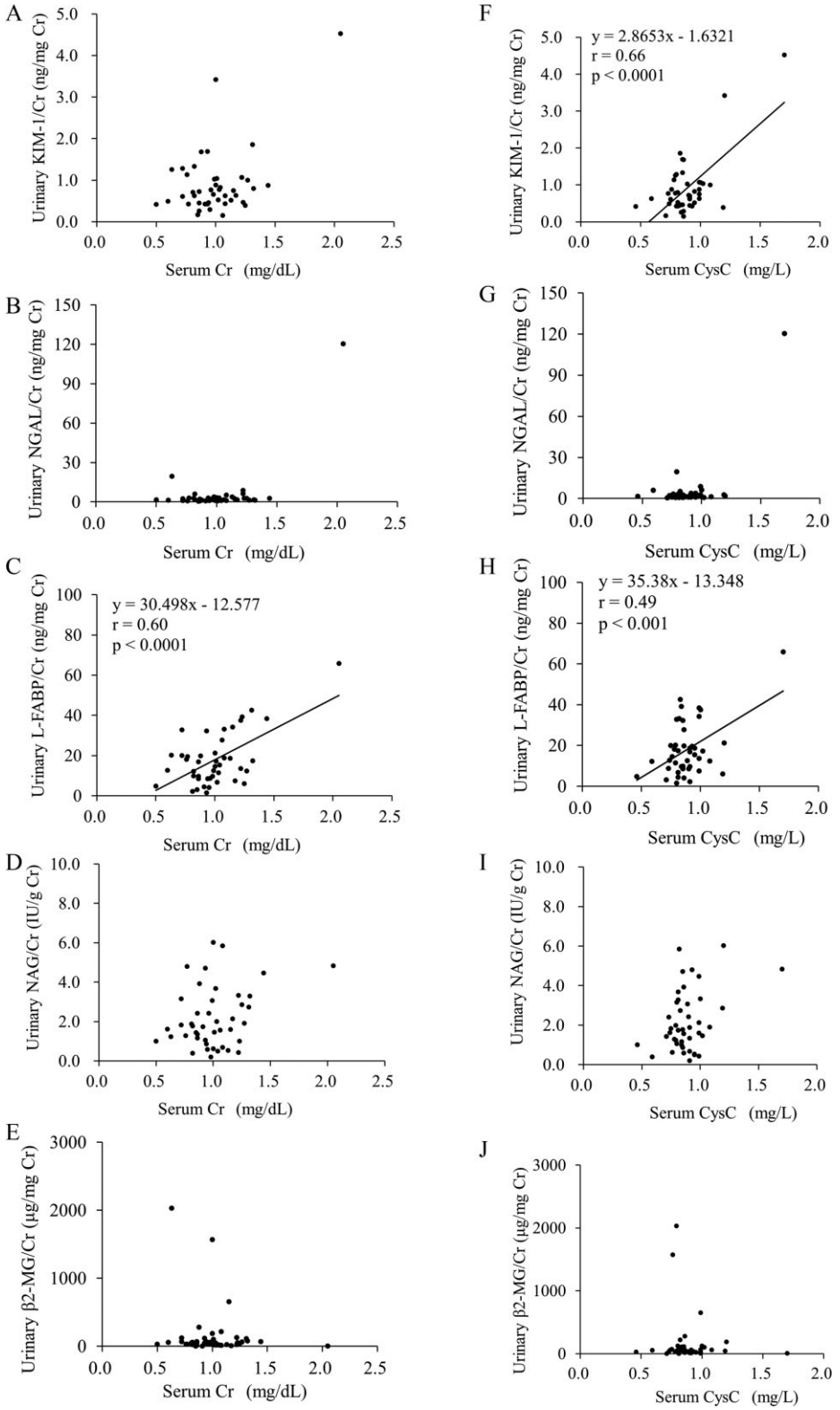


FIGURE 5: Relationships between urinary biomarkers and sCr and sCysC. Urinary L-FABP/Cr was positively and significantly correlated with sCr (Pearson's $r = 0.60$, $P < .0001$). Urinary KIM-1/Cr and L-FABP/Cr correlated significantly with the sCysC level ($r = 0.66$, $P < .0001$ for KIM-1; $r = 0.49$, $P < .001$ for L-FABP; **F**–**J**). Data were analysed using Pearson's correlation coefficient.

high as those reported in previous studies [9, 19]. This was likely because the severity of heat-related illness and kidney injury in our study was mild to moderate. Another reason may have been that the patients in our study were mainly healthy with no comorbidities. A previous study reported that a cut-off value of 486 ng/mg Cr for AKI yielded both a good sensitivity (0.71) and specificity (0.68), although they enrolled patients who underwent cardiac surgery, so some patients might have had a decreased renal blood flow due to cardiac failure before surgery [19]. A new cut-off value for exertional heatstroke and heatstroke-induced AKI in healthy people may be needed. Fourth, we defined kidney injury as sCr $\geq$ 1.2 mg/dL according to the JAAM-HS-WG criteria, as we did not measure the baseline sCr value. However, we confirmed that all patients with an sCr $\geq$1.2 mg/dL had a markedly decreased sCr ($\geq$0.3 mg/dL) during the follow-up period. Therefore they were presumably diagnosed with AKI according to the Kidney Disease: Improving Global Outcomes criteria [44]. Finally, since we enrolled all patients whom physicians diagnosed with heat-related illness, there may have been patients who had other diseases. Furthermore, although a high body temperature (BT) is an important factor in heat-related illness [45], the JAAM heat-related illness criteria did not make mention of BT. One reason for this is because it is not standard to measure the core BT in Japan. In addition, a recent study showed that the BT measured in the hospital was not an independent prognostic factor of mortality or the neurological outcome in patients with heat-related illness [46].

In conclusion, we conducted research on heat-related illness to assess the actual situation of exertional heatstroke onset in a clinical setting. Patients with mild to moderate heat-related illness, who did not seem to need hospitalization, could have kidney injury. L-FABP is useful for detecting severe heatstroke and heatstroke-induced AKI. A simple measurement kit, such as a dipstick for L-FABP, would be useful for monitoring athletes and laborers, including Japanese SDFs, to identify subjects who need to be treated immediately to prevent severe AKI. In addition, although the urinary KIM-1 levels were not elevated in patients with severe heat-related illness or heatstroke-induced AKI, there was a significant positive correlation between the sCysC and urinary KIM-1 levels, suggesting that urinary KIM-1 may detect heatstroke-induced AKI. Large cohort studies are needed to confirm the usefulness of these urinary biomarkers.

SUPPLEMENTARY DATA

Supplementary data are available at *ndt* online.

ACKNOWLEDGEMENTS

We thank the staff of the GSDF Fuji Hospital for their cooperation, Masashi Tsunoda (Department of Preventive Medicine and Public Health, National Defense Medical College) for his kind support and Keiko Komoda, Midori Sano and Mie Shirase for their expert secretarial assistance.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no relevant financial interests.

AUTHORS' CONTRIBUTIONS

H.G. designed and H.G. and M.N. performed the experiments under the supervision of M.K., S.T. and H.K. The manuscript was written by H.G., S.S., T.I., H.N., N.O., M.K., S.T. and H.K. and the final version was approved by all the authors.

FUNDING

This work was supported by grants from the Ministry of Defense, Japan.

DATA AVAILABILITY STATEMENT

The data underlying this article cannot be shared publicly, because this was not planned in the research ethics committee-approved protocol.

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Received: 7.1.2022; Editorial decision: 4.4.2022