

Consistently low levels of histidine-rich glycoprotein as a new prognostic biomarker for sepsis: a multicenter prospective observational study

Short title

Potential of histidine-rich glycoprotein as a novel prognostic biomarker for sepsis

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Abstract

Background

Few sepsis biomarkers accurately predict severity and mortality. Previously, we had reported that first-day histidine-rich glycoprotein (HRG) levels were significantly lower in patients with sepsis and were associated with mortality. Since the time trends of HRG are unknown, this study focused on the time course of HRG in patients with sepsis and evaluated the differences between survivors and non-survivors.

Methods

A multicenter prospective observational study was conducted involving 200 patients with sepsis in 16 Japanese hospitals. Blood samples were collected on days 1, 3, 5, and 7, and 28-day mortality was used for survival analysis. Plasma HRG levels were determined using a modified quantitative sandwich enzyme-linked immunosorbent assay.

Results

First-day HRG levels in non-survivors were significantly lower than those in survivors (mean, 15.7 [95% confidence interval (CI), 13.4–18.1] vs 20.7 [19.5–21.9] $\mu\text{g/mL}$; $P = 0.006$). Although there was no time $\times$ survivors/non-survivors interaction in the time courses of HRG ($P = 0.34$), the main effect of generalized linear mixed models was significant ($P < 0.001$). In

a univariate Cox proportional hazards model with each variable as a time-dependent covariate, higher HRG levels were significantly associated with a lower risk of mortality (hazard ratio, 0.85 [95% CI, 0.78–0.92]; $P < 0.001$). Furthermore, presepsin levels ($P = 0.02$) and Sequential Organ Function Assessment scores ($P < 0.001$) were significantly associated with mortality. Harrell's C-index values for the 28-day mortality effect of HRG, presepsin, procalcitonin, and C-reactive protein were 0.72, 0.70, 0.63, and 0.59, respectively.

Conclusions

HRG levels in non-survivors were consistently lower than those in survivors during the first seven days of sepsis. Repeatedly measured HRG levels were significantly associated with mortality. Furthermore, the predictive power of HRG for mortality may be superior to that of other singular biomarkers, including presepsin, procalcitonin, and C-reactive protein.

Keywords: Histidine-rich glycoprotein; Time course; Sepsis; Biomarker; Prognosis

Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. Globally, approximately 48.9 million patients suffer from sepsis annually, and 11.0 million (19.7%) of all deaths are sepsis-related [2]. Although the sepsis mortality rate has been decreasing every year owing to efforts in early diagnosis and treatment [2–4], sepsis remains a leading cause of death and a major public health concern [2,3]. The clinical course of sepsis is unpredictable, and the current situation has caused serious problems in predicting whether a patient will deteriorate or recover soon. To overcome this issue, better indicators of severity in patients with sepsis are needed. The first-day value and time-dependent changes in the Sequential Organ Function Assessment (SOFA) score are useful prognostic markers [1,5–8], however, the usefulness of presepsin (P-SEP), procalcitonin (PCT), and C-reactive protein (CRP) levels is controversial [7–10].

Histidine-rich glycoprotein (HRG) is a 75 kDa plasma glycoprotein that is mainly produced in the liver [11]; it is present at a concentration of approximately 60–150 $\mu\text{g/mL}$ in healthy individuals [12,13]. HRG binds to a broad range of ligands and is implicated in regulating coagulation, fibrinolysis, and immune response [13–15]. We chose HRG as a possible biomarker for sepsis because we assumed that decreased plasma HRG levels in sepsis would cause the dysregulation of coagulation, fibrinolysis, and immune response, resulting in disseminated intravascular coagulation and multiple organ dysfunction syndrome [14,16–18].

Previously, using a cecal ligation and puncture model, we showed that HRG levels decreased in septic mice and that replenishment of HRG improved their survival rate [18]. In our clinical study, we reported that first-day HRG levels were lower in patients with sepsis than in those without sepsis [12]; moreover, lower HRG levels were associated with an increased risk of septic mortality [19], suggesting that first-day HRG levels might be a biomarker for sepsis [12,16,17,19]. However, owing to a limited sample size and lack of knowledge on how HRG levels change, the prognostic value of HRG in sepsis was not fully evaluated. Herein, for the first time, we aimed to determine the trends of plasma HRG levels in patients with sepsis and evaluate the differences between survivors and non-survivors.

Materials and methods

Study design

A multicenter prospective observational study was conducted with the Institutional Review Board approval of all relevant institutions: the Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences (Ethical Number: 1801-020), Japanese Red Cross Okayama Hospital (R1-33), Okayama saiseikai General Hospital (180201), Onomichi Municipal Hospital (19-11), Kagawa Prefectural Central Hospital (786), National Hospital Organization Fukuyama Medical Center (H29-40), Tsuyama Chuo Hospital (421), Shimane University Hospital (3999), Japanese Red Cross Society Himeji Hospital (2019-30), Kagawa University Hospital (H30-006), Tottori University Hospital (18A014), Fukuyama City Hospital (365), Kawasaki Medical School General Medical Center (3006-2), Japanese Red Cross Kobe Hospital (154), Okayama City Hospital (1-116), and Kawasaki Medical School Hospital (3265). This study was registered with the UMIN Clinical Trials Registry on February 1, 2018 (UMIN000030037; the lead principal investigator is Hiroshi Morimatsu). This study was performed in accordance with the World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. This observational study was based on the Strengthening the Reporting of Observational Studies in Epidemiology guidelines [20].

Patients and data collection

Patients newly diagnosed with sepsis based on the Sepsis-3 definition [1] were prospectively enrolled in the study. SOFA scores were calculated from PaO₂/F_IO₂ ratio, use of mechanical ventilation, platelet count, total bilirubin, blood pressure, used inotropes and vasopressors, Glasgow Coma Scale, creatinine, and urine output [6]. The inclusion criteria included admission to the intensive care unit (ICU) with an increase in the SOFA score by 2 points or more caused by a dysregulated host response to infection. The exclusion criteria were as follows: age less than 20 years, pregnancy, and failure to provide written consent. After obtaining written consent from patients or their relatives, blood samples were collected to be analyzed later for HRG, P-SEP, and PCT levels on days 1 (within 24 h of diagnosis of sepsis), 3, 5, and 7, while the patients stayed in the ICU. Daily clinical and blood sampling data were also recorded using the Research Electronic Data Capture system in each hospital, and the Acute Physiology and Chronic Health Evaluation (APACHE) II score on day 1 [21] and SOFA scores on days 1, 3, 5, and 7 were calculated. The patients were then observed for 90 days through medical records and telephone follow-up in each hospital, and the 28-day mortality values were used for survival analysis.

Measurement methods

Blood samples were collected in tubes containing dipotassium-ethylenediaminetetraacetic acid (EDTA) and processed within 60 min of sampling. Samples were centrifuged at 3,000 rpm for

128 10 min, and the plasma was collected in polypropylene tubes. The samples were immediately
129 frozen at each hospital. Thereafter, the samples were transported from each hospital to Okayama
130 University, and a protease inhibitor cocktail (cOmplete, Mini, EDTA-free; Roche Diagnostics,
131 Basel, Switzerland) was added to the samples according to the manufacturer's instructions prior
132 to their storage at -80°C .

133 Plasma HRG levels were determined using a previously reported in-house modified
134 quantitative sandwich enzyme-linked immunosorbent assay in S3 Fig [12]. Briefly, a rat
135 monoclonal antibody against human HRG (in-house, #75-14) was used as the capture antibody,
136 and a nitrilotriacetate nickel (Ni^{2+})-activated derivative of horseradish peroxidase (HisProbe-
137 HRP Conjugate; Thermo Fisher Scientific, Waltham, MA) diluted by 1000-fold was used for
138 detection. Plasma samples were diluted by 200- and 400-fold with phosphate-buffered saline
139 containing 1% bovine serum albumin. A 96-well plate (Clear Flat-Bottom Immuno Nonsterile
140 96-Well Plates; Thermo Fisher Scientific) and a microplate washer (ImmunoWash 1575
141 Microplate Washer; Bio-Rad Laboratories, Hercules, CA) were used. O-phenylenediamine
142 (SIGMAFAST OPD tablet; Sigma-Aldrich, St. Louis, MO) and stop solutions (3 M H_2SO_4)
143 were used to develop the reaction. The absorbance was measured at 492 nm using a 96-well
144 plate reader (Nivo 5S Multimode Plate Reader; PerkinElmer, Waltham, MA), and a standard
145 curve was generated using serial dilutions of a known amount of purified HRG (manufactured
146 in-house). Plasma samples were measured in duplicate, and HRG levels were determined by

averaging two independent assays. P-SEP and PCT levels were determined using a chemiluminescent enzyme immunoassay (SRL, Tokyo, Japan).

Outcomes

The primary outcome of this study was all-cause mortality within 28 days of the initial sampling. The differences in the time courses of HRG levels between survivors and non-survivors were evaluated. The secondary outcome was the association between each variable (HRG, P-SEP, PCT, CRP, SOFA score, and APACHE II score) and mortality.

Statistical analysis

All the statistical methods were designed a priori, except for the statistical analysis shown in S1 Fig. This subgroup analysis was designed as a post-hoc analysis because it was conducted while exploring the data.

Continuous variables are presented as the median (interquartile range [IQR], 25th to 75th percentiles) or the mean (95% confidence interval [CI]), and differences between survivors and non-survivors were analyzed using the Mann–Whitney U test. Categorical variables are presented as proportions, and differences between survivors and non-survivors were analyzed using Fisher’s exact test. The time-dependent changes in each variable in survivors and non-survivors were compared using generalized linear mixed models (GLMMs) for repeated

measures. The Cox proportional hazards model and Kaplan–Meier method were used for survival analysis. The ability of each variable to predict 28-day mortality was evaluated using the Cox proportional hazards model with each variable as a time-dependent covariate and then adjusted for the APACHE II score to correct for the severity of disease. The hazard ratios (HRs) and 95% CIs were estimated. In addition, patients with sepsis were stratified according to background factors to evaluate the association between HRG levels and 28-day mortality. After dividing patients with sepsis into two groups according to the cutoff value, which was derived from receiver operating characteristic analysis in logistic regression and determined using the Youden index method, cumulative survival rates were estimated using the Kaplan–Meier method and tested using the log-rank test. The significance level of testing was set at 0.05 (two-sided p value). IBM SPSS Statistics (version 25.0; International Business Machines Corporation, Armonk, NY) was used to analyze the GLMM, Stata 17.0 (StataCorp LLC, College Station, TX) was used to analyze the Cox proportional hazards model with time-dependent covariates, and JMP Pro 14.0.0 (SAS Institute Inc., Cary, NC) was used for the other analyses.

Results

Patient characteristics

Patients were registered from August 2018 to September 2019 in 16 Japanese hospitals: 5 university and 11 general hospitals. During the study, 11,511 patients were admitted to the ICU and 502 patients (4.4%) were diagnosed with sepsis. Written informed consent was obtained from 201 patients, and one patient who failed to follow-up was excluded. Finally, 200 patients were analyzed in this study (Fig 1). Patient characteristics are shown in Table 1 and S1 Table. The median first-day APACHE II and SOFA scores were 25 (IQR, 20–31) and 10 (IQR, 7–12), respectively, and 132 patients (66.0%) were diagnosed with septic shock. The 28- and 90-day mortality rates were 11.5% (23 patients) and 19.0% (38 patients), respectively. The main causes of 28-day mortality were multiple organ dysfunction syndrome associated with exacerbation of the primary disease (18 cases), fatal arrhythmia (two cases), and others (three cases).

Fig 1. Patient flow chart.

Table 1. Patient characteristics in survivors and non-survivors.

Variables	Total (<i>n</i> = 200)	Survivors (<i>n</i> = 177)	Non-survivors (<i>n</i> = 23)	<i>P</i> value
Age, median (IQR), years	75 (67–83)	75 (67–83)	75 (67–86)	0.73
Sex, male	110 (55.0%)	94 (53.1%)	16 (69.6%)	0.18
Height, median (IQR), cm	160 (150–165)	160 (150–166)	158 (150–164)	0.74
Weight, median (IQR), kg	55 (46–65)	55 (47–65)	49 (46–59)	0.13

Surgical admission	61 (30.5%)	57 (32.2%)	4(17.4%)	0.23
APACHE II score*, median (IQR)	25 (20–31)	25 (19–30)	35 (24–41)	< 0.001
SOFA score*, median (IQR)	10 (7–12)	10 (7–12)	13 (11–16)	< 0.001
Septic shock	132 (66.0%)	112 (63.3%)	20 (87.0%)	0.03
Inotropes/vasopressors	160 (80.0%)	139 (78.5%)	21 (91.3%)	0.18
Acute kidney injury	109 (54.5%)	93 (52.5%)	16 (69.6%)	0.18
Renal replacement therapy	33 (16.5%)	24 (13.6%)	9 (39.1%)	0.005
Mechanical ventilation	99 (49.5%)	83 (46.9%)	16 (69.6%)	0.047
Source of infection				0.10
Lung	51 (25.5%)	39 (22.0%)	12 (52.2%)	
Urinary tract	38 (19.0%)	36 (20.3%)	2 (8.7%)	
Gastrointestinal tract	36 (18.0%)	33 (18.6%)	3 (13.0%)	
Hepatobiliary tract	26 (13.0%)	25 (14.1%)	1 (4.3%)	
Bone and soft tissue	20 (10.0%)	18 (10.2%)	2 (8.7%)	
Others	29 (14.5%)	26 (14.7%)	3 (13.0%)	

* On day 1

Time course of plasma HRG levels and other markers

S2 Table shows mean HRG levels on days 1, 3, 5, 7, and last observation till day 7 carried forward (LOCF) in survivors and non-survivors. HRG level in non-survivors was significantly lower than that in survivors on each day ($P = 0.006, 0.001, 0.004, 0.02$, and < 0.001 , respectively).

Fig 2 shows the comparative time courses of HRG in survivors and non-survivors obtained

using GLMMs. Although the HRG was unchanged at low levels up to 7 days in non-survivors and recovered from day 5 in survivors, there was no time $\times$ survivors/non-survivors interaction in their time-dependent changes ($P = 0.34$). However, the main effect of GLMMs was significant ($P < 0.001$), and HRG levels in non-survivors remained consistently lower than those in survivors.

Fig 2. Comparative time courses of plasma HRG levels in survivors and non-survivors.

GLMMs were used to compare the time-dependent changes in plasma HRG levels between survivors ($n = 177$) and non-survivors ($n = 23$) among patients with sepsis. There was no time $\times$ survivors/non-survivors interaction in the time-dependent changes in HRG ($P = 0.34$). However, the main effect of GLMMs was significant ($P < 0.001$), and HRG levels in non-survivors remained consistently lower than those in survivors. Contrast test in GLMMs; $*P = 0.004$, $***P < 0.001$.

Fig 3 shows differences in time courses of P-SEP, PCT, CRP, and SOFA scores between survivors and non-survivors using GLMMs. There was a significant time $\times$ survivors/non-survivors interaction in the time-dependent changes in P-SEP ($P < 0.001$), with an increase in non-survivors and a decrease in survivors up to seven days; the main effect of GLMMs was also significant ($P = 0.02$). There was no significant time $\times$ survivors/non-survivors interaction

in the time-dependent changes in PCT ($P = 0.23$), and the main effect of GLMMs was not significant ($P = 0.14$). There was a significant time $\times$ survivors/non-survivors interaction in the time-dependent changes in CRP ($P = 0.01$), but the main effect of GLMMs was not significant ($P = 0.08$). There was a significant time $\times$ survivors/non-survivors interaction in the time-dependent changes in SOFA scores ($P < 0.001$), which remained unchanged in non-survivors and decreased in survivors over time. The main effect of GLMMs was also significant ($P < 0.001$), and SOFA scores in non-survivors remained consistently higher than those in survivors.

Fig 3. Comparative time courses of each variable in survivors and non-survivors.

GLMMs were used to compare the time-dependent changes in each variable, including (A) P-SEP, (B) PCT, and (C) CRP levels, as well as (D) SOFA scores, between survivors ($n = 177$) and non-survivors ($n = 23$) among patients with sepsis. (A) There was a significant time $\times$ survivors/non-survivors interaction in the time-dependent changes in P-SEP ($P < 0.001$). The main effect of GLMMs was significant ($P = 0.02$), and P-SEP levels in non-survivors were higher than those in survivors. (B) There was no significant time $\times$ survivors/non-survivors interaction in the time-dependent changes in PCT ($P = 0.23$). The main effect of GLMMs was not significant ($P = 0.14$), and PCT levels in survivors and non-survivors were not different. (C) There was a significant time $\times$ survivors/non-survivors interaction in the time-dependent changes in CRP ($P = 0.01$). However, the main effect of GLMMs was not significant ($P = 0.08$),

and CRP levels in survivors and non-survivors were not different. (D) There was a significant time $\times$ survivors/non-survivors interaction in the time-dependent changes in SOFA scores ($P < 0.001$). The main effect of GLMMs was significant ($P < 0.001$), and SOFA scores in non-survivors remained consistently higher than those in survivors.

Association between markers and mortality

Table 2 shows the association between each variable and the 28-day mortality. In the univariate Cox proportional hazards model with each variable as a time-dependent covariate, higher HRG levels were significantly associated with a lower risk of mortality (HR, 0.85 [95% CI, 0.78–0.92]; $P < 0.001$). The Harrell’s C-index value of HRG for mortality was 0.72. P-SEP (HR, 1.01 [95% CI, 1.002–1.02]; $P = 0.02$) and SOFA scores (HR, 1.34 [95% CI, 1.24–1.45]; $P < 0.001$) were also significantly associated with a risk of mortality. However, PCT and CRP were not associated with mortality ($P = 0.20$ and 0.34 , respectively). After adjusting for the first-day APACHE II score, HRG levels (adjusted HR, 0.88 [95% CI, 0.81–0.96]; $P = 0.003$) and SOFA scores (adjusted HR, 1.34 [95% CI, 1.19–1.51]; $P < 0.001$) remained significant prognostic factors.

Table 2. Association between each variable and 28-day mortality.

	Univariate analysis	Adjusted for APACHE II score*
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Variables	HR (95% CI)	<i>P</i> value	Harrell's C-Index	Adjusted HR (95% CI)	<i>P</i> value
HRG	0.85 (0.78–0.92)	< 0.001	0.72	0.88 (0.81–0.96)	0.003
P-SEP (/100)	1.01 (1.002–1.02)	0.02	0.70	1.00 (0.99–1.02)	0.58
PCT	1.01 (0.99–1.03)	0.20	0.63	1.00 (0.99–1.02)	0.74
CRP	1.02 (0.98–1.07)	0.34	0.59	1.03 (0.99–1.08)	0.13
SOFA score	1.34 (1.24–1.45)	< 0.001	0.87	1.34 (1.19–1.51)	< 0.001
APACHE II score*	1.12 (1.07–1.17)	< 0.001	0.74		
Lactate*	1.15 (1.06–1.25)	0.001	0.71		

* On day 1

The Cox proportional hazards model with each variable as a time-dependent covariate was used to evaluate associations between each variable and 28-day mortality. APACHE II score and lactate were only used for first-day values. Adjusted HR denotes the HR adjusted for first-day APACHE II score.

The sensitivity, specificity, and positive and negative predictive values of the first-day HRG levels associated with mortality at the cutoff level of 14.03 µg/mL were 0.48, 0.83, 0.27, and 0.92, respectively. When patients with sepsis were divided into high- and low-HRG groups according to this cutoff level, the Kaplan–Meier survival curves (S2 Fig) showed that mortality in the low-HRG group was significantly higher than that in the high-HRG group (log-rank test, $P < 0.001$).

Subgroup analysis

274 S1 Fig shows the association between HRG levels and 28-day mortality in subgroups stratified
275 by the following background factors: APACHE II score, septic shock, creatinine, bilirubin, and
276 lung infection. In the univariate Cox proportional hazards model with time-dependent
277 covariates, higher HRG levels were significantly associated with a lower risk of mortality in all
278 subgroups, except in the group with bilirubin levels of 2 mg/dL or higher.

279

Discussion

We demonstrated that first-day HRG levels in non-survivor patients with sepsis were significantly lower than those in survivors and remained consistently lower during the first seven days in the ICU. Repeatedly measured HRG levels were significantly associated with mortality in the acute phase of sepsis. These characteristics of HRG were confirmed in patients with varying severity, organ damage, and source of infection.

Previously, we revealed that plasma HRG levels were markedly decreased due to a rapid reduction in HRG mRNA expression in the liver, deposition of HRG on immunothrombi, and degradation of HRG by thrombin in a sepsis state [18]. And we reported that first-day HRG levels in patients with sepsis ($n = 20$) were significantly lower than those in patients with non-infective systemic inflammatory response syndrome ($n = 50$) in a single-center [12]; HRG levels in non-survivors ($n = 16$) were significantly lower than those in survivors ($n = 83$) among patients with sepsis in 11 Japanese hospitals [19]. Consistent with our previous studies, first-day HRG levels in non-survivors were significantly lower than those in survivors in this study. Regarding the time-dependent change in HRG, survivors showed recovery from day 5, and HRG levels on days 5 and 7 were significantly higher than those on day 1. In contrast, HRG levels in non-survivors remained low and unchanged. HRG levels in non-survivors were consistently lower than those in survivors during the observational period. These characteristics were similar to those observed in the SOFA scores. Regarding other markers, first-day P-SEP

levels were similar between survivors and non-survivors, but there was a significant difference in its time-dependent changes, consistent with previous reports [8]. In the present study, PCT and CRP levels decreased similarly over time in both survivors and non-survivors as in previous studies [8,9]. We showed that the repeatedly measured HRG, P-SEP, and SOFA scores, and not PCT and CRP, were significantly associated with mortality in the acute phase of sepsis. Although several studies have reported the usefulness of P-SEP as a prognostic biomarker for sepsis, similar to the present study, it is difficult to predict the prognosis of sepsis based on PCT and CRP levels [7–9]. SOFA and APACHE II scores require the calculation of multiple items; these scores are well-established prognostic indicators for critically ill patients in the ICU, especially patients with severe sepsis [1,5,6]. The ability of HRG to predict mortality was inferior to that of the SOFA score but superior to that of other single biomarkers, including P-SEP, PCT, and CRP. To confirm the reliability of HRG as a prognostic biomarker, we performed additional analyses, such as APACHE II-adjusted and subgroup analyses, which demonstrated that HRG levels were efficient prognostic biomarkers in the acute phase of sepsis.

We demonstrated that HRG levels in non-survivor patients with sepsis were always different from those in survivors throughout the first 7 days in the ICU. This trend was significant only for HRG levels and SOFA scores. The initial levels of these two markers were different in survivors and non-survivors; they improved in survivors but not in non-survivors. These findings are important for assessing patient responses to treatment in clinical settings. Levels

of other biomarkers, including P-SEP, PCT, and CRP, showed a little difference between survivors and non-survivors on day 1, followed by a gradual increase in differences between the groups. Moreover, in a recent proteome study on patients with severe coronavirus disease 2019, the initial HRG level was one of the most useful prognostic factors, and HRG levels in non-survivors were consistently lower than those in survivors at multiple subsequent measurements [22]. These results indicate that, in addition to the markers used clinically, HRG provides more reliable information about the clinical course in the acute phase of sepsis and would enable us to discriminate more severely patients who truly need multidisciplinary treatment. Furthermore, in our previous animal study, we reported that replenishment of HRG in septic mice with reduced HRG levels improved their survival rate [18], which might also provide a novel therapeutic strategy for supplementation of HRG in the future treatment of sepsis.

This study has some limitations. First, we enrolled 200 patients with sepsis from 16 hospitals, and the 28-day mortality, median first-day APACHE II score, and SOFA score were 11.5%, 25, and 10, respectively. The mortality rate was lower than expected. In Japan, in severe sepsis and septic shock based on the Sepsis-2 definition [23], the 28-day mortality, median APACHE II score, and SOFA score were reported to be 18.9%, 21–25, and 8–9, respectively, in 59 ICUs [24], and 18.0%, 24–28, and 9–13, respectively, in 11 ICUs [19]. However, we believe that our patient group was appropriate, considering the severity of the disease based on the APACHE II

337 and SOFA scores. To confirm the capability of HRG as a prognostic biomarker for sepsis, it is
338 advisable to conduct a larger study. Second, we collected limited data after ICU discharge and,
339 therefore, lack data on the recovery period for survivors who were discharged early and data on
340 non-survivors who died early. To compensate for missing data, we compared the time-
341 dependent trends between survivors and non-survivors using GLMMs for repeated measures.
342 The data reported in this study may have missed higher HRG levels in recovered survivors and
343 lower HRG levels in non-survivors; hence, the premise of “missing at random” in GLMMs is
344 unsatisfactory and the mean HRG difference between survivors and non-survivors might be
345 biased. Because it was considered that the missing data might have led to an underestimation
346 of the difference in HRG between survivors and non-survivors in GLMMs, LOCF analysis was
347 performed to verify this issue. It was observed that the data were not underestimated because
348 the mean day 7 HRG difference in GLMMs and LOCF was 8.18 and 7.47 $\mu\text{g/mL}$, respectively.
349 Third, we did not have detailed data on confounding factors that affect HRG levels, such as age,
350 liver function, malignancy status, and steroid use [25–28]. Therefore, we performed additional
351 subgroup analyses to confirm the reliability of the HRG trends as a prognostic biomarker.
352 However, the influence of these factors cannot be completely excluded and hence might have
353 affected our data. Fourth, we revealed that HRG levels in non-survivor patients with sepsis were
354 consistently lower than those in survivors. However, the mechanism of time trends in HRG
355 after treatment of sepsis is not clear, therefore, further studies on HRG would be needed.

356

357 **Conclusions**

358 HRG levels in non-survivor patients with sepsis were significantly lower than those in survivors
359 on day 1 and remained consistently lower than those in survivors during the first 7 days in the
360 ICU. Repeatedly measured HRG was significantly associated with mortality. We suggest that
361 HRG is a useful diagnostic and prognostic biomarker in the acute phase of sepsis. However,
362 large validation studies are needed to confirm our findings.

363

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383

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Supporting Information

S1 Table. Patient characteristics in survivors and non-survivors.

S2 Table. Plasma HRG levels on days 1, 3, 5, 7, and LOCF in survivors and non-survivors.

S1 Fig. Association between plasma HRG levels and mortality in subgroups. The Cox proportional hazards model with time-dependent covariates was used to evaluate associations between HRG levels and 28-day mortality. Higher HRG levels were significantly associated with a lower risk of mortality in all subgroups, except in the group with bilirubin levels ≥ 2 mg/dL.

S2 Fig. Kaplan–Meier survival curves. Patients with sepsis were divided into high- and low-HRG groups according to the cutoff level of 14.04 $\mu\text{g/mL}$. The sensitivity and specificity

488 of first-day HRG levels associated with mortality were 0.48 and 0.83, respectively, at this
489 cutoff level.

490 **S3 Fig. Schematic diagram of enzyme-linked immunosorbent assay.**

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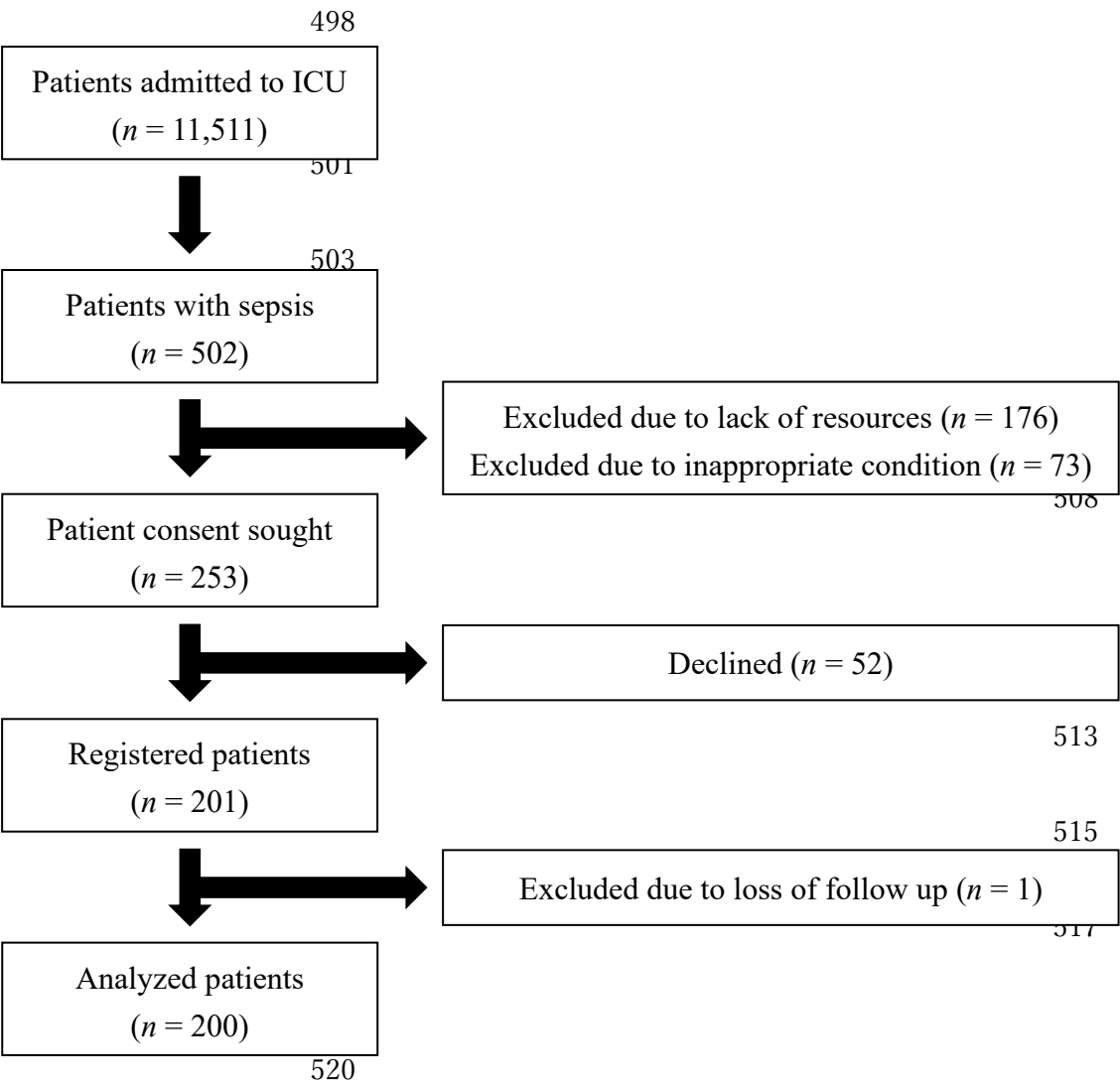
493 **Funding and disclosures**

494 This work was supported by grants from the Japan Agency for Medical Research and

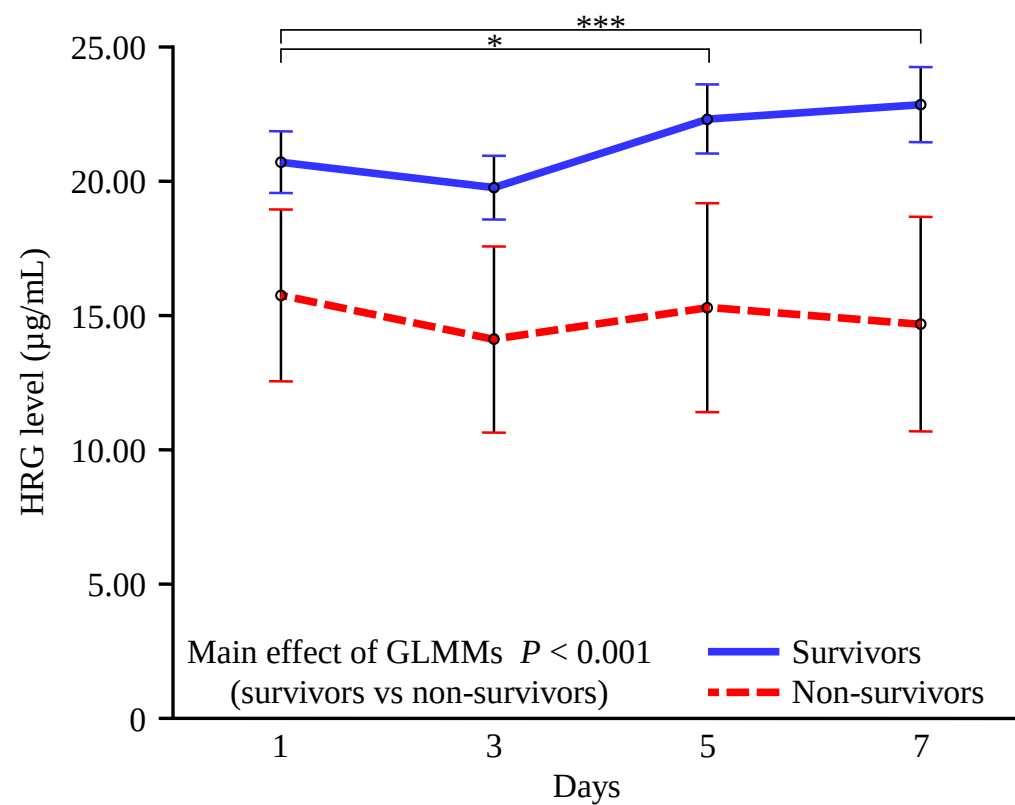
495 Development (AMED): AMED JP19im0210109 to MN and AMED JP22lk0201085 to HM.

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Figure 1



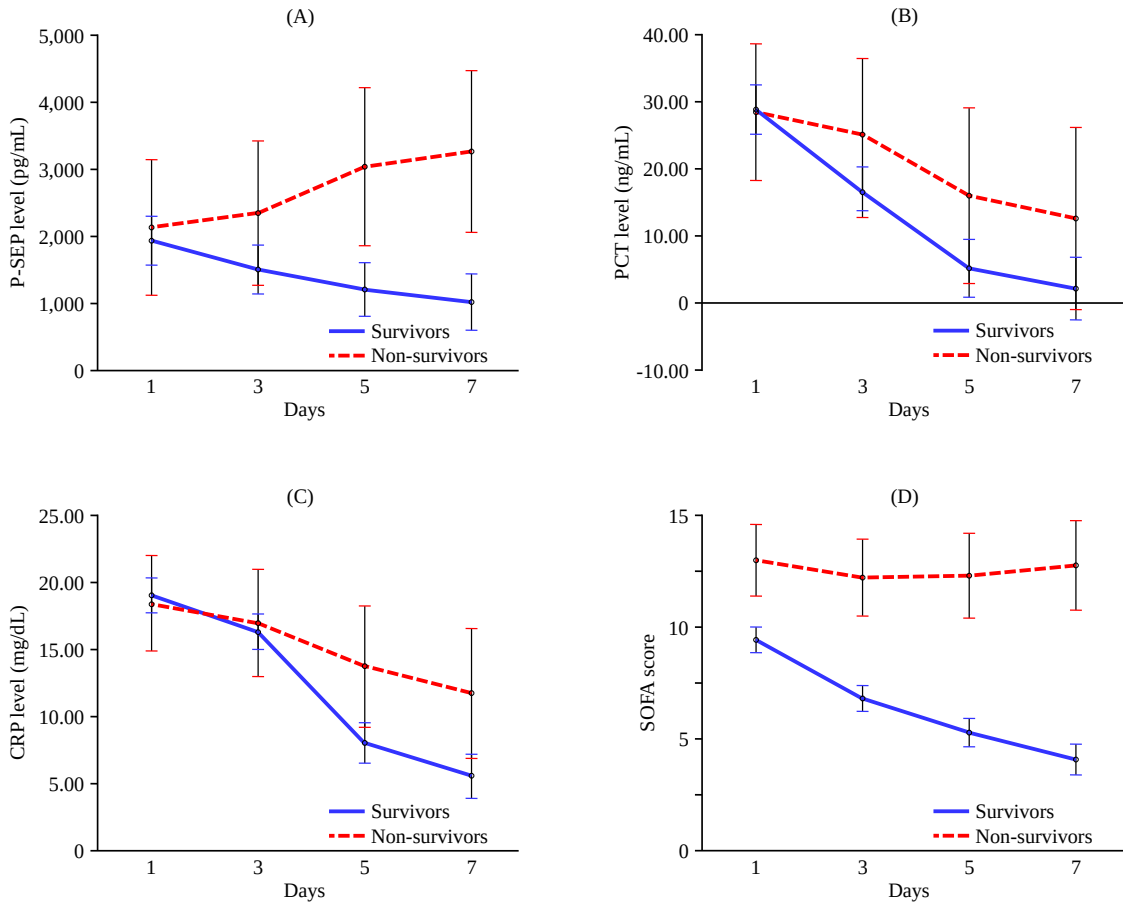
522 **Figure 2**



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Figure 3



Supplement

Consistently low levels of histidine-rich glycoprotein as a new prognostic biomarker for sepsis: a multicenter prospective observational study

Supplemental Table 1	Patient characteristics	page 2
Supplemental Table 2	Plasma HRG levels on each day	page 3
Supplemental Figure 1	Association between HRG and mortality in subgroups	page 4
Supplemental Figure 2	Kaplan–Meier survival curves	page 5

Supplemental Table 1. Patient characteristics in survivors and non-survivors (first-day blood sampling data)
[median (IQR)]

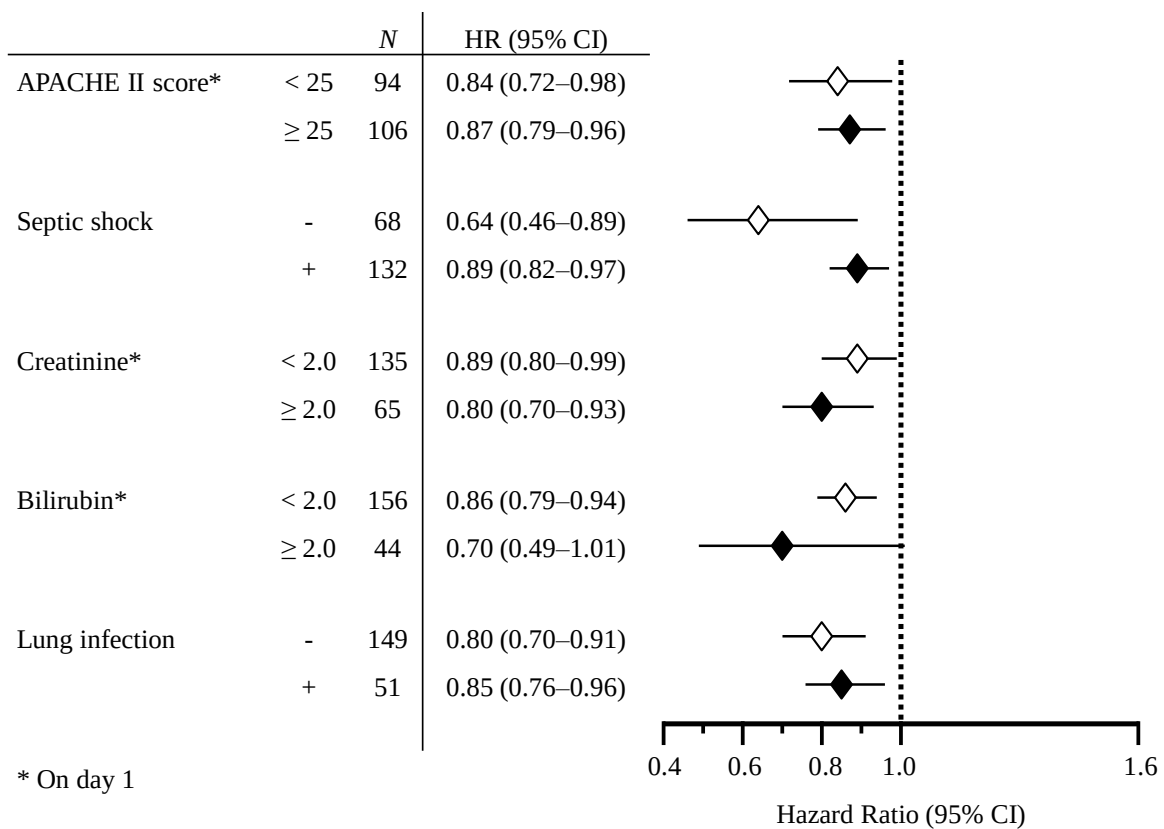
Variables	Total (<i>n</i> = 200)	Survivors (<i>n</i> = 177)	Non-survivors (<i>n</i> = 23)	<i>P</i> value
HRG (μg/mL)	18.7 (14.8–23.7)	18.9 (15.0–24.5)	15.0 (11.2–19.9)	0.006
P-SEP (pg/mL)	1,065 (532–2,143)	992 (507–1,950)	1,570 (767–3,010)	0.03
PCT (ng/mL)	14.3 (3.4–49.8)	14.3 (3.2–49.5)	14.1 (3.4–58.1)	0.89
WBC (/L)	15,150 (9,473–21,630)	15,300 (9,600–21,450)	12,100 (5,000–31,100)	0.92
Ht (%)	28.5 (24.2–33.9)	28.8 (24.5–34.1)	25.4 (21.5–30.7)	0.06
Plt (*10000/L)	12.6 (6.8–19.2)	12.8 (7.4–19.2)	7.0 (3.9–19.9)	0.19
CRP (mg/dL)	18.0 (12.3–25.9)	18.1 (12.4–25.7)	16.2 (11.6–28.5)	0.64
T.Bil (mg/dL)	1.0 (0.7–1.7)	1.0 (0.7–1.8)	0.8 (0.5–1.5)	0.19
AST (U/L)	43 (24–103)	42 (23–103)	60 (28–143)	0.23
Alb (g/dL)	2.4 (1.9–2.7)	2.4 (2.0–2.8)	1.9 (1.7–2.6)	0.04
BUN (mg/dL)	33.5 (21.4–52.6)	33.0 (20.6–50.6)	48.0 (24.0–55.0)	0.10
Cr (mg/dL)	1.5 (1.0–2.4)	1.4 (1.0–2.2)	2.1 (1.0–3.0)	0.14
PT-INR	1.3 (1.2–1.6)	1.3 (1.1–1.5)	1.5 (1.3–2.0)	0.002
APTT (sec)	40.6 (34.0–50.9)	40.3 (33.9–49.6)	43.8 (36.7–60.7)	0.16
Lac (mmol/L)	2.6 (1.8–5.2)	2.6 (1.8–4.4)	5.6 (2.6–8.8)	< 0.001

Abbreviations: IQR, interquartile range; Alb, albumin; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; CRP, C-reactive protein; HRG, histidine-rich glycoprotein; Ht, hematocrit; Lac, lactate; PCT, procalcitonin; Plt, platelet; P-SEP, presepsin; PT-INR, international normalized ratio of prothrombin time; T.Bil, total bilirubin; WBC, white blood cell.

Supplemental Table 2. Plasma HRG levels on days 1, 3, 5, 7, and LOCF in survivors and non-survivors [mean (95% CI)]

Time	Survivors (<i>n</i> = 177)	Non-survivors (<i>n</i> = 23)	<i>P</i> value
Day 1 (µg/mL)	20.7 (19.5–21.9)	15.7 (13.4–18.1)	0.006
Day 3 (µg/mL)	19.6 (18.5–20.7)	14.3 (10.9–17.7)	0.001
Day 5 (µg/mL)	20.9 (19.5–22.3)	15.9 (9.9–21.8)	0.004
Day 7 (µg/mL)	21.4 (19.8–22.9)	15.7 (11.1–20.2)	0.02
LOCF (µg/mL)	22.2 (21.0–23.4)	14.7 (12.1–17.3)	< 0.001

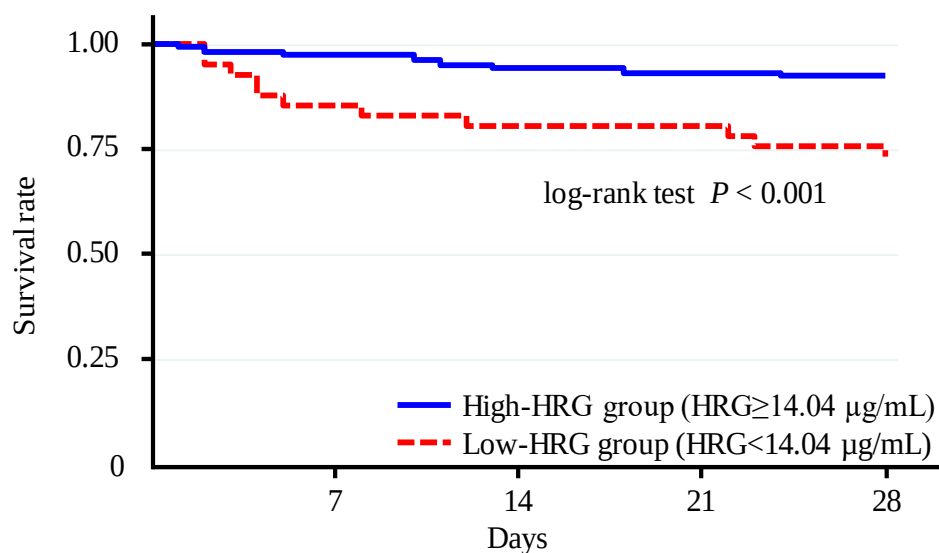
Abbreviations: CI, confidence interval; HRG, histidine-rich glycoprotein; LOCF, last observation till day seven carried forward.



Supplemental Figure 1. Association between plasma HRG levels and mortality in subgroups.

We used the Cox proportional hazards model with time-dependent covariates to evaluate associations between HRG levels and 28-day mortality. Higher HRG levels were significantly associated with a lower risk of mortality in all subgroups, except in the group with bilirubin levels ≥ 2 mg/dL.

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; CI, confidence interval; HR, hazard ratio; HRG, histidine-rich glycoprotein.



Number at risk

Group	Day 0	Day 7	Day 14	Day 21	Day 28
High-HRG group	159	155	150	148	147
Low-HRG group	41	35	33	33	30

Supplemental Figure 2. Kaplan–Meier survival curves.

Patients with sepsis were divided into high- and low-HRG groups according to the cutoff level of 14.04 $\mu\text{g/mL}$.

The sensitivity and specificity of first-day HRG levels associated with mortality were 0.48 and 0.83, respectively, at this cutoff level.

Abbreviation: HRG, histidine-rich glycoprotein.

Patient	Outcome	Study time	HRG				P-SEP				PCT				CRP				SOFA			
			Day1	Day3	Day5	Day7	Day1	Day3	Day5	Day7	Day1	Day3	Day5	Day7	Day1	Day3	Day5	Day7	Day1	Day3	Day5	Day7
1	survival	28	37.14	25.73			942	233			5.97	1.69			10.59	15.79			5	2		
2	survival	28	13.65	15.50	23.04	26.21	649	1050	525	353	5.03	4.86	0.85	0.32	8.75	7.89	4.24	2.05	12	11	9	6
3	death	23	14.03	14.49	11.90	12.62	2370	2740	2180	2280	8.45	3.26	2.03	1.21	13.07	18.97	8.49	7.56	12	16	14	16
4	survival	28	42.51	25.30			1790	1140			60.2	36.7			31.27	10.33			10	3		
5	death	12	12.81	14.46			5470	5640			1.73	0.97			17.38	6.07			7	6		
6	survival	28	9.14				2810				7.64				9.71				11			
7	survival	28	27.27	16.23	12.29	14.80	7860	6890	5660	6600	100	60.7	23.1	18.2	12.46	25.1	8.5	5.17	16	22	19	14
8	survival	28	14.94	20.63	17.65		406	175	179		1.29	0.34	0.12		18.38	5.34	1.68		10	7	4	
9	survival	28	22.81				423				6.17				24.94				2			
10	survival	28	17.13	19.30	23.99	22.37	710	1450	415	311	2.22	1.37	0.69	0.6	24.31	9.22	4.83	2.34	7	8	6	3
11	survival	28	22.22	13.98	13.94	20.55	2530	2880	561	570	100	29.1	7.68	2.3	15.41	40.6	11.31	5.28	12	11	10	7
12	survival	28	11.64	10.49	14.40	18.36	4080	2600	3560	4830	2.01	1.15	0.86	85.6	0.77	4.55	5.24	3.93	12	7	7	12
13	survival	28	18.86	25.42	21.49	32.12	1530	730	368	256	67.7	11	2.85	0.8	13.96	7.51	2.11	0.93	6	6	2	1
14	survival	28	18.47	19.94	25.70	20.29	428	204	253	266	0.21	0.07	0.05	0.07	12.35	4.87	3.09	2.37	6	4	3	4
15	survival	28	25.69	30.09			1060	557			21.9	6.03			27.07	15.63			11	4		
16	survival	28	15.07	10.06	9.25	13.26	1950	1320	1370	2280	58.3	27	8.57	2.08	14.54	22.35	6.47	5.53	12	10	11	11
17	survival	28	29.77	29.36	22.02	20.16	16000	10300	8270	7340	1.84	2.04	0.7	0.53	8.12	9.39	5.77	3.96	12	10	10	10
18	survival	28	11.78	12.36	11.58	10.77	922	1230	742	876	10.8	6.6	1.06	0.67	23.7	9.95	3.92	5.31	15	12	8	7
19	survival	28	14.32	13.82	18.26	28.83	2390	717	1470	848	72.5	78.3	38.7	10.7	26.7	9.38	10.39	5.8	9	12	6	3
20	survival	28	21.60	20.60	28.06	24.25	715	296	215	213	34.6	5.44	3.01	1.03	16.58	8.58	3.49	1.36	10	6	2	3
21	survival	28	15.00	17.36	14.36	22.61	4160	2390	1380	539	3.86	1.35	0.7	0.3	19.9	11.21	2.62	0.97	3	4	4	3
22	survival	28	24.61	21.59	32.44	27.54	223	407	628	860	0.4	0.24	0.11	0.09	11.41	5.21	8.14	8.56	8	7	5	5
23	survival	28	28.41	24.09	13.33	16.85	934	1120	2220	2420	4.14	2.7	4.43	2.1	28.23	10.99	21.55	13.22	12	7	14	14
24	survival	28	18.28	15.95	13.31	12.78	1320	4150	9410	7390	0.6	0.82	0.88	0.61	11.77	8.98	10.68	6.79	6	6	8	9
25	survival	28	20.53	17.75			542	327			54.4	13			25.56	10.49			9	5		

26	death	13	23.51	14.31	13.40	14.40	628	720	739	713	0.29	0.1	0.09	0.08	0.39	0.71	0.25	0.2	10	10	7	7
27	survival	28	21.41	21.60	19.54		2980	2030	1290		0.27	0.24	0.21		10.97	7.47	7.2		3	3	3	
28	survival	28	18.92	19.90	20.53	16.51	2360	2470	1990	1740	1.88	1.58	0.4	0.28	16.09	5.85	2.87	1.28	9	13	11	11
29	survival	28	30.20				1260				50				30.46				6			
30	survival	28	13.27	12.84	11.13	11.85	1230	841	1230	1400	10.7	4	7.31	12.4	13.45	9.37	4.84	7.41	13	13	12	9
31	survival	28	29.43	25.35			2120	1820			100	27.6			22.02	8.89	2.36		9	10	6	
32	survival	28	18.54	18.32	19.68	19.77	1230	672	842	1670	1.04	0.24	0.19	0.22	8.1	5.59	3.83	4.13	10	1	0	0
33	survival	28	9.78				2500				14.9				29.48				9			
34	survival	28	19.06	14.48			1350	1680			48.5	17.3			22.32	29.47			8	2		
35	survival	28	13.60	14.17	15.31	10.75	884	722	862	1410	7.74	2.59	0.66	19.3	37	11.88	5.33	19.06	12	5	5	7
36	survival	28	14.76	12.42	12.88	11.45	549	741	725	692	7.68	5.49	3.79	1.71	17.77	17.18	13.1	13.91	10	5	5	3
37	survival	28	10.33	6.79	7.77	13.32	1560	1760	2200	2260	6.61	11.7	4.47	1.53	13.22	17.32	4.94	8.77	15	18	20	15
38	survival	28	22.92	23.61	15.20		259	335	273		19.7	5.44	1.61		16.78	10.61	2.27		10	7	5	
39	survival	28	18.42	20.32			272	500			3.83	1.13			29.13	23.48			12	3		
40	survival	28	15.82	19.88	18.71	16.83	2400	1170	652	624	64.9	43.2	12	2.97	27.13	9.7	3.5	3.46	13	5	5	5
41	survival	28	32.11	23.51			298	231			0.5	0.15			1.3	0.6			6	0		
42	survival	28	18.40	13.01			1140	717			20.1	10.3			34.15	23.3			7	3		
43	survival	28	20.84	13.78			1680	772			100	59.8			9.57	22.65			11	5		
44	survival	28	24.44	25.65	22.81	31.24	776	771	531	420	0.06	0.09	0.06	0.15	3.44	4.26	0.71	0.08	7	4	3	3
45	survival	28	16.08	13.48	23.66	19.60	571	526	231	245	5.58	1.42	0.25	0.08	2.46	8.43	1.56	0.67	10	7	7	4
46	survival	28	18.51	18.36			305	258			23.9	9.24			12.33	9.04	5.82	5.47	6	3	1	1
47	survival	28	24.58	25.02	33.68	34.26	5110	1350	490	459	16.5	10.3	2.62	1.04	19.29	23.27	5.76	2.38	12	9	5	2
48	survival	28	22.37	13.58	16.78	16.28	1300	1150	843	514	50.9	39	19.3	5.64	15.23	20.87	2.72	1.1	12	8	0	1
49	survival	28	18.27	20.27			531	238			51.7	22.2			23.59	5.24			10	6		
50	survival	28	16.60	10.95	12.74		585	780	331		78.9	29.1	8.69		25.96	35.4	26.95		5	4	3	
51	survival	28	15.13	15.77	16.21	16.44	1950	1050	576	956	67.8	39.9	15.3	4.07	10.67	24.11	13.96	7.84	12	8	7	7
52	survival	28	15.86	24.43			412	374			11.9	4.39			25.6	18.55			5	1		

53	survival	28	20.46	18.88	33.63		352	376	194		0.56	0.38	0.17		18.09	15.13	6.84		8	1	2	
54	survival	28	15.50	14.40	25.17	17.93	965	457	322	328	14.5	6.81	2.78	0.96	6.17	2.86	1.71	0.74	4	3	3	2
55	death	10	14.99	14.18	18.40	22.57	653	415	363	415	2.17	4.09	0.93	0.22	8.64	5.78	3.54	1.87	12	5	5	5
56	survival	28	12.80	12.87			12200	13300			4.73	3.96			8.73	6.17	4.14		9	7	7	
57	survival	28	16.20				324				14.2				24				2			
58	survival	28	11.37	6.95	11.32	21.32	1350	1210	2500	717	28.1	26.5	13.4	3.8	25.24	21.79	8	3.75	15	19	10	7
59	survival	28	7.93	8.88	9.61		14000	12200	8700		0.6	0.57	0.28		14.82	10.7	6.12		13	11	10	
60	survival	28	26.83	25.16	27.44	14.34	1650	554	422	116	100	37.4	10.1	1.48	34.7	11.54	3.28	1.43	11	5	3	1
61	death	18	19.12	11.89	18.09	21.37	1380	742	699	552	5.58	1.55	0.24	0.12	28.49	15.36	10.62	7.61	12	7	5	5
62	survival	28	22.45	14.95	14.17	18.94	372	472	852	690	17.4	50.2	16.8	5.73	12.51	26.69	24.9	20.23	10	9	5	3
63	survival	28	14.43	22.62	26.52	21.36	6880	9610	3840	2020	100	59.9	8.72	2.47	39.16	22.71	9.79	9.36	15	9	7	5
64	survival	28	16.28	17.85			2330	1420			100	77			14.49	4.11			10	6		
65	survival	28	18.17	16.54	20.24	19.86	1340	1730	871	1060	6.29	3.34	0.63	0.24	40.04	27.95	9.63	8.71	6	2	1	1
66	survival	28	17.54	15.10			5440	4990			8.82	16.7			33.36	25.53			12	9		
67	survival	28	10.20	12.01	27.25		1190	566	211		100	78.7	0.93		16.55	8.57	6.25	2.17	13	7	4	1
68	survival	28	14.97	10.75	20.38		1450	1370	1180		41.6	19.1	4.5		15.88	35.34	13.6		12	6	6	
69	survival	28	21.21	18.56	19.76	18.67	409	239	208	190	0.25	0.12	0.06	0.05	20.78	5.51	3.7	1.42	15	5	3	2
70	survival	28	13.84				1470				4.67				11.63	14.76			8	5		
71	survival	28	17.16	17.02	16.55	20.41	521	640	380	363	8.08	3.35	0.9	0.4	21.3	13.49	10.57	9.23	10	5	3	2
72	death	18	16.23	10.43	14.32	16.20	767	1350	2210	3440	13.2	7.72	4.41	3	16.24	9.74	6.28	2.99	13	12	14	9
73	survival	28	20.56	23.39			247	364			1.97	0.89			1.93	6.79			9	5		
74	survival	28	48.33	49.95			256	224			2.72	3.34			5.87	4.68			5	5		
75	survival	28	46.37	41.95			592	286			4.4	1.99			3.98	14.14			7	3		
76	survival	28	12.09	13.88	24.18	21.39	17500	12700	12600	6030	16.9	8.16	4.62	1.78	20.88	13.51	8.04	3.66	19	19	15	10
77	survival	28	19.65	18.06			534	443			20.9	13.8			20.89	19.3			3	1		
78	survival	28	23.78	21.89	23.45	26.51	471	321	232	224	29.9	7.34	2.23	0.9	21.01	17.59	7.8	7.7	9	4	2	0
79	death	10	19.47	11.45			2590	1520			82.9	79.4			18.98	31.58			8	5		

80	death	28	8.82	10.70	12.62		1350	877	669		39.5	9.68	4.24		27.88	17.56	13.04		8	6	5	
81	survival	28	39.96	42.53	40.95	38.67	1380	591	415	200	39.6	18.1	3.98	1.01	30.46	18.28	10.43	2.87	5	1	1	1
82	survival	28	21.72	19.44			3150	912			1.46	0.57			1.37	0.96			13	6		
83	survival	28	25.45	22.42			371	167			48.3	13.2			24.97	14.1			5	4		
84	survival	28	17.93	15.07	15.12	17.80	729	625	717	737	0.68	0.43	0.27	0.12	12.56	12.4	8.21	4.17	9	8	7	3
85	survival	28	23.47	27.27			1460	945			12.6	5.12			13.87	7.38			11	3		
86	survival	28	17.33	29.54			274	185			0.86	0.32			13	10.36			9	6		
87	survival	28	26.54	22.27	26.77	28.52	981	1140	732	491	11	10.4	3.75	1.02	20	23.3	9.75	6.11	12	10	8	6
88	survival	28	20.76	24.49			707	552			5.69	2.66			14.4	19.8			7	6		
89	survival	28	12.09	15.35	24.58		3460	3640	1780		4.27	4.4	1.12		21.3	19.74	7.8		12	12	8	
90	survival	28	23.73	22.69	25.61	17.01	413	249	233	268	0.62	0.57	0.15	0.11	15	9.21	2.83	3	10	9	8	8
91	survival	28	29.92	35.78			306	219			0.53	0.17			4.8	6			10	3		
92	death	11	24.41	25.04	13.54	12.11	1570	1930	3300	3830	19.2	20	13.7	8.29	8.2	23.5	27	21	15	15	19	19
93	survival	28	24.64	17.48	21.49	19.85	810	850	620	349	25.2	19.9	5.94	1.69	26.7	24	15	8	8	7	7	6
94	survival	28	24.08	23.82	16.48	20.44	407	612	646	520	2.36	1.93	0.89	0.38	27.4	29.7	26	24.2	9	9	9	9
95	survival	28	24.57	23.02			409	299			6.5	3.67			21.9	18.7			9	7		
96	survival	28	19.09	18.01	25.29		1210	1070	751		4.01	2.16	0.72		19.4	14	4.61		12	8	3	
97	survival	28	34.72				164				10.1				12.6				3			
98	survival	28	20.01	19.05			636	462			100	79.7			22.7	10.73			10	5		
99	survival	28	24.46	27.84			803	193			18.8	4.72			10.7	16			8	7		
100	survival	28	22.83	17.50	16.83	15.01	422	398	234	211	0.84	2.17	0.62	0.19	18.25	22.67	5.66	2.27	6	7	5	6
101	survival	28	19.97	20.33	24.17	22.03	1650	4060	4450	5960	100	100	100	23.3	25.8	26	9.8	4.31	19	20	18	14
102	survival	28	13.32	13.15	15.26	17.07	2260	998	511	381	15.1	11.6	2.99	1.19	29	32	8.43	5.51	10	8	6	1
103	survival	28	43.48	42.96			1080	578			0.32	0.24			18	8.63			7	4		
104	survival	28	21.46	27.80			842	297			22.6	9.21			4.45	5.25			5	3		
105	survival	28	19.03	23.63			272	194			2.79	1.21			18	14.35			3	2		
106	survival	28	14.04	14.45			2720	2470			21.9	9.86			26.9	16.59			11	10		

107	survival	28	12.22	10.86	14.24	21.49	760	675	324	308	32.6	13.7	3.08	0.7	10.74	11.85	2.66	0.84	12	9	8	4
108	survival	28	13.71				757				0.36				12				4			
109	survival	28	11.91	11.99			1070	724			24.9	8.62			13.27	23.47			10	4		
110	survival	28	15.84	15.41	21.88		500	454	319		12.3	6.93	2.33		30.42	24.7	11		10	8	2	
111	survival	28	17.01	17.80	24.07		1360	770	443		52.7	29.2	14.1		26.7	24.53	7.43		14	11	10	
112	survival	28	37.87				190				0.09				5.61				6			
113	survival	28	28.82	33.32	27.12	28.44	1020	476	409	247	0.61	0.31	0.16	0.08	24.03	23.32	14.88	3.72	11	7	6	5
114	survival	28	26.18	26.15			899	359			28.9	9.66			24.42	10.18			6	3		
115	survival	28	25.99	28.15			99	177			0	0.03			6.21	4.78			3	3		
116	survival	28	19.80	20.03	24.79		508	373	269		0.55	0.37	0.14		8.28	3.72	1.72		7	4	4	
117	death	11	23.62	30.21	41.20	28.02	233	264	242	380	0.36	0.35	0.2	0.21	12.29	18.61	16.91	15.98	11	9	10	10
118	survival	28	45.49				266				0.58				16.47				5			
119	survival	28	23.41	17.30	21.07		353	344	275		15.2	3.82	0.74		8.17	2.77	1.4		7	7	3	
120	survival	28	17.95	13.81	26.57	40.16	403	410	286	184	7.48	2.86	0.89	0.27	4.6	23.9	11.4	4.1	11	11	8	4
121	survival	28	31.99	22.91			695	530			1.6	1.18			0.09	11.24			6	7		
122	survival	28	17.44	14.74			541	419			38.2	19.4			29.9	24.8			11	9		
123	survival	28	20.31				395				1.79				22.6	22.4			9	9		
124	death	24	18.81	12.15	11.10	11.86	3010	6820	12600	11500	25.5	90.6	52.6	20.9	16	17.23	8.3	6.23	17	19	19	20
125	survival	28	33.44	19.92			1030	1010			67.3	23.2			21.86	24.4			8	6		
126	survival	28	31.64	28.03	32.01		266	322	189		4.64	3.29	1.24		19.63	23.1	7.23		10	8	2	
127	survival	28	30.68	13.05	22.11		514	501	381		28.6	24.5	7.82		14.6	29.6	21.4		9	8	3	
128	survival	28	22.27	25.43			258	369			18.1	6.26			15.9	5.71			8	3		
129	survival	28	28.52				991				0.91				40.4				5			
130	survival	28	25.08	18.56	23.73	27.88	502	564	585	478	28.3	40.1	7.02	1.72	22.75	26.5	24.8	12.4	3	5	10	5
131	survival	28	19.19	17.17	18.13		281	261	230		75.9	56.4	14.8		20.3	19.8	8.8		10	5	3	
132	survival	28	32.54	34.11	39.86		350	189	140		1.09	0.31	0.17		3.25	9	2.02		10	8	4	
133	survival	28	13.82	17.88	19.62	18.60	2550	2330	1180	2280	4.22	2.49	0.66	0.28	24.25	22.19	8.75	5.54	10	7	6	5

134	survival	28	11.60	13.65	17.43	19.79	2520	1070	879	525	87.2	46.2	12.9	4.72	17.59	10.37	3.34	1.61	3	4	1	0
135	survival	28	14.08	12.68	13.92	16.92	1350	2420	2180	1920	13.4	7.35	3.3	1.32	3.18	33.77	16.05	14.54	15	9	10	8
136	survival	28	26.14	24.47	28.05	24.34	393	239	208	243	9.61	4.53	1.29	0.38	19.72	7.91	4.58	4.67	5	5	2	1
137	survival	28	14.93	14.68	19.86	22.87	1190	677	486	338	0.94	0.38	0.12	0.06	8.28	17.25	5.08	1.54	13	5	6	5
138	survival	28	18.41	22.26	30.40	34.75	1620	561	575	345	65	72	21.2	6.15	30.61	19.57	9.05	3.39	7	4	2	1
139	survival	28	17.51	15.79	18.00	24.35	3600	1850	770	536	32.5	22	4.2	1.33	31	10.92	5.52	1.28	16	6	5	4
140	death	1	18.91				740				12.7				5.22				16			
141	death	5	21.75	24.71			1510	614			6.42	1.97			30.5	13.93	21.06		12	9	15	
142	survival	28	16.22	16.27	20.46	19.79	9840	14800	11600	13000	92.5	100	61.4	23.4	5.19	11.96	6.31	4.98	11	19	15	7
143	death	2	11.16				1080				62.6				28.65				16			
144	survival	28	23.56	27.98	29.36	38.71	10800	7370	2200	745	100	100	24.7	5.03	17.34	13.55	7.66	5.01	13	10	9	6
145	survival	28	25.15	29.18	44.41	36.72	1220	310	183	163	3.5	0.81	0.19	0.08	17.75	3.27	1.21	0	8	2	1	
146	survival	28	18.22	14.30	14.27		1190	1370	1050		77.5	58.1	19.7		16.99	28.08	25.07	19.42	15	11	6	2
147	survival	28	35.00	24.38			351	294			64.3	36.5			7.11	5.97			6	2		
148	survival	28	27.03				720				20.2				13.65				5			
149	survival	28	18.08	22.70	32.53	32.34	558	517	282	255	1.72	1.69	0.63	0.22	12.46	14.84	7.16	2.26	4	6	5	1
150	death	4	13.59	15.18			1130	2150			97.4	100			16.35	24.89			13	18		
151	survival	28	20.22	42.14			943	525			1.8	3.76			25.97	19.67			2	2		
152	death	2	19.89				277				0.78				5.5				13			
153	survival	28	59.88	40.20	40.62	23.67	930	1130	591	405	0.47	2.43	0.71	0.18	24.45	38.13	0.35	2	8	10	5	2
154	survival	28	8.56	17.00			593	671			19.1	5.18			45.54	35.02			14	8		
155	death	2	21.05				3240				37.1				35				14			
156	survival	28	30.64	27.68			4380	1250			30.6	8.89			10.19	13.83			8	7		
157	survival	28	7.29	6.38	7.11	5.82	2270	3240	3060	3040	100	80.6	33.2	12.5	2.35	14.77	12.11	5.26	16	14	15	14
158	survival	28	22.14	21.33	20.94	26.34	288	243	219	143	8.61	3.35	1.1	0.39	30.31	27.53	5.88	5.75	15	9	2	2
159	death	22	6.72	9.92	10.73	9.77	1590	2710	2150	3490	58.1	40.2	18.8	22.7	37.05	28.14	29.9	31.66	13	14	15	16
160	survival	28	8.66	12.74	14.09	13.64	4780	4920	5670	6060	59.7	46.6	22.1	55.8	30	15.99	10.22	7.59	13	9	9	8

161	survival	28	16.53	17.19			8690	4010			15.1	6.1			18.08	17.05			13	11		
162	survival	28	17.48	16.14	17.43	18.19	1350	1230	720	388	78.2	24.4	4.42	0.88	23.34	14.9	7.44	2.91	9	5	3	2
163	survival	28	21.40	18.67	24.17	19.70	6650	1970	1020	470	68.3	88.1	23.6	6.78	38.84	26.72	3.51	2.49	13	11	8	6
164	death	3	12.36				5310				63.6				12.63				21			
165	death	4	10.96	4.60			2050	2230			3.38	1.19			20.42	5.09			17	18		
166	survival	28	31.38	18.20	35.50	24.50	2150	1810	1650	1490	100	43.5	13.8	5.4	38.88	40.11	24.74	26.08	7	5	2	1
167	survival	28	17.23	19.56	17.88	20.53	1000	401	529	1010	1.4	0.74	0.23	0.37	24.3	11.2	7.61	12.76	4	3	3	3
168	survival	28	17.45	15.58			991	408			28.2	5.32			45.36	31.4			8	5		
169	survival	28	23.39	16.33	17.35	22.89	992	484	424	415	5.01	1.45	0.3	0.13	15.37	9.38	7.79	3.92	12	9	8	7
170	death	8	10.04	10.27	9.37	8.04	4050	3220	3930	3100	40.2	19.5	12.1	6.96	13.77	9.14	7.92	4.54	12	10	10	17
171	survival	28	16.05	12.64	23.18	28.22	3610	724	2050	685	93.1	23.2	6.41	1.61	21.02	8.76	2.23	1.24	13	7	8	5
172	survival	28	22.44	17.07	18.81	24.05	652	464	353	321	14.1	2.87	0.87	0.39	15.42	12.8	6.57	2.38	8	8	6	6
173	survival	28	30.94	24.48	23.05	35.91	8980	1950	648	633	100	100	48.1	13.1	16.29	12.06	3.01	2.59	12	11	9	4
174	survival	28	15.90	13.53	15.69	15.58	727	488	478	439	29.2	6.86	1.76	0.6	21.23	19.58	10.58	8.76	11	4	5	5
175	survival	28	18.64	20.77	24.13	20.16	11400	10800	7600	8230	35.6	9.91	3.6	4.3	24.43	6.85	4.07	5.21	16	7	5	6
176	survival	28	16.97	17.16	21.19		506	266	310		96.2	34.6	9.5		28.14	19.62	6.33		10	4	6	
177	survival	28	14.78	11.07	12.52	14.83	1210	304	558	239	17.1	3.66	9.85	1.55	17.11	14.96	7.88	7.61	13	9	10	6
178	survival	28	13.05	12.09	11.21	13.77	1940	2040	2780	2870	35.5	10.1	3.62	1.06	15.7	21.42	6.41	3.19	16	12	9	8
179	survival	28	17.93	16.68	28.60		540	488	420		2.8	2.87	0.99		13.9	17	11.84		11	7	3	
180	survival	28	9.98	8.94	16.63		850	517	986		66	5.56	0.9		11.56	38.72	9.87		12	10	5	
181	survival	28	11.89	8.05	7.58	10.14	3190	2530	5980	2750	42	27.3	8.01	2.81	21.48	38.21	34.86	8.33	13	11	9	12
182	survival	28	7.82	10.37			1370	801			13.3	2.21			10.5	8.71			10	7		
183	survival	28	20.62	24.37			878	815			79	16.7			33.3	15.3			9	7		
184	survival	28	16.40	12.58	20.00	26.06	1230	1040	708	500	13.3	7.53	1.45	0.54	28	26.86	14.35	14.9	11	10	10	7
185	survival	28	20.52	23.38	32.20		460	323	243		7.19	2.07	1.21		28.5	15.7	9.2		13	8	1	
186	survival	28	14.14	14.25			1950	540			78.2	29			32.7	17.2			10	5		
187	survival	28	9.72	15.04	19.42	19.18	7750	6700	2180	1710	25.7	7.98	2.88	1.5	35	24.76	7.39	2.21	16	15	6	7

188	survival	28	13.15	16.96	16.36	16.26	1990	1420	814	675	10.7	4.52	1.4	0.7	26.8	15.4	7.41	0	5	3	3	
189	survival	28	18.98	20.72	24.34	29.30	379	417	549	518	14.3	4.34	1.54	0.7	33.81	29.56	25.07	12.27	4	7	6	6
190	death	5	13.81	9.13			2590	3370			58.9	33.5			38.48	47.8			18	21		
191	survival	28	18.05	25.13			1470	459			0.91	0.31			16.7	17.6			7	1		
192	survival	28	25.23	29.47			3370	2340			5.29	1.2			21.3	21.98			9	5		
193	death	2	6.15				5320				14.1				11.57				9			
194	survival	28	27.12	17.27			243	305			49	13.4			8.97	19.89			9	8		
195	survival	28	14.22	16.08	13.52		721	320	219		100	67.2	18.5		26.26	20.84	3.97		6	6	4	
196	survival	28	14.06	16.20	20.27		1380	1010	541		5.23	1.42	0.39		15	5.8	1.83		5	5	2	
197	survival	28	16.68	18.24			2320	1100			60.3	24.7			18.27	10.88			11	4		
198	survival	28	23.13	21.18			3900	2420			2.46	0.64			31.3	19.04	6.02		13	8	3	
199	survival	28	19.93	23.88			1210	389			18.9	5.19			19.71	13.86			9	3		
200	survival	28	14.74	10.98	14.11	12.64	1050	2180	1390	987	84.3	48.7	16.7	15.8	15.56	26.33	9.7	7.46	9	13	7	6