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ORIGINAL ARTICLE

Elevated serum alkaline phosphatase as an indicator of bacteremia in haemodialysis outpatients —a multicentre retrospective cohort study

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ABSTRACT (298 words)

Objectives: Elevated baseline serum alkaline phosphatase (ALP) may correlate with higher medium- to long-term mortality in the general population and in chronic kidney disease (CKD) patients. There are few data on the association between serum ALP and the short-term prognosis of patients on haemodialysis (HD). We verified the association of ALP levels and bacteremia or death in maintenance HD patients suspected of bacteremia in an outpatient setting.

Setting: This study involved 315 consecutive HD patients suspected of having bacteremia with two sets of blood cultures drawn upon admission to either of two tertiary-care university medical centres from January 2013 to December 2015.

Participants: We enrolled consecutive cases on maintenance HD who were of age ≥ 18 years. Cases of hospitalised patients who had been transferred from another hospital, who had a vintage of dialysis < 2 months, who were also undergoing peritoneal dialysis (PD), and who were receiving HD less than once a week were excluded.

Primary and secondary outcome measures: The primary outcome measure was bacteraemia and the secondary outcome was in-hospital death.

Results: After the sampling, 315 cases that met the eligibility criteria were included in the study. The high-ALP group had a higher incidence of bacteremia. In multivariate analysis, there was a statistically significant association between higher ALP in hospital visit and bacteremia (OR: 2.37, 95%CI: 1.17 to 4.83, p=0.02). However, there were no statistically significant associations between higher ALP and in-hospital death (OR: 1.20, 95%CI: 0.57 to 2.54, p=0.63). A sensitivity analysis of 187 patients with no missing ALP values also demonstrated a significant association between ALP and bacteremia, but no significant association between ALP and in-hospital death.

Conclusions: Elevated ALP is a predictor of bacteremia. In HD patients suspected of bacteremia in outpatient settings, increased ALP levels heighten its likelihood.

Trial registration: none

Strengths and limitations of this study:

・This is the first multicentre investigation of the association between ALP levels and bacteremia or death in patients on maintenance HD.

・Elevated serum ALP levels in haemodialysis patients suspected of bacteremia could allow for early recognition and may potentially allow for earlier medical intervention.

・Our findings should facilitate further research to investigate any causal association of ALP elevation with bacteremia in complex biological systems.

・Although the study sample consisted of patients on maintenance HD from three geographically diverse hospitals in Japan, our inferences may not be generalisable to patients on maintenance HD in other clinical settings.

Keywords: alkaline phosphatase, bacteremia, haemodialysis, mortality, prognostic indicator

(2615 word)

INTRODUCTION

In patients on haemodialysis (HD), it is well known that the second most common cause of death after cardiovascular events is infection, especially sepsis or bacteremia [1,2]. The prevalence of bacteremia in patients with HD is 10 to 40 times that in the general population [3,4] with a 50-fold increase in mortality [5–7].

Multiple studies have shown a positive relationship between serum alkaline phosphatase (ALP) and medium- to long-term mortality in the general population and in chronic kidney disease (CKD) patients, including those on haemodialysis and peritoneal

dialysis [8–16]. The explanation is that elevated levels of serum ALP may reflect arterial stiffness, renal osteodystrophy, and inflammation [11,12,17–19].

In addition to the relationship between serum ALP and mid- to long-term prognosis, observational studies have identified other risk factors for bacteremia in dialysis patients, including leukocyte dysfunction, malnutrition, parathyroid hormone derangements, and vitamin D deficiency [8,20–22].

We focused on ALP, an enzyme that hydrolyses phosphate monoester. It is a dimer consisting of two identical molecules, and is expressed as four isoenzymes (placental, germ cell, intestinal, and tissue-nonspecific [liver/bone/kidney]) [23]. ALP is known as an indicator of renal osteodystrophy given its close relationship with bone, parathyroid gland function, the GI tract, and overall mineral balance [24]. Historically, high ALP levels have been considered related to renal osteodystrophy.

Damera et al. reported that ALP is one of the inflammatory markers independent of 25-OH vitamin D levels in CKD [25]. In addition, the 'BAC-HD' (Body temperature ≥ 38.3°C, ALP > 360 U/L, C-reactive protein [CRP] ≥ CRP 10 mg/dL, Heart rate ≥ 125 beats/min, Drugs: no prior antibiotic use for 1 week) score [26], which we previously

developed, is a clinical prediction algorithm for bacteremia among patients with HD that includes ALP levels as scoring factor.

Tung et al. showed extremely high ALP levels (ALP > 1000 U/L) to be associated with bacteremia [27]. However, this study had a very small sample size of 16. In other words, there are few studies showing an association between serum ALP and short-term prognosis of bacteraemia and in-hospital mortality.

Our aim was to verify the association of ALP levels and bacteremia or death in maintenance HD patients suspected of bacteremia in an outpatient setting.

METHODS

This study was approved by the ethics committees of Aso Iizuka Hospital (No. 17167), Okinawa Chubu Hospital (H28_No. 51), and Saku Central Hospital (201701-01), and was conducted in accordance with the ethical standards of the Declaration of Helsinki. In the present study, the Department of Nephrology, Aso Iizuka Hospital had collected anonymous data from the participating facilities. In addition, since all patient information analysed in this study was retrospective, the consent of participants was not obtained. The study results are reported according to the Strengthening the Reporting of Observational Studies in

Epidemiology (STROBE) guidelines for cohort studies [28]. The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Study design and participants

We performed a retrospective cohort study at three academic medical institutions in Japan. Data were collected from medical records from January 2013 to December 2015 in each facility. We enrolled consecutive cases on maintenance HD who were of age ≥ 18 years that had had two sets of blood cultures drawn at admission to assess for the presence of bacteremia. Cases of hospitalised patients who had been transferred from another hospital, who had a vintage of dialysis < 2 months, who were also undergoing peritoneal dialysis (PD), and who were receiving HD less than once a week were excluded (Fig. 1).

ALP levels

Admission ALP levels were dichotomized using the upper limit of normal range of 360 U/L as the cut-off value.

Outcomes

The primary outcome was bacteremia, which was diagnosed based on the results of admission blood cultures. To avoid misclassification of the primary outcome, an external consensus panel of infectious disease physicians who have more than ten years clinical experience and Japanese board of infectious disease determined whether a culture was contaminated or not based on the conventional definition of contamination and their clinical expertise. Contamination was defined as: only one of the two sets of culture bottles was positive; or the presence of certain species of bacteria, such as diphtheroids, *Bacillus* spp., *Propionibacterium* sp., *Micrococci*, *Corynebacterium* spp., and coagulase-negative staphylococci. The secondary outcome was in-hospital death.

Other Covariates

Clinical information collected on hospital admission included age, sex, body temperature, systolic blood pressure, diastolic blood pressure, pulse rate, respiratory rate, haemodialysis vintage, presence or absence of diabetes mellitus, and use of vitamin D analogues. In addition, white blood cell counts (WBC), aspartate aminotransferase (AST), total bilirubin (T-BIL), corrected calcium (cCa), phosphate (P), and C-reactive protein (CRP) were obtained from medical records.

Statistical analysis

The patients' baseline characteristics stratified by ALP categorised with the cut-off of normal range of 360 U/L at diagnosis were expressed as medians (quartile) or numbers (%). Multivariate analysis was performed using five models for bacteremia, the primary outcome, adjusted for age, sex, aspartate aminotransferase (AST), vitamin D analogue use, and haemodialysis vintage. Six models were used for the secondary outcome, in-hospital death, adjusted for age, sex, AST, T-BIL, vitamin D analogue use, cCa, P, haemodialysis vintage, and presence of bacteremia using a logistic regression model. We selected variables for multivariate analysis through a literature review and based on clinical experience. To minimize the bias from missing data, all missing values were imputed using multiple imputation by chained equation (MICE) treated as missing at random including ALP; ten imputed datasets were created [29]. On multivariate analysis, these ten datasets were combined with Rubin's rules and analysed. Analyses were assessed at two-tailed alpha = 0.05. We used commercial software (STATA 15.0, StataCorp LP, College Station, TX, USA) for statistical analysis.

Sample Size

We estimated the prevalence of bacteremia in maintenance HD patients suspected to have bacteremia to be 16% based on a previous report [26]. Since we planned a logistic regression analysis with five explanatory variables, we estimated that the number of bacteremia cases was required to be 50, following the rule of requiring ten outcomes per explanatory variable [30]. From these, it was estimated that a total of 312 subjects were needed.

Sensitivity analysis

To demonstrate the robustness of our inferences, we conducted a complete case analysis for ALP as a sensitivity analysis, which meant excluding participants missing admission ALP.

RESULTS

After the sampling, 315 cases that met the eligibility criteria were included in the study, as shown in Figure 1. Table 1 shows the baseline characteristics of the cohort.

Table 1. Baseline characteristics

	ALP ≤ 360 U/L N =133	ALP > 360 U/L N = 54	Total N = 315	Missing (N)
Age, years mean (range)	73 (66, 80)	72 (62, 79)	73 (63, 80)	0
Sex	-	-	-	0
males, n (%)	77 (57.9)	26 (48.1)	178 (56.5)	
females, n (%)	56 (42.1)	28 (51.9)	137 (43.5)	

Diabetes mellitus, n (%)	64 (48.1)	27 (50.0)	159 (50.5)	0
Mean systolic blood pressure, mmHg, (range)	134 (110, 150)	134 (11, 150)	134 (110, 150)	2
Mean diastolic blood pressure, mmHg, (range)	70 (60, 80)	70 (60, 80)	70 (60, 80)	22
Mean pulse rate, beats/minute, (range)	90 (78, 102)	92 (84, 108)	90 (78, 102)	4
Mean respiratory rate, per minute, (range)	20 (18, 24)	20 (18, 24)	20 (18, 24)	43
Mean body temperature, °C, (range)	37.3 (36.5, 38.0)	37.6 (36.9, 38.3)	37.2 (36.5, 38.0)	6
Laboratory data				
Mean WBC × 10^3/μL, range	8.7 (6.2, 12.4)	8.6 (6.1, 11.3)	8.4 (6.2, 12.0)	2
Mean AST (U/L), range	17 (12, 25)	24 (18, 55)	18 (13, 25)	7
Mean ALT (U/L) , range	10 (7, 15)	18 (12, 38)	11 (7.5, 17)	7
Mean T-Bill (mg/dl) , range	0.5 (0.3,0.6)	0.6 (0.4, 1.5)	0.5 (0.3, 0.7)	17
Mean Ca (mg/dl) , range	8.8 (8.4, 9.3)	8.7 (8.3, 9.4)	8.8 (8.4, 9.4)	93
Mean P (mg/dl) , range	4.4 (3.3, 5.8)	5.3 (4.1, 6.6)	4.7 (3.8, 6.1)	284
Mean CRP (mg/dL) , range	5.2 (2.1, 11.2)	6.0 (1.5, 12.3)	5.5 (2.1, 12.1)	31
Mean haemodialysis vintage, months, (range)	51 (17.5, 114)	58 (18, 139)	55 (20, 115)	14
Vitamin D analogue use, n (%)	60 (45.1)	25 (46.3)	134 (42.5)	2
Vascular access				
arteriovenous fistula, n (%)	86 (64.7)	44 (81.5)	130 (41.3)	0
arteriovenous graft, n (%)	11 (8.3)	2 (3.7)	13 (4.1)	0
arteriovenous shunt, n (%)	5 (3.8)	2 (3.7)	7 (2.2)	0
temporary catheter, n (%)	30 (22.6)	6 (11.1)	36 (11.4)	0

This table shows the baseline characteristics of the cohort.

Abbreviation: ALP, alkaline phosphatase; WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin; Ca, calcium; P, phosphorus; CRP, C-reactive protein

Occurrence of Outcomes

Table 2 shows the incidence of bacteremia and in-hospital deaths in the total and groups stratified by ALP. The high-ALP group had a higher incidence of bacteremia.

Table 2. Incidence of bacteraemia and in-hospital death in the total and groups stratified by ALP

	ALP ≤ 360 U/L N = 133	ALP > 360 U/L N = 54	total N = 315	Missing (N)
Bacteraemia, n (%)	20 (15.0)	19 (35.2)	50 (15.9)	0
In-hospital death, n (%)	17 (12.8)	9 (16.7)	48 (15.2)	0

This table shows the incidence of bacteremia and in-hospital deaths in the total and groups stratified by ALP. The high-ALP group had a higher incidence of bacteremia.

Abbreviations: ALP, alkaline phosphatase

Association of ALP in hospital visit and bacteremia

In multivariate analysis shown in Figure 2, there was a statistically significant association between higher ALP in hospital visit and bacteremia in all four models.

Association of ALP in hospital visit and in-hospital death

As shown in Figure 2, there were no statistically significant associations between higher ALP and in-hospital death in all five models.

Sensitivity Analysis

To examine the robustness of the findings, we conducted a complete case analysis for ALP excluding participants who were missing ALP values. A sensitivity analysis of 187 patients with no missing ALP values also demonstrated a significant association between ALP and bacteremia, but no significant association between ALP and in-hospital death (Figs. 2).

DISCUSSION

This study showed a statistically significant positive correlation between ALP levels and bacteremia in HD patients suspected of having bacteremia in the outpatient setting. Few studies examining the association between serum ALP and short-term prognosis have been reported. This is the first multicentre investigation of the association between ALP levels and bacteremia or death in patients on maintenance HD.

Based on the results of this study, elevated serum ALP levels in haemodialysis patients suspected of bacteremia could allow for early recognition and may potentially allow for earlier medical intervention.

We considered two reasons why elevated ALP levels were associated with bacteramia.

The first is a hypothesis that hepatobiliary infections such as cholangitis cause bacteremia or sepsis, leading to elevated ALP levels [31,32]. However, since the main cause of bacteremia in HD patients is bloodstream infection with staphylococci, it is considered that bacteremia due to biliary tract infection does not significantly affect ALP levels in this population. In addition, we adjusted for the liver enzyme AST in multivariate analysis, but the changes in the OR of bacteremia were small. These findings suggest that the increase in ALP levels in HD patients is due to factors other than hepatobiliary infection.

We then considered a biological response to bacteremia. Previous studies have shown that ALP acts on inflammatory mediators, such as bacterial endotoxin and extracellular adenosine triphosphate, and may detoxify them via dephosphorylation [10,12,33–35]. Previous studies using sepsis in animal models (mice, rats, sheep, piglets) have reported that treatment with ALP reduced systemic inflammation and organ dysfunction, and improved survival [36–41]. There are also reports suggesting that ALP may be effective in the treatment of sepsis in HD patients [42]. Sepsis-related AKI is a result of a combination of inflammatory, nephrotoxic, and ischemic injuries and is believed to cause rapid development of renal damage. Pickkers et al. showed that treatment with ALP improved creatinine clearance; as well as the need for, and duration of, dialysis in patients with AKI due to sepsis [43].

From these studies, it is clear that elevation of ALP is a response to inflammation and bacteremia, suggested the relationship between ALP and sepsis.

It is known that percutaneous bloodstream infections mainly caused by gram-positive cocci (GPC) are common in HD patients [44]. However, previous meta-analysis review reported that about 20% of hemodialysis catheter-related bacteremia

were caused by gram-negative rods (GNR) as well as coagulase-negative staphylococci and *staphylococcus aureus* [45].

In our study, GNR-induced sepsis accounted for 34% of cases, which may have been associated with ALP levels. However, the median values (quartiles) of ALP in bacteremia due to GPC and GNR were 302 (217, 455) U/L and 388 (225-530) U/L, and there may be reasons other than this hypothesis. Second, given the mechanism by which GPC inactivates inflammatory mediators, ALP can be elevated not only by GNR but also by GPC-induced sepsis [46]. From the above, it is considered that ALP is associated with bacteremia in HD patients regardless of the category of bacteremia.

We found no significant association of ALP with mortality in the analysis for secondary outcome, different from previous studies [10,15,47]. In previous study, HD patients with elevated ALP levels had an approximately 50% higher risk of sepsis compared to those with normal ALP levels [15]. It is possible that the overall good prognosis among patients on maintenance HD in Japan influenced the results.

Our study has several potential limitations. First, there may be unmeasured confounding factors, a limit of observational studies. However, it was designed to optimise the selection of the adjusted confounding factors and to minimise their effect as compared

with previous studies. Second, since it is a cross-sectional study, the effect of causal reversal cannot be denied. However, high ALP levels were shown to be a predictor of bacteremia. Third, this was a retrospective study, and the uncertainty of the data extracted from medical records cannot be ruled out. Fourth, while we conducted a multicentre study, the sample size was relatively small. This study should facilitate further validation studies to confirm the association of ALP elevation and bacteremia in patients on maintenance HD. Lastly, although the study sample consisted of patients on maintenance HD from three geographically diverse hospitals in Japan, our inferences may not be generalisable to patients on maintenance HD in other clinical settings (e.g., patients with hospitalisation at index dates). Nonetheless, our inferences should remain relevant for over 340,000 patients on maintenance HD in Japan, a vulnerable population with high mortality, about 14 times compared to the general population, from bacteremia [48].

CONCLUSIONS

By conducting a multicentre retrospective observational study, we identified elevation of ALP levels as an independent predictor of bacteremia among maintenance HD outpatients suspected of having sepsis. The association remained consistent after adjusting for other

potential predictors for bacteremia. For clinicians, our data could provide an evidence base for the early identification of patients with bacteremia and their resultant prompt hospitalisation. Our findings should facilitate further research to investigate any causal association of ALP elevation with bacteremia in complex biological systems.

Contributors: All authors have read and approved the submission of the manuscript; the manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language, except as an abstract. Sho Sasaki (SS) created the study design. SS, Yoshihiko Raita, Shungo Yamamoto, Kentaro Tochitani, Minoru Murakami, and Ryo Nishioka performed data collection. Aya Katasako and SS analysed data, and wrote the article. All authors reviewed the manuscript. Kiichiro Fujisaki approved the submission of the article.

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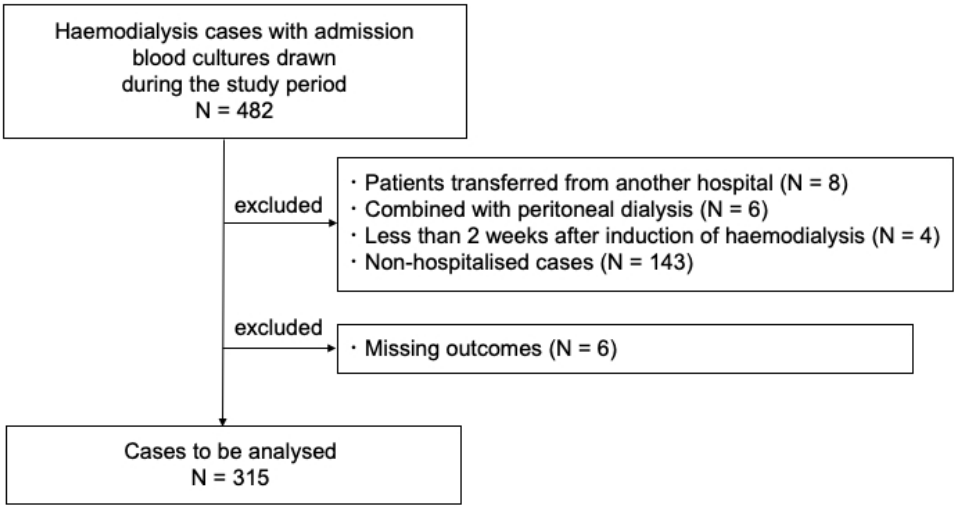
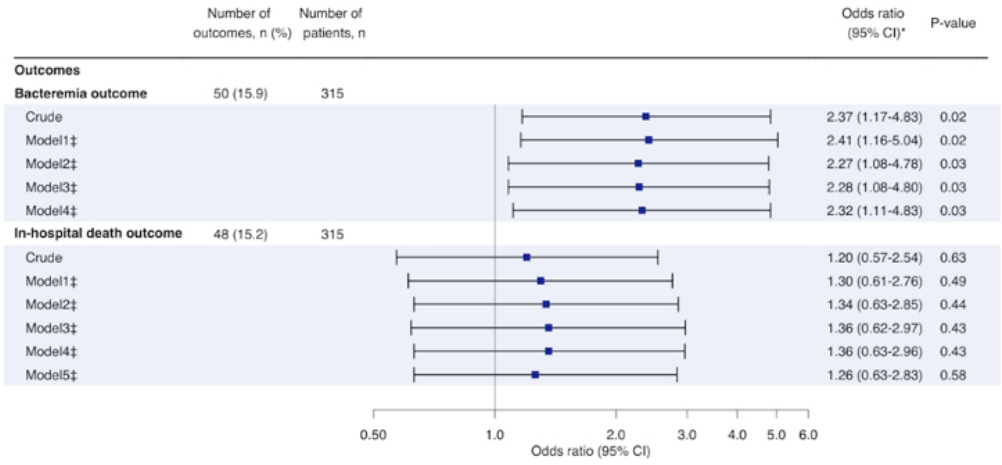
Figure 1. Study Flow



After the sampling, 315 cases that met the eligibility criteria were included.

254x190mm (72 x 72 DPI)

Figure 2. Association between ALP and bacteremia or In-hospital death : logistic regression model



Multivariate analysis shown in this Figure. There were no relationship between higher ALP and in-hospital death, however a statistically significant association between higher ALP and bacteremia.

(Bacteremia outcome) Model 1, adjusted for age, sex; Model 2, Model 1 + aspartate aminotransferase, Model 3, Model 2 + vitamin D analogue use; Model 4, Model 3 + hemodialysis vintage
(In-hospital death outcome) Model 1, adjusted for age, sex; Model 2, Model 1 + aspartate aminotransferase; Model 3, Model 2 + vitamin D analogue use; Model 4, Model 3 + hemodialysis vintage; Model 5, Model 4 + presence of bacteremia

254x190mm (72 x 72 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	*(a)* Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	9-10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9-10
		(*b*) Describe any methods used to examine subgroups and interactions	9-10
		(*c*) Explain how missing data were addressed	9
		(*d*) If applicable, explain how loss to follow-up was addressed	9
		(*e*) Describe any sensitivity analyses	10
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10-12
		(b) Indicate number of participants with missing data for each variable of interest	10-12
		(c) Summarise follow-up time (eg, average and total amount)	10-12
Outcome data	15*	Report numbers of outcome events or summary measures over time	13

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	13 13
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14-17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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The association between serum alkaline phosphatase and bacteraemia in haemodialysis outpatients: A multicentre retrospective cross-sectional study

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ORIGINAL ARTICLE

The association between serum alkaline phosphatase and bacteraemia in haemodialysis outpatients: A multicentre retrospective cross-sectional study

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ABSTRACT (292 words)

Objectives: Elevated baseline serum alkaline phosphatase (ALP) may correlate with higher medium- to long-term mortality in the general population and in chronic kidney disease (CKD) patients. There are few data on the association between serum ALP and the short-term prognosis of patients on haemodialysis (HD). We verified the association of ALP levels and bacteraemia or death in maintenance HD patients suspected of bacteraemia in an outpatient setting.

Design: We analysed 315 consecutive HD patients suspected of having bacteraemia with two sets of blood cultures drawn upon admission.

Setting: Patients were admitted to one of two tertiary-care university medical centres from January 2013 to December 2015.

Participants: We enrolled consecutive cases on maintenance HD who were aged ≥ 18 years. Cases of hospitalised patients who had been transferred from another hospital, had a dialysis vintage < 2 months, were also undergoing peritoneal dialysis (PD), and/or were receiving HD less than once a week were excluded.

Primary and secondary outcome measures: The primary outcome measure was bacteraemia and the secondary outcome was in-hospital death.

Results: Among 315 cases included in the study, 187 had baseline-measured ALP levels. The high-ALP group had a higher incidence of bacteraemia. In multivariate analysis, there was a statistically significant association between a higher ALP in hospital visit and bacteraemia (OR: 2.37, 95% CI: 1.17 to 4.83). However, there were no statistically significant associations between higher ALP and in-hospital death (OR: 1.20, 95% CI: 0.57 to 2.54). A sensitivity analysis of 187 patients with no missing ALP values also demonstrated a significant association between elevated ALP and bacteraemia, but no significant association between ALP and in-hospital death.

Conclusions: Elevated ALP is a predictor of bacteraemia. In HD patients suspected of bacteraemia in outpatient settings, increased ALP levels heighten its likelihood.

Trial registration: none

Strengths and limitations of this study:

- This is the first multicentre investigation of the association between ALP levels and bacteraemia or death in patients on maintenance HD.
- Elevated serum ALP levels in haemodialysis patients suspected of bacteraemia could lead to earlier diagnosis and may potentially allow for earlier medical intervention.

・Our findings should facilitate further research to investigate any causal association of ALP elevation with bacteraemia in complex biological systems.

・Although the study sample consisted of patients on maintenance HD from two geographically diverse hospitals in Japan, our inferences may not be generalisable to patients on maintenance HD in other clinical settings.

Keywords: alkaline phosphatase, bacteraemia, haemodialysis, mortality, prognostic indicator

INTRODUCTION

In patients on haemodialysis (HD), it is well known that the second-most common cause of death after cardiovascular events is infection, especially sepsis or bacteraemia[1,2]. The prevalence of bacteraemia in patients with HD is 10 to 40 times that in the general population[3,4] with a 50-fold increase in mortality[5–7].

Multiple studies have shown a positive relationship between serum alkaline phosphatase (ALP) and medium- to long-term mortality in the general population and in chronic kidney disease (CKD) patients, including those on haemodialysis and peritoneal dialysis[8–16]. The explanation is that elevated levels of serum ALP may reflect

abnormalities such as arterial stiffness, renal osteodystrophy, and inflammation[11,12,17–19].

In addition to the relationship between serum ALP and mid- to long-term prognosis, observational studies have identified other risk factors for bacteraemia in dialysis patients, including leukocyte dysfunction, malnutrition, parathyroid hormone derangements, and vitamin D deficiency[8,20–22].

We focused on ALP, an enzyme that hydrolyses phosphate monoester. It is a dimer consisting of two identical molecules, and is expressed as four isoenzymes (placental, germ cell, intestinal, and tissue-nonspecific [liver/bone/kidney])[23]. ALP is known as an indicator of renal osteodystrophy, associated with its close relationship with bone, parathyroid gland function, the GI tract, and overall mineral balance[24]. Historically, high ALP levels have been considered related to renal osteodystrophy.

Damera et al. reported that ALP is one of the inflammatory markers which are independent of 25-OH vitamin D levels in CKD[25]. In addition, the 'BAC-HD' (Body temperature ≥ 38.3°C, ALP > 360 U/L, C-reactive protein [CRP] ≥ CRP 10 mg/dL, Heart rate ≥ 125 bpm, Drugs: no prior antibiotic use for 1 week) score[26], which we previously developed, is a clinical prediction algorithm for bacteraemia among patients with HD.

Tung et al. showed that extremely high ALP levels (ALP > 1000 U/L) were associated with bacteraemia[27]. However, that study had a very small sample size of 16. In other words, there are few studies showing an association between serum ALP and short-term prognosis of bacteraemia and in-hospital mortality.

ALP levels can be measured easily and are a less burdensome test for the patient. In addition, bacteraemia is an important outcome for haemodialysis patients because of its high morbidity and mortality. Therefore, it is important to investigate serum ALP levels as predictive markers of bacteraemia. Our aim was to verify the association of ALP levels and bacteraemia or death in maintenance HD patients suspected of bacteraemia in an outpatient setting.

METHODS

This study was approved by the ethics committees of Aso Iizuka Hospital (No. 17167), Okinawa Chubu Hospital (H28_No. 51), and Saku Central Hospital (201701-01), and was conducted in accordance with the ethical standards of the Declaration of Helsinki. In the present study, the Department of Nephrology of Aso Iizuka Hospital had collected anonymous data from the participating facilities. Since this study was retrospective, the

consent of participants was not obtained. The study results are reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies[28].

Study design and participants

We performed a cross-sectional study at the three academic medical institutions mentioned above. Data were collected from medical records from January 2013 to December 2015 in each facility. We enrolled consecutive cases of patients on maintenance HD who were aged ≥ 18 years and had had two sets of blood cultures drawn at admission to assess for the presence of bacteraemia. Cases of hospitalised patients who had been transferred from another hospital, had a vintage of dialysis < 2 months, were also undergoing peritoneal dialysis (PD), or were receiving HD less than once a week were excluded (Fig. 1).

ALP levels

Logistic regression analysis was performed with bacteraemia as the dependent variable and ALP as the explanatory variable. Based on the ROC analysis, the value with the highest discriminatory power was used as the cut-off point.

Outcomes

The primary outcome was bacteraemia, which was diagnosed based on the results of admission blood cultures. To avoid misclassification of the primary outcome, an external consensus panel of infectious disease physicians with more than ten years' clinical experience and Japanese board certification in infectious disease determined whether a culture was contaminated or not based on the conventional definition of contamination and their clinical expertise. Contamination was defined as: only one of the two sets of culture bottles was positive; or the presence of certain species of bacteria, such as diphtheroids, *Bacillus* spp., *Propionibacterium* spp., *Micrococci spp.*, *Corynebacterium* spp., and coagulase-negative staphylococci. The secondary outcome was in-hospital death.

Other Covariates

Clinical information collected on hospital admission included age, sex, body temperature, systolic and diastolic blood pressure, pulse rate, respiratory rate, haemodialysis vintage, presence or absence of diabetes mellitus, and use of vitamin D analogues. In addition, white blood cell counts (WBC), aspartate aminotransferase (AST), total bilirubin (T-BIL),

corrected calcium (cCa), phosphate (P), and C-reactive protein (CRP) were obtained from medical records.

Statistical analysis

The serum ALP levels at diagnosis were stratified by the cut-off value based on ROC analysis, and patients' baseline characteristics were expressed as medians (quartile) or numbers (%). Multivariate analysis was performed for the primary outcome of bacteraemia in four models adjusted for age, sex, aspartate aminotransferase (AST), vitamin D analogue use, and haemodialysis vintage. Five models were used for the secondary outcome: in-hospital death, adjusted for age, sex, AST, T-BIL, vitamin D analogue use, cCa, P, haemodialysis vintage, and presence of bacteraemia using a logistic regression model (Fig. 2). We selected variables for multivariate analysis through a literature review and based on clinical experience. To minimise the bias from missing data, all missing values were imputed using multiple imputation by chained equation (MICE) treated as missing at random including ALP; ten imputed datasets were created[29]. On multivariate analysis, these ten datasets were combined with Rubin's rules and analysed. Analyses were assessed at a two-tailed alpha =

0.05. We used commercial software (STATA 15.0, StataCorp LP, College Station, TX, USA) for statistical analysis.

Sample Size

We estimated the prevalence of bacteraemia in maintenance HD patients suspected to have bacteraemia to be 16% based on a previous report[26]. Since we planned a logistic regression analysis with five explanatory variables, we estimated that the number of bacteraemia cases was required to be 50, following the rule of requiring ten outcomes per explanatory variable[30]. From these, it was estimated that a total of 312 subjects was needed.

Sensitivity analysis

To demonstrate the robustness of our inferences, we conducted a complete case analysis for ALP as a sensitivity analysis, which meant excluding participants missing admission ALP. In addition, we added CRP, which is not a confounding factor but is a strong prognostic factor, and performed a sensitivity analysis.

Patient and public involvement

No current patients or members of the public were directly involved in this study.

RESULTS

The cut-off value for ALP was 360 U/L based on ROC analysis. Among the 315 cases included in the study (Figure 1), 187 had baseline measured ALP levels (133 with normal levels ≤ 360 U/L and 54 with ALP levels > 360 U/L). Table 1 shows the baseline characteristics of the cohort.

Table 1. Baseline characteristics

	ALP ≤ 360 U/L N =133	ALP > 360 U/L N = 54	Total N = 315	Missing (N)
Age, years, median (IQR)	73 (66, 80)	72 (62, 79)	73 (63, 80)	0
Sex				0
males, n (%)	77 (57.9)	26 (48.1)	178 (56.5)	
females, n (%)	56 (42.1)	28 (51.9)	137 (43.5)	
Diabetes mellitus, n (%)	64 (48.1)	27 (50.0)	159 (50.5)	0
Systolic blood pressure, mmHg, median (IQR)	134 (110, 150)	134 (11, 150)	134 (110, 150)	2
Diastolic blood pressure, mmHg, median (IQR)	70 (60, 80)	70 (60, 80)	70 (60, 80)	22
Pulse rate, beats/minute, median (IQR)	90 (78, 102)	92 (84, 108)	90 (78, 102)	4
Respiratory rate, per minute, median (IQR)	20 (18, 24)	20 (18, 24)	20 (18, 24)	43

Body temperature, °C, median (IQR)	37.3 (36.5, 38.0)	37.6 (36.9, 38.3)	37.2 (36.5, 38.0)	6
Laboratory data				
WBC ($\times 10^3/\mu L$), median (IQR)	8.7 (6.2, 12.4)	8.6 (6.1, 11.3)	8.4 (6.2, 12.0)	2
ALP (U/L), median (IQR)	-	-	271 (219, 376)	128
AST (U/L), median (IQR)	17 (12, 25)	24 (18, 55)	18 (13, 25)	7
ALT (U/L) , median (IQR)	10 (7, 15)	18 (12, 38)	11 (7.5, 17)	7
T-Bill (mg/dl) , median (IQR)	0.5 (0.3,0.6)	0.6 (0.4, 1.5)	0.5 (0.3, 0.7)	17
Ca (mg/dL) , median (IQR)	8.8 (8.4, 9.3)	8.7 (8.3, 9.4)	8.8 (8.4, 9.4)	93
P (mg/dL) , median (IQR)	4.4 (3.3, 5.8)	5.3 (4.1, 6.6)	4.7 (3.8, 6.1)	284
CRP (mg/dL) , median (IQR)	5.2 (2.1, 11.2)	6.0 (1.5, 12.3)	5.5 (2.1, 12.1)	31
Haemodialysis vintage, months, median (IQR)	51 (17.5, 114)	58 (18, 139)	55 (20, 115)	14
Vitamin D analogue use, n (%)	60 (45.1)	25 (46.3)	134 (42.5)	2
Vascular access				
arteriovenous fistula, n (%)	86 (64.7)	44 (81.5)	130 (41.3)	0
arteriovenous graft, n (%)	11 (8.3)	2 (3.7)	13 (4.1)	0
arteriovenous shunt, n (%)	5 (3.8)	2 (3.7)	7 (2.2)	0
temporary catheter, n (%)	30 (22.6)	6 (11.1)	36 (11.4)	0

This table shows the baseline characteristics of the cohort.

Abbreviation: ALP, alkaline phosphatase; WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin; Ca, calcium; P, phosphorus; CRP, C-reactive protein; IQR Interquartile range

Occurrence of Outcomes

Table 2 shows the incidence of bacteraemia and in-hospital deaths in the total and groups stratified by ALP. The high-ALP group had a higher incidence of bacteraemia.

Table 2. Incidence of bacteraemia and in-hospital death in the total and groups stratified by ALP

	ALP ≤ 360 U/L N = 133	ALP > 360 U/L N = 54	total N = 315	Missing (N)
Bacteraemia, n (%)	20 (15.0)	19 (35.2)	50 (15.9)	11
In-hospital death, n (%)	17 (12.8)	9 (16.7)	48 (15.2)	22

This table shows the incidence of bacteraemia and in-hospital deaths in the total and groups stratified by ALP. The high-ALP group had a higher incidence of bacteraemia.

Abbreviations: ALP, alkaline phosphatase

Association of ALP in hospital visit and bacteraemia

In the multivariate analysis shown in Figure 2, there was a statistically significant association between higher ALP in hospital visit and bacteraemia in all four models.

Association of ALP in hospital visit and in-hospital death

As shown in Figure 2, there were no statistically significant associations between higher ALP and in-hospital death in all five models.

Sensitivity Analysis

To examine the robustness of the findings, we conducted a complete case analysis for ALP excluding participants who were missing ALP values. A sensitivity analysis of the 187 patients with no missing ALP values also demonstrated a significant association between ALP and bacteraemia, but no significant association between ALP and in-hospital death (Fig. 2). In a sensitivity analysis with the addition of CRP, it did not show a significant association between bacteraemia and ALP levels in analysis adjusted for age, sex, AST, CRP, vitamin D analogue use, or haemodialysis vintage (OR: 1.97, 95% CI: 0.97 to 4.01) as shown in Supplementary Figure.

DISCUSSION

This study showed a statistically significant positive correlation between ALP levels and bacteraemia in HD patients suspected of having bacteraemia in the outpatient setting. Few

studies examining the association between serum ALP and short-term prognosis have been reported. This is the first multicentre investigation of the association between ALP levels and bacteraemia or death in patients on maintenance HD.

Based on the results of this study, elevated serum ALP levels in haemodialysis patients with suspected bacteraemia could allow for early recognition and may potentially allow for earlier medical intervention.

Association between ALP and bacteraemia

We considered two reasons why elevated ALP levels were associated with bacteraemia. First is the involvement of hepatobiliary infections such as cholangitis. We hypothesise that it may cause bacteraemia or sepsis, leading to elevated ALP levels[31,32]. However, since the main cause of bacteraemia in HD patients is bloodstream infection with staphylococci, it is considered that bacteraemia due to biliary tract infection does not significantly affect ALP levels in this population. In addition, we adjusted for the liver enzyme AST in multivariate analysis, but the changes in the OR of bacteraemia were small. These findings suggest that the increase in ALP levels in HD patients was due to factors other than hepatobiliary infection.

Second, we considered a biological response to bacteraemia. Previous studies have shown that ALP acts on inflammatory mediators, such as bacterial endotoxin and extracellular adenosine triphosphate, and may detoxify them via dephosphorylation[10,12,33–35]. In animal models of sepsis (mice, rats, sheep, piglets), it has been reported that treatment with ALP reduced systemic inflammation and organ dysfunction, and improved survival[33,36–40]. There are also reports suggesting that ALP t is effective in the treatment of sepsis in HD patients[41]. Sepsis-related AKI is thought to be the result of a combination of inflammatory, nephrotoxic, and ischemic injury with rapid progression of renal damage. Pickkers et al. showed that treatment with ALP improved creatinine clearance, as well as the need for and duration of dialysis in patients with sepsis-related AKI[42].

The above two points suggest that the increase in ALP may be a response to inflammation or bacteraemia.

In maintenance haemodialysis patients with a high risk of infection, the therapeutic strategy, including antimicrobials, is often distressing until the results of blood culture are available. Unnecessary administration of antimicrobials can be harmful to the patient, because antimicrobial resistance is a serious problem for them. However, it has also known

that delayed administration of empiric antimicrobial therapy leads to increased mortality.[43] We need to decide the timing of administration of therapy and choice of antimicrobial agents appropriately. Serum ALP levels have been reported as one example of a simple clinical prediction rule in the bacteraemia 'BAC-HD score'.[44] In maintenance HD outpatients suspected of sepsis, elevated serum ALP levels may indicate the presence of bacteraemia and may aid in the decision to begin early antimicrobial therapy and in the choice of the antimicrobial agent.

ALP isozymes

Intestinal isozyme may be of possible relevance to sepsis-related treatment.[34, 41] However, no association has been found between specific isozymes and bacteraemia or sepsis, and we do not recommend the measurement of isozymes at this time in clinical practice. If the above two points are resolved, it may be useful to measure ALP isozymes in the future.

The species associated with bacteraemia

It is known that percutaneous bloodstream infections caused primarily by gram-positive cocci (GPC) are common in HD patients[45]. However, a previous meta-analysis reported that

about 20% of haemodialysis catheter-related bacteraemias were caused by gram-negative rods (GNR) as well as coagulase-negative staphylococci and *Staphylococcus aureus*[46].

In our study, GNR-induced sepsis accounted for 34% of cases, which may have been associated with ALP levels. However, the median quartile values of ALP in bacteraemia due to GPC and GNR were 302 (range, 217, 455) U/L and 388 (range, 225, 530) U/L, respectively, and there may be reasons other than this hypothesis. Second, given the mechanism by which GPC inactivates inflammatory mediators, ALP can be elevated not only by GNR but also by GPC-induced sepsis[47]. From the above, it is considered that ALP is associated with bacteraemia in HD patients regardless of the category of the offending bacterium.

Association between ALP and mortality

We found no significant association of ALP with mortality in the analysis for secondary outcome, in contrast to previous studies[10,15,48]. In one study, HD patients with elevated ALP levels had an approximately 50% higher risk of infection-related mortality compared to those with normal ALP levels[15]. One reason for the significant difference in bacteraemia

but not in mortality may be that the overall prognosis for maintenance HD patients in Japan is good.

Limitations

Our study has several limitations. First, there may be unmeasured confounding factors, a limit of observational studies. However, it was designed to optimise the selection of the adjusted confounding factors and to minimise their effect as compared with previous studies. Second, since it is a cross-sectional study, the possibility of reverse causation cannot be denied. However, high ALP levels were shown to be a predictor of bacteraemia. Third, this was a retrospective study, and the uncertainty of the data extracted from medical records cannot be ruled out. Fourth, while we conducted a multicentre study, the sample size was relatively small and there were substantial missing data. In patients with ALP data, there was a statistically significant association between ALP and bacteraemia, but no association between ALP and in-hospital mortality. We consider the small sample size as a reason why we could not show an association with mortality, unlike previous reports. This is the first study suggesting that serum ALP is one of several independent predictors of bacteraemia in HD patients. Our study should facilitate further validation studies to confirm the association

of ALP elevation and bacteraemia in maintenance HD patients. Fifth, it cannot be determined in this study whether serum ALP levels were elevated before illness or due to bacteraemia. However, baseline serum ALP levels are often unknown in clinical practice. Therefore we consider it may be clinically acceptable. Lastly, the study sample consisted of patients on maintenance HD from three geographically diverse hospitals in Japan, and our findings may not be generalisable to patients on maintenance HD in other clinical settings (e.g., patients with hospitalisation at index dates). Nonetheless, our inferences should remain relevant for over 340,000 patients on maintenance HD in Japan, a vulnerable population with high mortality from bacteraemia, at about 14 times that of the general population [49].

CONCLUSIONS

By conducting a multicentre retrospective observational study, we identified elevation of ALP levels as an independent predictor of bacteraemia among maintenance HD outpatients suspected of having sepsis. The association remained consistent after adjusting for other potential predictors for bacteraemia. For clinicians, our data could provide an evidence base for the early identification of patients with bacteraemia and their resultant prompt

hospitalisation. Our findings should facilitate further research to investigate any causal association of ALP elevation with bacteraemia in complex biological systems.

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Contributors: All authors have read and approved the submission of the manuscript; the manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language, except as an abstract. Sho Sasaki (SS) created the study design. SS, Yoshihiko Raita, Shungo Yamamoto, Kentaro Tochitani, Minoru Murakami, and Ryo Nishioka performed data collection. Aya Katasako and SS analysed data, and wrote the article. All authors reviewed the manuscript. Kiichiro Fujisaki approved the submission of the article.

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Figure legends

Figure 1. Study flow

After the sampling, 315 cases that met the eligibility criteria were included.

Figure 2. Association between ALP and bacteraemia or In-hospital death: logistic regression model

Multivariate analysis shown in this Figure. There was no relationship between higher ALP and in-hospital death, however there was a statistically significant association between higher ALP and bacteraemia.

Bacteraemia outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase, Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage

In-hospital death outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage; Model 5, adjusted for Model 4 + presence of bacteraemia

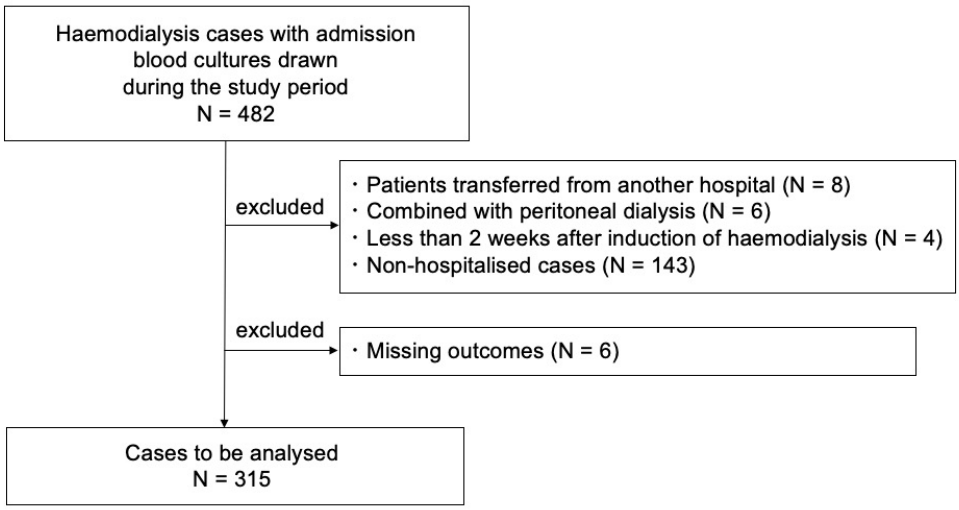

Figure 1. Study flow
After the sampling, 315 cases that met the eligibility criteria were included.

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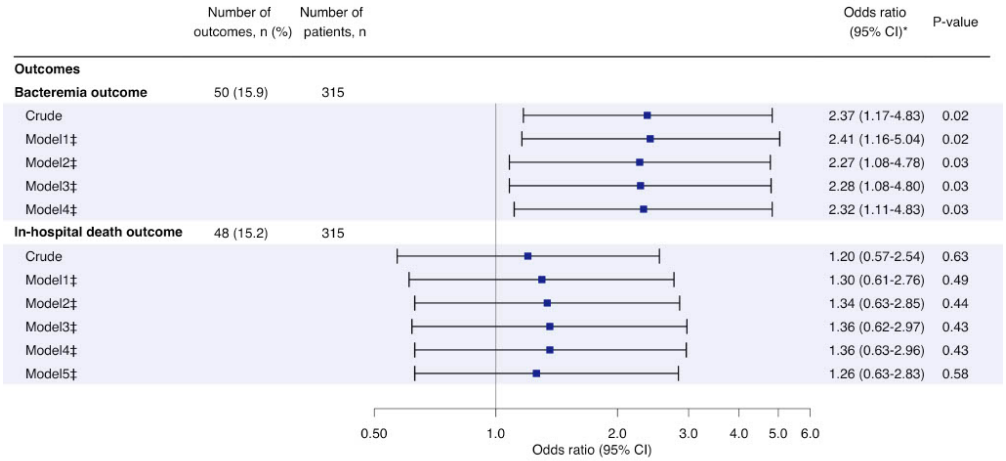

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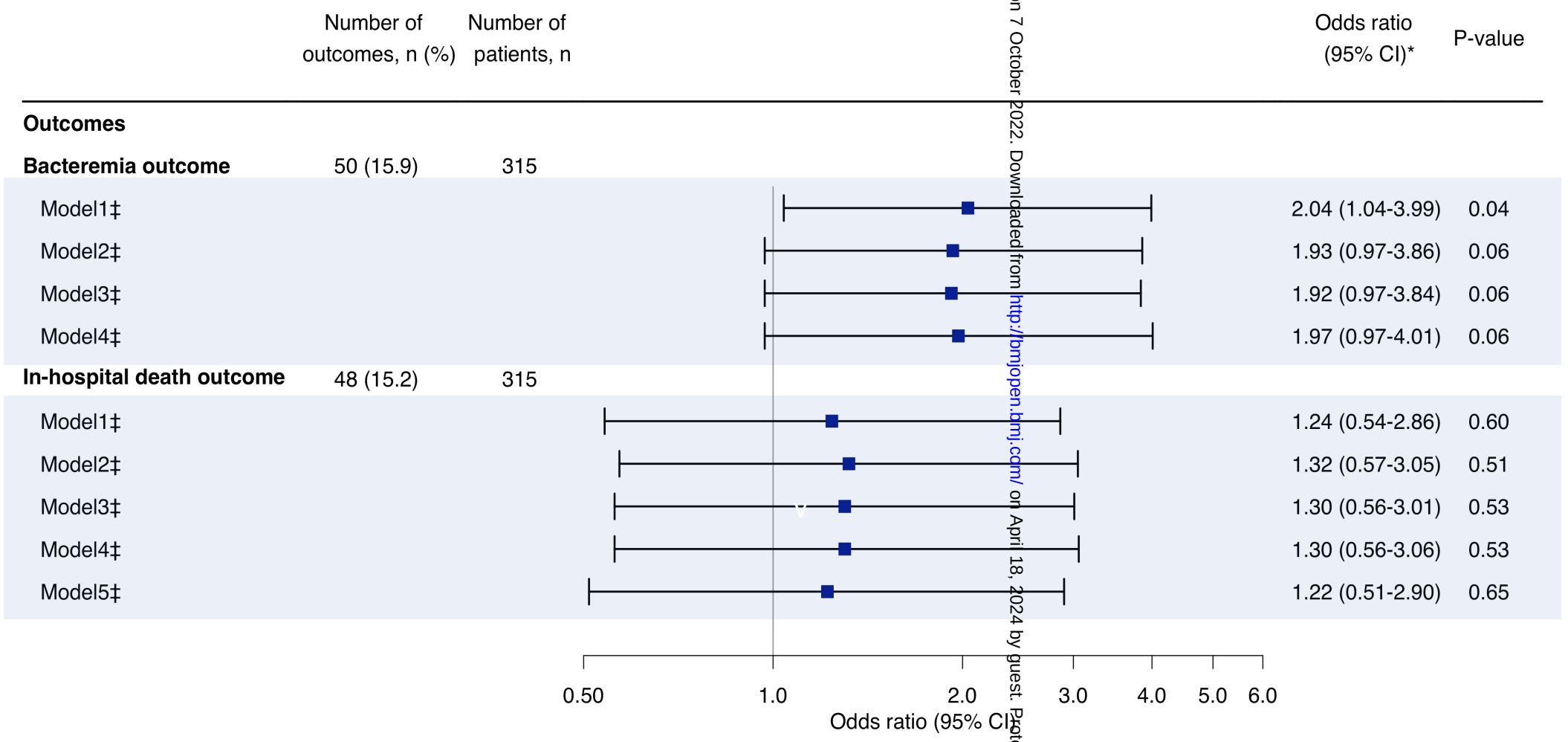
Supplementary Figure



Bacteraemia outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase + CRP; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage

In-hospital death outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase + CRP; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage; Model 5, adjusted for Model 4 + presence of bacteraemia

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-11
Bias	9	Describe any efforts to address potential sources of bias	9-10
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10-11
		(*b*) Describe any methods used to examine subgroups and interactions	10-11
		(*c*) Explain how missing data were addressed	10-11
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	10-11
		(*e*) Describe any sensitivity analyses	11
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	12
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-13
		(b) Indicate number of participants with missing data for each variable of interest	12-13
Outcome data	15*	Report numbers of outcome events or summary measures	13-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-15

		(*b*) Report category boundaries when continuous variables were categorized	-
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	15
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	20-21
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-21
Generalisability	21	Discuss the generalisability (external validity) of the study results	17-18, 20-21
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	22

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The association between serum alkaline phosphatase and bacteraemia in haemodialysis outpatients: A multicentre retrospective cross-sectional study

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ORIGINAL ARTICLE

The association between serum alkaline phosphatase and bacteraemia in haemodialysis outpatients: A multicentre retrospective cross-sectional study

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ABSTRACT (292 words)

Objectives: Elevated baseline serum alkaline phosphatase (ALP) may correlate with higher medium- to long-term mortality in the general population and in chronic kidney disease (CKD) patients. There are few data on the association between serum ALP and the short-term prognosis of patients on haemodialysis (HD). We verified the association of ALP levels and bacteraemia or death in maintenance HD patients suspected of bacteraemia in an outpatient setting.

Design: We analysed 315 consecutive HD patients suspected of having bacteraemia with two sets of blood cultures drawn upon admission.

Setting: Patients were admitted to one of two tertiary-care university medical centres from January 2013 to December 2015.

Participants: We enrolled consecutive cases on maintenance HD who were aged ≥ 18 years. Cases of hospitalised patients who had been transferred from another hospital, had a dialysis vintage < 2 months, were also undergoing peritoneal dialysis (PD), and/or were receiving HD less than once a week were excluded.

Primary and secondary outcome measures: The primary outcome measure was bacteraemia and the secondary outcome was in-hospital death.

Results: Among 315 cases included in the study, 187 had baseline-measured ALP levels. The high-ALP group had a higher incidence of bacteraemia. In multivariate analysis, there was a statistically significant association between a higher ALP in hospital visit and bacteraemia (OR: 2.37, 95% CI: 1.17 to 4.83). However, there were no statistically significant associations between higher ALP and in-hospital death (OR: 1.20, 95% CI: 0.57 to 2.54). A sensitivity analysis of 187 patients with no missing ALP values also demonstrated a significant association between elevated ALP and bacteraemia, but no significant association between ALP and in-hospital death.

Conclusions: Elevated ALP is a predictor of bacteraemia. In HD patients suspected of bacteraemia in outpatient settings, increased ALP levels heighten its likelihood.

Trial registration: none

Strengths and limitations of this study:

- This is the first multicentre investigation of the association between ALP levels and bacteraemia or death in patients on maintenance HD.
- Elevated serum ALP levels in haemodialysis patients suspected of bacteraemia could lead to earlier diagnosis and may potentially allow for earlier medical intervention.

・Our findings should facilitate further research to investigate any causal association of ALP elevation with bacteraemia in complex biological systems.

・Although the study sample consisted of patients on maintenance HD from two geographically diverse hospitals in Japan, our inferences may not be generalisable to patients on maintenance HD in other clinical settings.

Keywords: alkaline phosphatase, bacteraemia, haemodialysis, mortality, prognostic indicator

INTRODUCTION

In patients on haemodialysis (HD), it is well known that the second-most common cause of death after cardiovascular events is infection, especially sepsis or bacteraemia[1,2]. The prevalence of bacteraemia in patients with HD is 10 to 40 times that in the general population[3,4] with a 50-fold increase in mortality[5–7].

Multiple studies have shown a positive relationship between serum alkaline phosphatase (ALP) and medium- to long-term mortality in the general population and in chronic kidney disease (CKD) patients, including those on haemodialysis and peritoneal dialysis[8–15]. The explanation is that elevated levels of serum ALP may reflect

abnormalities such as arterial stiffness, renal osteodystrophy, and inflammation[11,12,16–18].

In addition to the relationship between serum ALP and mid- to long-term prognosis, observational studies have identified other risk factors for bacteraemia in dialysis patients, including leukocyte dysfunction, malnutrition, parathyroid hormone derangements, and vitamin D deficiency[8,19–21].

We focused on ALP, an enzyme that hydrolyses phosphate monoester. It is a dimer consisting of two identical molecules, and is expressed as four isoenzymes (placental, germ cell, intestinal, and tissue-nonspecific [liver/bone/kidney])[22]. ALP is known as an indicator of renal osteodystrophy, associated with its close relationship with bone, parathyroid gland function, the GI tract, and overall mineral balance[23]. Historically, high ALP levels have been considered related to renal osteodystrophy.

Damera et al. reported that ALP is one of the inflammatory markers which are independent of 25-OH vitamin D levels in CKD[24]. In addition, the 'BAC-HD' (Body temperature ≥ 38.3°C, ALP > 360 U/L, C-reactive protein [CRP] ≥ CRP 10 mg/dL, Heart rate ≥ 125 bpm, Drugs: no prior antibiotic use for 1 week) score[25], which we previously developed, is a clinical prediction algorithm for bacteraemia among patients with HD.

Tung et al. showed that extremely high ALP levels (ALP > 1000 U/L) were associated with bacteraemia[26]. However, that study had a very small sample size of 16. In other words, there are few studies showing an association between serum ALP and short-term prognosis of bacteraemia and in-hospital mortality.

ALP levels can be measured easily and are a less burdensome test for the patient. In addition, bacteraemia is an important outcome for haemodialysis patients because of its high morbidity and mortality. Therefore, it is important to investigate serum ALP levels as predictive markers of bacteraemia. Our aim was to verify the association of ALP levels and bacteraemia or death in maintenance HD patients suspected of bacteraemia in an outpatient setting.

METHODS

This study was approved by the ethics committees of Aso Iizuka Hospital (No. 17167), Okinawa Chubu Hospital (H28_No. 51), and Saku Central Hospital (201701-01), and was conducted in accordance with the ethical standards of the Declaration of Helsinki. In the present study, the Department of Nephrology of Aso Iizuka Hospital had collected anonymous data from the participating facilities. Since this study was retrospective, the

consent of participants was not obtained. The study results are reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies[27].

Study design and participants

We performed a cross-sectional study at the three academic medical institutions mentioned above. Data were collected from medical records from January 2013 to December 2015 in each facility. We enrolled consecutive cases of patients on maintenance HD who were aged ≥ 18 years and had had two sets of blood cultures drawn at admission to assess for the presence of bacteraemia. Cases of hospitalised patients who had been transferred from another hospital, had a vintage of dialysis < 2 months, were also undergoing peritoneal dialysis (PD), or were receiving HD less than once a week were excluded (Fig. 1).

ALP levels

Logistic regression analysis was performed with bacteraemia as the dependent variable and ALP as the explanatory variable. Based on the ROC analysis, the value with the highest discriminatory power was used as the cut-off point.

Outcomes

The primary outcome was bacteraemia, which was diagnosed based on the results of admission blood cultures. To avoid misclassification of the primary outcome, an external consensus panel of infectious disease physicians with more than ten years' clinical experience and Japanese board certification in infectious disease determined whether a culture was contaminated or not based on the conventional definition of contamination and their clinical expertise. Contamination was defined as: only one of the two sets of culture bottles was positive; or the presence of certain species of bacteria, such as diphtheroids, *Bacillus* spp., *Propionibacterium* spp., *Micrococci spp.*, *Corynebacterium* spp., and coagulase-negative staphylococci. The secondary outcome was in-hospital death.

Other Covariates

Clinical information collected on hospital admission included age, sex, body temperature, systolic and diastolic blood pressure, pulse rate, respiratory rate, haemodialysis vintage, presence or absence of diabetes mellitus, and use of vitamin D analogues. In addition, white blood cell counts (WBC), aspartate aminotransferase (AST), total bilirubin (T-BIL),

corrected calcium (cCa), phosphate (P), and C-reactive protein (CRP) were obtained from medical records.

Statistical analysis

The serum ALP levels at diagnosis were stratified by the cut-off value based on ROC analysis, and patients' baseline characteristics were expressed as medians (quartile) or numbers (%). Multivariate analysis was performed for the primary outcome of bacteraemia in four models adjusted for age, sex, aspartate aminotransferase (AST), vitamin D analogue use, and haemodialysis vintage (Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage). Five models were used for the secondary outcome: in-hospital death, adjusted for age, sex, AST, T-BIL, vitamin D analogue use, cCa, P, haemodialysis vintage, and presence of bacteraemia using a logistic regression model (Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage; Model 5, adjusted for Model 4 + presence of bacteraemia, Fig. 2). We selected variables for multivariate analysis through a literature review and based on clinical

experience. To minimise the bias from missing data, all missing values were imputed using multiple imputation by chained equation (MICE) treated as missing at random including ALP; ten imputed datasets were created[28]. On multivariate analysis, these ten datasets were combined with Rubin's rules and analysed. Analyses were assessed at a two-tailed alpha = 0.05. We used commercial software (STATA 15.0, StataCorp LP, College Station, TX, USA) for statistical analysis.

Sample Size

We estimated the prevalence of bacteraemia in maintenance HD patients suspected to have bacteraemia to be 16% based on a previous report[25]. Since we planned a logistic regression analysis with five explanatory variables, we estimated that the number of bacteraemia cases was required to be 50, following the rule of requiring ten outcomes per explanatory variable[29]. From these, it was estimated that a total of 312 subjects was needed.

Sensitivity analysis

To demonstrate the robustness of our inferences, we conducted a complete case analysis for ALP as a sensitivity analysis, which meant excluding participants missing admission ALP.

In addition, we added CRP, which is not a confounding factor but is a strong prognostic factor, and performed a sensitivity analysis.

Patient and public involvement

No current patients or members of the public were directly involved in this study.

RESULTS

The cut-off value for ALP was 360 U/L based on ROC analysis (AUC 0.60, sensitivity 0.49, specificity 0.76) in complete cases of ALP. Among the 315 cases included in the study (Figure 1), 187 had baseline measured ALP levels (133 with normal levels ≤ 360 U/L and 54 with ALP levels > 360 U/L). Table 1 shows the baseline characteristics of the cohort.

Table 1. Baseline characteristics

	ALP ≤ 360 U/L N =133	ALP > 360 U/L N = 54	Total N = 315	Missing (N)
Age, years, median (IQR)	73 (66, 80)	72 (62, 79)	73 (63, 80)	0
Sex				0
males, n (%)	77 (57.9)	26 (48.1)	178 (56.5)	
females, n (%)	56 (42.1)	28 (51.9)	137 (43.5)	
Diabetes mellitus, n (%)	64 (48.1)	27 (50.0)	159 (50.5)	0
Systolic blood pressure,	134 (110, 150)	134 (11, 150)	134 (110, 150)	2

mmHg, median (IQR)				
Diastolic blood pressure, mmHg, median (IQR)	70 (60, 80)	70 (60, 80)	70 (60, 80)	22
Pulse rate, beats/minute, median (IQR)	90 (78, 102)	92 (84, 108)	90 (78, 102)	4
Respiratory rate, per minute, median (IQR)	20 (18, 24)	20 (18, 24)	20 (18, 24)	43
Body temperature, °C, median (IQR)	37.3 (36.5, 38.0)	37.6 (36.9, 38.3)	37.2 (36.5, 38.0)	6
Laboratory data				
WBC ($\times 10^3/\mu L$), median (IQR)	8.7 (6.2, 12.4)	8.6 (6.1, 11.3)	8.4 (6.2, 12.0)	2
ALP (U/L), median (IQR)	237 (203, 280)	502 (404, 780)	271 (219, 376)	128
AST (U/L), median (IQR)	17 (12, 25)	24 (18, 55)	18 (13, 25)	7
ALT (U/L) , median (IQR)	10 (7, 15)	18 (12, 38)	11 (7.5, 17)	7
T-Bill (mg/dl) , median (IQR)	0.5 (0.3,0.6)	0.6 (0.4, 1.5)	0.5 (0.3, 0.7)	17
Ca (mg/dL) , median (IQR)	8.8 (8.4, 9.3)	8.7 (8.3, 9.4)	8.8 (8.4, 9.4)	93
P (mg/dL) , median (IQR)	4.4 (3.3, 5.8)	5.3 (4.1, 6.6)	4.7 (3.8, 6.1)	284
CRP (mg/dL) , median (IQR)	5.2 (2.1, 11.2)	6.0 (1.5, 12.3)	5.5 (2.1, 12.1)	31
Haemodialysis vintage, months, median (IQR)	51 (17.5, 114)	58 (18, 139)	55 (20, 115)	14
Vitamin D analogue use, n (%)	60 (45.1)	25 (46.3)	134 (42.5)	2
Vascular access				
arteriovenous fistula, n (%)	86 (64.7)	44 (81.5)	130 (41.3)	0
arteriovenous graft, n (%)	11 (8.3)	2 (3.7)	13 (4.1)	0
arteriovenous shunt, n (%)	5 (3.8)	2 (3.7)	7 (2.2)	0
temporary catheter, n (%)	30 (22.6)	6 (11.1)	36 (11.4)	0

1

2 This table shows the baseline characteristics of the cohort.

Abbreviation: ALP, alkaline phosphatase; WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin; Ca, calcium; P, phosphorus; CRP, C-reactive protein; IQR Interquartile range

Occurrence of Outcomes

Table 2 shows the incidence of bacteraemia and in-hospital deaths in the total and groups stratified by ALP. The high-ALP group had a higher incidence of bacteraemia.

Table 2. Incidence of bacteraemia and in-hospital death in the total and groups stratified by ALP

	ALP ≤ 360 U/L N = 133	ALP > 360 U/L N = 54	total N = 315	Missing (N)
Bacteraemia, n (%)	20 (15.0)	19 (35.2)	50 (15.9)	11
In-hospital death, n (%)	17 (12.8)	9 (16.7)	48 (15.2)	22

This table shows the incidence of bacteraemia and in-hospital deaths in the total and groups stratified by ALP. The high-ALP group had a higher incidence of bacteraemia.

Abbreviations: ALP, alkaline phosphatase

Association of ALP in hospital visit and bacteraemia

In the multivariate analysis shown in Figure 2, there was a statistically significant association between higher ALP in hospital visit and bacteraemia in all four models.

Association of ALP in hospital visit and in-hospital death

As shown in Figure 2, there were no statistically significant associations between higher ALP and in-hospital death in all five models.

Sensitivity Analysis

To examine the robustness of the findings, we conducted a complete case analysis for ALP excluding participants who were missing ALP values. A sensitivity analysis of the 187 patients with no missing ALP values also demonstrated a significant association between ALP and bacteraemia, but no significant association between ALP and in-hospital death (Fig. 2). In a sensitivity analysis with the addition of CRP, results showed no significant association between bacteraemia and ALP levels in analysis adjusted for age, sex, AST, CRP,

vitamin D analogue use, or haemodialysis vintage (OR: 1.97, 95% CI: 0.97 to 4.01) as shown in the Supplementary Figure.

DISCUSSION

This study showed a statistically significant positive correlation between ALP levels and bacteraemia in HD patients suspected of having bacteraemia in the outpatient setting. Few studies examining the association between serum ALP and short-term prognosis have been reported. This is the first multicentre investigation of the association between ALP levels and bacteraemia or death in patients on maintenance HD.

Based on the results of this study, elevated serum ALP levels in haemodialysis patients with suspected bacteraemia could allow for early recognition and may potentially allow for earlier medical intervention.

Association between ALP and bacteraemia

We considered two reasons why elevated ALP levels were associated with bacteraemia. First is the involvement of hepatobiliary infections such as cholangitis. We hypothesise that it may cause bacteraemia or sepsis, leading to elevated ALP levels[30,31]. However, since the main

cause of bacteraemia in HD patients is bloodstream infection with staphylococci, it is considered that bacteraemia due to biliary tract infection does not significantly affect ALP levels in this population. In addition, we adjusted for the liver enzyme AST in multivariate analysis, but the changes in the OR of bacteraemia were small. These findings suggest that the increase in ALP levels in HD patients was due to factors other than hepatobiliary infection.

Second, we considered a biological response to bacteraemia. Previous studies have shown that ALP acts on inflammatory mediators, such as bacterial endotoxin and extracellular adenosine triphosphate, and may detoxify them via dephosphorylation[10,12,32–34]. In animal models of sepsis (mice, rats, sheep, piglets), it has been reported that treatment with ALP reduced systemic inflammation and organ dysfunction, and improved survival[32,35–39]. There are also reports suggesting that ALP is effective in the treatment of sepsis in HD patients[40]. Sepsis-related AKI is thought to be the result of a combination of inflammatory, nephrotoxic, and ischemic injury with rapid progression of renal damage. Pickkers et al. showed that treatment with ALP improved creatinine clearance, as well as the need for and duration of dialysis in patients with sepsis-related AKI[41].

The above two points suggest that the increase in ALP may be a response to inflammation or bacteraemia.

In maintenance haemodialysis patients with a high risk of infection, the therapeutic strategy, including antimicrobials, is often distressing until the results of blood culture are available. Unnecessary administration of antimicrobials can be harmful to the patient, because antimicrobial resistance is a serious problem for them. However, it has also known that delayed administration of empiric antimicrobial therapy leads to increased mortality.[42] We need to decide the timing of administration of therapy and choice of antimicrobial agents appropriately. Serum ALP levels have been reported as one example of a simple clinical prediction rule in the bacteraemia 'BAC-HD score'.[43] In maintenance HD outpatients suspected of sepsis, elevated serum ALP levels may indicate the presence of bacteraemia and may aid in the decision to begin early antimicrobial therapy and in the choice of the antimicrobial agent.

ALP isozymes

Intestinal isozyme may be of possible relevance to sepsis-related treatment.[33, 40] However, no association has been found between specific isozymes and bacteraemia or sepsis, and we

do not recommend the measurement of isozymes at this time in clinical practice. If the above two points are resolved, it may be useful to measure ALP isozymes in the future.

The species associated with bacteraemia

It is known that percutaneous bloodstream infections caused primarily by gram-positive cocci (GPC) are common in HD patients[44]. However, a previous meta-analysis reported that about 20% of haemodialysis catheter-related bacteraemias were caused by gram-negative rods (GNR) as well as coagulase-negative staphylococci and *Staphylococcus aureus*[45].

In our study, GNR-induced sepsis accounted for 34% of cases, which may have been associated with ALP levels. However, the median quartile values of ALP in bacteraemia due to GPC and GNR were 302 (range, 217, 455) U/L and 388 (range, 225, 530) U/L, respectively, and there may be reasons other than this hypothesis. Second, given the mechanism by which GPC inactivates inflammatory mediators, ALP can be elevated not only by GNR but also by GPC-induced sepsis[46]. From the above, it is considered that ALP is associated with bacteraemia in HD patients regardless of the category of the offending bacterium.

Association between ALP and mortality

We found no significant association of ALP with mortality in the analysis for secondary outcome, in contrast to previous studies[10,14,47]. In one study, HD patients with elevated ALP levels had an approximately 50% higher risk of infection-related mortality compared to those with normal ALP levels[14]. One reason for the significant difference in bacteraemia but not in mortality may be that the overall prognosis for maintenance HD patients in Japan is good.

Limitations

Our study has several limitations. First, there may be unmeasured confounding factors, a limit of observational studies. However, the study was designed to optimise the selection of adjusted confounding factors and to minimise their effect as compared with previous studies. It is possible that intact PTH was a residual confounding factor. However, we could not test this possibility because we did not measure intact PTH in this study, for two reasons: first, because intact PTH may not contribute significantly to outcomes for bacteremia or mortality [48]; and second, since ALP reflects factors of origin other than bone, we considered that the association between PTH and ALP in the acute phase, such as the subject of this study, might

be still unclear. Nevertheless, there are reports of increased mortality in patients with PTH outside the normal range in the non-acute phase, [49] and further validation is needed. Second, since it is a cross-sectional study, the possibility of reverse causation cannot be denied. However, high ALP levels were shown to be a predictor of bacteraemia. Third, this was a retrospective study, and the uncertainty of the data extracted from medical records cannot be ruled out. Fourth, while we conducted a multicentre study, the sample size was relatively small and there were substantial missing data. In patients with ALP data, there was a statistically significant association between ALP and bacteraemia, but no association between ALP and in-hospital mortality. We consider the small sample size as a reason why we could not show an association with mortality, unlike previous reports. This is the first study suggesting that serum ALP is one of several independent predictors of bacteraemia in HD patients. Our study should facilitate further validation studies to confirm the association of ALP elevation and bacteraemia in maintenance HD patients. Fifth, it cannot be determined in this study whether serum ALP levels were elevated before illness or due to bacteraemia. However, baseline serum ALP levels are often unknown in clinical practice. Therefore we consider it may be clinically acceptable. Lastly, the study sample consisted of patients on maintenance HD from three geographically diverse hospitals in Japan, and our findings may

not be generalisable to patients on maintenance HD in other clinical settings (e.g., patients with hospitalisation at index dates). Nonetheless, our inferences should remain relevant for over 340,000 patients on maintenance HD in Japan, a vulnerable population with high mortality from bacteraemia, at about 14 times that of the general population [50].

CONCLUSIONS

By conducting a multicentre retrospective observational study, we identified elevation of ALP levels as an independent predictor of bacteraemia among maintenance HD outpatients suspected of having sepsis. The association remained consistent after adjusting for other potential predictors for bacteraemia. For clinicians, our data could provide an evidence base for the early identification of patients with bacteraemia and their resultant prompt hospitalisation. Our findings should facilitate further research to investigate any causal association of ALP elevation with bacteraemia in complex biological systems.

Contributors: All authors have read and approved the submission of the manuscript; the manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language, except as an abstract. Sho Sasaki (SS) created the study

design. SS, Yoshihiko Raita, Shungo Yamamoto, Kentaro Tochitani, Minoru Murakami, and Ryo Nishioka performed data collection. Aya Katasako and SS analysed data, and wrote the article. All authors reviewed the manuscript. Kiichiro Fujisaki approved the submission of the article.

Competing interests: All of authors declare that they have no relevant financial interests.

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Data sharing statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient consent for publication: Not required

Ethics statement: This study was approved by the ethics committees of Aso Iizuka Hospital (No. 17167), Okinawa Chubu Hospital (H28_No. 51), and Saku Central Hospital (201701-01).

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Figure legends

Figure 1. Study flow

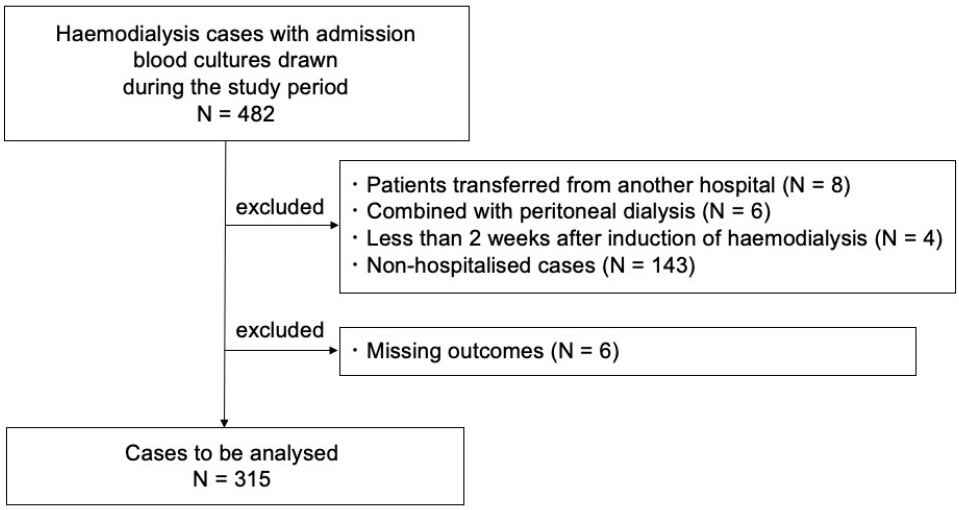
After the sampling, 315 cases that met the eligibility criteria were included.

Figure 2. Association between ALP and bacteraemia or In-hospital death: logistic regression model

Multivariate analysis shown in this Figure. There was no relationship between higher ALP and in-hospital death, however there was a statistically significant association between higher ALP and bacteraemia.

Bacteraemia outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase, Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage

In-hospital death outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage; Model 5, adjusted for Model 4 + presence of bacteraemia



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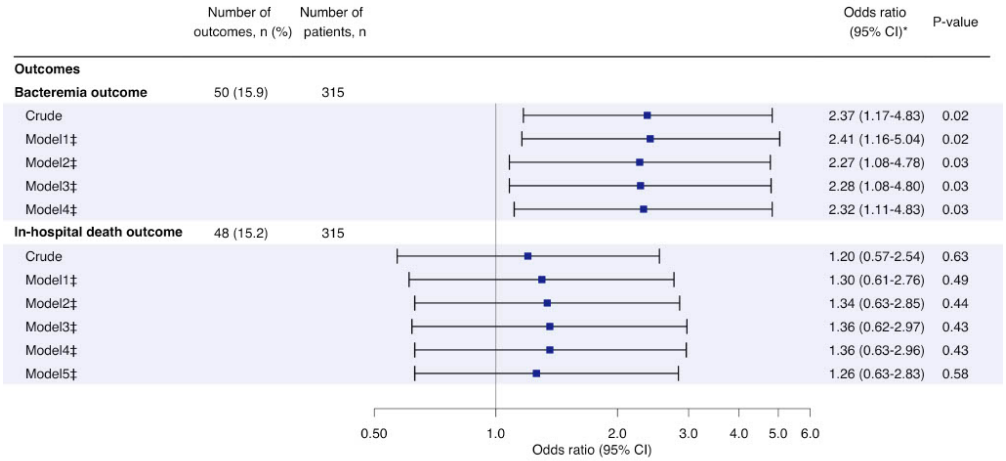

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Multivariate analysis shown in this Figure. There was no relationship between higher ALP and in-hospital death, however there was a statistically significant association between higher ALP and bacteraemia.
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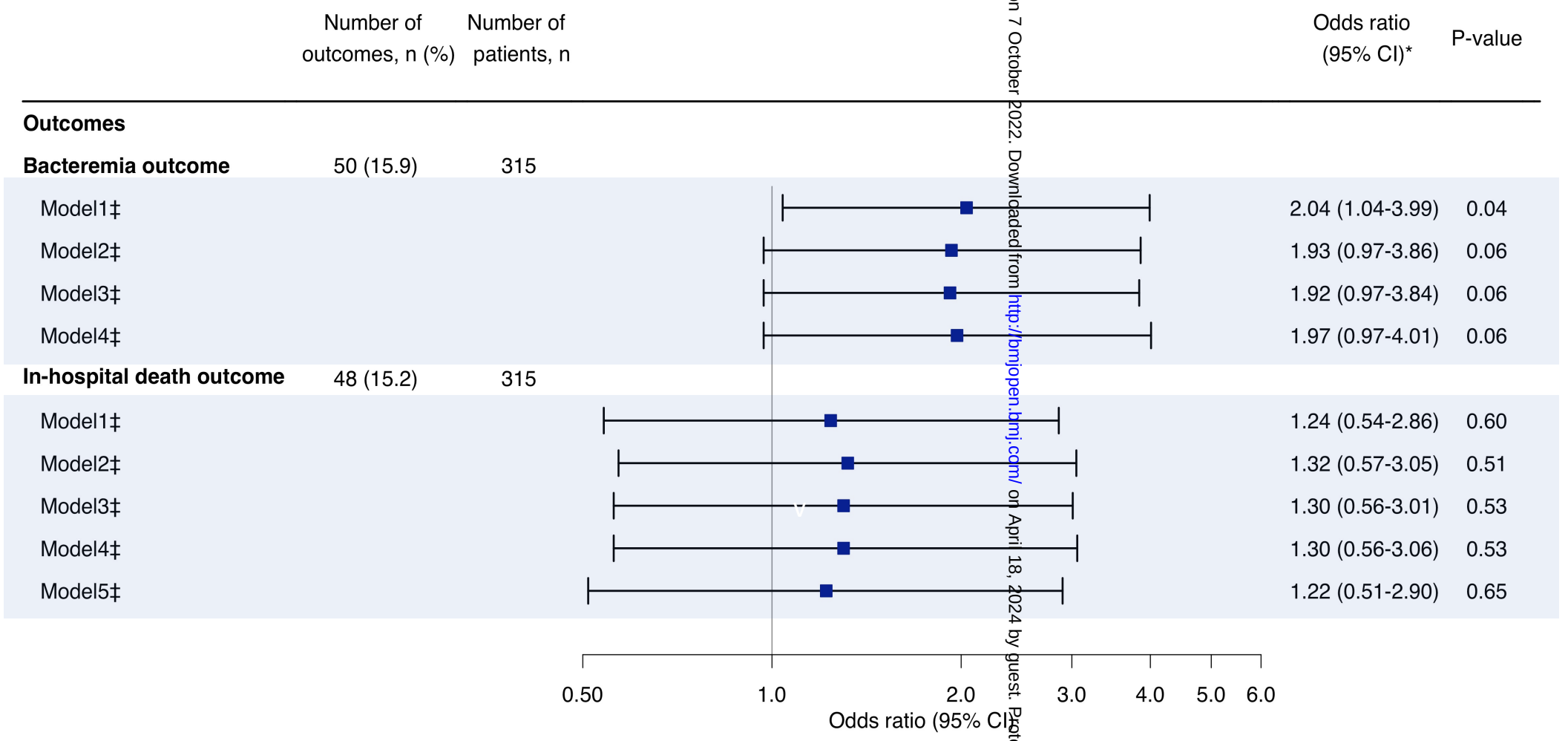
In-hospital death outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage; Model 5, adjusted for Model 4 + presence of bacteraemia

361x203mm (72 x 72 DPI)

Supplementary Figure: Sensitivity analysis with the addition of CRP



Bacteraemia outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase + CRP; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage

In-hospital death outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase + CRP; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage; Model 5, adjusted for Model 4 + presence of bacteraemia

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-11
Bias	9	Describe any efforts to address potential sources of bias	9-10
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10-11
		(*b*) Describe any methods used to examine subgroups and interactions	10-11
		(*c*) Explain how missing data were addressed	10-11
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	10-11
		(*e*) Describe any sensitivity analyses	11
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	12
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-13
		(b) Indicate number of participants with missing data for each variable of interest	12-13
Outcome data	15*	Report numbers of outcome events or summary measures	13-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-15

		(*b*) Report category boundaries when continuous variables were categorized	-
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	15
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	20-21
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-21
Generalisability	21	Discuss the generalisability (external validity) of the study results	17-18, 20-21
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	22

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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The association between serum alkaline phosphatase and bacteraemia in haemodialysis outpatients: A multicentre retrospective cross-sectional study

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ORIGINAL ARTICLE

The association between serum alkaline phosphatase and bacteraemia in haemodialysis outpatients: A multicentre retrospective cross-sectional study

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ABSTRACT (295 words)

Objectives: Elevated baseline serum alkaline phosphatase (ALP) may correlate with higher medium- to long-term mortality in the general population and in chronic kidney disease (CKD) patients. However, few data are available on the association between serum ALP and the short-term prognosis of patients on haemodialysis (HD). We verified the association of ALP levels and bacteraemia or death in maintenance HD patients suspected of bacteraemia in an outpatient setting.

Design: We analysed 315 consecutive HD patients suspected of having bacteraemia with two sets of blood culture drawn upon admission.

Setting: Admission to two tertiary-care university medical centres from January 2013 to December 2015.

Participants: Consecutive cases on maintenance HD aged ≥ 18 years. Cases of hospitalised patients who had been transferred from another hospital, had a dialysis vintage < 2 months, were also undergoing peritoneal dialysis (PD), and/or were receiving HD less than once a week were excluded.

Primary and secondary outcome measures: Primary outcome measure was bacteraemia and secondary outcome was in-hospital death.

Results: Among 315 cases included in the study, 187 had baseline-measured ALP levels, with a cut-off value on ROC analysis of 360 U/L (AUC 0.60, sensitivity 0.49, specificity 0.76). In multivariate analysis, there was a statistically significant association between a higher ALP in hospital visit and bacteraemia (OR: 2.37, 95% CI: 1.17 to 4.83). However, there were no statistically significant associations between higher ALP and in-hospital death (OR: 1.20, 95% CI: 0.57 to 2.54). A sensitivity analysis of 187 patients with no missing ALP values also demonstrated a significant association between elevated ALP and bacteraemia, but no significant association between ALP and in-hospital death.

Conclusions: Elevated ALP is a predictor of bacteraemia. In HD patients suspected of bacteraemia in outpatient settings, increased ALP levels were associated with increased likelihood of confirmed disease.

Trial registration: none

Strengths and limitations of this study:

- This is the first multicentre investigation of the association between ALP levels and bacteraemia or death in patients on maintenance HD.

- Elevated serum ALP levels in haemodialysis patients suspected of bacteraemia could lead to earlier diagnosis and may potentially allow for earlier medical intervention.
- Our findings should facilitate further research to investigate any causal association of ALP elevation with bacteraemia in complex biological systems.
- Although the study sample consisted of patients on maintenance HD from two geographically diverse hospitals in Japan, our inferences may not be generalisable to patients on maintenance HD in other clinical settings.

Keywords: alkaline phosphatase, bacteraemia, haemodialysis, mortality, prognostic indicator

INTRODUCTION

In patients on haemodialysis (HD), it is well known that the second-most common cause of death after cardiovascular events is infection, especially sepsis or bacteraemia[1,2]. The prevalence of bacteraemia in patients with HD is 10 to 40 times that in the general population[3,4] with a 50-fold increase in mortality[5–7].

Multiple studies have shown a positive relationship between serum alkaline phosphatase (ALP) and medium- to long-term mortality in the general population and in

chronic kidney disease (CKD) patients, including those on haemodialysis and peritoneal dialysis[8–15]. The explanation is that elevated levels of serum ALP may reflect abnormalities such as arterial stiffness, renal osteodystrophy, and inflammation[11,12,16–18].

In addition to the relationship between serum ALP and mid- to long-term prognosis, observational studies have identified other risk factors for bacteraemia in dialysis patients, including leukocyte dysfunction, malnutrition, parathyroid hormone derangements, and vitamin D deficiency[8,19–21].

We focused on ALP, an enzyme that hydrolyses phosphate monoester. It is a dimer consisting of two identical molecules, and is expressed as four isoenzymes (placental, germ cell, intestinal, and tissue-nonspecific [liver/bone/kidney])[22]. ALP is known as an indicator of renal osteodystrophy, associated with its close relationship with bone, parathyroid gland function, the GI tract, and overall mineral balance[23]. Historically, high ALP levels have been considered related to renal osteodystrophy.

Damera et al. reported that ALP is one of the inflammatory markers which are independent of 25-OH vitamin D levels in CKD[24]. In addition, the 'BAC-HD' (Body temperature ≥ 38.3°C, ALP > 360 U/L, C-reactive protein [CRP] ≥ CRP 10 mg/dL, Heart rate

≥ 125 bpm, Drugs: no prior antibiotic use for 1 week) score[25], which we previously developed, is a clinical prediction algorithm for bacteraemia among patients with HD.

Tung et al. showed that extremely high ALP levels (ALP > 1000 U/L) were associated with bacteraemia[26]. However, that study had a very small sample size of 16. In other words, there are few studies showing an association between serum ALP and short-term prognosis of bacteraemia and in-hospital mortality.

ALP levels can be measured easily and are a less burdensome test for the patient. In addition, bacteraemia is an important outcome for haemodialysis patients because of its high morbidity and mortality. Therefore, it is important to investigate serum ALP levels as predictive markers of bacteraemia. Our aim was to verify the association of ALP levels and bacteraemia or death in maintenance HD patients suspected of bacteraemia in an outpatient setting.

METHODS

This study was approved by the ethics committees of Aso Iizuka Hospital (No. 17167), Okinawa Chubu Hospital (H28_No. 51), and Saku Central Hospital (201701-01), and was conducted in accordance with the ethical standards of the Declaration of Helsinki. In the

present study, the Department of Nephrology of Aso Iizuka Hospital had collected anonymous data from the participating facilities. Since this study was retrospective, the consent of participants was not obtained. The study results are reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies[27].

Study design and participants

We performed a cross-sectional study at the three academic medical institutions mentioned above. Data were collected from medical records from January 2013 to December 2015 in each facility. We enrolled consecutive cases of patients on maintenance HD who were aged ≥ 18 years and had had two sets of blood cultures drawn at admission to assess for the presence of bacteraemia. Cases of hospitalised patients who had been transferred from another hospital, had a vintage of dialysis < 2 months, were also undergoing peritoneal dialysis (PD), or were receiving HD less than once a week were excluded (Fig. 1).

ALP levels

Logistic regression analysis was performed with bacteraemia as the dependent variable and ALP as the explanatory variable. Based on the ROC analysis, the value with the highest discriminatory power was used as the cut-off point.

Outcomes

The primary outcome was bacteraemia, which was diagnosed based on the results of admission blood cultures. To avoid misclassification of the primary outcome, an external consensus panel of infectious disease physicians with more than ten years' clinical experience and Japanese board certification in infectious disease determined whether a culture was contaminated or not based on the conventional definition of contamination and their clinical expertise. Contamination was defined as: only one of the two sets of culture bottles was positive; or the presence of certain species of bacteria, such as diphtheroids, *Bacillus* spp., *Propionibacterium* spp., *Micrococci spp.*, *Corynebacterium* spp., and coagulase-negative staphylococci. The secondary outcome was in-hospital death.

Other Covariates

Clinical information collected on hospital admission included age, sex, body temperature, systolic and diastolic blood pressure, pulse rate, respiratory rate, haemodialysis vintage, presence or absence of diabetes mellitus, and use of vitamin D analogues. In addition, white blood cell counts (WBC), aspartate aminotransferase (AST), total bilirubin (T-BIL), corrected calcium (cCa), phosphate (P), and C-reactive protein (CRP) were obtained from medical records.

Statistical analysis

The serum ALP levels at diagnosis were stratified by the cut-off value based on ROC analysis, and patients' baseline characteristics were expressed as medians (quartile) or numbers (%). Multivariate analysis was performed for the primary outcome of bacteraemia in four models adjusted for age, sex, aspartate aminotransferase (AST), vitamin D analogue use, and haemodialysis vintage (Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage). Five models were used for the secondary outcome: in-hospital death, adjusted for age, sex, AST, T-BIL, vitamin D analogue use, cCa, P, haemodialysis vintage, and presence of bacteraemia using a logistic regression model

(Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage; Model 5, adjusted for Model 4 + presence of bacteraemia, Fig. 2). We selected variables for multivariate analysis through a literature review and based on clinical experience. To minimise the bias from missing data, all missing values were imputed using multiple imputation by chained equation (MICE) treated as missing at random including ALP; ten imputed datasets were created[28]. On multivariate analysis, these ten datasets were combined with Rubin's rules and analysed. Analyses were assessed at a two-tailed alpha = 0.05. We used commercial software (STATA 15.0, StataCorp LP, College Station, TX, USA) for statistical analysis.

Sample Size

We estimated the prevalence of bacteraemia in maintenance HD patients suspected to have bacteraemia to be 16% based on a previous report[25]. Since we planned a logistic regression analysis with five explanatory variables, we estimated that the number of bacteraemia cases was required to be 50, following the rule of requiring ten outcomes per explanatory variable[29]. From these, it was estimated that a total of 312 subjects was needed.

Sensitivity analysis

To demonstrate the robustness of our inferences, we conducted a complete case analysis for ALP as a sensitivity analysis, which meant excluding participants missing admission ALP. In addition, we added CRP, which is not a confounding factor but is a strong prognostic factor, and performed a sensitivity analysis.

Patient and public involvement

No current patients or members of the public were directly involved in this study.

RESULTS

The cut-off value for ALP was 360 U/L based on ROC analysis (AUC 0.60, sensitivity 0.49, specificity 0.76) in complete cases of ALP. Among the 315 cases included in the study (Figure 1), 187 had baseline measured ALP levels (133 with normal levels ≤ 360 U/L and 54 with ALP levels > 360 U/L). Table 1 shows the baseline characteristics of the cohort.

Table 1. Baseline characteristics

	ALP ≤ 360 U/L N =133	ALP > 360 U/L N = 54	Total N = 315	Missing (N)
Age, years, median (IQR)	73 (66, 80)	72 (62, 79)	73 (63, 80)	0
Sex				0
males, n (%)	77 (57.9)	26 (48.1)	178 (56.5)	
females, n (%)	56 (42.1)	28 (51.9)	137 (43.5)	
Diabetes mellitus, n (%)	64 (48.1)	27 (50.0)	159 (50.5)	0
Systolic blood pressure, mmHg, median (IQR)	134 (110, 150)	134 (11, 150)	134 (110, 150)	2
Diastolic blood pressure, mmHg, median (IQR)	70 (60, 80)	70 (60, 80)	70 (60, 80)	22
Pulse rate, beats/minute, median (IQR)	90 (78, 102)	92 (84, 108)	90 (78, 102)	4
Respiratory rate, per minute, median (IQR)	20 (18, 24)	20 (18, 24)	20 (18, 24)	43
Body temperature, °C, median (IQR)	37.3 (36.5, 38.0)	37.6 (36.9, 38.3)	37.2 (36.5, 38.0)	6
Laboratory data				
WBC (× 10^3/μL), median (IQR)	8.7 (6.2, 12.4)	8.6 (6.1, 11.3)	8.4 (6.2, 12.0)	2
ALP (U/L), median (IQR)	237 (203, 280)	502 (404, 780)	271 (219, 376)	128
AST (U/L), median (IQR)	17 (12, 25)	24 (18, 55)	18 (13, 25)	7
ALT (U/L) , median (IQR)	10 (7, 15)	18 (12, 38)	11 (7.5, 17)	7
T-Bill (mg/dl) , median (IQR)	0.5 (0.3,0.6)	0.6 (0.4, 1.5)	0.5 (0.3, 0.7)	17
Ca (mg/dL) , median (IQR)	8.8 (8.4, 9.3)	8.7 (8.3, 9.4)	8.8 (8.4, 9.4)	93
P (mg/dL) , median (IQR)	4.4 (3.3, 5.8)	5.3 (4.1, 6.6)	4.7 (3.8, 6.1)	284
CRP (mg/dL) , median (IQR)	5.2 (2.1, 11.2)	6.0 (1.5, 12.3)	5.5 (2.1, 12.1)	31
Haemodialysis vintage, months, median (IQR)	51 (17.5, 114)	58 (18, 139)	55 (20, 115)	14
Vitamin D analogue use, n (%)	60 (45.1)	25 (46.3)	134 (42.5)	2
Vascular access				
arteriovenous fistula, n (%)	86 (64.7)	44 (81.5)	130 (41.3)	0
arteriovenous graft, n (%)	11 (8.3)	2 (3.7)	13 (4.1)	0

arteriovenous shunt, n (%)	5 (3.8)	2 (3.7)	7 (2.2)	0
temporary catheter, n (%)	30 (22.6)	6 (11.1)	36 (11.4)	0

This table shows the baseline characteristics of the cohort.

Abbreviation: ALP, alkaline phosphatase; WBC, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin; Ca, calcium; P, phosphorus; CRP, C-reactive protein; IQR Interquartile range

Occurrence of Outcomes

Table 2 shows the incidence of bacteraemia and in-hospital deaths in the total and groups stratified by ALP. The high-ALP group had a higher incidence of bacteraemia.

Table 2. Incidence of bacteraemia and in-hospital death in the total and groups stratified by ALP

	ALP ≤ 360 U/L N = 133	ALP > 360 U/L N = 54	total N = 315	Missing (N)
Bacteraemia, n (%)	20 (15.0)	19 (35.2)	50 (15.9)	11
In-hospital death, n (%)	17 (12.8)	9 (16.7)	48 (15.2)	22

This table shows the incidence of bacteraemia and in-hospital deaths in the total and groups stratified by ALP. The high-ALP group had a higher incidence of bacteraemia.

Abbreviations: ALP, alkaline phosphatase

Association of ALP in hospital visit and bacteraemia

In the multivariate analysis shown in Figure 2, there was a statistically significant association between higher ALP in hospital visit and bacteraemia in all four models.

Association of ALP in hospital visit and in-hospital death

As shown in Figure 2, there were no statistically significant associations between higher ALP and in-hospital death in all five models.

Sensitivity Analysis

To examine the robustness of the findings, we conducted a complete case analysis for ALP excluding participants who were missing ALP values. A sensitivity analysis of the 187 patients with no missing ALP values also demonstrated a significant association between ALP and bacteraemia, but no significant association between ALP and in-hospital death (Fig.

2). In a sensitivity analysis with the addition of CRP, results showed no significant association between bacteraemia and ALP levels in analysis adjusted for age, sex, AST, CRP, vitamin D analogue use, or haemodialysis vintage (OR: 1.97, 95% CI: 0.97 to 4.01) as shown in the Supplementary Figure.

DISCUSSION

This study showed a statistically significant positive correlation between ALP levels and bacteraemia in HD patients suspected of having bacteraemia in the outpatient setting. Few studies examining the association between serum ALP and short-term prognosis have been reported. This is the first multicentre investigation of the association between ALP levels and bacteraemia or death in patients on maintenance HD.

Based on the results of this study, elevated serum ALP levels in haemodialysis patients with suspected bacteraemia could allow for early recognition and may potentially allow for earlier medical intervention.

Association between ALP and bacteraemia

We considered two reasons why elevated ALP levels were associated with bacteraemia. First is the involvement of hepatobiliary infections such as cholangitis. We hypothesise that it may cause bacteraemia or sepsis, leading to elevated ALP levels[30,31]. However, since the main cause of bacteraemia in HD patients is bloodstream infection with staphylococci, it is considered that bacteraemia due to biliary tract infection does not significantly affect ALP levels in this population. In addition, we adjusted for the liver enzyme AST in multivariate analysis, but the changes in the OR of bacteraemia were small. These findings suggest that the increase in ALP levels in HD patients was due to factors other than hepatobiliary infection.

Second, we considered a biological response to bacteraemia. Previous studies have shown that ALP acts on inflammatory mediators, such as bacterial endotoxin and extracellular adenosine triphosphate, and may detoxify them via dephosphorylation[10,12,32–34]. In animal models of sepsis (mice, rats, sheep, piglets), it has been reported that treatment with ALP reduced systemic inflammation and organ dysfunction, and improved survival[32,35–39]. There are also reports suggesting that ALP is effective in the treatment of sepsis in HD patients[40]. Sepsis-related AKI is thought to be the result of a combination of inflammatory, nephrotoxic, and ischemic injury with rapid

progression of renal damage. Pickkers et al. showed that treatment with ALP improved creatinine clearance, as well as the need for and duration of dialysis in patients with sepsis-related AKI[41].

The above two points suggest that the increase in ALP may be a response to inflammation or bacteraemia.

In maintenance haemodialysis patients with a high risk of infection, the therapeutic strategy, including antimicrobials, is often distressing until the results of blood culture are available. Unnecessary administration of antimicrobials can be harmful to the patient, because antimicrobial resistance is a serious problem for them. However, it has also known that delayed administration of empiric antimicrobial therapy leads to increased mortality.[42] We need to decide the timing of administration of therapy and choice of antimicrobial agents appropriately. Serum ALP levels have been reported as one example of a simple clinical prediction rule in the bacteraemia 'BAC-HD score'.[43] In maintenance HD outpatients suspected of sepsis, elevated serum ALP levels may indicate the presence of bacteraemia and may aid in the decision to begin early antimicrobial therapy and in the choice of the antimicrobial agent.

ALP isozymes

Intestinal isozyme may be of possible relevance to sepsis-related treatment.[33, 40] However, no association has been found between specific isozymes and bacteraemia or sepsis, and we do not recommend the measurement of isozymes at this time in clinical practice. If the above two points are resolved, it may be useful to measure ALP isozymes in the future.

The species associated with bacteraemia

It is known that percutaneous bloodstream infections caused primarily by gram-positive cocci (GPC) are common in HD patients[44]. However, a previous meta-analysis reported that about 20% of haemodialysis catheter-related bacteraemias were caused by gram-negative rods (GNR) as well as coagulase-negative staphylococci and *Staphylococcus aureus*[45].

In our study, GNR-induced sepsis accounted for 34% of cases, which may have been associated with ALP levels. However, the median quartile values of ALP in bacteraemia due to GPC and GNR were 302 (range, 217, 455) U/L and 388 (range, 225, 530) U/L, respectively, and there may be reasons other than this hypothesis. Second, given the mechanism by which GPC inactivates inflammatory mediators, ALP can be elevated not only by GNR but also by GPC-induced sepsis[46]. From the above, it is considered that ALP is

associated with bacteraemia in HD patients regardless of the category of the offending bacterium.

Association between ALP and mortality

We found no significant association of ALP with mortality in the analysis for secondary outcome, in contrast to previous studies[10,14,47]. In one study, HD patients with elevated ALP levels had an approximately 50% higher risk of infection-related mortality compared to those with normal ALP levels[14]. One reason for the significant difference in bacteraemia but not in mortality may be that the overall prognosis for maintenance HD patients in Japan is good.

Limitations

Our study has several limitations. First, there may be unmeasured confounding factors, a limit of observational studies. However, the study was designed to optimise the selection of adjusted confounding factors and to minimise their effect as compared with previous studies. It is possible that intact PTH was a residual confounding factor. However, we could not test this possibility because we did not measure intact PTH in this study, for two reasons: first,

because intact PTH may not contribute significantly to outcomes for bacteremia or mortality [48]; and second, since ALP reflects factors of origin other than bone, we considered that the association between PTH and ALP in the acute phase, such as the subject of this study, might be still unclear. Nevertheless, there are reports of increased mortality in patients with PTH outside the normal range in the non-acute phase, [49] and further validation is needed. Second, since it is a cross-sectional study, the possibility of reverse causation cannot be denied. However, high ALP levels were shown to be a predictor of bacteraemia. Third, this was a retrospective study, and the uncertainty of the data extracted from medical records cannot be ruled out. Fourth, while we conducted a multicentre study, the sample size was relatively small and there were substantial missing data. In patients with ALP data, there was a statistically significant association between ALP and bacteraemia, but no association between ALP and in-hospital mortality. We consider the small sample size as a reason why we could not show an association with mortality, unlike previous reports. This is the first study suggesting that serum ALP is one of several independent predictors of bacteraemia in HD patients. Our study should facilitate further validation studies to confirm the association of ALP elevation and bacteraemia in maintenance HD patients. Fifth, it cannot be determined in this study whether serum ALP levels were elevated before illness or due to bacteraemia.

However, baseline serum ALP levels are often unknown in clinical practice. Therefore we consider it may be clinically acceptable. Lastly, the study sample consisted of patients on maintenance HD from three geographically diverse hospitals in Japan, and our findings may not be generalisable to patients on maintenance HD in other clinical settings (e.g., patients with hospitalisation at index dates). Nonetheless, our inferences should remain relevant for over 340,000 patients on maintenance HD in Japan, a vulnerable population with high mortality from bacteraemia, at about 14 times that of the general population [50].

CONCLUSIONS

By conducting a multicentre retrospective observational study, we identified elevation of ALP levels as an independent predictor of bacteraemia among maintenance HD outpatients suspected of having sepsis. The association remained consistent after adjusting for other potential predictors for bacteraemia. For clinicians, our data may support the early identification of patients with bacteraemia and their resultant prompt hospitalisation. Our findings may facilitate further research to investigate any causal association of ALP elevation with bacteraemia in complex biological systems.

Contributors: All authors have read and approved the submission of the manuscript; the manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language, except as an abstract. Sho Sasaki (SS) created the study design. SS, Yoshihiko Raita, Shungo Yamamoto, Kentaro Tochitani, Minoru Murakami, and Ryo Nishioka performed data collection. Aya Katasako and SS analysed data, and wrote the article. All authors reviewed the manuscript. Kiichiro Fujisaki approved the submission of the article.

Competing interests: All of authors declare that they have no relevant financial interests.

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Data sharing statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient consent for publication: Not required

Ethics statement: This study was approved by the ethics committees of Aso Iizuka Hospital (No. 17167), Okinawa Chubu Hospital (H28_No. 51), and Saku Central Hospital (201701-01).

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Figure legends

Figure 1. Study flow

After the sampling, 315 cases that met the eligibility criteria were included.

Figure 2. Association between ALP and bacteraemia or In-hospital death: logistic regression model

Multivariate analysis shown in this Figure. There was no relationship between higher ALP and in-hospital death, however there was a statistically significant association between higher ALP and bacteraemia.

Bacteraemia outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase, Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage

In-hospital death outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage; Model 5, adjusted for Model 4 + presence of bacteraemia

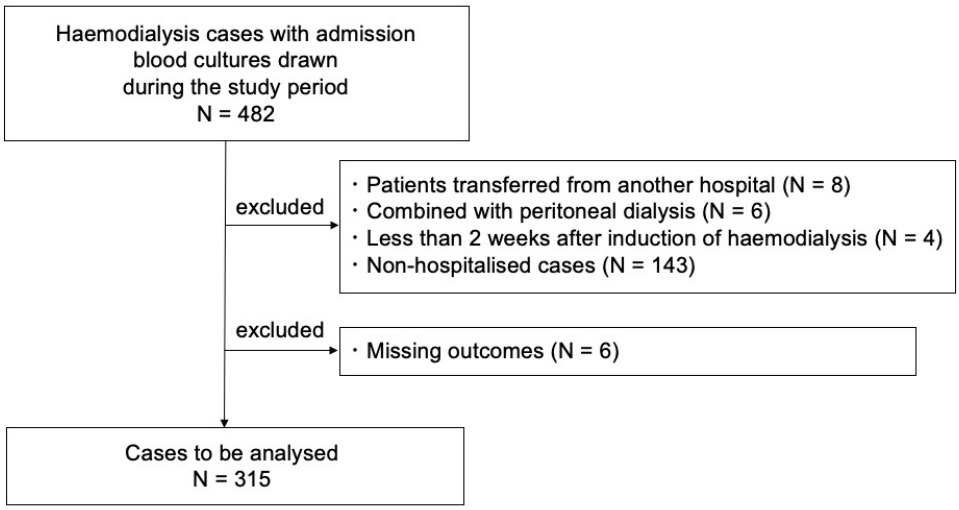
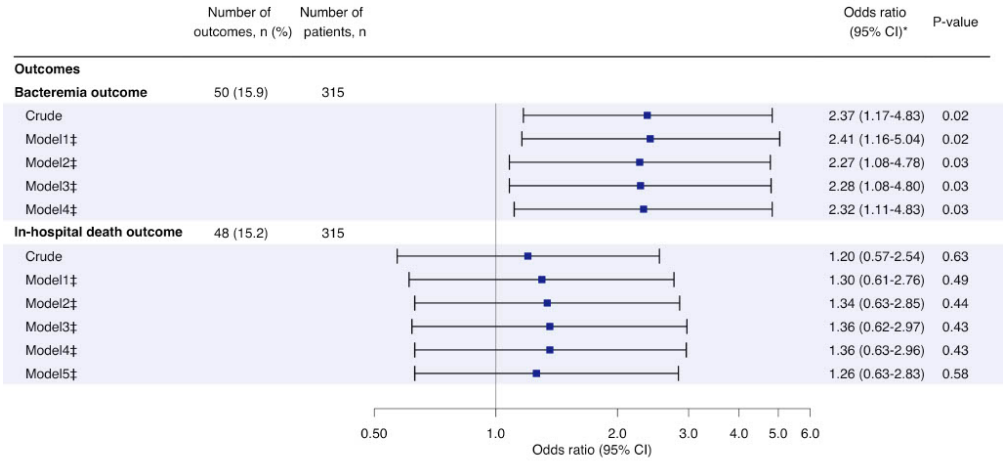


Figure 1. Study flow
After the sampling, 315 cases that met the eligibility criteria were included.

254x190mm (96 x 96 DPI)



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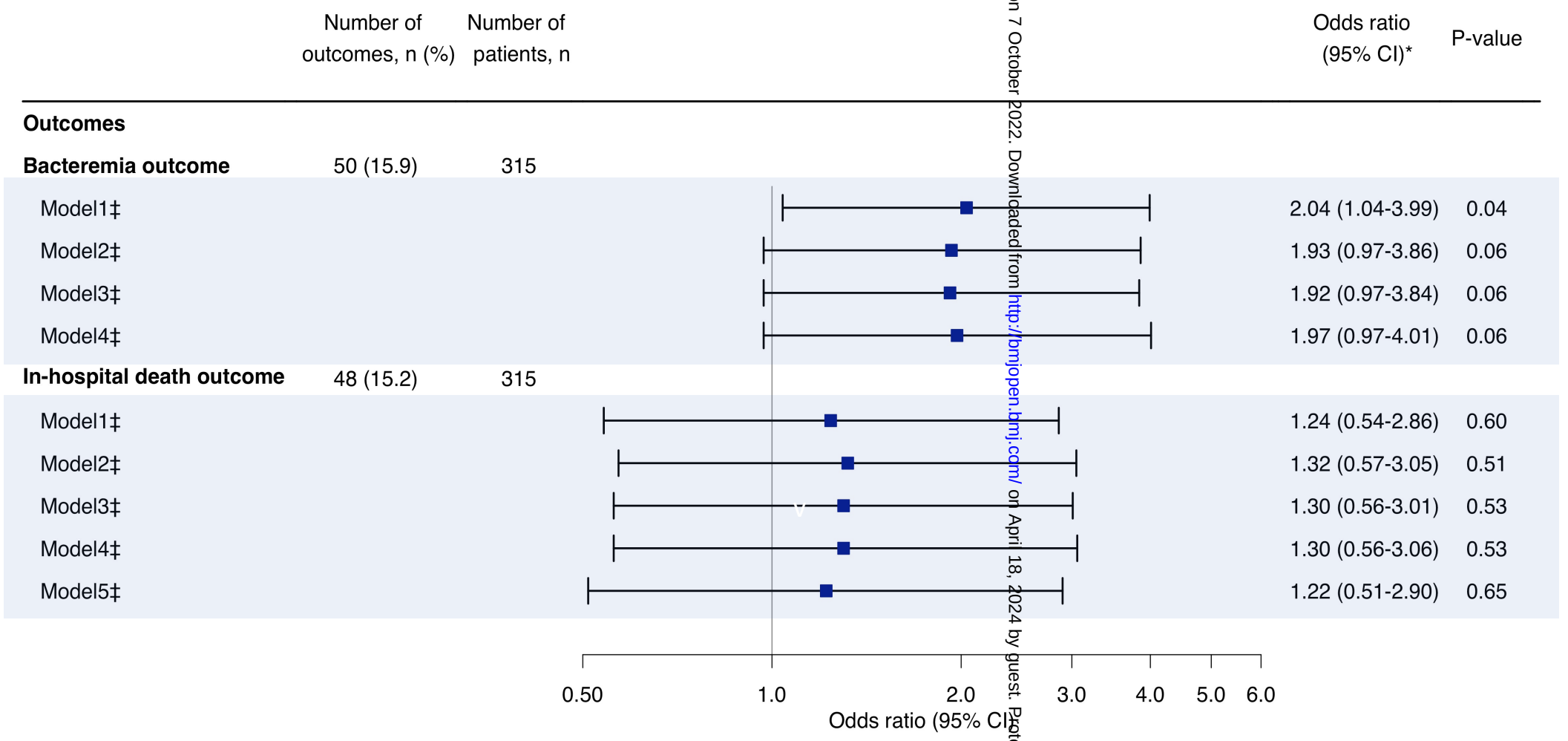
Supplementary Figure: Sensitivity analysis with the addition of CRP



Bacteraemia outcome: Model 1, adjusted for age, sex; Model 2, adjusted for Model 1 + aspartate aminotransferase + CRP; Model 3, adjusted for Model 2 + vitamin D analogue use; Model 4, adjusted for Model 3 + haemodialysis vintage

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3-5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-11
Bias	9	Describe any efforts to address potential sources of bias	9-10
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10-11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10-11
		(*b*) Describe any methods used to examine subgroups and interactions	10-11
		(*c*) Explain how missing data were addressed	10-11
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	10-11
		(*e*) Describe any sensitivity analyses	11
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	12
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-13
		(b) Indicate number of participants with missing data for each variable of interest	12-13
Outcome data	15*	Report numbers of outcome events or summary measures	13-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-15

		(*b*) Report category boundaries when continuous variables were categorized	-
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	15
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	20-21
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-21
Generalisability	21	Discuss the generalisability (external validity) of the study results	17-18, 20-21
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	22

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.