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Posted Date: 20 June 2023

doi: 10.20944/preprints202306.1442.v1

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## Article

# What about alkaline phosphatase in endocarditis?

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**Abstract:** (1) Infective endocarditis is a severe inflammatory disease associated with substantial mortality and morbidity. Alkaline phosphatase (AP) levels have been shown to change significantly during sepsis additionally we previously found higher initial AP drop after cardiac surgery is associated with unfavorable outcomes. Therefore, the course of AP after surgery for endocarditis is of special interest. (2) 314 patients with active isolated left-sided infective endocarditis at the Department of Cardiac Surgery (Medical University of Vienna, Austria) between 2009 and 2018 were enrolled in this retrospective analysis. Blood samples were analyzed at different time points (baseline, postoperative day 1-7, postoperative days 14, and 30). Patients were categorized according to relative alkaline phosphatase drop ( $\geq 30\%$  vs.  $< 30\%$ ). (3) The rate of postoperative renal replacement therapy with or without prior renal replacement therapy (7.4 vs. 21.8%;  $p=0.001$  and 6.7 vs. 15.6%;  $p=0.015$ , respectively) and extracorporeal membrane oxygenation (2.2 vs. 19.0%;  $p=0.000$ ) was observed after a higher initial alkaline phosphatase drop. Short-term (30-day mortality 3.0 vs. 10.6%;  $p=0.010$ ) and long-term mortality ( $p=0.008$ ) were significantly impaired after a higher initial alkaline phosphatase drop. (4) The higher initial alkaline phosphatase drop was accompanied by impaired short and long-term outcomes after cardiac surgery for endocarditis. Future risk assessment scores for cardiac surgery should consider alkaline phosphatase.

**Keywords:** alkaline phosphatase; cardiac surgery; cardio-pulmonary bypass; endocarditis; kidney

## Introduction

Infective endocarditis (IE) is a rare but severe manifestation of valvular disease associated with significant morbidity and mortality. [1] The Duke criteria are used for diagnostic purposes. [2] Besides extended antimicrobial therapy, surgery should be performed in case of heart failure, uncontrolled infection and in order to prevent embolism. If indicated, surgery should be performed as early as possible. [3, 4]

Transient bacteremia, as it may occur after dental, gynecologic, gastrointestinal, or urologic procedures or even after daily activities such as brushing teeth, may lead to bacterial colonization of heart valves resulting in sepsis, causing a spectrum of end-organ damage. [5] With up to two-thirds developing acute kidney injury (AKI) in the setting of IE, end-organ damage of the kidney is one of the most common. [6] Renal inflammation and resulting hypoxia are considered to be the causative. [7] The binding of pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) to toll-like receptor 4 leads to the further release of inflammatory mediators. [8] The pervasive alkaline phosphatase (AP) neutralizes LPS and converts proinflammatory molecules to anti-inflammatory ones (such as adenosine). [7, 9, 10] (Severely) elevated AP levels have been considered a surrogate parameter for infection or bacteremia. [11-13] Consequently, AP has been considered a treatment target for sepsis patients in improving AKI or survival (in animal models). [7, 14, 15]

Additionally, to the inflammatory process caused by endocarditis, cardiac surgery induces some systemic inflammation due to the heart-lung machine (HLM). In up to 30%, a systemic inflammatory response syndrome (SIRS) occurs, associated with an elevated risk of end-organ injury. [16-18] No sole mechanism is responsible, but various factors are considered contributing [7, 19-22] Increased consumption of AP during cardiac surgery or extracorporeal membrane oxygenation is associated with a higher morbidity and mortality rate. [23-26]

This study aims to evaluate the AP metabolism in the setting of an inflammatory state in IE patients undergoing surgery. To our knowledge, no study evaluating the course of AP following cardiac surgery for IE has been published previously.

## 2. Results

### *Patient Population and Cut-off Values*

After reviewing the exclusion criteria 314 patients were included in the retrospective analysis. According to an initial drop in AP of  $\geq 30\%$  (with an area under curve (AUC) 0.638; sensitivity 71%, specificity 53% for 1-year mortality) two groups were created– with 179 patients with an AP drop  $\geq 30\%$  and 135 patients with an AP drop  $< 30\%$ . The selection of cut-off values was guided by previous research findings. [25, 26] In the following sections, the first value always refers to the lower initial AP drop cohort.

### *Preoperative Characteristics*

The higher initial AP drop cohort was at significantly higher surgical risk (EuroScore II 9.1 (15.9;3.5) vs. 16.1 (34.3;6.4) %;  $p=0.000$ ), had a significantly higher rate of preoperative dialysis (5.9 vs. 13.4%;  $p=0.030$ ) and prosthetic valve endocarditis (PVE) (20.0 vs. 40.2%;  $p=0.000$ ). All other patient characteristics were similar among the cohorts (Table 1).

**Table 1.** Preoperative characteristics.

	Overall n=314	AP drop < 30% n=135	AP drop ≥ 30% n=179	p-value <sup>a</sup>	
Age	62 (70;48)	59 (69;45)	62 (70;49)	0.230	
Female	85 (27.1)	37 (27.4)	48 (26.8)	0.907	
BMI	25.3 (28.9;22.8)	25.1 (28.9;22.9)	25.6 (28.7;22.5) <sup>†</sup>	0.703	
EuroScore II	11.7 (26.4;5.5)	9.1 (15.9;3.5)	16.1 (34.3;6.4)	0.000*	
NYHA III	78 (24.8)	34 (25.2)	44 (24.6)	0.902	
NYHA IV	89 (28.3)	35 (25.9)	54 (30.2)	0.409	
LVEF	60 (60;55)	60 (60;55)	60 (60;55)	0.042*	
Hypertension	200 (63.7)	79 (58.5)	121 (67.6)	0.098	
Atrial fibrillation	90 (28.7)	38 (28.1)	52 (29.1)	0.861	
IDDM	15 (4.8)	6 (4.4)	9 (5.0)	0.810	
Preoperative dialysis	32 (10.2)	8 (5.9)	24 (13.4)	0.030*	
Cancer	19 (6.1)	10 (7.4)	9 (5.0)	0.381	
h/o stroke	123 (39.2)	55 (40.7)	68 (38.0)	0.621	
Modified Duke Criteria					
Major criteria	Positive BC	259 (82.5)	107 (79.3)	152 (84.9)	0.192
	Vegetation	266 (84.7)	114 (84.4)	152 (84.9)	0.908
	Annular abscess	131 (41.7)	55 (40.7)	76 (42.5)	0.760
Minor criteria	IV drug abuse	24 (7.6)	11 (8.1)	13 (7.3)	0.770
	Fever > 38°C	201 (64.0)	81 (60)	120 (67.0)	0.198
	Vascular phenomena	161 (51.3)	67 (49.6)	94 (52.5)	0.613
	Immunologic phenom- ena	19 (6.1)	11 (8.1)	8 (4.5)	0.176
PVE	99 (31.5)	27 (20.0)	72 (40.2)	0.000*	

Preoperative ventilation	142 (45.2)	64 (47.4)	78 (43.6)	0.499
Preoperative inotropic support	88 (28.0)	32 (23.7)	56 (31.3)	0.139
CPR	13 (4.1)	5 (3.7)	8 (4.5)	0.736
Lactate value	0.9 (1.3;0.7)	0.8 (1.2;0.7)	1.0 (1.3;0.7)	0.013*

All values are referred in median (Q3;Q1) or in total number (n) and percentage (%) if not stated otherwise | <sup>†</sup> normal distributed, median taken for better comparison | <sup>a</sup> If not stated otherwise, the Mann-Whitney *U* Test and Pearson's chi-squared test, respectively, were used; values marked with an asterisk (\*) achieved statistically significance | age [years]; BC – blood culture; BMI – body mass index [kg/m<sup>2</sup>]; CPR – cardiopulmonary resuscitation; lactate [mmol/l]; EuroSCORE II: European System for Cardiac Operative Risk Evaluation [%]; h/o – history of; IDDM – insulin dependent diabetes mellitus; IV – intravenous; NYHA – new york heart association classification; LVEF – left ventricular ejection fraction [%]; PVE – prosthetic valve endocarditis.

### Procedural Data

A significantly higher rate of re-sternotomy (21.5 vs. 40.8%;  $p=0.000$ ) and double valve replacement (8.9 vs. 22.9%;  $p=0.001$ ) were observed after an initial higher AP drop. Surgical times were prolonged in the initial higher AP cohort: total surgery time (240 (300;195) vs. 348 (455;255) min;  $p=0.000$ ), total cardiopulmonary bypass time (110 (155;90) vs. 162 (237;117) min;  $p=0.000$ ) and total aortic cross clamp time (81 (113;62) vs. 111 (159;79) min;  $p=0.000$ ). Procedural data is illustrated in Table 2.

**Table 2.** Procedural data.

	Overall n=314	AP drop < 30% n=135	AP drop ≥ 30% n=179	p-value <sup>a</sup>
Urgent operation	262 (83.4)	118 (87.4)	144 (80.4)	0.100
Emergency operation	49 (15.6)	16 (11.9)	33 (18.4)	0.111
Salvage operation	3 (1.0)	1 (0.7)	2 (1.1)	0.734
Full sternotomy	303 (96.5)	128 (94.8)	175 (97.8)	0.159
Re-sternotomy	102 (32.5)	29 (21.5)	73 (40.8)	0.000*
Isolated AVR	168 (53.5)	84 (62.2)	84 (46.9)	0.007*
Isolated MVR	93 (29.6)	39 (28.9)	54 (30.2)	0.806
Double valve replacement	52 (16.9)	12 (8.9)	41 (22.9)	0.001*
Surgery time	276 (390;220)	240 (300;195)	348 (455;255)	0.000*
CPB	139 (204;104)	110 (155;90)	162 (237;117)	0.000*
ACC	100 (144;70)	81 (113;62)	111 (159;79)	0.000*

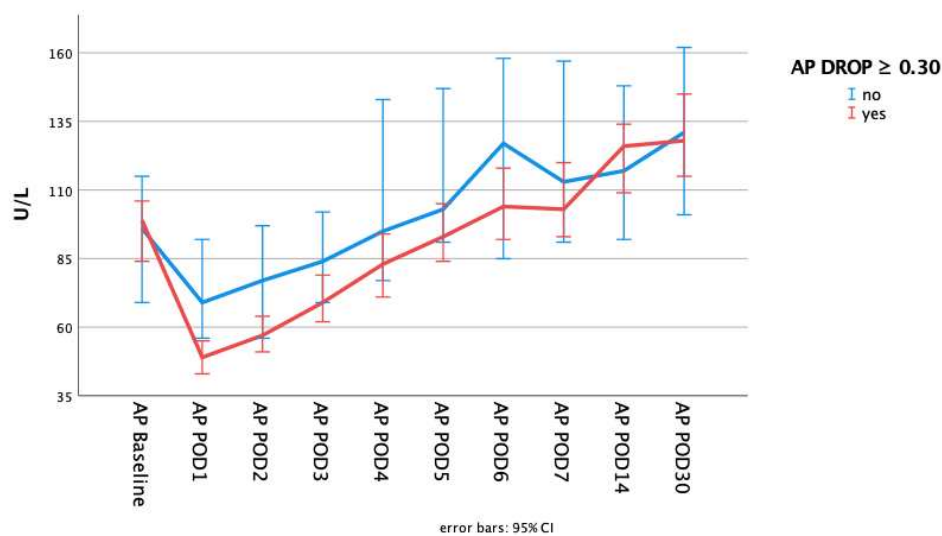
All values are referred in median (Q3;Q1) or in total number (n) and percentage (%) | <sup>a</sup> If not stated otherwise, the Mann-Whitney *U* Test and Pearson's chi-squared test, respectively, were used; values marked with an asterisk (\*) achieved statistically significance | ACC – aortic cross clamp time in minutes; AVR – aortic valve replacement; CPB – cardiopulmonary bypass in minutes; MVR – mitral valve replacement.

### Laboratory data

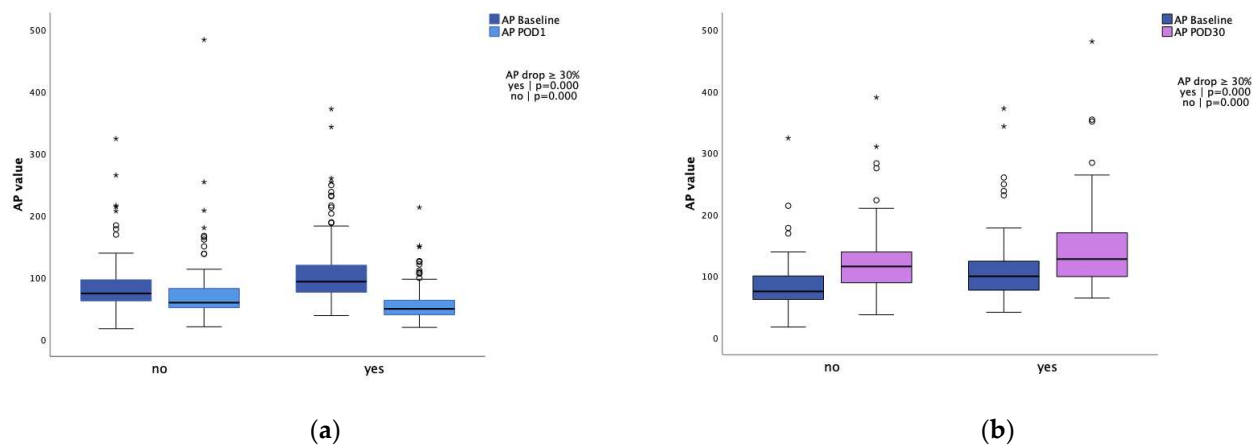
While AP values at baseline were significantly higher in the higher initial AP drop cohort (74 (96;62) vs. 93 (120;76) U/L;  $p=0.000$ ), there was no significant difference at 30 days (116 (141;89) vs. 128 (172;99) U/L;  $p=0.089$ ). Patients with the higher initial AP drop required a longer time to reach baseline values (days till baseline value surpassed: 4 (6;3) vs. 6 (7;4) days;  $p=0.000$ ; baseline within 3 days: 43.7 vs. 33.0%;  $p=0.052$ ; baseline within 5 days: 74.1 vs 60.3 %;  $p=0.011$ ). The postoperative course of AP dependent on the initial AP drop is depicted in Figure 1. On postoperative day (POD) 30 AP levels were significantly higher compared to baseline in both cohorts ( $p=0.000$  for both). Figure 2 illustrates the differences of AP levels at baseline to POD 1 and POD 30.

Although there was no statistic significant difference at baseline CRP values (3.9 (8.3;1.7) vs. 4.5 (11.7;1.5) mg/dL;  $p=0.456$ ), a significantly higher value after 30 days in the higher initial AP drop

cohort was observed (2.1 (5.7;0.7) vs. 4.6 (10.6;1.8) mg/dL; p=0.001). Laboratory values are provided in Table 3.



**Figure 1.** Alkaline phosphatase blood values dependent in the initial AP drop at baseline, on each consecutive day until POD 7 as well as on POD 14 and POD30.



**Figure 2.** Comparison of alkaline phosphatase levels at baseline to different postoperative timepoints dependent on the initial drop of alkaline phosphatase. P-values calculated for the difference in alkaline phosphatase between the timepoints using Wilcoxon signed rank test. (a) Comparison AP baseline to POD 1 (b) Comparison AP baseline to POD 30.

**Table 3.** Laboratory data.

	Overall n=314	AP drop < 30% n=135	AP drop ≥ 30% n=179	p-value <sup>a</sup>
Baseline AP value	85 (112;69)	74 (96;62)	93 (120;76)	0.000*
Baseline CRP value	4.2 (10.7;1.6)	3.9 (8.3;1.7)	4.5 (11.7;1.5)	0.456
AP Drop	32.5 (45.2;23.0)	21.4 (25.8;11.5)	42.7 (54.7;36.4)	0.000*
First day baseline AP value surpassed <sup>a</sup>	5 (7;4)	4 (6;3)	6 (7;4)	0.000*
Baseline within 3 days	118 (37.6)	59 (43.7)	59 (33.0)	0.052

Baseline within 5 days	208 (66.2)	100 (74.1)	108 (60.3)	0.011*
30day AP value <sup>f</sup>	121 (157;95)	116 (141;89)	128 (172;99)	0.089
30day CRP value <sup>g</sup>	3.4 (7.8;1.1)	2.1 (5.7;0.7)	4.6 (10.6;1.8)	0.001*

All values are referred in median (Q3;Q1) or in total number (n) and percentage (%) |<sup>a</sup> If not stated otherwise, the Mann-Whitney U Test and Pearson's chi-squared test, respectively, were used; values marked with an asterisk (\*) achieved statistically significance | AP in U/L | AP Drop – defined as 1 – AP POD 1 / AP Baseline | CRP – C-reactive protein in mg/dL. <sup>a</sup> available for 254 patients (overall), 115 (AP drop < 30%) and 139 (AP drop ≥ 30%) <sup>f</sup> available for 140 patients (overall), 62 (AP drop < 30%) and 78 (AP drop ≥ 30%). <sup>g</sup> available for 159 patients (overall), 71 (AP drop < 30%) and 88 (AP drop ≥ 30%)

#### Adverse Events and mortality

An overview of adverse events and mortality is available in Table 4. A higher rate of revision due to bleeding (7.4 vs. 15.6%; p=0.027), postoperative renal replacement therapy with or without previous one (7.4 vs 21.8 %; p=0.001 and 6.7 vs. 15.6%; p=0.015), extracorporeal membrane oxygenation (2.2 vs. 19.0%; p=0.000) was seen after higher initial AP drop. While there was a strong trend towards prolonged hospitalization (p=0.054), a prolonged ICU stay was significantly more likely in the higher initial AP drop cohort (31.9 vs. 48.0%; p=0.004).

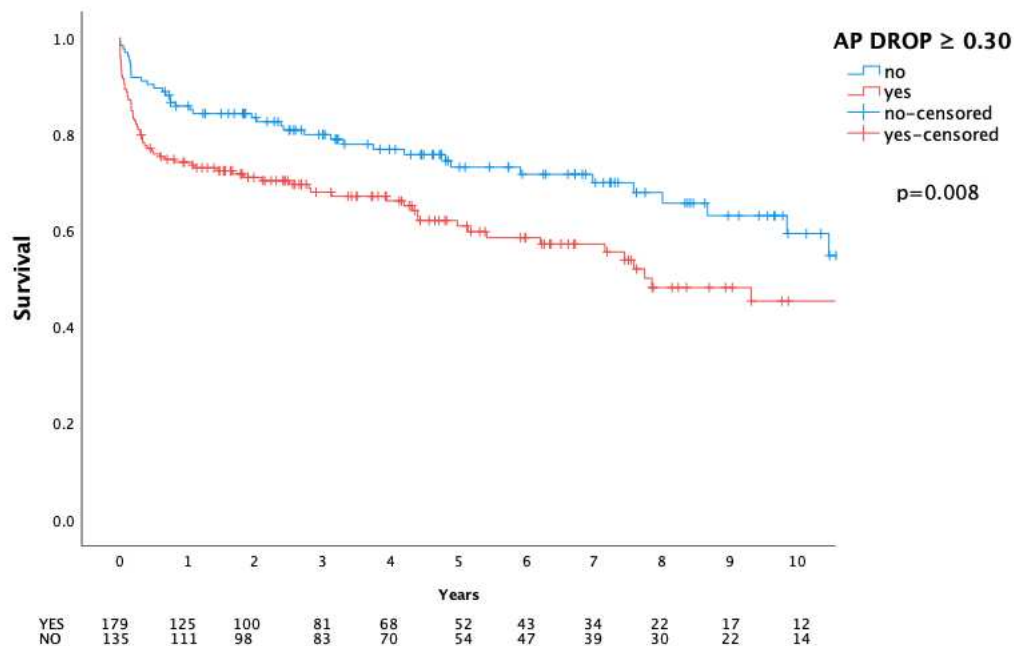
Short-term mortality (30day: 3.0 vs. 10.6; p=0.010; in-hospital: 5.9 vs. 17.3%; p=0.002; 1-year: 14.1 vs. 25.7%; p=0.012) and long-term mortality (p=0.008, see Figure 3) were significantly higher in the higher initial AP drop cohort.

**Table 4.** Adverse events and mortality.

	Overall n=314	AP drop < 30% n=135	AP drop ≥ 30% n=179	p-value <sup>a</sup>
Bleeding revision	38 (12.1)	10 (7.4)	28 (15.6)	0.027*
Need for any renal replacement therapy	49 (15.6)	10 (7.4)	39 (21.8)	0.001*
Need for any renal replacement therapy without preoperative	37 (11.8)	9 (6.7)	28 (15.6)	0.015*
Need for ECMO	37 (11.8)	3 (2.2)	34 (19.0)	0.000*
Prolonged intubation	107 (34.1)	38 (28.1)	69 (38.5)	0.054
Prolonged ICU stay	129 (41.1)	43 (31.9)	86 (48.0)	0.004*
Prolonged hospital stay	62 (19.7)	22 (16.3)	40 (22.3)	0.182
Mortality				
30 day	23 (7.3)	4 (3.0)	19 (10.6)	0.010*
In-hospital	39 (12.4)	8 (5.9)	31 (17.3)	0.002*
1 year	65 (20.7)	19 (14.1)	46 (25.7)	0.012*

All values are referred in median (Q3;Q1) or in total number (n) and percentage (%) |<sup>a</sup> If not stated otherwise, the Mann-Whitney U Test and Pearson's chi-squared test, respectively, were used; values marked with an asterisk (\*) achieved statistically significance |.

ECMO – extracorporeal membrane oxygenation; prolonged hospital stay longer than 30days; prolonged ICU (intensive care unit) stay defined as more than 7 days; prolonged intubation defined as reintubated, longer as 48h, tracheostoma;



**Figure 3.** 10-year survival. Long-term survival comparison dependent on initial drop of alkaline phosphatase. P-value calculated using log-rank test.

### 3. Discussion

In line with previous studies by Schaefer et al. (mitral valve patients with impaired left ventricular function) and Poschner et al. (postcardiotomy VA-ECMO patients), we were able to demonstrate a relation between higher initial AP drop and morbidity and mortality in patients undergoing cardiac surgery for left-sided IE. [25, 26] Not only short-term (i.e., hospital mortality) but also long-term survival (over ten years) was significantly impaired following a higher initial AP drop ( $p=0.008$ ).

Prosthetic valve endocarditis occurs in up to 20% of all endocarditis cases and is associated with a worse prognosis. [27, 28] This finding could also be highlighted in our study cohort. Indeed, PVE was approximately twice as common in the higher initial AP drop cohort (40.2% vs. 20.0%;  $p=0.000$ ). Even though preoperative AP levels were within the normal range in both cohorts, AP levels were significantly higher in the higher initial AP drop cohort (see Table 3). Interestingly, baseline AP values, as well as baseline CRP values, showed no significant difference in PVE versus native IE (baseline AP in PVE 87 (109; 71) vs. native 84 (113; 68) U/L;  $p=0.465$ ; CRP in PVE 4.3 (12.6; 1.3) vs. native 4.2 (8.7; 1.8);  $p=0.617$ ). Hence, we may conclude that PVE seems to be not related to a more pertinent systemic inflammation and that the significant impaired outcome was instead primarily due to the longer surgical times (total surgical time: PVE 389 (485;320) vs. native 245 (319;205) min; total cardiopulmonary bypass time PVE 192 (266;147) vs. native 118 (172;94) min and total aortic cross-clamp time PVE 121 (173;95) vs. native 82 (120;62) min all with a  $p=0.000$ ). As demonstrated by Doenst T et al., Nissinen J et al., and others, longer operation times are an independent predictor of worse outcomes. [29, 30]. Increased permeability in the gastrointestinal tract induced by HLM leads to a wash-in of endotoxins into the bloodstream, inducing the release of proinflammatory mediators. [20, 22] AP-mediated dephosphorylation may convert a portion into anti-inflammatory substances. [31] This process leads to the consumption of systemic alkaline phosphatase. [23, 32] Thus, prolonged duration of surgery is also likely to lead to an increased decrease in AP due to prolonged exposure to endotoxins and associated mediators.

Iso-forms of AP are present in the proximal tubule cells of the kidney. Without a complete understanding of the role of AP in the kidney, animal studies have demonstrated increased levels of AP in the urine after ischemia-reperfusion injury and administration of LPS, thus, suggesting injury to the proximal tubule brush border in these situations. [33, 34] In the receiver operating characteristics (ROC) analysis in our cohort, the drop in alkaline phosphatase was associated with the need for

postoperative dialysis with an AUC of 0.668. After an initial more significant drop, the need for postoperative dialysis was significantly more frequent ( $p=0.001$ ). Interestingly, the rate of postoperative dialysis was significantly higher with and without preoperative dialysis in the cohort with a higher initial AP drop. This suggests that patients with and without injured renal parenchyma are significantly more likely to have AKI due to SIRS (represented by the higher AP drop). Patients with AKI have significantly worse outcomes after cardiac surgery. [35]

Zhang et al. highlighted the alkaline phosphatase on the surface membrane of neutrophils as a good tool for differentiating between SIRS patients with or without bacteremia. [36] Similarly, Kerner A. et al. and Tung C.B. et al. showed (severely) elevated AP levels as a surrogate parameter for bacteremia. [11, 12] However, these findings could not be confirmed in this study. In 82.5% of the total cohort, positive blood cultures were present, which were associated with a significantly elevated CRP level (CRP positive BC 4.9 (11.9; 1.9) vs. negative BC 2.7 (5.7;0.8) mg/dL;  $p=0.001$ ); however, without any differences in AP (AP positive BC 86 (114;70) vs. negative BC 79 (106;64) U/L;  $p=0.116$ ).

An impaired barrier function of the gastrointestinal tract (GIT) leads to a wash-in of LPS. Hamarneh et al. demonstrated an impaired barrier function of the GIT in patients deprived of enteral feeding due to a loss of AP expression reversible to AP supplementation. [37] Given the critical state of IE patients, they often lack adequate enteral feeding. Hence, considering additionally the inflammatory state of endocarditis patients with the high rate of need for postoperative dialysis (and the likely even higher rate of any AKI) and the further AP consumption during cardiac surgery, supplementation of AP may be especially promising in IE patients. [8] Currently, clinical trials (e.g., AP-PIRED III – ClinicalTrials.gov NCT03050476) are evaluating the external administration of AP during (elective) cardiac surgery and are expected to reduce morbidity after cardiac surgery.

Our study found a significantly higher rate of ECMO implantation after a higher initial AP drop (2.2% vs. 19.0%;  $p=0.000$ ), presumably due to the longer operation times and the higher preoperative risk found in this cohort. In-hospital mortality after postcardiotomy ECMO support is reported in up to two-thirds of patients. [38, 39] The significantly worse outcome after a higher initial AP drop may thus be attributed to or at least influenced by the higher rate of ECMO implantation (30-day mortality 3.0% vs. 10.6%;  $p=0.010$ ; in-hospital mortality 5.9% vs. 17.3%;  $p=0.002$ ; 1-year mortality 14.1% vs. 25.7%;  $p=0.012$ ). However, as already shown in previous work, ECMO support did not increase AP consumption, evident in reaching baseline values within five days in both studies. [25]

#### 4. Materials and Methods

##### *Patients*

All patients who required valve surgery for isolated left-sided IE between January 2009 and October 2022 at the Department of Cardiac Surgery (Vienna General Hospital, Austria) were included into this retrospective data analysis. The ethics committee of the Medical University of Vienna approved this retrospective study (2101/2022). Modified Duke criteria were used for IE diagnosis. [40] Exclusion criteria included IE involvement of the right side, surgical valve repair, homograft implantation and missing laboratory values at baseline or on POD 1. After reviewing the exclusion criteria 314 patients were analyzed.

##### *Laboratory Data*

Blood samples were drawn at baseline and on each of the first seven POD as well as on POD 14 ( $\pm 3$ ) and 30 ( $\pm 5$ ). Baseline was defined as a preoperative blood draw within a maximum of 7 days prior surgery. The normal range of alkaline phosphatase is between 40 and 130 U/L and is determined as a routine parameter at our department. To determine the plasma concentrations of alkaline phosphatase, enzyme kinetic measurements are performed on native or heparinized blood samples.

##### *Follow-Up*

To evaluate the individual postoperative outcome and adverse events, the local patient documentation system AKIM was used. Additionally, federal statistics (Statistics Austria, Vienna, Austria) was used to determine the survival rates.

### Statistical Analysis

Categorical variables are presented by numbers and percentages. For continuous variables, the mean  $\pm$  standard deviation (SD) is reported if the data follows a normal distribution; otherwise, the median and interquartile range (IQR) are provided. The normal distribution assumption is tested using the Kolmogorov-Smirnov test. The patients were grouped according to the initial AP drop.

$$\text{AP drop} = 1 - \text{AP POD 1} / \text{AP Baseline}$$

Cut-offs were determined using a ROC analysis for 1-year mortality. Furthermore, the first day when alkaline phosphatase returned or exceeded baseline level was determined.

The Mann-Whitney-U-Test was used to assess differences between the continuous variables with non-normal distributions. For the comparison of categorical variables, the chi-square test was applied. Differences between AP at Baseline and AP POD 1 and AP POD 30, respectively, were determined using the Wilcoxon signed-rank test. The findings are visually presented using boxplots. Survival was visualized with Kaplan Meier Curves and the differences between the groups were tested using the log rank test. All analyses were performed using SPSS version 27.0 (IBM Corp, Armonk, NY, USA). A two-sided p-value of less than 0.05 was considered to indicate statistical significance.

## 5. Conclusions

A significant higher morbidity and an impaired outcome after a higher initial drop of alkaline phosphatase at 30-days, 1-year and in the long-term survival was evident. Future risk assessment scores for cardiac surgery should consider alkaline phosphatase.

**Author Contributions:** Conceptualization, A.K., T.P., P.A., M.A., D.H., R.B., G.L., D.W.; methodology, A.K., T.P., D.W.; formal analysis, A.K., T.P., D.W.; data curation; A.K., T.P., A.S., L.A.; writing—original draft preparation; A.K., T.P., D.W.; writing—review and editing, A.K., T.P., A.S., P.A., L.A., M.A., D.H., R.B., G.L., D.W.; visualization, A.K., T.P.; supervision, M.A., G.L., D.W.; project administration, T.P., D.W.; All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethics Committee (2101/2022).

**Informed Consent Statement:** Patient consent was waived given the retrospective nature of this study.

**Conflicts of Interest:** A.K. – non to declare; T.P. – Investigator of the APPIRED III study; A.S. – non to declare; P.A. – non toe declare; L.A. – non to declare; M.A. – Investigator of the APPIRED III study; D.H. – Investigator of the APPIRED III study; R.B. – CEO of Alloksys Life Sciences BV (Wageningen, Netherlands); G.L. – Investigator of the APPIRED III study; D.W. – Investigator of the APPIRED III study

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