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The Effect of Pseudomonas Aeruginosa Infection as A Cause of Increased Procalcitonin Levels on Colon Anastomotic Leakage in Post-Laparotomy Wistar Rats

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ABSTRACT

Background: Anastomotic leakage is a serious complication with a 6–39% mortality rate. Pseudomonas aeruginosa impairs healing, while procalcitonin rises in systemic inflammation. This study assesses procalcitonin as an early predictor of leakage post-colorectal anastomosis. ***Objective:*** To evaluate the role of Pseudomonas aeruginosa infection in increasing procalcitonin levels and anastomotic leakage after laparotomy in Wistar rats. ***Method:*** An experimental study on 20 male Wistar rats post-anastomosis, with Pseudomonas aeruginosa given to the treatment group (n=10). Procalcitonin levels were measured on days 1, 3, and 5, followed by relaparotomy to assess leakage. Data were analyzed using SPSS 26. ***Results:*** Procalcitonin levels progressively increased in both groups, with significantly higher levels in the Pseudomonas aeruginosa-infected group on postoperative day 5 (mean difference: 2.786 ng/mL, p = 0.001). Significant differences were observed between the treatment and control groups (p = 0.001) and within each group over time (control: p = 0.017; treatment: p = 0.009). Chi-square analysis showed that elevated procalcitonin levels (>2 ng/mL) increased the risk of anastomotic leakage (OR: 15, 95% CI: 1.652–136.17, p = 0.015). A significant association was also found between procalcitonin levels and hypertension risk (OR: 7, 95% CI: 1.14–42.97, p = 0.033), with a relative risk of 5 in the control group (95% CI: 1.87–28.86, p = 0.024). ***Conclusion:*** Samples infected with Pseudomonas aeruginosa exhibited higher procalcitonin levels and a greater risk of anastomotic leakage following colonic laparotomy, particularly on postoperative day 5.

Keywords: anastomosis leakage; leakage; procalcitonin; pseudomonas aeruginosa.

INTRODUCTION

Anastomotic leakage is one of the most severe complications that can occur following gastrointestinal surgical procedures. The incidence of anastomotic leakage varies between 3% and 26%, with associated mortality rates ranging from 6% to 39% [1]. Recent prevalence data indicate that approximately 9% of patients experience intestinal anastomotic leakage [2]. The high mortality associated with this complication is primarily due to delays in detecting and intervening in anastomotic failure [3]. Therefore, early diagnosis of anastomotic leakage is crucial to reducing morbidity and mortality associated with this condition. However, to date, there is no clear consensus on the most effective method for the rapid and accurate detection of anastomotic leakage in clinical practice. Anastomotic leakage is influenced by multiple factors, including surgical technique, patient conditions, and the presence of infections.

Pseudomonas aeruginosa is a common pathogen responsible for nosocomial infections and plays a role in increasing the risk of anastomotic leakage. Studies have shown that products released by hypoxic intestinal epithelial cells, such as adenosine and dynorphin, can activate the quorum sensing system of P. aeruginosa, leading to phenotypic changes that make the bacteria more aggressive, enhancing collagen degradation, and increasing intestinal tight junction permeability [4]. These conditions hinder wound healing and contribute to an increased risk of anastomotic leakage.

The choice of anastomotic technique also plays a crucial role in determining surgical success. One commonly used technique in intestinal surgery is End-to-End Anastomosis, which offers advantages such as a more straightforward procedure and direct reconnection of the distal and proximal intestinal ends without creating additional openings or relocating bowel segments.

However, the primary complication associated with this technique remains anastomotic leakage, which can lead to the leakage of intestinal contents into the peritoneal cavity, thereby increasing the risk of infection, peritonitis, and other life-threatening conditions.

Early detection of anastomotic leakage remains a significant clinical challenge. Conventional diagnostic methods, including physical examination, conventional radiography, and laboratory evaluations, often lack sufficient sensitivity and specificity for the rapid and accurate detection of leakage. Computed tomography (CT) scan with intraluminal contrast has been recognized as a valuable diagnostic modality for detecting anastomotic leakage [5]. However, its application is limited by technical constraints and high costs, making it less accessible in certain healthcare settings. The sensitivity and specificity of CT scans for detecting anastomotic leakage have been reported to be 59% and 88%, respectively [6]. Moreover, CT specificity in diagnosing anastomotic leakage may be lower than that of procalcitonin (PCT) due to residual postoperative intra-abdominal gas [7]. Studies have indicated that in patients ultimately confirmed to have anastomotic leakage, nearly one-quarter of initial CT scans yielded false-negative results, potentially leading to delays in diagnosis and appropriate management [8]. Therefore, alternative diagnostic methods that are more efficient, accessible, and highly accurate are needed.

A promising approach for the early detection of anastomotic leakage is the use of biomarkers, such as Procalcitonin (PCT). PCT is a precursor of calcitonin, produced by thyroid C cells and various other cell types in response to bacterial infections. Research has demonstrated that PCT levels can increase significantly in patients with anastomotic leakage, even before the appearance of overt clinical symptoms [9].

As an infection biomarker, PCT has been widely utilized in clinical settings, particularly in intensive care units (ICUs) and emergency departments [10] In colorectal surgery, PCT levels exceeding 5.27 ng/mL on the third postoperative day have been identified as a reliable indicator for early detection of anastomotic leakage, outperforming other methods [11]. Additionally, prior studies suggest that PCT elevation occurs more rapidly than C-reactive protein (CRP) and has superior prognostic value in assessing disease severity and mortality risk [12].

Despite its potential as a biomarker for anastomotic leakage, the clinical application of PCT remains limited. Some human studies have shown that PCT can serve as a useful tool to rule out anastomotic leakage following elective colorectal surgery; however, its utility as a standalone test for definitive detection remains uncertain [13]. Furthermore, a recent meta-analysis revealed that the sensitivity and specificity of PCT for detecting anastomotic leakage vary depending on the chosen cutoff value and timing of postoperative measurement [12]. Thus, further research is necessary to evaluate the accuracy and efficacy of PCT in more controlled settings.

To address the limitations of human studies, the use of animal models, such as Wistar rats, offers a more practical, cost-effective, and ethically viable alternative. Wistar rats share genetic and physiological similarities with humans, allowing for high-accuracy disease modeling and biological response studies. Therefore, this study aims to evaluate the role of PCT as a biomarker for the early detection of anastomotic leakage using a Wistar rat model, to improve diagnostic effectiveness and clinical interventions in the future.

METHOD

Study Design, time, and place of study

This study is an experimental study with a True Experimental design, involving two randomly assigned groups: a control group and a treatment group. The treatment group received an intervention in the form of *Pseudomonas aeruginosa* application to the anastomosis area to induce anastomotic leakage after laparotomy anastomosis surgery, while the control group underwent laparotomy and anastomosis without bacterial exposure. The study was conducted at the Integrated Biomedical Laboratory of the Faculty of Medicine, Udayana University, Denpasar, and the Faculty of Veterinary Medicine, Udayana University. The study duration was approximately six months, following approval from the Research Ethics Committee of the Faculty of Medicine, Udayana University.

Samples Characteristic

This study investigates the impact of *Pseudomonas aeruginosa* on anastomotic leakage and procalcitonin levels in male Wistar rats aged 2–3 months (250–300g). Using 20 rats divided into two groups, colorectal anastomosis was performed following aseptic techniques, with one group exposed to *P. aeruginosa*. Anastomotic leakage was assessed macroscopically, while procalcitonin levels were measured via electrochemiluminescence on postoperative days 1, 3, and 5. A threshold of >5.27 ng/mL on day 3 indicated leakage. The study aims to evaluate the bacterial influence on anastomotic integrity and its correlation with procalcitonin as a biomarker.

Research Procedure

The study followed a structured procedure, beginning with ethical approval from the Research Ethics Committee of the Faculty of Medicine, Udayana University, and research permits from RSUP Sanglah Denpasar before sample collection. Twenty healthy adult Wistar rats were acclimatized for one week under standard laboratory conditions (21ºC, 12-hour light/dark cycle) with ad libitum access to food and water. A standardized pellet diet was provided, and any sick animals were excluded.

All subjects underwent colorectal anastomosis under aseptic conditions. They were fasted for 12 hours preoperatively, with unrestricted water access. Anesthesia was induced using intramuscular ketamine (5-10 mg/kg), and prophylactic ceftriaxone (100 mg/kg) was administered. A 3 cm midline abdominal incision was made, followed by sigmoid colon resection (1 cm segment, 3 cm from the peritoneal reflection) while preserving vascularization. End-to-end anastomosis was performed using continuous 6/0 prolene sutures, and the abdominal layers were closed with continuous 3/0 vicryl sutures.

The treatment group received a 200 µL suspension of *Pseudomonas aeruginosa* (10^7 CFU) at the anastomosis site, while the control group underwent standard laparotomy without bacterial inoculation. Postoperatively, no antibiotics were administered, and oral feeding commenced on day one. Ketoprofen (3 mg/kg) was given subcutaneously once daily for analgesia. Blood samples (4 mL) were collected in EDTA tubes on postoperative days 1, 3, and 5 for procalcitonin measurement using ELISA.

Anastomotic leakage was assessed through daily monitoring for local infection, perforation, and peritonitis until day five. On the fifth day, euthanasia was performed using high-dose sodium thiopental (200 mg/kg), followed by laparotomy to evaluate anastomotic integrity. In cases of mortality before day five, a re-laparotomy was conducted to assess anastomotic leakage before disposal via incineration. Macroscopic signs of leakage, including anastomotic dehiscence, peritonitis, fecal contamination, or abscess formation, were documented.

Data Analysis

Data analysis was conducted using SPSS version 25.0, including data entry, cleaning, and verification. Descriptive statistics summarized sample characteristics, presenting categorical data as frequencies and percentages, and numerical data as mean ± standard deviation (normal distribution) or median with interquartile range (non-normal distribution). Normality was tested using Kolmogorov-Smirnov (>50 samples) or Shapiro-Wilk (≤50 samples). Analytical tests compared anastomotic leakage proportions based on procalcitonin levels using odds ratio (OR) with a significance threshold of $p<0.05$ and a 95% confidence interval. Chi-square or Fisher's exact test was applied depending on data distribution. Ethical approval was obtained from the Research and Development Unit, ensuring privacy, anonymity, and confidentiality.

RESULTS

Univariate Analysis

This study involved 20 male Wistar rats that underwent colorectal anastomosis. Several variables were examined, including procalcitonin levels, body weight, age, post-anastomosis leakage, and the presence of Pseudomonas aeruginosa infection. Data for each variable were compiled and analyzed.

In the univariate analysis, the basic characteristics of these variables were outlined to support the hypothesis that increased procalcitonin levels serve as an early predictor of colorectal post-anastomosis leakage. Procalcitonin levels were measured on days 1, 3, and 5. Table 1 below provides an overview of these levels in the control group (without Pseudomonas aeruginosa) versus the treatment group (with Pseudomonas aeruginosa) over time. Laboratory analysis revealed that the highest procalcitonin level was 5.31 ng/mL in the treatment group on day 5, while the lowest level, 0.61 ng/mL, was observed in control sample 10 on day 3.

TABLE 1: Description of Procalcitonin Levels on POD 1, POD3 and POD 5**.**

Procalcitonin Levels in the Group with *Pseudomonas aeruginosa* Infection (ng/mL)			**Procalcitonin Levels in the Group without *Pseudomonas aeruginosa* Infection (ng/mL)**		
POD 1	**POD 3**	**POD 5**	**POD 1**	**POD 3**	**POD 5**
1.82	1.85	1.78	2.28	3.20	3.82
2.32	2.87	5.22	1.92	0.98	0.91
3.01	5.01	4.95	1.72	1.33	1.42
2.08	4.28	4.89	1.84	1.62	1.29
1.33	3.01	1.92	1.30	2.04	1.04
1.07	1.92	1.89	2.03	1.85	0.98
1.92	1.84	1.40	1.97	1.92	2.98
2.05	1.21	2.41	1.62	2.05	2.81
1.88	3.09	5.31	1.55	1.27	1.36
1.02	1.39	4.92	1.78	0.61	2.51

POD: Postoperative Day.

Data normality was assessed using the Shapiro-Wilk test, which is appropriate for studies with a sample size of fewer than 50. The results, shown in Table 2, indicate that only the procalcitonin levels on day 1 followed a normal distribution (p = 0.353). In contrast, the data for body weight and the procalcitonin levels on days 3 and 5 did not follow a normal distribution, with p-values less than 0.05 (0.039, 0.039, and 0.007, respectively). Consequently, only the procalcitonin variable from day 1 will be presented using mean values, as it meets the criteria for parametric testing.

TABLE 2: Normality Test Results (Shapiro-Wilk).

Variable	p-Value	Description
Body Weight	0.039	Data not normally distributed
Procalcitonin POD 1	0.353	Data normally distributed
Procalcitonin POD 3	0.039	Data not normally distributed

This study conducted an analysis comparing the values of each variable between the control and treatment groups. The data analysis revealed that there was no significant difference between the groups in terms of body weight and rat age (p = 0.513). Regarding the progression of procalcitonin levels over time, the study demonstrated a significant difference on day 5 between the control and treatment groups, with median values of 1.39 (0.91–3.82) ng/mL versus 3.65 (1.40–5.31) ng/mL (p = 0.028). Furthermore, the analysis of post-anastomosis leakage showed that leakage occurred in only 30% of the group without Pseudomonas aeruginosa compared to 50% in the samples with Pseudomonas aeruginosa. These results are presented in Table 3 below.

TABLE 3: Comparison of Each Variable Between Control and Treatment Groups.

Variable	Control (Without P. aeruginosa)	Treatment (With P. aeruginosa)	p-Value
Body Weight (grams)	285 (250 – 310)	290 (250 – 310)	0.513
Rat Age (months)	3	3	
Procalcitonin Levels (ng/mL)			
POD 1	1.80 ± 0.27	1.85 ± 0.59	0.817
POD 3	1.74 (0.61 – 3.20)	2.39 (1.21 – 5.01)	0.130
POD 5	1.39 (0.91 – 3.82)	3.65 (1.40 – 5.31)	0.028
Anastomotic Leakage			
Leak	3 (30%)	5 (50%)	0.325
No Leak	7 (70%)	5 (50%)	

Mean Difference Analysis of Procalcitonin Levels in Anastomotic Leakage

The study analyzed mean differences in procalcitonin levels over time, correlating them with the incidence of post-anastomosis leakage. Table 4 presents the overall procalcitonin levels. Findings indicate that in groups experiencing post-anastomosis leakage, procalcitonin levels showed a significant increase starting from day 3, with a mean difference of 1.349 ng/mL (p = 0.005), and further rising on day 5 to a mean difference of 2.786 ng/mL (p = 0.001). For day 1, an unpaired t-test was employed due to the normal distribution of data, while the Mann-Whitney U test was utilized for days 3 and 5, given the non-normal distribution of numerical data.

The procalcitonin levels over time in the control group without Pseudomonas aeruginosa. In this group, a significant mean difference of 1.004 ng/mL (p = 0.033) was observed on day 3, which increased to 1.844 ng/mL (p = 0.017) on day 5, with higher levels in samples exhibiting post-anastomosis leakage compared to those without leakage (Table 4).

Further analysis was conducted on the treatment group exposed to Pseudomonas aeruginosa, as detailed (Table 4). The results revealed that procalcitonin levels were significantly higher in the leakage group, particularly on day 5, with median values of 4.95 (4.89–5.31) ng/mL compared to 1.89 (1.40–2.41) ng/mL in the non-leakage group, reflecting a mean difference of 3.178 ng/mL (p = 0.009).

TABLE 4: Comparison of Mean Differences in Procalcitonin Levels on Day 1, Day 3, and Day 5 in Samples with and Without Anastomotic Leakage.

POD	Group	Leakage	No Leakage	Mean Difference	p-value
1	All Samples	2.02 ± 0.57	1.69 ± 0.31	0.328	0.116
	Control (Without *Pseudomonas aeruginosa*)	1.96 ± 0.33	1.73 ± 0.24	0.222	0.264
	Treatment (With *Pseudomonas aeruginosa*)	2.06 ± 0.72	1.63 ± 0.41	0.424	0.289
3	All Samples	2.98 (1.39 – 5.01)	1.73 (0.61 – 3.01)	1.349	0.005
	Control (Without *Pseudomonas aeruginosa*)	2.05 (1.92 – 3.02)	1.33 (0.61 – 2.04)	1.004	0.033
	Treatment (With *Pseudomonas aeruginosa*)	3.09 (1.39 – 5.01)	1.85 (1.21 – 3.01)	1.362	0.117
5	All Samples	4.91 (2.81 – 5.31)	1.41 (0.91 – 2.51)	2.786	0.001
	Control (Without *Pseudomonas aeruginosa*)	2.98 (2.81 – 3.82)	1.29 (0.91 – 2.51)	1.844	0.017
	Treatment (With *Pseudomonas aeruginosa*)	4.95 (4.89 – 5.31)	1.89 (1.40 – 2.41)	3.178	0.009

Comparison Analysis of Procalcitonin Levels in Anastomotic Leakage

A comparability analysis was conducted using 2x2 cross-tabulation, followed by Chi-square testing and risk estimation, to predict the risk of high procalcitonin levels leading to post-anastomosis leakage in the study samples. High procalcitonin was defined as levels exceeding 2 ng/mL, as outlined in the methodology. The analysis of all cases, encompassing both control and treatment groups, is presented in Table 5. The data indicate that elevated procalcitonin levels on day 3 were significantly associated with post-anastomosis leakage. Specifically, among the 8 samples with leakage, 6 exhibited high procalcitonin levels, yielding an odds ratio (OR) of 15 (95% CI: 1.652–136.17; p = 0.015). By day 5, all samples with elevated procalcitonin levels experienced leakage, with an OR of 6.00 (95% CI: 1.693–21.26; p < 0.001).

The control group without Pseudomonas aeruginosa, high procalcitonin levels on day 5 were a significant risk factor for post-anastomosis leakage (Table 5). Specifically, 75% of samples with elevated procalcitonin experienced leakage, whereas 6 out of 7 samples with lower levels did not. This corresponds to an OR of 7 (95% CI: 1.14–42.97; p = 0.033).

In the treatment group exposed to Pseudomonas aeruginosa, high procalcitonin levels also emerged as an early predictor of post-anastomosis leakage. As shown in Table 5, by day 5, 83% of samples with elevated procalcitonin levels experienced leakage, while none with lower levels did. This resulted in an OR of 5 (95% CI: 1.87–28.86; p = 0.024).

These findings align with previous research indicating that elevated serum procalcitonin levels are a reliable marker for predicting anastomotic leaks in colorectal surgery. For instance, a study found that a postoperative day 3 procalcitonin level exceeding 5.27 ng/mL had 100% sensitivity and 85% specificity for detecting leaks.

TABLE 5: Comparison of High Procalcitonin Levels as a Risk Factor for Post-Anastomotic Leakage.

POD	Group	PCT Level	Leakage (n, %)	No Leakage (n, %)	p-value	OR	95% CI
1	All Group	High	3 (15%)	2 (10%)	0.296	3.00	0.372 – 24.17
		Low	5 (25%)	10 (50%)			
	Control Group	High	1 (10%)	1 (10%)	0.533	3.00	0.122 – 73.64
		Low	2 (20%)	6 (60%)			
	Treatment Group	High	2 (20%)	1 (10%)	0.500	2.67	0.158 – 45.14
		Low	3 (30%)	4 (40%)			
3	All Group	High	6 (30%)	2 (10%)	0.015	15.00	1.652–136.17
		Low	2 (10%)	10(50%)			
	Control Group	High	2 (20%)	1 (10%)	0.183	12.00	0.489–294.57
		Low	1 (10%)	6 (60%)			
	Treatment Group	High	4 (40%)	1 (10%)	0.103	16.00	0.722–354.80
		Low	1 (10%)	4 (60%)			
5	All Group	High	8 (40%)	2 (10%)	<0.001	6.00	1.693 – 21.26
		Low	0 (0%)	10(50%)			
	Control Group	High	3 (30%)	1 (10%)	0.033	7.00	1.14 – 42.97
		Low	0 (0%)	6 (60%)			
	Treatment Group	High	5 (50%)	1 (10%)	0.024	5.00	1.87 – 28.86
		Low	0 (0%)	4 (40%)			

The analysis showed high procalcitonin levels on day 3 were significantly associated with post-anastomotic leakage (OR = 15, 95% CI: 1.652–136.17; p = 0.015). By day 5, all samples with high procalcitonin levels experienced leakage (OR = 6.00, 95% CI: 1.693–21.26; p < 0.001). In the control group without *Pseudomonas aeruginosa*, a significant association was found on day 5 (OR = 7, 95% CI: 1.14–42.97; p = 0.033). In the treatment group with *Pseudomonas aeruginosa*, high procalcitonin levels on day 5 were also a strong predictor of leakage (OR = 5, 95% CI: 1.87–28.86; p = 0.024).

DISCUSSION

Univariate analysis showed an increasing trend in procalcitonin levels over time in the treatment group, with a significant difference compared to the control group on day 5. This increase, triggered by bacterial infection, confirms the role of procalcitonin as an effective inflammatory marker for detecting post-surgical infections, consistent with previous studies [14,15]. A meta-analysis by Nicolotti et al. also supports the use of procalcitonin as an infection predictor in cardiac surgery. Infection plays a crucial role in anastomotic leakage, with the treatment group experiencing more leakage events, although the difference was not statistically significant. A retrospective study by Hu et al. [16] also reported an association between elevated procalcitonin levels and anastomotic leakage.

This study demonstrated that procalcitonin (PCT) levels were significantly higher in the group with anastomotic leakage compared to those without leakage. On postoperative day 5, the mean difference became more pronounced, reaching 2.786 ng/mL (p < 0.001). The increase in PCT levels, as part of the systemic response to infection and inflammation, supports the underlying hypothesis of this study.

The predictive role of PCT in anastomotic leakage is reinforced by its consistency with previous findings. A study by Hu et al. [16] evaluated C-reactive protein (CRP) and PCT levels in colorectal anastomotic leakage. Their results showed a significant difference in mean PCT levels between patients with and without leakage, with the leakage group exhibiting mean PCT levels of 12.56 ± 9.52 ng/mL compared to 2.82 ± 0.75 ng/mL in the non-leakage group on postoperative day 3 (p < 0.01) [16]. Additionally, Sánchez-Iglesias et al. (2024) reported that increased PCT levels could predict anastomotic leakage in bowel resection patients with good sensitivity (83.3%) and specificity (81.3%), as indicated by an area under the curve (AUC) of 0.823 (Sánchez-Iglesias et al., 2024). A meta-analysis by Xu et al. [17] further supported the use of PCT as a predictor of anastomotic leakage in patients undergoing laparoscopic surgery [17]. The consistency of our findings with previous studies highlights the reproducibility of this result across different patient populations, strengthening the validity of our conclusions.

Procalcitonin is a precursor of calcitonin, primarily produced under physiological conditions by C cells of the thyroid gland and neuroendocrine tissues. Under normal circumstances, PCT circulates at very low levels. However, in systemic inflammatory responses, particularly bacterial infections, PCT levels rise significantly, making it a valuable biomarker for acute inflammation and sepsis [18]. In cases of severe infection or inflammatory processes, such as trauma, burns, or major surgery, PCT production increases systemically, involving tissues such as the liver, lungs, and intestines. This response is mediated by inflammatory cytokines, including interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α) [19]. Although other factors can contribute to elevated PCT levels, its specificity as a systemic infection marker has been well documented. For instance, Lee [20] reported that PCT has a specificity of approximately 79% for systemic infections.

In evaluating the predictive value of PCT for anastomotic leakage, this study found significant associations in a 2x2 comparative analysis. The temporal relationship, in which increased PCT levels preceded anastomotic leakage, supports its role as a predictive marker. The analysis showed that high PCT levels (>2 ng/mL) were associated with an increased risk of anastomotic leakage, with an odds ratio (OR) of 15 (95% CI: 1.652–136.17; $p = 0.015$) on postoperative day 3 and 6.00 (95% CI: 1.693–21.26; $p < 0.001$) on postoperative day 5. The significance of elevated PCT as a predictor of anastomotic leakage has been identified in previous research by Hayati et al. [21]. Their study assessed the sensitivity and specificity of PCT for detecting anastomotic leakage compared to the gold standard, computed tomography (CT) scan. The findings indicated that PCT exhibited high specificity and sensitivity, reaching 72% and 67%, respectively, on day 0, and 85% and 100% on day 3. Moreover, elevated PCT levels were significantly correlated with CT-confirmed anastomotic leakage ($p = 0.005$) [21].

Beyond anastomotic leakage, PCT has been widely used in clinical settings as an inflammatory biomarker. Becker et al. [22] evaluated the association between PCT levels and bacterial infections, reporting an 89% sensitivity and 90% specificity at a cut-off of 0.5 ng/mL. Furthermore, a meta-analysis by Wacker et al. [23] demonstrated that PCT had an 85% sensitivity and 83% specificity for sepsis detection in intensive care unit (ICU) patients, further establishing its reliability as a biomarker [22,23].

During septicemia, alternative pathways leading to PCT production are activated in non-thyroid tissues, including leukocytes, the spleen, kidneys, pancreas, colon, adipocytes, and the brain. Bacterial endotoxins stimulate rapid PCT synthesis, which is detectable in circulation within 3-4 hours and peaks at 8-24 hours (21 In bacterial infections, damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) stimulate cells to produce PCT, leading to a significant rise in serum levels. Similarly, following major abdominal, vascular, or thoracic surgery, serum PCT levels tend to increase during the first two postoperative days, whereas levels remain low in patients undergoing minor aseptic procedures.

Overall, this study, in conjunction with prior research, meets the Bradford Hill criteria for establishing a causal relationship between PCT levels and anastomotic leakage. The strength of the association is demonstrated by high odds ratios, while consistency across multiple studies reinforces its validity. The specificity of PCT as an inflammatory biomarker, the clear temporal relationship between increased PCT levels and leakage, and the biologically plausible inflammatory response mechanism further support this hypothesis [24].

This study reinforces the potential role of PCT in the early detection and risk stratification of anastomotic leakage. Patients at high risk of leakage due to infection may benefit from adequate antibiotic prophylaxis, thereby reducing morbidity and mortality [25].

RESEARCH LIMITATION

This study has several limitations. It only assessed procalcitonin in post-anastomotic leakage without evaluating other relevant markers or mediators. Multivariate analysis was not conducted to determine procalcitonin's independent effect. Additionally, long-term monitoring and clinical outcomes, including mortality, were not evaluated. Future studies should incorporate these aspects for a more comprehensive analysis.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study. No financial, personal, or professional affiliations influenced the design, execution, analysis, or interpretation of the research findings.

REFERENCES

[1] Schiff A, Roy S, Pignot M, Ghosh SK, Fegelman EJ. Diagnosis and Management of Intraoperative Colorectal Anastomotic Leaks: A Global Retrospective Patient Chart Review Study. Surg Res Pract. 2017;2017:3852731. doi: 10.1155/2017/3852731. Epub 2017 Jun 14. PMID: 28695192; PMCID: PMC5488233.

[2] Lyu, D., Su, L., Han, G., Yan, P., Xie, L., 2015. Prognostic Value of *Procalcitonin* in Adult Patients with Sepsis: A Systematic Review and Meta-Analysis. PLOS ONE 10, e0129450. https://doi.org/10.1371/journal.pone.0129450

[3] Blumetti J, Abcarian H. Management of low colorectal anastomotic leak: Preserving the anastomosis. World J Gastrointest Surg. 2015 Dec 27;7(12):378-83. doi: 10.4240/wjgs.v7.i12.378. PMID: 26730283; PMCID: PMC4691718.

[4] Olivas AD, Shogan BD, Valuckaite V, Zaborin A, Belogortseva N, Musch M, Meyer F, Trimble WL, A G, Gilbert J, Zaborina O, Alverdy JC. Intestinal tissues induce an SNP mutation in Pseudomonas aeruginosa that enhances its virulence: possible role in anastomotic leak. PLoS One. 2012;7(8):e44326. doi: 10.1371/journal.pone.0044326. Epub 2012 Aug 31. PMID: 22952955; PMCID: PMC3432121.

[5] Samji, K. B., Kielar, A. Z., Connolly, M., Fasih, N., Doherty, G., Chung, A., et al. (2018) 'Anastomotic Leaks After Small- and Large-Bowel Surgery: Diagnostic Performance of CT and the Importance of Intraluminal Contrast Administration', American Journal of Roentgenology, 210(6), pp. 1259–1265

[6] Kornmann, V.N.N., van Ramshorst, B., Smits, A.B., Bollen, T.L., Boerma, D., 2014. Beware of false-negative CT scan for anastomotic leakage after colonic surgery. Int J Colorectal Dis 29, 445–451. https://doi.org/10.1007/s00384-013-1815-5

[7] Kornmann, V.N.N., Treskes, N., Hoonhout, L.H.F., Bollen, T.L., van Ramshorst, B. and Boerma, D., 2013. A systematic review on the value of CT scanning in the diagnosis of anastomotic leakage after colorectal surgery. *International Journal of Colorectal Disease*, 28, pp.437-445. Available at: https://doi.org/10.1007/s00384-012-1623-3

[8] Gessler, B., Eriksson, O. and Angenete, E., 2017. Diagnosis, treatment, and consequences of anastomotic leakage in colorectal surgery. *International Journal of Colorectal Disease*, 32, pp.549-556. Available at: https://doi.org/10.1007/s00384-016-2744-x

[9] Gray M, Marland JRK, Murray AF, Argyle DJ, Potter MA. Predictive and Diagnostic Biomarkers of Anastomotic Leakage: A Precision Medicine Approach for Colorectal Cancer Patients. J Pers Med. 2021 May 25;11(6):471. doi: 10.3390/jpm11060471. PMID: 34070593; PMCID: PMC8229046

[10] Hayati Firdaus et al.Serum Procalcitonin Predicts Anastomotic Leaks in Colorectal Surgery.2017.Asian Pacific Journal of Cancer Prevention : APJCP are provided here courtesy of West Asia Organization for Cancer Prevention. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5648385

[11] Garcia-Granero, A., Frasson, M., Flor-Lorente, B., Blanco, F., Puga, R., Carratalá, A., Garcia-Granero, E., 2013. *Procalcitonin* and C-reactive protein as early predictors of anastomotic leak in colorectal surgery: a prospective observational study. Dis Colon Rectum 56, 475–483. https://doi.org/10.1097/DCR.0b013e31826ce825

[12] Su'a, B., Tutone, S., MacFater, W., Barazanchi, A., Xia, W., Zeng, I. and Hill, A.G., 2019. Diagnostic accuracy of procalcitonin for the early diagnosis of anastomotic leakage after colorectal surgery: a meta-analysis. ANZ Journal of Surgery. https://doi.org/10.1111/ans.15291

[13] Su'a BU, Mikaere HL, Rahiri JL, Bissett IB, Hill AG. Systematic review of the role of biomarkers in diagnosing anastomotic leakage following colorectal surgery. Br. J. Surg. 2017; 104: 503–12

[14] Wirz, Y., Meier, M.A., Bouadma, L. *et al.* Effect of procalcitonin-guided antibiotic treatment on clinical outcomes in intensive care unit patients with infection and sepsis patients: a patient-level meta-analysis of randomized trials. *Crit Care* 22, 191 (2018). https://doi.org/10.1186/s13054-018-2125-7.

[15] Tyagi N, Gawhale S, Patil MG, Tambolkar S, Salunkhe S, Mane SV. Comparative Analysis of C-reactive Protein and Procalcitonin as Biomarkers for Prognostic Assessment in Pediatric Sepsis. Cureus. 2024 Jul 26;16(7):e65427. doi: 10.7759/cureus.65427. PMID: 39184737; PMCID: PMC11344993.

[16] Hu Y, Ren J, Lv Z, Liu H, Qiu X. Procalcitonin and C-reactive protein as early predictors in patients at high risk of colorectal anastomotic leakage. Journal of International Medical Research. 2024;52(6). doi:10.1177/03000605241258160

[17] Xu, Z., Zong, R., Zhang, Y., Chen, J., & Liu, W. (2022). Diagnostic accuracy of *procalcitonin* on POD3 for the early diagnosis of anastomotic leakage after colorectal surgery: A meta-analysis and systematic review. *International journal of surgery (London, England)*, *100*, 106592. https://doi.org/10.1016/j.ijsu.2022.106592

[18] Bona D, Danelli P, Sozzi A, Sanzi M, Cayre L, Lombardo F, Bonitta G, Cavalli M, Campanelli G, Aiolfi A. C-reactive Protein and Procalcitonin Levels to Predict Anastomotic Leak After Colorectal Surgery: Systematic Review and Meta-analysis. J Gastrointest Surg. 2023 Jan;27(1):166-179. doi: 10.1007/s11605-022-05473-z. Epub 2022 Sep 29. PMID: 36175720.

[19] Choghakhori R, Abbasnezhad A, Hasanvand A, Amani R. Inflammatory cytokines and oxidative stress biomarkers in irritable bowel syndrome: Association with digestive symptoms and quality of life. Cytokine. 2017 May;93:34-43. doi: 10.1016/j.cyto.2017.05.005. Epub 2017 May 12. PMID: 28506572.

[20] Lee H. *Procalcitonin* as a biomarker of infectious diseases. Korean J Intern Med. 2013 May;28(3):285-91. doi: 10.3904/kjim.2013.28.3.285. Epub 2013 May 1. PMID: 23682219; PMCID: PMC3654123

[21] Hayati, F., Azman, Z.A.M., Nasuruddin, D.N., Mazlan, L., Zakaria, A.D., Sagap, I., 2017. Serum Procalcitonin Predicts Anastomotic Leaks in Colorectal Surgery. Asian Pac J Cancer Prev 18, 1821–1825. https://doi.org/10.22034/APJCP.2017.18.7.1821

[22] Becker, K. L., et al. (2004). "*Procalcitonin* and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors." Journal of Clinical Endocrinology & Metabolism

[23] Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis. 2013 May;13(5):426-35. doi: 10.1016/S1473-3099(12)70323-7. Epub 2013 Feb 1. PMID: 23375419.

[24] Fedak KM, Bernal A, Capshaw ZA, Gross S. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerg Themes Epidemiol. 2015 Sep 30;12:14. doi: 10.1186/s12982-015-0037-4. PMID: 26425136; PMCID: PMC4589117

[25] Hernandez A, Frasson M, García-Granero A, Hervás Marín D, Laiz Marro B, Alonso Pardo R, et al; the EDEN study group. Diagnostic accuracy of C-reactive protein, procalcitonin and neutrophils for the early detection of anastomotic leakage after colorectal resection: a multicentric, prospective study. Colorectal Dis. 2021; 23: 2723–2730. https://doi.org/10.1111/codi.15845