ASSOCIATION BETWEEN FIBRINOGEN LEVELS AND OTHER INFLAMMATORY PARAMETERS IN A COHORT OF PATIENT TREATED IN ICU LONGER THEN 7 DAYS

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The graduation thesis was graded on June 24th in Rijeka, before the Committee composed of the following members:

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The graduation thesis contains 22 pages, 3 figures, 7 tables, 29 references.

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LIST OF ABBREVIATIONS AND ACRONYMS

CRP- C-reactive protein

CI- C-reactive protein on the first day

CIII- C-reactive protein on the third day

CV- C-reactive protein on the fifth day

CVII- C-reactive protein on the seventh day

FI- fibrinogen on the first day

FIII- fibrinogen on the third day

FV- fibrinogen on the fifth day

FVII- fibrinogen on the seventh day

ICU- Intensive Care Unit

LI- leukocytes on the first day

LIII- leukocytes on the third day

LV- leukocytes on the fifth day

LVII- leukocytes on the seventh day

PCT- procalcitonin

PI- procalcitonin on the first day

PIII- procalcitonin on the third day

PV- procalcitonin on the fifth day

PVII- procalcitonin on the seventh day

1 INTRODUCTION

Inflammation is a response of vascularized tissues to infections and tissue injury that transfers cells and molecules of host defense from the circulation to the sites where they are needed, to eliminate the offending agents (1). The process of inflammation involves a coordinated response from the immune system and is characterized by four primary signs: redness (rubor), heat (calor), swelling (tumor), and pain (dolor). Sometimes, a fifth sign, loss of function (functio laesa), is also included (2). The inflammatory process begins with the recognition of harmful stimuli. These stimuli can be infectious agents like bacteria, viruses, fungi, or non-infectious factors such as physical injury, chemical toxins, or autoimmune reactions. Immune cells, like macrophages, dendritic cells, and mast cells, recognize these harmful stimuli through pattern recognition receptors. These receptors detect pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), triggering the immune response (3). Once the inflammatory response is initiated, vasodilation and higher vascular permeability facilitate the movement of immune cells to the site of injury or infection. The cellular response involves the migration of leukocytes to the site of injury. Firstly, leukocytes go toward higher concentrations of chemotactic signals released by damaged tissues (chemotaxis). Secondly, they adhere to endothelial cell lining in the blood vessels (margination) and then pass through the vessel wall (diapedesis) to reach the inflamed tissue. Finally, by phagocytosis neutrophils and macrophages digest pathogens (3). The resolution phase of inflammation involves the removal of dead cells and pathogens, followed by tissue repair and healing. Anti-inflammatory signals and cytokines help to dampen the inflammatory response and promote the regeneration of healthy tissue. To restore normal tissue architecture and function fibroblasts are activated to produce extracellular matrix components such as collagen, which provides structural support for new tissue (4). Understanding the mechanisms of inflammation has paved the way for the identification and utilization of various biomarkers to diagnose, monitor, and manage inflammatory conditions. In contemporary clinical practice, biomarkers such as C-reactive protein, leukocytes, and procalcitonin are routinely measured to identify and evaluate inflammation in patients. However, the role of fibrinogen, a glycoprotein involved in coagulation, has expanded significantly concerning inflammation, particularly in Intensive Care Unit settings. This retrospective observational study will explore the hypothesis that fibrinogen when measured alongside traditional biomarkers like CRP, procalcitonin, and leukocyte counts, can enhance the accuracy of inflammation assessment and inform better clinical interventions. The potential of fibrinogen to act as an early indicator of infection and inflammation could

revolutionize patient management, especially in critical care settings where prompt and accurate diagnosis is crucial. To facilitate timely decision-making about therapeutic modifications, useful results on the correlation of fibrinogen, C-reactive protein, leukocytes, and procalcitonin among patients who spent more than seven days in the Intensive Care Unit have been gathered. To provide a comprehensive understanding of these biomarkers, the following sections will detail each parameter.

1.1 FIBRINOGEN

Fibrinogen is a glycoprotein found in plasma. Synthesized in the liver under the influence of cytokines, especially interleukin-6 (IL-6), which act as signaling molecules during inflammatory processes (5). When the organism comes into contact with any stimulus, such as an infection or injury, the liver begins to produce fibrinogen intensively. Fibrinogen is a key component in blood coagulation. It regulates blood loss through two main mechanisms after vascular injury; it acts as an adhesive protein for platelet aggregation and generates a fibrin clot during the blood coagulation process (6). Beyond its role in hemostasis, fibrinogen is also considered an acute phase reactant, meaning its levels in blood increase in response to inflammation, tissue injury, or infection. Binding to its integrin receptor on leukocyte surfaces, also encourages a chemotactic response, thereby playing a significant role in the inflammatory process (7). As cellular and molecular mechanisms for fibrinogen functions in tissues are identified, the role of fibrinogen is evolving from a marker of vascular rupture to a multi-faceted signaling molecule with a wide spectrum of functions that can tip the balance between hemostasis and thrombosis, coagulation and fibrosis, protection from infection and extensive inflammation, and eventually life and death (7).

1.2 C-REACTIVE PROTEIN

C-reactive protein (CRP) is another acute-phase protein whose levels rapidly rise as a reaction to infection, inflammation, and tissue damage, making it a widely used marker for these conditions (8). Primarily synthesized in liver hepatocytes but could also be by endothelial cells, smooth muscle cells, macrophages, lymphocytes, and adipocytes (9). Due to its specificity for phosphocholine, a constituent part of pathogens, CRP is able to identify a variety of compromised cells. Moreover, CRP centers on the stimulation of the C1q molecule in the complement pathway and serves as an opsonin. Additionally, interacting with Fc receptors of

IgG starts a cell-mediated pathway. Consequently, the principal biological function of CRP is to defend the host against pathogens and facilitate the removal of apoptotic and necrotic cells (10).

1.3 PROCALCITONIN

Procalcitonin is a precursor of the hormone calcitonin, formed by the parafollicular C cells of the thyroid gland (11). In healthy individuals, procalcitonin is minimally produced, baseline level is <0.1 μg/L. It undergoes cleavage to form calcitonin, which plays a role in lowering blood calcium levels by suppressing osteoclast activity and increasing calcium excretion in the kidney. During systemic inflammation, particularly bacterial infections, procalcitonin synthesis is upregulated, and it is produced in various tissues and cells throughout the body. More precisely, the extrathyroidal sites that have been mentioned are neuroendocrine cells of the intestine and lungs (12). Its production is driven by pro-inflammatory cytokines as well as by direct stimulation from bacterial endotoxins. Distinguishing them from viral infections and other non-infectious causes of inflammation, procalcitonin levels are particularly elevated in bacterial infections, contributing to the guidance of antibiotic therapy (13).

1.4 LEUKOCYTES

White blood cells, also known as leukocytes, are an essential component of the immune system and are classified into two categories according to the presence or absence of granules in the cytoplasm (14). Granulocytes are defined by the existence of granules in their cytoplasm and can further be divided into neutrophils, eosinophils, and basophils. They all contain toll-like receptors, which let them recognize pathogen-associated molecular patterns (PAMPS) and granules which are made of enzymes that ease the process of digestion of pathogens (14). Neutrophils are making the most of circulating leukocytes and are primarily responding to microbial infections. Agranulocytes, on the other hand, lack cytoplasmic granules and are divided into lymphocytes and monocytes (15). Moreover, leukocytes are produced in the bone marrow by a cascade of events, and various chemical mediators recruiting them to the injury site play a vital part in the organism's immunological response to infection, providing essential information about the type and severity of the inflammatory condition (15). Leukocyte migration to areas of inflammation is a complicated, multi-step process that involves a series of interactions between leukocytes and endothelial cells lining the blood vessels. This intricate

process begins with the capture of leukocytes by endothelial cells, facilitated by L-selectin and very late antigen 4 (VLA-4) on the surface of leukocytes that interact with their corresponding receptors on endothelial cells (16). These interactions ensure that leukocytes are efficiently captured from the bloodstream. Following capture, leukocytes begin to roll down the endothelial surface by E-selectin and P-selectin, which bind to their ligands on the leukocyte surface. This rolling is essential for positioning leukocytes correctly along the endothelial layer. Once rolling has positioned the leukocytes appropriately, they are activated by signals from the endothelial cells. This activation involves the activation of integrins on the leukocyte surface, transforming them into a high-affinity state capable of strong adhesion. This strong adhesion is mediated by intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), which ensure that leukocytes are securely attached to the endothelial layer (16). Furthermore, leukocytes undergo transmigration, where they traverse the endothelial cell layer to enter the underlying tissue to reach the site of inflammation. This migration within the tissue is directed by interactions between integrins on surface of the leukocyte and components of the extracellular matrix, such as collagen and laminin (16). These interactions guide the leukocytes to the precise location of the inflammatory response, ensuring an effective immune reaction.

2 AIMS AND OBJECTIVES

The primary aim of this retrospective observational study is to assess the potential of fibrinogen as a predictor of infection within a convenience sample of the patients treated at the Department of Anaesthesiology and Intensive Care, Clinical Hospital Center Sušak in the period from January 1, 2022, to October 31, 2023. Specifically, the study seeks to investigate the variability of the concentrations of fibrinogen levels taken on the first, third, fifth, and seventh day of hospitalized patients during the above-mentioned period. Furthermore, we aim to analyse the associations between fibrinogen levels and C-reactive protein, procalcitonin, and leukocytes, each measured on days one, three, five, and seven, to reveal whether there is a statistically significant association between these parameters of interest. As prolonged stays in the Intensive Care Unit pose unique challenges, both physical and psychological, we seek suitable parameters that would urgently alert us about the patient's condition and point out the necessity of therapy modification. As part of the body's acute phase response, fibrinogen levels rise significantly during inflammatory processes, making it a valuable biomarker for various inflammatory conditions. Interest in fibrinogen as an inflammation biomarker has increased because to its significant connection with development and progression of cardiovascular diseases (17), COPD (18) and infections. However, the research study where association between fibrinogen levels and other inflammatory markers in patients who stay in ICU for extended stay has not yet been conducted except in the context of cardiovascular events and intracerebral hemorrhage. One such study found that elevated fibrinogen levels are individually associated with death rates in severely ill patients with exacerbation of chronic heart failure by data used from the Medical Information Mart for Intensive Care III database (17). Another study, population-based, found that circulating inflammatory indicators, such as fibrinogen, were associated with risks of cardiovascular and non-cardiovascular events in Intensive Care Unit patients (19). Fibrinogen was found to have a strong relationship with cardiovascular disease risk, which is somewhat lower compared to the prevalence of established risk factors (tobacco usage, hypertension, cholesterol) and likely contribute towards their predictive power. Comparatively, the retrospective cohort study was conducted between C-reactive protein and fibrinogen and intracerebral hemorrhage. The study showed that C-reactive protein and fibrinogen were not linked with a significantly greater exposure of intracerebral hemorrhage in Intensive Care Unit patients (20). Overall, the research studies on the association between fibrinogen and other inflammatory markers in ICU patients constructed divergent findings, encouraging future analysis of the association between the discussed parameters.

3 MATERIALS AND METHODS

3.1 DATA COLLECTION AND SAMPLE

We performed a retrospective observational study. For the purpose of the study, the medical documentation of all the patients taken into the Intensive Care Unit of Clinical Hospital Center Sušak was anonymized and ensured with discretion. In this study, we used the medical records of patients treated at the Department of Anaesthesiology and Intensive Care, Clinical Hospital Center Sušak in the period from January 1, 2022, to October 31, 2023. From this data, 51 patients were selected to be included in this paper. The convenient sample comprised 39 male patients and 12 female patients. The following data has been collected: fibrinogen, C-reactive protein (CRP), procalcitonin (PCT), and leukocytes, measured on the first, third, fifth, and seventh days of the patients' ICU stay.

3.2 ETHICAL ASPECTS OF RESEARCH

This retrospective observational study was approved by the Ethics Committee of Clinical Hospital Center Rijeka. During the collection of data individuals' private health information is kept confidential, protecting them from potential misuse or unauthorized access to their data.

3.3 STATISTICAL ANALYSIS

In this study, the dana was analyzed using descriptive statistics and presented as mean, median and standard deviations. In addition, we used Pearson correlation analysis to investigate correlations between variables of interest, with significance threshold set at 5%. The correlation coefficient, denoted as r, assesses the strength and the direction of the relationship between two variables. A positive r-value indicates that as one variable increases, the other variable also increases. Conversely, a negative r-value indicates that as one variable increases, the other decreases, suggesting an inverse relationship. The p-value will be reported to assess the statistical significance of the correlation coefficients. A p-value less than 0.05 is deemed statistically significant, implying that the probability of the correlation occurring by random chance is less than 5%.

4 RESULTS

Each parameter of interest (fibrinogen, C-reactive protein, procalcitonin, and leukocytes) were collected on days one, three, five, and seven. To ease the understanding of the results, we consistently use the labelling for each day, as follows: F indicates fibrinogen (FI on day 1; FIII on day 3; FIV on day 5; FVII on day 7); C refers to C-reactive protein fibrinogen (CI on day 1; CIII on day 3; CIV on day 5; CVII on day 7); L denotes leucocytes (LI on day 1; LIII on day 3; LIV on day 5; LVII on day 7) and P refers to procalcitonin (PI on day 1; PIII on day 3; PIV on day 5; PVII on day 7). We start off by presenting the results regarding each of the parameters of interest, followed by the exploration of the associations between fibrinogen and the inflammatory markers.

4.1 FIBRINOGEN

Table 1 of results-the concentration of fibrinogen levels (F), g/L, measured on the first (FI), third (FIII), fifth (FV), and seventh (FVII) day

TABLE 1	FI	FIII	FV	FVII
Median	3.89	5.38	5.99	5.79
Mean	4.44	5.76	5.73	5.19
Standard deviation	2.34	1.82	1.90	1.80

On the initial day, the mean fibrinogen (FI) concentration was 4.44 g/L, accompanied by a standard deviation of 2.34 g/L, indicating higher variability compared to subsequent days (Table 1). The median value of 3.89 g/L suggests a right-skewed distribution, with a tail extending towards higher values. Conversely, on the third day, the mean fibrinogen (FIII) concentration increased to 5.76 g/L, with a reduced standard deviation of 1.82 g/L, indicative of lower variability. The median value of 5.38 g/L supports this course, suggesting a balanced distribution of data points. Similarly, on the fifth day, the mean fibrinogen (FV) concentration was 5.73 g/L, with a reduced standard deviation of 1.90 g/L and a median value of 5.99 g/L, reflecting a symmetry in the distribution of results. Lastly, on the seventh day, the mean fibrinogen (FVII) concentration slightly decreased to 5.19 g/L, with a standard deviation of 1.80 g/L and a median value of 5.79 g/L, indicating proximity to the average and symmetry akin to the fifth day. The normal range for fibrinogen levels typically falls between 1.80 and 4.00 g/L, providing context for interpreting the observed variability in fibrinogen concentrations. Throughout the observed seven-day period, it was consistently noted that fibrinogen levels surpassed the established reference value.

4.2 C-REACTIVE PROTEIN

Table 2 of results- the concentration of C-reactive protein levels (C), mg/L, measured on the first (CI), third (CIII), fifth (CV), and seventh (CVII) day

TABLE 2	CI	CIII	CV	CVII
Median	80.8	206.4	153	133.7
Mean	141.2	214.04	162.56	135.04
Standard deviation	135.88	111.93	85.32	82.64

On the first day, C-reactive protein (CI) presents with a mean 141.2 mg/L and a substantially lower median of 80.8 mg/L, suggesting a right-skewed distribution and high standard deviation of 135.88 mg/L indicating considerable variability in the data around the mean (Table 2). Nonetheless, C-reactive protein on the third day (CIII) with a mean of 214.04 mg/L and a median of 206.4 mg/L shows relatively symmetric distribution with notable moderate variability of the standard deviation of 111.93 mg/L. The fifth day, the symmetric distribution of C-reactive protein (CV) with a mean of 162.56 mg/L and median of 153 mg/L is seen as they are close to one another. The standard deviation, 85.32 mg/L is lower compared to other days, indicating less variability. Similar to CV, the mean of 135.04 mg/L and the median of 133.7 mg/L on the seventh day (CVII) show the symmetric distribution and with a low standard deviation of 82.64 mg/L less variability. All mentioned values referring to C-reactive protein indicate significantly elevated levels, as the reference value, set at <5 mg/L, is notably exceeded. Furthermore, CI shows the highest variability with a large standard deviation, while CV and CVII exhibit the least variability with lower standard deviations.

4.3 PROCALCITONIN

Table 3 of results - the concentration of procalcitonin levels (P), µg/L, measured on the first (PI), third (PIII), fifth (PV), and seventh (PVII) day

TABLE 3	PI	PIII	PV	PVII
Median	1.35	1.32	0.811	0.45
Mean	20.99	12.44	6.56	3.02
Standard deviation	67.37	38.45	15.96	6.82

The data for the first day of procalcitonin (PI) shows a relatively high mean of 20.99 µg/L and a standard deviation of 67.37 µg/L compared to the median of 1.35 µg/L, indicating potential skewness or outliers in the data (Table 3). Moreover, the third day of procalcitonin (PIII) shows a moderate mean of 12.44 µg/L and a standard deviation of 38.45 µg/L. Fifth-day procalcitonin (PV) has a lower mean of 6.56 µg/L and a standard deviation of 15.96 µg/L, indicating a wide

spread of data around the mean. A more concentrated distribution of values can be seen on the seventh day of procalcitonin (PVII) with lowest mean of 3.02 µg/L and a standard deviation of 6.82 µg/L than previous days. The referent interval for procalcitonin, commonly established as <0.046µg/L and <0.05 µg/L, is indicative of low sepsis risk. Across the span of seven days, procalcitonin levels outpace the reference threshold.

4.4 LEUKOCYTES

Table 4 of results - the concentration of leukocytes levels (L), x10^9/L, measured on the first (LI), third (LIII), fifth (LV), and seventh (LVII) day

TABLE 4	LI	LIII	LV	LVII
Median	10.1	9.6	10.2	10.3
Mean	10.09	10.88	10.34	11.53
Standard deviation	4.81	5.85	4.67	5.86

Leukocytes (LI), first day, present with a mean of 10.09 x10^9/L and a median of 10.1 x10^9/L indicating a symmetric distribution (Table 4). The standard deviation, 4.81 x10^9/L, is relatively high, suggesting moderate variability in the data around the mean. Moreover, on the third-day leukocytes (LIII) median 9.6 x10^9/L is lower than the mean 10.88 x10^9/L, pointing to a right-skewed distribution. The standard deviation is 5.85 x10^9/L, higher than on the first day, indicating increased variability of the data. Similar to LI, the mean 10.34 x10^9/L and the median 10.2 x10^9/L on the fifth day (LV) are close, showing a symmetric distribution. Compared to other days, the standard deviation, 4.67 x10^9/L is lower implying there is lesser variability in data. Seventh-day leukocytes (LVII) the mean is 11.53 x10^9/L and the median is 10.3 x10^9/L, slightly lower indicating a right skewed distribution. The standard deviation 5.86 x10^9/L points to moderate variability in the data. These results demonstrate that observed leukocytes do not overpass the referent value, 3.4-9.7 x10^9/L in a considerable manner , as parameters previously mentioned above. Overall, LV appears to have the least variability, as indicated by its lower standard deviation. Days LI and LVII show moderate variability, while Day LIII exhibits the highest variability among the seven days.

4.5 CORRELATION BETWEEN FIBRINOGEN AND INFLAMMATORY MARKERS

The associations between fibrinogen and the three inflammatory markers are, generally speaking, weak.

Table 5- statistical correlation of fibrinogen (F) and leukocytes (L) measured on the first,third,fifth and seventh day

Table 5	FI r(p)	FIII r(p)	FV r(p)	FVII r(p)
LI	-0.17 (0.23)	-0.02(0.92)	0.09 (0.54)	0.09(0.54)
LIII	0.06 (0.70)	-0.11 (0.44)	-0.20 (0.16)	-0.26 (0.06)
LV	0.20 (0.16)	-0.07 (0.62)	-0.34 (0.01)	-0.31 (0.03)
LVII	0.14 (0.32)	-0.00 (0.95)	-0.27 (0.06)	-0.20 (0.16)

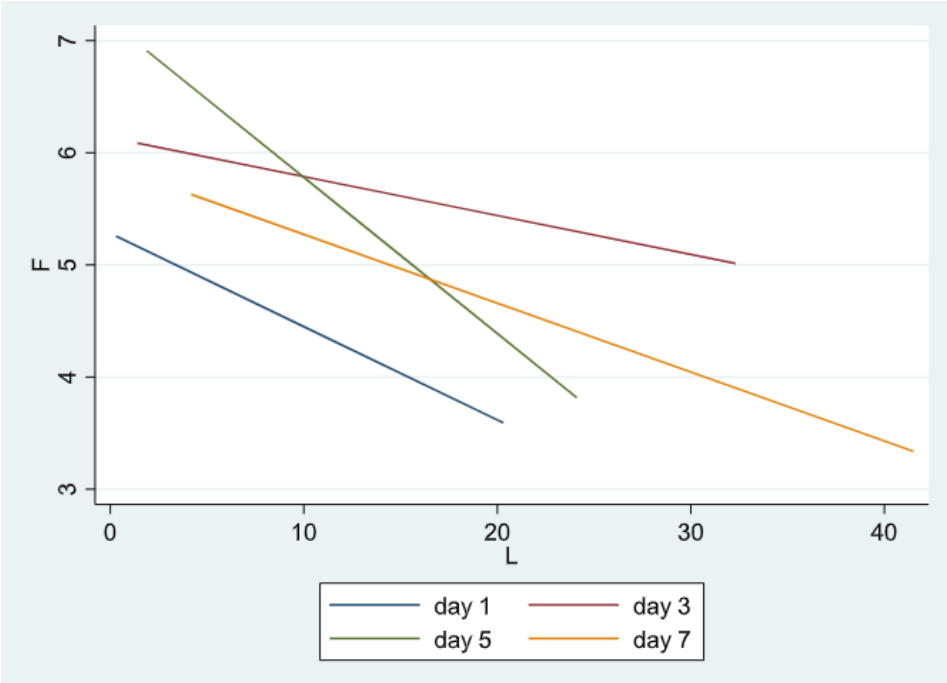

Figure 1- correlation between fibrinogen (F), g/L, and leukocytes (L),), x10^9/L, on day 1, day 3, day 5 and day 7

Although there is an overall negative correlation between fibrinogen and leucocytes (Figure 1), their correlations on each measurement days are statistically not significant (Table 5), suggesting a weak relationship between fibrinogen and leukocyte levels over time.

Table 6- statistical correlation of fibrinogen (F) and procalcitonin (P) measured on the first,third,fifth and seventh day

Table 6	FI r(p)	FIII r(p)	FV r(p)	FVII r(p)
PI	0.22(0.11)	-0.02 (0.88)	-0.27 (0.06)	-0.37 (0.01)
PIII	0.20(0.17)	0.03(0.83)	-0.15 (0.30)	-0.28 (0.05)
PV	0.13(0.36)	-0.01 (0.90)	-0.14(0.32)	-0.26 (0.07)
PVII	-0.02(0.86)	-0.07 (0.64)	-0.1(0.51)	-0.26(0.07)

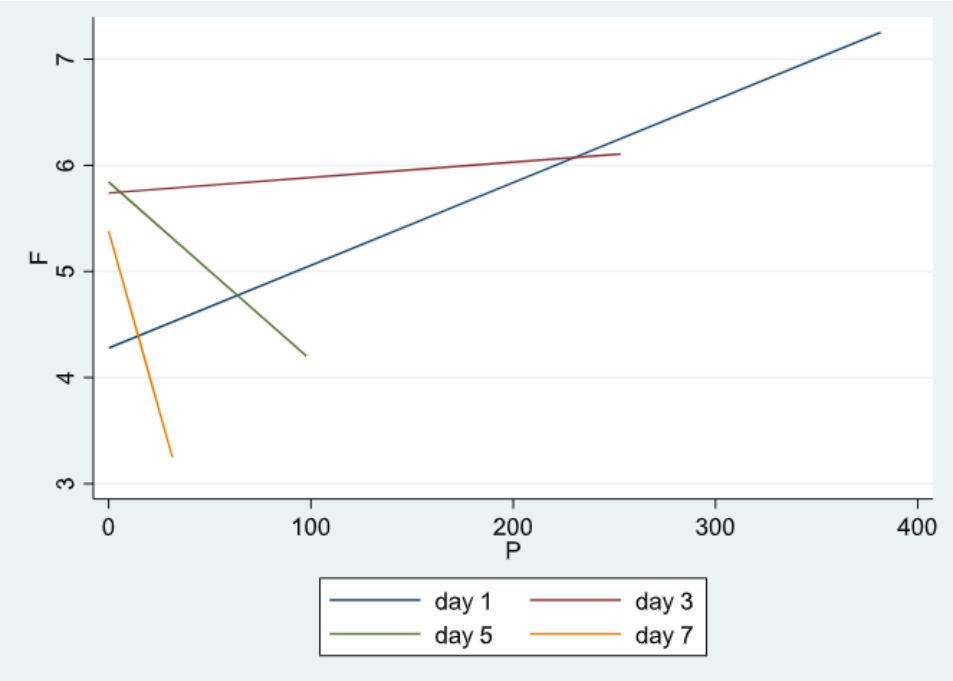

Figure 2- correlation of fibrinogen (F),g/L, and procalcitonin (P), µg/L, on day 1, day 3, day 5, day 7

In contrast to the correlation previously outlined between fibrinogen and leukocytes, a brief positive association of fibrinogen and procalcitonin can be discerned in Figure 2 on day 1 where the other measurement days, this association is largely negative. Statistical significance of these associations, however, is not observed (Table 6).

Table 7-statistical correlation of fibrinogen(F) and C-reactive protein (CRP) measured on the first,third,fifth and seventh day

Table 7	FI r(p)	FIII r(p)	FV r(p)	FVII r(p)
CI	0.82(0.00)	0.38(0.19)	-0.19(0.18)	-0.39(0.01)
CIII	0.38(0.01)	0.60(0.00)	0.26(0.07)	-0.02(0.91)
CV	0.12(0.40)	0.39(0.00)	0.39(0.00)	0.27(0.06)
CVII	-0.19(0.17)	0.12(0.39)	0.49(0.00)	0.56(0.00)

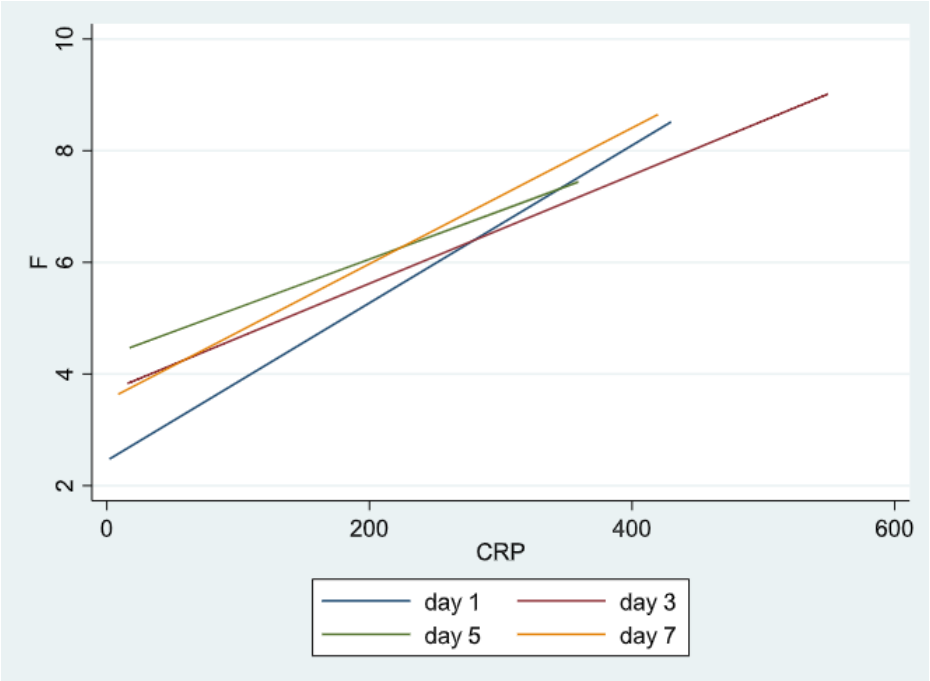

Figure 3- correlation of fibrinogen (F), g/L, and C-reactive protein (CRP),mg/L, on day 1, day 3, day 5, day 7

Contrary to the correlations presented between fibrinogen and leukocytes, and fibrinogen and procalcitonin, the correlation between fibrinogen and C-reactive protein (CRP) demonstrated a consistently positive relationship throughout all days of the study (Figure 3). This is evidenced by the statistical significance (Table 7), with p-values consistently below 0.05, marking a significant association between fibrinogen and CRP levels. This persistent and statistically significant correlation suggests that as CRP levels increase, fibrinogen levels also increase significantly, highlighting the potential interactive role these markers play in the inflammatory response. Fibrinogen on the first day (FI) shows positive correlation with C-reactive protein on the first day (CI, r = 0.82), the third day (CIII, r = 0.38), and the fifth day (CV, r=0.12), however on the seventh day (CVII) exhibits negative correlation, r= -0.19 (Table 7). Indicating statistical significance with only CI (p =0.00) and CIII (p=0.01), a notable difference in the strength of their relationship is marked as the p-value noted at CV (p= 0.40) and CVII (p=0.17) is >0.05. A significant association is observed between fibrinogen on day three (FIII) and C-reactive protein (CIII, CV). The correlation coefficient and statistical significance of CIII (r = 0.60, p = 0.00) and CV (r= 0.39, p = 0.00) suggest that this correlation is unlikely to be due to random chance (Table 7). Therefore, as FIII levels increase, CIII and CV levels tend to increase which is also the case between FI and CI,CIII. In addition, fibrinogen on the fifth day (FV) exhibits moderate positive correlation and true statistical significance with both CV (r= 0.39, p =0.00) and CVII (r= 0.49, p =0.00). Consistent with observations of FV, similar correlations are

observed between fibrinogen on the seventh day (FVII) and CVII (r=0.56, p = 0.00) pointing to the potential interplay between these variables. These findings emphasize the unique and significant relationship between fibrinogen and CRP in the context of inflammation.

5 DISCUSSION

The correlation between fibrinogen and other inflammatory parameters, like leukocytes, C-reactive protein, and procalcitonin, has been thoroughly analyzed with the particular objective of determining whether fibrinogen can serve as a reliable predictor of infection development, in combination with other parameters of infection, in individuals admitted to the Intensive Care Unit (ICU) for an extended period of time. Fibrinogen is an acute-phase protein that grows in response to tissue damage or infection, contributing to the formation of blood clots and inflammatory response (5). Its inflammatory role was found in vivo, and later on in vitro, studies demonstrating how it affects leukocyte migration by regulating the inflammatory response of leukocytes and endothelial cells through an elevated cytokine/chemokine response (21). Due to its dual role as both an acute phase protein and as a vital component in the coagulation system fibrinogen was considered as an appropriate parameter to analyze. Given its ability, we explored the correlation between fibrinogen and other inflammatory parameters such as C-reactive protein, procalcitonin, and leukocytes, as well. Moreover, given the distinct regulatory mechanisms and specific stimuli driving procalcitonin and leukocyte response, we did not anticipate a strong correlation between fibrinogen and procalcitonin, and fibrinogen and leukocytes. However, we anticipated fibrinogen and C-reactive protein to correlate due to their shared regulation by pro-inflammatory cytokines. This anticipation is based on their roles as acute phase proteins, where both markers increase in response to systemic inflammation. In our retrospective observational study, we observed this expected correlation in the results, demonstrating that fibrinogen and CRP levels rise concurrently, thereby providing a dual perspective on the inflammatory process: CRP indicating overall inflammation severity and fibrinogen reflecting coagulation activity in response to inflammatory stimuli. Driven by a complex interplay of inflammation, disease progression, individual patient differences, and treatment effects the results have shown greater variability in each parameter presented, fibrinogen, C-reactive protein, procalcitonin, and leukocytes. Patients' extended ICU stays provide unique challenges and risks, emphasizing the importance of identifying reliable and predictive markers that can improve patient outcomes through early detection and treatment. In the results section, detailed tables and graphs are presented by illustrating the association between fibrinogen and leukocytes, procalcitonin, and C-reactive protein, examined separately. These graphic illustrations offer a thorough grasp of the interactions and variability between the parameters. At first, the relationship between fibrinogen and leukocytes presents an enigma within the complex interaction of physiological markers, with a negative overall correlation that

is statistically non-significant. The observed variability and limited significance of this correlation across consecutive days may stem from the small research sample size, the individual physiological status at the time of measurement, and additional factors such as age, sex, and comorbidities. As noticed, a statistically insignificant negative correlation was observed between fibrinogen and leukocytes, and can be seen between fibrinogen and procalcitonin with a brief positive association evident only on the first day. The variability in these correlations, spanning from weak positive to weak negative, can be attributed to the distinct biological functions and regulatory mechanisms of the markers. Fibrinogen plays a key role in inflammation, coagulation, and wound healing, whereas procalcitonin is a precursor to calcitonin, which is produced in response to bacterial infection. These different regulatory pathways lead to asynchronous fluctuations in their levels. Despite these differences, the correlations of fibrinogen with both leukocytes and procalcitonin remain statistically insignificant, highlighting the complexity of their interactions and the necessity for further research to fully understand these relationships within the inflammatory response. Contrary to the non-significant correlations observed between fibrinogen and leukocytes, and fibrinogen and procalcitonin, the correlation between fibrinogen and C-reactive protein (CRP) demonstrated a consistently positive relationship throughout all days of the study, with notable statistical significance. The significant p-value obtained suggests that fibrinogen and CRP can be reliably used as correlating markers in the detection and treatment of inflammatory conditions in Intensive Care Unit (ICU) patients with prolonged stays. CRP is a biomarker of inflammation, an acute phase protein. Synthesized in the liver and released into the bloodstream in response to the presence of pathogens or damaged tissue (8). The production of both fibrinogen and CRP is upregulated by pro-inflammatory cytokines. When inflammation occurs, these cytokines stimulate the liver to produce more acute-phase reactants. What's more, these proteins are notably elevated in various medical scenarios, including rheumatoid arthritis, cardiovascular diseases, and infections, Implementing a regimen for frequent fibrinogen tests in conjunction with CRP can considerably improve the ability of critically ill patients to detect and manage infections and other inflammatory disorders. This technique not only promotes a more tailored treatment strategy, but also helps to construct firm predictive models for infection risk, which leads to better clinical results in the ICU surroundings. Moreover, despite the small sample size of this study, the findings provide a valuable foundation for future research in similar ICU settings. Given these results, it is advisable for future studies to stratify patients based on their specific conditions, such as sepsis and trauma, which are known to cause significant increases in fibrinogen levels. By following the trajectory of fibrinogen in these

patient groups, we can gain deeper insights into its role and potential as a predictor for the development of infections in the ICU. This categorization could show more detailed patterns and improve fibrinogen's prognostic accuracy, resulting in better patient outcomes through more targeted and timely therapies. Fibrinogen, as such, has not been frequently mentioned as a potentially reliable marker for diagnosing infection. It has been proven to exhibit great sensitivity for the recognition of infected nonunions, alongside traditional biomarkers that include CRP, erythrocyte sedimentation rate, and leukocyte count, which have been the most widely utilized markers. Infected nonunion constitutes a significant difficulty that mostly emerges after open reduction and internal fixation, impeding the healing process and potentially resulting in lifelong functional disability or amputation of the affected limb, key factor of effective treatment is accurately diagnosing whether the nonunion is infected (22). Fibrinogen's diagnostic performance suggested that it may have significant potential as a practical and cost-efficient biomarker for the diagnosis of infected nonunion (23). What's more, fibrinogen was found as a useful marker for identifying periprosthetic joint infection, a complication that arises after total joint arthtroplasty (24). Several studies have demonstrated that fibrinogen outperforms D-dimer and fibrin degradation product and exhibits diagnostic value comparable to or exceeding that of erythrocyte sedimentation rate and C-reactive protein collectively emphasizing its future as a predictive marker of infection (25). In like manner, it has been associated with various inflammatory conditions, including periodontitis (26), malaria (27), sepsis (28) and appendicitis (29). Nonetheless, in medical literature, CRP has been established as a reliable biomarker of inflammation, and now, with statistical significance reinforcing the association between fibrinogen and CRP, it can stand alongside CRP as an equally important marker in clinical practice. Further investigation into fibrinogen's role could considerably improve its practicality in ICU settings, particularly for quick decision-making and management of long-term patients. This type of work also opens up multiple possibilities for future researchers to further divide patients based on diagnosis, comorbidities, age, and/or initiated treatments, thereby strengthening and extending fibrinogen's therapeutic relevance as an infection diagnostic marker.

6 CONCLUSION

This study aimed to investigate whether fibrinogen can be used as a predictor of infection development in the ICU. Initially, the results table showed high fibrinogen values that exceeded the reference range, but these were not statistically significant, as fibrinogen exhibits the greatest variability, already, on the first day. This variability is likely due to numerous factors influencing fibrinogen levels, including individual differences, specific diagnoses, and disease progressions. Moreover, after conducting correlations between fibrinogen levels and other inflammatory parameters, including C-reactive protein (CRP), leukocytes, and procalcitonin, we found that the correlation and statistical significance were most effectively presented between fibrinogen and CRP while others were negligible. It should be noted that this study involved a relatively small number of participants, which can affect and reduce the reliability of the results. In conclusion, fibrinogen can not be used as a reliable predictor of infection development in the ICU. However, these findings highlight an opportunity for future research to further stratify patients by their underlying conditions, particularly focusing on sepsis and trauma, which are most commonly seen in the ICU. By doing so, researchers can better understand the role of fibrinogen in the inflammatory processes associated with these critical conditions. This approach could lead to more precise and effective predictive models for managing inflammation and infection in Intensive Care settings.

7 SUMMARY

In summary, this retrospective observational study aimed to evaluate the potential of fibrinogen as a predictor of infection in patients who remain in the Intensive Care Unit (ICU) for longer than seven days. Our findings indicate that fibrinogen can not be reliably used as a predictor of infection development in this patient population. The data showed a significant correlation between fibrinogen and C-reactive protein (CRP), but no significant correlation was found between fibrinogen and leukocytes or procalcitonin (PCT). The small sample size of this study restricts the generalizability of the results. However, these findings provide a valuable foundation for further research. Future studies with a greater sample sizes are necessary to explore these relationships more comprehensively. Such research is crucial for developing better strategies to predict and manage infections in patients with prolonged ICU stays, ultimately improving patient outcomes in critical care settings.

Keywords: C-reactive protein, Fibrinogen, Leukocytes, Procalcitonin

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9 CURRICULUM VITAE

Karla Tenžera was born in Zagreb, Croatia, on October 21, 1998. In 2018, she began her integrated undergrated and graduate studies at the Faculty of Medicine and became a part of the second generation of students to study medicine in English at the University of Rijeka. From the second year of her studies, she actively engaged as a member of the Rotaract Club Rijeka, which operates as part of Rotary International. Assuming the role of secretary for a tenure of two years, she contributed to the club's organizational functions and initiatives. Participation in the Rotaract club provided her with opportunities to cultivate both personal and professional skills through extensive volunteering efforts and involvement in community service projects. Furthermore, it provided her to explore numerous destinations and engage with a multitude of cultures. Notably, she participated twice in the SCOPE exchange program, CROMSIC, complemented by internships at the Department of Gastroenterology in Poland and the Department of Anesthesiology and Intensive Care in Malta.