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RESEARCH ARTICLE

The role of ferritin and carboxyhemoglobin as inflammatory biomarkers in sepsis and septic shock

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Background: While increased carbon monoxide production in sepsis is well-documented, the role of carboxyhemoglobin blood level fluctuations as a potential indicator of disease progression in sepsis remains unclear.

Objective: This study evaluates carboxyhemoglobin as a biomarker in sepsis and septic shock, comparing it with ferritin, C-reactive protein, and procalcitonin while assessing its potential as a bedside indicator for disease severity and mortality.

Material and Methods: We conducted a pilot, prospective, and observational study involving 52 patients diagnosed with sepsis or septic shock based on the SEPSIS 3 Consensus criteria. Clinical and laboratory parameters were monitored on days 1 and 5 following inclusion in the study.

Results: We observed a statistically significant variation in C-reactive protein and the severity scores for the sepsis and septic shock groups, and in carboxyhemoglobin, procalcitonin and one severity score for the survivor and non-survivor groups. In the survivor group we observed a statistically significant correlation between ferritin and the C-reactive protein, while for non-survivors, ferritin correlated with the APACHE II severity score. For all the studied groups we observed a statistically significant correlation between both studied severity scores.

Conclusions: Carboxyhemoglobin shows potential as a biomarker for monitoring sepsis progression, with its trends offering more clinical value than absolute cutoff values. Ferritin remains a dependable marker of inflammation and, when analyzed alongside carboxyhemoglobin and other known inflammatory biomarkers, provides a comprehensive view of sepsis progression, aiding in effective management.

Keywords: sepsis, carboxyhemoglobin, ferritin, procalcitonin, severity scores

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Introduction

Recent advancements in understanding the mechanisms of sepsis and septic shock have led to the identification of several promising biomarkers. These biomarkers provide valuable insights into the pathophysiological processes of sepsis and can assist in early diagnosis, risk stratification, monitoring disease progression, and evaluating the effectiveness of therapeutic interventions. However, novel sepsis parameters may pose challenges, particularly regarding costs and dynamic monitoring. Therefore, recent research focuses on identifying simple, point-of-care, and cost-effective tools that assist physicians in monitoring the progression of sepsis.

Carbon monoxide (CO) is a naturally occurring gas present in exhaled human breath, whose exploration dates to the 1920s, initially linked to pollution and smoking and subsequently to gut microbiota metabolism [1]. Its roles in inflammation, cellular death, and metabolic regulation are well-documented. Predominantly, it is synthesized in the liver through heme breakdown via the heme oxygenase-1 (HO-1) pathway, along with byproducts like free ferrous iron and biliverdin [2]. Myoglobin, cytochromes, peroxidases, and catalase are alternative sources and contribute around 20–25% to the total endogenous CO levels. In sepsis, impairment of hepatic microcirculation leads to increased heme degradation and elevated carbon monoxide production [3, 4].

The presence of CO is detected in the blood as carboxyhemoglobin (COHb) and through CO excretion in breath. Carboxyhemoglobin concentrations in the blood reflect internal carbon monoxide synthesis and are often measured during standard blood gas analyses. While increased endogenous CO production has been observed in sepsis, COHb levels have not been thoroughly investigated as a potential biomarker for sepsis [5]. Although COHb is not specific to any particular pathology, its variations may offer early prognostic insights into both bacterial and viral infections. Measuring COHb provides several advantages, including cost-effectiveness, ease of measurement, and bedside accessibility, making it a convenient parameter for real-time assessment.

Cells require iron to perform specialized functions. Beyond serving as the central component of hemoglobin and myoglobin for oxygen binding, iron plays crucial roles in various metabolic processes of both the host and pathogens. Maintaining iron homeostasis in the human body is vital for basic metabolism [6].

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Tissue ferritin is a crucial site for the physiological storage of iron in a nontoxic form that remains bioavailable to the body. Although a mitochondrial version of ferritin has been recently identified and nuclear localization and functions have been hypothesized, ferritin is predominantly found in the cytosol of most tissues [7]. Serum ferritin is generally regarded as a reliable indicator of iron stores under most conditions. Elevated serum ferritin levels are closely associated with inflammation and are regulated by proinflammatory cytokines. While the regulation of ferritin by inflammatory cytokines has been extensively studied, the underlying reasons for increased circulating ferritin during inflammation and its potential role in chronic injury remain unclear [8]. Ferritin is recognized as a marker of inflammation and macrophage activation [9].

Since 2003, procalcitonin (PCT) and C-reactive protein (CRP) have remained widely used biomarkers for diagnosing and managing sepsis. While PCT is more specific and dynamic for bacterial sepsis, CRP is a valuable general inflammation marker. In current practice, both biomarkers are often used in conjunction with clinical evaluation and other laboratory findings (e.g., blood cultures, lactate) to improve diagnostic accuracy and therapeutic decision-making in sepsis management.

Scoring systems play a crucial role in intensive care by providing objective tools to stratify patient groups based on disease severity, estimate individual mortality probabilities, and facilitate "adjusted" analyses of morbidity and mortality within patient cohorts. These systems emerged from the necessity to establish standardized reference points that clinicians can use to guide therapeutic decisions, resource allocation, and outcome evaluations [10]. The most widely used scores in clinical practice in intensive care are the Sequential Organ Failure Assessment Score (SOFA) and Acute Physiology, Age, Chronic Health Evaluation II score (APACHE II). The SOFA score is particularly useful for assessing ongoing organ dysfunction and sepsis severity, while APACHE II offers a comprehensive early assessment of patient prognosis at ICU admission. Even though prognostic scores are not key elements of treatment, they are essential to clinical decision-making and identifying patients with unexpected outcomes.

This study aims to evaluate the utility of carboxyhemoglobin as a biomarker in sepsis and septic shock, comparing its performance with established inflammatory markers such as ferritin, C-reactive protein, and procalcitonin. The research also aimed to assess carboxyhemoglobin's potential as a practical bedside indicator for disease severity and mortality prediction in these critical conditions.

Methods

This prospective observational single-center study enrolled 52 consecutive patients admitted to the County Emergency Clinical Hospital ICU in Târgu Mureș, Romania, between July 2021 and March 2023.

The study was conducted in accordance with the Helsinki Declaration of 1975. Ethical approval for this study was obtained from the Ethics Committee of the George Emil Palade University of Medicine and Pharmacy, Science, and Technology of Târgu Mureș, Mureș, Romania (approval no 1425/01.07.2021). Informed consent for study participation and data publication was obtained from each patient or their legal guardian.

Study Cohort

Participants included individuals aged 18 and above diagnosed with sepsis or septic shock according to the Sepsis-3 Consensus criteria [8]. The exclusion criteria were as follows: current cancer with chemotherapy or radiation therapy, ongoing treatment with corticosteroids or immunosuppressive medication, and evidence of autoimmune disorders.

The patients were categorized into four subgroups based on their diagnosis (sepsis or septic shock) and survival status (survivors and non-survivors).

Evaluated Parameters

The parameters examined in the study included age, gender, PCT, CRP, and ferritin levels. COHb was determined by an arterial puncture using a standard heparinized syringe (Stat Profile Prime Plus, Manufacturer: Nova Biomedical, Waltham, MA 02454-9141 USA, year of manufacture 2018). The APACHE II and SOFA scores were calculated daily. We assessed the parameters on the first (D1) and fifth days (D5) after confirmation of either sepsis or septic shock in the intensive care unit (ICU).

Statistical analysis

The information was input into MS Excel (Microsoft® Excel® for Microsoft 365 MSO. Subsequent statistical analyses, encompassing descriptive and inferential processing, were conducted using GraphPad Prism version 8.4.3 (GraphPad Software, San Diego, CA, USA). The Kolmogorov-Smirnov test was performed to verify the normality of data distribution. The mean was determined for data exhibiting a normal distribution, while for non-Gaussian distributions, the median and the interquartile range were calculated. We used either the Student's t-test or the Wilcoxon test for paired data, depending on whether the distribution was Gaussian or non-Gaussian.

Results

In the present study, we enrolled 52 consecutive patients diagnosed with sepsis (N=35, 67.3%) or septic shock (N=17, 32.69%), with a mean age of 67 years. More than half of the patients were male (N=31, 59.61%). The patients spent an average of 20 days in the ICU. We observed more in-hospital deaths (N=34, 65.38%) than survivors (N=18, 34.61%).

Tables 1 and 2 present the descriptive statistics of the determined biomarkers and prognostic scores in the sepsis

Table 1. Descriptive statistics of the determined biomarkers and prognostic scores for the group of sepsis patients for day 1 and day 5.

	Sepsis (n = 35)						
	Day	Minimum	Maximum	Percentiles			Mean
				25th	50th	75th	
PCT ng/mL	1	0.1	64.2	0.32	0.79	5.76	6.49
	5	0.07	93.09	0.32	0.79	2.04	6.32
CRP mg/dL	1	9.04	363.4	86.1	138.3	224.8	159.6
	5	14.0	360.8	42.6	108.6	169.3	121.8
Ferritin (pg/ml)	1	9.53	3060	225	385	666	591.2
	5	20.6	4580	233	386	783	705.1
COHb (%)	1	0	2.5	0.7	1.1	1.6	1.137
	5	0	3.2	0.6	1	1.4	1.106
APACHE II points	1	8	44	13	22	27	1.42
	5	5	37	13	18	23	18.69
SOFA points	1	1	18	5	8	12	8.4
	5	0	17	2	7	10	6.943

Legend: APACHE II: Acute Physiology, Age, Chronic Health Evaluation II score, COHb: Carboxyhemoglobin; CRP: C-reactive protein; PCT: Procalcitonin; SOFA: Sequential Organ Failure Assessment Score.

Table 2. Descriptive statistics of the determined biomarkers and prognostic scores for the lot of septic shock patients for day 1 and day 5.

	Septic shock (n = 17)						
	Day	Minimum	Maximum	Percentiles			Mean
				25th	50th	75th	
PCT ng/mL	1	1.76	51.5	2.07	3.7	22.39	12.59
	5	0.32	41.69	0.91	2.08	15.3	9.04
CRP mg/dL	1	45.1	648.0	75.68	164.9	249.6	194.1
	5	13.03	212.1	37	142.4	156.2	113.6
Ferritin (pg/ml)	1	54.9	5590	166.5	871	1305	1029
	5	110	3050	199	499	1200	812.4
COHb (%)	1	0	2.3	0.35	1.2	1.75	1.07
	5	0.2	2.6	0.6	1	1.85	1.22
APACHE II points	1	7	37	8.5	19	29.5	19.71
	5	5	37	11	18	32	20.47
SOFA points	1	2	16	5.5	9	12.5	9.05
	5	0	16	2	7	12.5	7.47

Legend: APACHE II: Acute Physiology, Age, Chronic Health Evaluation II score, COHb: Carboxyhemoglobin; CRP: C-reactive protein; PCT: Procalcitonin; SOFA: Sequential Organ Failure Assessment Score.

and septic shock groups for day 1 and day 5. According to the SEPSIS-3 Consensus, day 1 is defined as the day of establishing the diagnosis of either sepsis or septic shock.

We analyzed the variation in the studied biomarkers and prognostic scores on day 1 versus day 5 in the sepsis group presented as median values with interquartile ranges (IQR). The statistical analysis revealed a significant difference in the median value of CRP between day 1 and day 5 (p = 0.01). As expected, we also found a significant difference in the median values of SOFA and APACHE II scores between day 1 and day 5 (p = 0.001; p=0.03) (Table 3). In the septic shock group, analyzing the determined biomarkers on day 1 and day 5 revealed statistical significance only for CRP (p = 0.04) and SOFA score (p = 0.01) (Table 3).

We also analyzed the variation in the studied biomarkers and prognostic scores on day 1 versus day 5 in survivors versus non-survivor patients. In the survivor group, the median values of COHb (p = 0.0328), PCT (p = 0.0156), and SOFA score (p = 0.0295) varied significantly, while in the non-survivor group, only the median values of CRP (p

Table 3. The variation of the biomarkers and prognostic scores studied on day 1 versus day 5 in the sepsis and septic shock group (median value and IQR).

Parameter	Sepsis		p[a] value	Septic shock		p[a] value
	Day 1	Day 5		Day 1	Day 5	
COHb, (%)	1.1 (0.9)	1 (0.8)	0.6942	1.2 (1.4)	1 (1.25)	0.5388
CRP, (mg/L)	138.3 (138.7)	108.6 (126.7)	0.0102	164.9 (173.92)	142.4 (119.2)	0.0479
PCT, (ng/mL)	1.8 (4.6)	0.79 (1.72)	0.3303	3.7 (20.31)	2.08 (14.39)	0.1563
Ferritin	385 (441)	386 (550)	0.2516	871 (1138.5)	499 (1001)	0.6441
SOFA, points	8 (7)	7 (8)	0.0017	9 (7)	7 (10.5)	0.0115
APACHE II, points	22 (14)	18 (10)	0.0344	19 (21)	18 (21)	0.4854

Legend: [a]Wilcoxon test. Bold type indicates significance. APACHE II: Acute Physiology, Age, Chronic Health Evaluation II score, COHb: Carboxyhemoglobin; CRP: C-reactive protein; PCT: Procalcitonin; SOFA: Sequential Organ Failure Assessment Score.

Table 4. The variation of the biomarkers and prognostic scores studied on day 1 versus day 5 in survivors versus non-survivor patients. (median value and IQR).

Parameter	Survivors		p[a] value	Non-survivors		p[a] value
	Day 1	Day 5		Day 1	Day 5	
COHb, %	1.4 (1.15)	0.9 (1.05)	0.0328	1.1 (1.13)	1 (0.9)	0.1367
CRP, (mg/L)	180.5 (231.14)	91.45 (153.65)	0.2661	144.3 (127.4)	126.1 (114.12)	0.0025
PCT, (ng/mL)	3.27 (3.9)	0.32 (0.77)	0.0156	2.01 (6.18)	1.29 (8.5)	0.6257
Ferritin, (pg/L)	377.5 (417.8)	329 (371)	0.9323	582.5 (849.5)	454 (860.4)	0.5888
SOFA, points	5 (5.25)	2 (5.5)	0.0295	10.5 (6)	9 (8)	0.0006
APACHE II, points	13 (10)	12 (6.75)	0.2473	25.5 (11.5)	22.5 (16.25)	0.3101

Legend: [a]Wilcoxon test. Bold type indicates significance. APACHE II: Acute Physiology, Age, Chronic Health Evaluation II score, COHb: Carboxyhemoglobin; CRP: C-reactive protein; PCT: Procalcitonin; SOFA: Sequential Organ Failure Assessment Score.

= 0.0025) and SOFA (p = 0.0006) scores varied statistically considerably (Table 4).

We correlated the median values of the studied biomarkers and prognostic scores for each group: sepsis and septic shock, survivors and non-survivors on day 1 and day 5.

In the sepsis group on day 1, we only found a statistically significant positive correlation between the SOFA and APACHE II scores (r = 0.7622, p < 0.00010). On day 5, we found a statistically significant positive correlation between the PCT and SOFA scores (r = 0.4461, p = 0.0487) and also between SOFA and APACHE II scores (r = 0.7239, p < 0.0001) (Table 5).

In the septic shock group on day 1, we only found a statistically significant positive correlation between the SOFA and APACHE II score (r = 0.8398, p < 0.0001). On day 5, we found a statistically significant positive correlation between the same parameters as on day 1: SOFA and APACHE II score (r = 0.8327, p < 0.0001) (Table 6).

In the survivor group on day 1, we found only a statistically significant positive correlation between the CRP and Ferritin (r = 0.6348, p = 0.0074) and between SOFA and APACHE II score (r = 0.6752, p = 0.0021). On day 5, we also found a statistically significant positive correlation between the SOFA and APACHE II scores (r = 0.6860, p = 0.0017) (Table 7).

In the non-survivor group on day 1, we found a statistically significant positive correlation between the SOFA and APACHE II score (r = 0.6882, p < 0.0001). On day 5, we found a statistically significant positive correlation between PCT and APACHE II score (r = 0.4492, p = 0.0411), Ferritin and APACHE II score (r = 0.4115, p = 0.0156), SOFA and APACHE II score (r = 0.6922, p < 0.0001) (Table 8).

Discussions

Extensive research has been conducted over the years to understand the role of the liver in sepsis, which has become indispensable in unraveling the intricate pathophysiology

Table 5. Correlations between the median values of the studied biomarkers and prognostic scores for the sepsis group on day 1 and day 5.

		CRP (mg/L)	PCT (ng/mL)	Ferritin (pg/L)	SOFA, points	APACHE II, points
COHb (%)	Day 1	r = 0.1377 (-0.2158 to 0.4593) p[b] = 0.4449	r = 0.1904 (-0.3334 to 0.6243) p[a] = 0.4607	r = 0.04688 (-0.3003 to 0.3831) p[a] = 0.7891	r = 0.01729 (-0.3178 to 0.3485) p[b] = 0.9215	r = -0.1416 (-0.4534 to 0.2012) p[b] = 0.4173
	Day 5	r = -0.1417 (-0.4951 to 0.2519) p[b] = 0.4808	r = 0.08943 (-0.3797 to 0.5220) p[a] = 0.7077	r = 0.1728 (-0.1802 to 0.4863) p[a] = 0.3210	r = 0.2142 (-0.1282 to 0.5109) p[b] = 0.2167	r = 0.2900 (-0.04791 to 0.5683) p[b] = 0.0911
CRP (mg/L)	Day 1		r = 0.4414 (-0.06519 to 0.7671) p[a] = 0.0773	r = 0.2303 (-0.1331 to 0.5391) p[a] = 0.1973	r = -0.2393 (-0.5384 to 0.1133) p[b] = 0.1798	r = -0.2336 (-0.5341 to 0.1193) p[b] = 0.1908
	Day 5		r = 0.2788 (-0.2304 to 0.6681) p[a] = 0.2626	r = -0.2827 (-0.6059 to 0.1207) p[a] = 0.1531	r = -0.1763 (-0.5214 to 0.2184) p[b] = 0.3791	r = -0.04667 (-0.4192 to 0.3394) p[b] = 0.8172
PCT (ng/mL)	Day 1			r = 0.2367 (-0.2895 to 0.6530) p[a] = 0.3578	r = -0.1071 (-0.5696 to 0.4068) p[a] = 0.6797	r = -0.06006 (-0.5367 to 0.4456) p[a] = 0.8177
	Day 5			r = 0.07223 (-0.3944 to 0.5093) p[a] = 0.7622	r = 0.4461 (-0.009587 to 0.7484) p[a] = 0.0487	r = 0.3835 (-0.08510 to 0.7131) p[a] = 0.0951
Ferritin (pg/L)	Day 1				r = 0.02038 (-0.3242 to 0.3602) p[a] = 0.9075	r = 0.1572 (-0.1956 to 0.4740) p[a] = 0.3671
	Day 5				r = 0.1329 (-0.2194 to 0.4546) p[a] = 0.4466	r = 0.3056 (-0.04098 to 0.5866) p[a] = 0.0742
SOFA, points	Day 1					r = 0.7622 (0.5751 to 0.8736) p[b] < 0.0001
	Day 5					r = 0.7239 (0.5148 to 0.8517) p[b] < 0.0001

Legend: [a]Spearman test, [b]Pearson test. Bold type indicates significance. APACHE II: Acute Physiology, Age, Chronic Health Evaluation II score, COHb: Carboxyhemoglobin; CRP: C-reactive protein; PCT: Procalcitonin; SOFA: Sequential Organ Failure Assessment Score.

Table 6. Correlations between the median values of the studied biomarkers and prognostic scores for the septic shock group on day 1 and day 5

		CRP (mg/L)	*PCT (ng/mL)	Ferritin (pg/L)	SOFA, points	APACHE II, points
COHb, %	Day 1	r = -0.2188 (-0.6446 to 0.3106) p^b = 0.4156	r = 0.7714 p^a = 0.1028	r = -0.05893 (-0.5358 to 0.4465) p^a = 0.8219	r = -0.2871 (-0.6746 to 0.2245) p^b = 0.2639	r = -0.3227 (-0.6955 to 0.1869) p^b = 0.2065
	Day 5	r = 0.2545 (-0.3609 to 0.7155) p^a = 0.3984	r = -0.3891 p^a = 0.2658	r = -0.3985 (-0.7448 to 0.1169) p^a = 0.1135	r = 0.1445 (-0.3746 to 0.5947) p^a = 0.5765	r = 0.01108 (-0.4840 to 0.5008) p^a = 0.9669
CRP (mg/L)	Day 1		r = 0.8000 p^a = 0.1333	r = 0.3971 (-0.1386 to 0.7530) p^a = 0.1289	r = 0.2205 (-0.3090 to 0.6456) p^b = 0.4119	r = 0.2205 (-0.3090 to 0.6456) p^b = 0.4120
	Day 5		r = 0.2619 p^a = 0.5364	r = -0.1593 (-0.6634 to 0.4442) p^a = 0.6040	r = 0.3028 (-0.2979 to 0.7317) p^b = 0.3146	r = 0.3206 (-0.2798 to 0.7407) p^b = 0.2856
*PCT (ng/mL)	Day 1			r = -0.4857 p^a = 0.3556	r = 0.4414 p^a = 0.4333	r = -0.3714 p^a = 0.4972
	Day 5			r = 0.6322 p^a = 0.0551	r = 0.4554 p^a = 0.1873	r = 0.4939 p^a = 0.1495
Ferritin (pg/L)	Day 1				r = 0.02948 (-0.4698 to 0.5145) p^a = 0.9113	r = 0.1156 (-0.3996 to 0.5753) p^a = 0.6565
	Day 5				r = 0.2027 (-0.3219 to 0.6321) p^a = 0.4315	r = 0.3986 (-0.1167 to 0.7449) p^a = 0.1132
SOFA, points	Day 1					r = 0.8398 (0.6023 to 0.9407) p^b < 0.0001
	Day 5					r = 0.8327 (0.5869 to 0.9379) p^b < 0.0001

Legend: [a]Spearman test, [b]Pearson test. Bold type indicates significance. APACHE II: Acute Physiology, Age, Chronic Health Evaluation II score, COHb: Carboxyhemoglobin; CRP: C-reactive protein; PCT: Procalcitonin; SOFA: Sequential Organ Failure Assessment Score. *correlations with PCT contained a small number of pairs, such that a 95% confidence interval could not be calculated

of this condition. The liver regulates the overall immune response by orchestrating diverse local immune cell groups that produce immune-modulating cytokines, thus stimulating adaptive immunity. [11]. The decline in hepatocyte function affects global metabolism and innate and adaptive immune responses due to their release of pro- and anti-inflammatory proteins. The extent of liver function impairment is indicated by reduced bilirubin clearance and

Table 7. Correlations between the median values of the studied biomarkers and prognostic scores for survivor patients on day 1 and day 5

		CRP (mg/L)	PCT (ng/mL)	Ferritin (pg/L)	SOFA, points	APACHE II, points
COHb, %	Day 1	r = -0.02163 (-0.4971 to 0.4638) p^b = 0.9343	r = 0.4201 (-0.4870 to 0.8912) p^b = 0.3480	r = 0.01861 (-0.4640 to 0.4927) p^a = 0.9416	r = -0.2843 (-0.6632 to 0.2105) p^b = 0.2528	r = -0.3720 (-0.7148 to 0.1148) p^b = 0.1284
	Day 5	r = -0.1828 (-0.6950 to 0.4525) p^a = 0.5671	* r = 0.3590 p^a = 0.3420	r = -0.1180 (-0.5646 to 0.3820) p^a = 0.6409	r = 0.1950 (-0.3127 to 0.6160) p^a = 0.4382	r = 0.1671 (-0.3384 to 0.5978) p^a = 0.5075
CRP (mg/L)	Day 1		r = 0.04407 (-0.7960 to 0.8261) p^b = 0.9339	r = 0.6348 (0.2071 to 0.8588) p^a = 0.0074	r = -0.1944 (-0.6173 to 0.3158) p^b = 0.4548	r = -0.1385 (-0.5805 to 0.3665) p^b = 0.5960
	Day 5		* r = 0.02857 p^a > 0.9999	r = -0.4476 (-0.8192 to 0.1887) p^a = 0.1474	r = 0.09628 (-0.5198 to 0.6465) p^a = 0.7663	r = -0.1393 (-0.6604 to 0.4724) p^b = 0.6659
PCT (ng/mL)	Day 1			* r = 0.1429 p^a = 0.7825	r = 0.4942 (-0.4123 to 0.9090) p^b = 0.2596	r = 0.4097 (-0.4965 to 0.8886) p^b = 0.3613
	Day 5			* r = -0.0169 p^a = 0.9818	* r = 0.3448 p^a = 0.3701	* r = -0.0344 p^a = 0.9479
Ferritin (pg/L)	Day 1				r = -0.1030 (-0.5542 to 0.3950) p^a = 0.6843	r = -0.2790 (-0.6683 to 0.2302) p^a = 0.2622
	Day 5				r = -0.02528 (-0.4977 to 0.4588) p^a = 0.9207	r = -0.09860 (-0.5511 to 0.3987) p^a = 0.6971
SOFA, points	Day 1					r = 0.6752 (0.3042 to 0.8683) p^b = 0.0021
	Day 5					r = 0.6860 (0.3089 to 0.8767) p^a = 0.0017

Legend: [a]Spearman test, [b]Pearson test. Bold type indicates significance. APACHE II: Acute Physiology, Age, Chronic Health Evaluation II score, COHb: Carboxyhemoglobin; CRP: C-reactive protein; PCT: Procalcitonin; SOFA: Sequential Organ Failure Assessment Score. *correlations with PCT contained a small number of pairs, such that a 95% confidence interval could not be calculated

Table 8. Correlations between the median values of the studied biomarkers and prognostic scores for non-survivor patients on day 1 and day 5

		CRP (mg/L)	PCT (ng/mL)	Ferritin (pg/L)	SOFA, points	APACHE II, points
COHb, %	Day 1	r = -0.08303 (-0.4196 to 0.2736) p^a = 0.6514	r = 0.07584 (-0.4492 to 0.5619) p^b = 0.7786	r = 0.07047 (-0.2838 to 0.4078) p^b = 0.6921	r = 0.2121 (-0.1358 to 0.5134) p^a = 0.2285	r = 0.08429 (-0.2613 to 0.4108) p^a = 0.6355
	Day 5	r = 0.1329(-0.2527 to 0.4821) p^b = 0.5002	r = -0.07045 (-0.4977 to 0.3843) p^a = 0.7615	r = -0.04887 (-0.3896 to 0.3036) p^a = 0.7837	r = 0.1654 (-0.1830 to 0.4769) p^b = 0.3498	r = 0.2058 (-0.1423 to 0.5086) p^b = 0.2430
CRP (mg/L)	Day 1		r = 0.4636 (-0.05775 to 0.7862) p^a = 0.0721	r = 0.2727 (-0.09462 to 0.5747) p^a = 0.1310	r = 0.05930 (-0.2955 to 0.3997) p^b = 0.7471	r = 0.08596 (-0.2709 to 0.4220) p^b = 0.6399
	Day 5		r = 0.3729 (-0.09728 to 0.7070) p^a = 0.1053	r = -0.1237 (-0.4838 to 0.2722) p^a = 0.5306	r = -0.04703 (-0.4129 to 0.3319) p^b = 0.8121	r = 0.1520 (-0.2344 to 0.4969) p^b = 0.4400
PCT (ng/mL)	Day 1			r = 0.2163 (-0.3274 to 0.6524) p^a = 0.4181	r = 0.09549 (-0.4332 to 0.5753) p^a = 0.7228	r = -0.09443 (-0.5746 to 0.4341) p^a = 0.7253
	Day 5			r = 0.2922 (-0.1729 to 0.6508) p^a = 0.1987	r = 0.2679 (-0.1984 to 0.6353) p^a = 0.2404	r = 0.4492 (0.008122 to 0.7440) p^a = 0.0411
Ferritin (pg/L)	Day 1				r = 0.03423 (-0.3169 to 0.3771) p^a = 0.8476	r = 0.2328 (-0.1246 to 0.5367) p^a = 0.1852
	Day 5				r = 0.1403 (-0.2177 to 0.4650) p^a = 0.4287	r = 0.4115 (0.07479 to 0.6639) p^a = 0.0156
SOFA, points	Day 1					r = 0.6882 (0.4562 to 0.8326) p^b < 0.0001
	Day 5					r = 0.6922 (0.4623 to 0.8350) p^b < 0.0001

Legend: [a]Spearman test, [b]Pearson test. Bold type indicates significance. APACHE II: Acute Physiology, Age, Chronic Health Evaluation II score, COHb: Carboxyhemoglobin; CRP: C-reactive protein; PCT: Procalcitonin; SOFA: Sequential Organ Failure Assessment Score.

increased transaminase levels. A noteworthy aspect is the substantial production of carbon monoxide (CO) by the liver during sepsis through the catabolism of heme via the heme oxygenase-1 (HO-1) pathway [12, 13, 14]. HO-1 is an enzyme induced by various factors, such as oxidative stress, hypoxia, cytokines, endotoxins, and inflammatory mediators. Most HO-1 isoforms are found in the spleen and liver [5]. During sepsis, all endogenous CO sources become activated, primarily due to the upregulation of HO-1 expression, driven by tissue hypoxia, liver dysfunction, oxidative stress, and bacteremia [15].

Our study examined the variation of COHb from day 1 to day 5 in survivors and non-survivors and sepsis and septic shock patients and found that a significant variation of COHb might predict sepsis survival (p = 0.03). Probably, due to the relatively small number of patients included in the study, the COHB value does not correlate with any of the biomarkers or scores studied, in any of the groups analyzed.

Arterial puncture for COHb assessment offers a rapid, straightforward, and cost-effective means of gauging liver function impairment due to tissue hypoxia and compromised microcirculation. Since smokers typically have higher absolute COHb levels than non-smokers, we focused on the variation of COHb levels rather than the absolute values. Boehm et al. demonstrated that refraining from smoking for over 12 hours or smoking fewer than 20 cigarettes daily reduced COHb levels [16]. However, ongoing debates persist regarding the utility of COHb measurement in detecting exogenous CO poisoning, as studies have produced conflicting results, with some supporting its efficacy and others refuting it [1, 2].

The ferritin was another biomarker examined in our study. As reported before in other studies, ferritin participates in sepsis response due to interleukin – 1 and tumor necrosis factor – alfa induced nuclear factor kappa B activation, an acute phase reactant. Its role has been reported to be sequestrating iron from iron–loving bacteria and preventing oxidative stress.

Our study did not observe any variations in the median value of ferritin from day 1 to day 5 in survivors and non-survivors or in sepsis and septic shock patients. In our study, we found a statistically significant correlation between ferritin and APACHE II score on day 5 in the non–survivor group of our patients, suggesting precautious use of this biomarker alone as a prognosis factor of mortality in sepsis and septic shock.

Our findings do not correlate with the findings of Yi-Peng Fang et al., who demonstrated in a retrospective cohort study that high soluble ferritin was significantly associated with poor outcomes in septic patients [6]. Their study results showed that ferritin levels were even higher in the septic shock subgroup; however, these findings are not consistent with ours. Additionally, they observed a positive correlation between ferritin levels and the SOFA score, as well as a linear correlation between ferritin and in-hospital mortality in septic patients, but only among those with positive cultures and anemia. Ferritin was also associated with an increased risk of developing sepsis-related complications, such as acute kidney injury (AKI)

and the need for vasopressors. This suggests that AKI and anemia are significant interactive factors. However, the predictive value of ferritin is diminished in these patients [6].

In a recent study, ferritin demonstrated greater statistical significance on day 1 compared to the APACHE II score. The study also concluded that when serial biomarker values are considered, they provide far more predictive value than scoring systems alone [8].

Our findings regarding the presence of elevated ferritin levels in sepsis and septic shock highlight its correlation with a widely used mortality score in the non-surviving group. This observation underscores the potential of reconsidering ferritin as a valuable prognostic marker in these conditions.

In contrast to other studies in the literature, we found statistically significant ferritin values on later days after the onset of sepsis. Utilizing point-of-care ferritin determination could serve as an intermediate approach to harness its role as a prognostic factor for mortality in sepsis and septic shock, though not as a standalone tool. While it is clear that ferritin plays a role in sepsis and septic shock, the discrepancies between findings in the literature and our study highlight the need for further investigation into its significance and potential applications.

This study has several limitations. The relatively small sample size made it challenging to draw definitive conclusions. Also, as a single-center study, there was possible bias in assessing the pathology. The authors plan to broaden the study group and further evaluate the examined parameters in a larger, more diverse patient cohort.

Conclusion

Monitoring carboxyhemoglobin levels in sepsis and septic shock demonstrates promise as a biomarker for tracking disease progression. The statistically significant variations observed in survivors highlight its potential for outcome assessment, particularly when multiple daily measurements are conducted. A key advantage of carboxyhemoglobin monitoring is its ease of measurement; however, the trend in its variation, rather than absolute cutoff values, should alert clinicians to potential deterioration.

Ferritin remains a reliable inflammatory biomarker for evaluating sepsis progression. When correlated with carboxyhemoglobin, C-reactive protein, procalcitonin, and severity scores, it provides an extensive perspective on the clinical course of sepsis, enhancing the ability to monitor and manage the condition effectively.

Authors' contribution

B.L.G. (Conceptualization; Data curation; Methodology; Project administration; Writing—original draft; Funding acquisition)
O.C. (Data curation; Formal analysis; Investigation; Resources; Software)
A.H. (Investigation)
R.Ș.F. (Conceptualization; Methodology; Project administration; Supervision; Validation; Writing—review and editing) All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

None to declare.

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