Hemostatic balance in acute-on-chronic liver failure

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Survival in septic shock associated with thrombocytopenia

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Introduction

Sepsis is a syndrome of infection-induced physiological, pathological, and biochemical abnormalities; while, septic shock is a septic process associated with circulatory, cellular, and metabolic deleterious changes with a higher risk of mortality compared to the unique presence of sepsis.[1,2] Severe sepsis and septic shock are two clinical entities that have a significant impact on intra-hospital morbidity and mortality, as well as on cost in health systems.[3,4] Sepsis can progress to septic shock, multiple organ failure, and death if not recognized early.[5] Clinically, septic shock includes patients who meet the criteria for sepsis and who, despite adequate fluid resuscitation, require vasopressors to maintain a mean arterial pressure greater than 65 mmHg and serum lactate concentrations greater than 2 mmol/L.[6] The factors that contribute to septic shock are vasodilation, increased permeability due to loss of vascular smooth muscle reactivity secondary to cellular and humoral mediators that condition endothelial dysfunction, hypovolemia, and bilateral ventricular dysfunction.[6]

Sepsis continues to be a challenge worldwide due to the high morbidity and mortality it represents.[7] Progressive advances in the management of sepsis have led to a decrease in mortality rates in recent decades. The estimated frequency of septic shock in Europe and North America ranges between 8.3% and 10.4%.[8] Mortality in septic shock, although very similar, varies as a function of the place and time; consequently, the intensive care unit (ICU) mortality is 37.3%, the hospital mortality 39.0%, and the 28-/30-day mortality 36.7%.[8] Mortality rates have decreased from 45% in 1993 to 37% in 2003, to 29% in 2007, and 18.4% in 2012.[7] Therefore, it is important to know the factors associated with mortality in septic shock, thus allowing to gain more knowledge of this entity, to create better therapeutic schemes. More than 50% of patients with sepsis develop severe sepsis and 25% develop septic shock; these figures represent 15% of all admissions to the ICU.[9]

Platelets are the smallest blood cells, derived from cytoplasmatic fragments of megakaryocytes; furthermore, they play a critical role in vascular, immunological, and endothelial hemostasis, as well as contribute to thrombotic disorders.[10–12] Platelets are activated in patients with systemic inflammation, the release of cytokines and mediators stored in dense and alpha granules after stimulation, and in sepsis; hence platelet sequestration occurs within the microcirculation and consequently gives rise to thrombocytopenia.[13-16] Thrombocytopenia can be classified as mild (i.e. 100,000/μL to 149,000 /μL), moderate (i.e. 50,000/μL to 99,999/μL), and severe (i.e. <50,000/μL).[17] Worldwide, between 22% and 58% of patients with sepsis can present thrombocytopenia;[18–22] meanwhile, 20% to 50% of patients at the ICU present thrombocytopenia.[11] The presence of thrombocytopenia in septic shock has been associated with a higher lethality, having a case-fatality rate of 22.8–35.6% for platelets >150,000/mm,[3] 25.1–42.1% for mild thrombocytopenia, 37.2–46.0% for moderate thrombocytopenia, and 54.1–60.0% for severe thrombocytopenia for septic shock at 28 to 30 days after admission to the ICU.[23–26]

We report a prospective study according to the STROBE Statement[27] that included 440 patients diagnosed with septic shock who required management in an intensive care unit at a university teaching hospital. The patients were classified according to the degree of thrombocytopenia, while demographical, prognostic scales and biological markers were recorded. While previous work has shown that thrombocytopenia is a risk factor for mortality in patients with sepsis and septic shock, this study provides insight into the association between septic shock and thrombocytopenia. The main objective of the study was to compare the fatality distribution at 30-, 60-, and 90-days due to septic shock between patients with and without thrombocytopenia upon ICU admission. Once differences were observed in

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the mortality distribution, a regression analysis was performed to assess the predictor variables associated with death at each time point; thus, a secondary objective was to explore the factors associated with a higher case-fatality rate and thrombocytopenia at 30-, 60-, and 90-days.

Methods

A longitudinal, observational, prospective study that included 440 patients diagnosed with septic shock from the intensive care unit at a university teaching hospital (Hospital General León, León, México) from April 2018 through December 2019. The inclusion criteria included: age between 18 and 80 years, both sexes, diagnosis of septic shock, patients with 30-, 60- and 90-day follow-up. The exclusion criteria included: the presence of thrombocytosis; diagnosis of hematologic or solid neoplasm; hepatic cirrhosis diagnosis; previous diagnosis of autoimmune disease; previous splenectomy; diagnosis of drug-induced thrombocytopenia. The elimination criteria included: incomplete 30-, 60-, and 90-day follow-up; diagnosis of septic and another type of shock (i.e. hypovolemic, cardiogenic, anaphylactic, or neurogenic shock); sustaining an ischemic cerebrovascular event; readmission to the ICU.

Upon ICU admission, full blood and biochemistry workup was requested. Patients were classified into four groups based on their platelet levels upon admission to the ICU: (1) no thrombocytopenia (i.e. 450,000/μL > x >150,000/μL); (2) mild (i.e. 100,000/μL to 149,999 /μL); (3) moderate (i.e. 50,000/μL to 99,999/μL); and (4) severe (i.e. <50,000/μL). The patient selection flow diagram is displayed in Fig. 1. Septic shock was defined according to the Sepsis-3 definition, where a septic shock is a subset of sepsis in which the underlying circulatory and cellular or metabolic abnormalities cause persistent hypotension requiring vasopressors to maintain mean arterial pressure greater than or equal to 65 mmHg and lactate greater than or equal to 18 mg/dL (2 mmol/L) despite adequate fluid resuscitation.[1] Sepsis severity was assessed with the chronic health status as evaluated (Charlson comorbidity index), the Sequential Organ Failure Assessment (SOFA), and the Acute Physiology and Chronic Health Evaluation (APACHE IV and APACHE IV APS). Antibiotic therapy was assigned empirically based on the primary infection site and suspected microorganism; however, once the microorganism was isolated via blood, secretion, urine, or cerebrospinal fluid culture-specific targeted therapy was initiated.

Demographical variables such as sex, age, weight, height, body-mass index, as well as clinical variables such as length of stay in the ICU, intra-hospital stay, vital signs, PaO_2/FiO_2, comorbidities, heart failure, acute kidney failure, acute respiratory failure, and therapeutic management (e.g. use of vasopressors and inotropes, renal replacement therapy, steroid use, mechanical ventilation) were recorded in the medical file. This study was approved by the Institutional Review Board of our hospital (Hospital General León Bioethics and Research Committee) and registered at ClinicalTrials.gov with identifier NCT03617965. Upon hospital admission, the patient signed an informed consent permitting the use of her clinical file information for didactic, research, and publication purposes. Abiding by the Declaration of Helsinki, patient anonymity was guaranteed.

Statistical analysis was performed using SPSS 25 (SPSS Inc., Chicago, IL). Data were screened for outliers and normality assumptions. The normality of continuous variables (i.e. age, BMI, length of stay in ICU, CCI, SOFA, APACHE IV, and APACHE IV APS) was assessed with the Shapiro-Wilk normality test and visually using histograms and Q-Q plots. Demographical and clinical factors are summarized using proportions and percentages. The variable sex, a categorical demographical variable, and the time to death, a clinical continuous variable, were assessed for statistical inference with a Mann-Whitney test. The clinical continuous variables (i.e. age, BMI, length of stay in ICU, CCI, SOFA, APACHE IV, and APACHE IV APS) were assessed for statistical inference individually using independent-sample t-tests and Levene's test for equality of variances to test for homoscedasticity. Statistical significance was set at $p \leq 0.0019$ after a Bonferroni correction for multiple comparisons. The Kaplan-Meier method was used to calculate survival distributions; meanwhile, the Gehan-Breslow-Wilcoxon method was used to compare the equality of survival distributions, as it gives more weight to deaths at early time points. Both an overall (i.e. differences between all four groups) and a between-group (i.e. differences found between-groups accounting for all between-group comparisons) survival analysis was performed. A multivariable Cox proportional hazard model was used to determine the association between differences in platelet count levels upon ICU admission and the case-fatality rate at 30-, 60-, and 90-days. A multiple Cox regression model with backward stepwise elimination was performed with the following covariables included: sex, age, body mass index, APACHE score, SOFA score, Charlson comorbidity index, two interaction variables to account for overlapping factors incorporated between the scales (i.e. APACHE_score*SOFA_score and APACHE_score* Charlson_comorbidity_index), serum lactate, and serum procalcitonin levels. Overall, tests for the equality of survival times, as well as pairwise comparisons (i.e. between-group comparisons) were performed with statistical significance set at $p \leq 0.05$.

Results

Seven hundred and ten patients with septic shock were identified in the 21-month recruitment phase of this study. One hundred and thirty patients were excluded, leaving 580 patients of which 140 were eliminated from the analysis. Fig. 1 displays the patient selection flow diagram in this study with the reason and the number of patients excluded and eliminated from the analysis. Four hundred and forty patients were included in the final analysis of which 157 had a septic shock but no thrombocytopenia (i.e. control group), 113 had mild thrombocytopenia, 107 had moderate thrombocytopenia, and 63 had severe thrombocytopenia. No statistical differences were found between each thrombocytopenia group and the control group for the demographical variables (i.e. sex, age, BMI, and length of ICU stay). Demographic patient characteristics can be found in Table 1. The means of all four intensive care unit assessment scale scores (i.e. CCI, SOFA, APACHE IV, and APACHE IV APS) were statistically different between controls and patients with severe thrombocytopenia; severe thrombocytopenia was associated with higher CCI, SOFA, APACHE IV, and APACHE IV APS scores. Meanwhile, patients with moderate thrombocytopenia had a higher SOFA score than controls. Clinical participant characteristics can be found in Table 2.

Survival analysis

An overall and between-group survival analysis was performed at 30-, 60-, and 90-days using the Kaplan-Meier method. The survival pairwise comparison analysis at 30-, 60-, and 90-days is found in Table 3. At 30 days, 122 patients died (i.e. 35 controls and 87 with thrombocytopenia; 28 with mild, 25 with moderate, and 34 with severe thrombocytopenia); furthermore, the case-fatality rate at 30 days was 22.29% for the control group, while 24.78%, 23.36%, and 53.97% for mild, moderate, and severe thrombocytopenia respectively. The survival distributions across the four groups (i.e. control, mild, moderate, and severe thrombocytopenia) at 30 days were statistically different ($p \leq 0.001$, χ^2=35.301, global mean survival=68.61%, 95% CI [65.36, 71.86]) and are displayed in Fig. 2a. The between-group survival distribution differences were statistically significant between the patients with severe thrombocytopenia (i.e. mean survival=47.11%) and the three other groups included (i.e. control, $p \leq 0.001$, χ^2=26.497, mean survival=72.80%, 95% CI [67.76, 77.85]; mild thrombocytopenia, $p \leq 0.001$, χ^2=18.332, mean survival=71.27%, 95% CI [65.21, 77.34]; moderate thrombocytopenia,

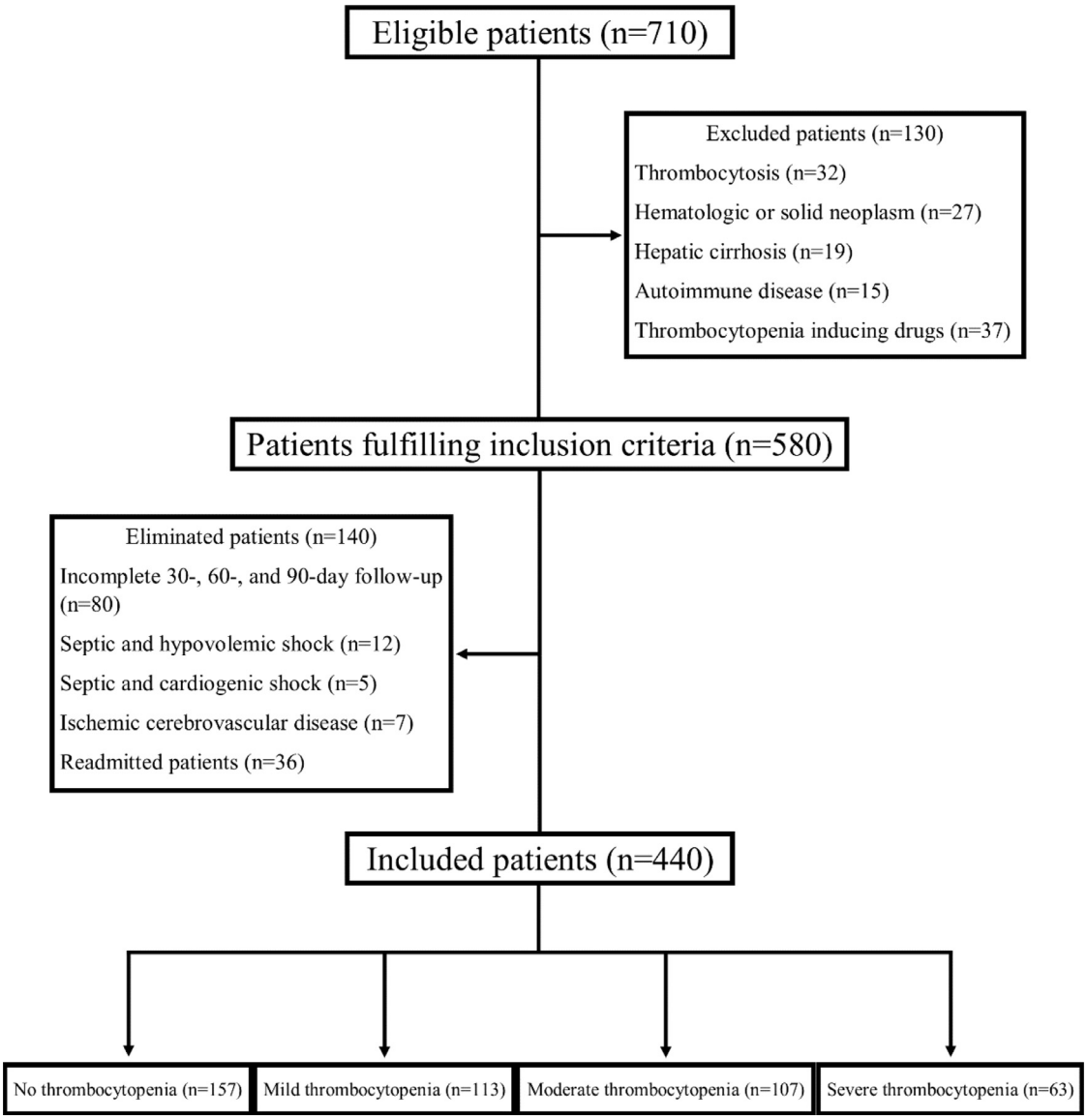

Fig. 1. Patient selection flow diagram. Patient selection flow diagram displaying a description of the patients included for data analyses, as well as a description of the patients excluded and eliminated from statistical analyses.

$p \leq 0.001$, χ^2=20.482, mean survival=72.30%, 95% CI [66.18, 78.42]) at 30 days.

At 60 days, 63 patients died (i.e. 20 controls and 43 with thrombocytopenia; specifically, 15 with mild, 19 with moderate, and 9 with severe); additionally, the case-fatality rate at 60 days was 35.03% for the control group and 38.05%, 41.12%, 68.25% for mild, moderate, and severe thrombocytopenia respectively. The survival distributions across the four groups were not statistically significantly different (Fig. 2b). Meanwhile, at 90 days, 35 patients died (i.e. 6 controls and 29 with thrombocytopenia; 9 with mild, 12 with moderate, and 8 with severe); meanwhile, the case-fatality rate at 90 days was 38.85% for the control group and 46.02%, 52.34%, and 80.95% for mild,

Table 1
Demographics participant characteristics upon admission to the ICU.

		Demographical							
Diagnosis	N	Sex	p	Age	p [CI 95]	BMI	p [CI 95]	Length of ICU stay	p [CI 95]
Overall	440	231 Male (52.5%)		62.90 ± 11.06		28.66 ± 3.19		5.88 ± 1.71	
No thrombocytopenia	157	85 Male (54.1%)		60.63 ± 11.26		28.77 ± 3.13		6.13 ± 1.94	
Mild thrombocytopenia	113	58 Male (51.3%)	0.648	63.27 ± 11.57	0.061 [−5.41, 0.123]	28.91 ± 3.16	0.721 [−0.90, 0.62]	5.63 ± 1.53	0.024 [0.68, 0.93]
Moderate thrombocytopenia	107	58 Male (54.2%)	0.992	64.92 ± 10.66	0.002 [−7.00, −1.57]	28.21 ± 2.95	0.144 [−0.19, 1.32]	5.95 ± 1.81	0.463 [−0.29, 0.64]
Severe thrombocytopenia	63	33 Female (52.4%)	0.382	64.44 ± 9.36	0.011 [−6.75, −0.88]	28.69 ± 3.73	0.872 [−0.89, 1.05]	5.59 ± 1.06	0.009 [0.03, 1.05]

ICU: intensive care unit. N: number. CI 95: 95% confidence interval. BMI: body mass index.

Table 2
Clinical participant characteristics upon admission to the ICU.

		Clinical									
Diagnosis	N	Time to death in days	p	CCI	p[CI 95]	SOFA	p[CI 95]	APACHE IV	p[CI 95]	APACHE IV APS	p[CI 95]
Overall	440	26		4.87 ± 2.82		7.17 ± 2.20		44.23 ± 15.09		35.10 ± 13.00	
No thrombocytopenia	157	20		4.55 ± 2.70		6.53 ± 2.05		42.77 ± 12.39		35.13 ± 10.16	
Mild thrombocytopenia	113	29.5	0.655	4.06 ± 2.52	0.135	6.64 ± 1.88	0.658	41.45 ± 11.02	0.358	31.54 ± 9.37	0.003
					[−0.15, 1.13]		[−0.59, 0.37]		[−1.50, 4.14]		[1.20, 5.98]
Moderate thrombocytopenia	107	35	0.059	4.91 ± 2.74	0.293	7.83 ± 2.07	≤0.001*	43.18 ± 12.66	0.795	33.07 ± 9.90	0.102
					[−1.03, 0.31]		[−1.81, −0.80]		[−3.49, 2.68]		[−0.41, 4.55]
Severe thrombocytopenia	63	14	0.181	7.06 ± 2.65	≤0.001*	8.59 ± 2.34	≤0.001*	54.67 ± 24.49	≤0.001*	44.86 ± 22.03	0.001*
					[−3.3, −1.73]		[−2.69, −1.43]		[−18.35, −5.44]		[−15.49, −3.96]

ICU: intensive care unit. N: number. CI 95: 95% confidence interval. CCI: Charlson Comorbidity Index. SOFA: Sequential Organ Failure Assessment score. APACHE IV: Acute Physiology And Chronic Health Evaluation IV. SAPS 3: Simplified Acute Physiology Score 3. Statistical significance after a Bonferroni correction marked by *.

moderate, and severe thrombocytopenia and septic shock. Overall, there were statistically significant differences in survival time distribution across the four groups ($p \leq 0.001$, χ^2=19.663, global mean survival=87.77%, 95% CI [87.01, 88.53]) and are displayed in Fig. 2c. The between-group survival time distributions were statistically different between the control group (i.e. mean survival=89.07%) and the moderate thrombocytopenia group (p = 0.008, χ^2=7.001, mean survival=87.03%, 95% CI [85.18, 88.89]); as well as, between the control group (i.e. mean survival=89.07%) and the severe thrombocytopenia group ($p \leq 0.001$, χ^2=21.565, mean survival=83.45%, 95% CI [79.70, 87.20]). Furthermore, between-group survival time differences were found between the mild (p = 0.006, χ^2=7.505, mean survival=87.77%, 95% CI [86.35, 89.19]) and severe thrombocytopenia groups (i.e. mean survival=83.45%).

Regression analysis

Multivariate Cox proportional hazard models were performed to determine the association between differences in platelet count levels upon ICU admission and the case-fatality rate at 30-, 60-, and 90-days. This survival analysis independently compared patients with sepsis and mild, moderate, and severe thrombocytopenia to the control group (i.e. patients with sepsis and a normal platelet count). Only severe thrombocytopenia was associated with increased mortality at 90 days (HR=3.74, 95% CI [1.04, 13.42]). Males had twice the risk of death than females at 60 days in the moderate (HR=2.34, 95% CI [1.21, 4.53]) and severe thrombocytopenia groups (HR=2.16, 95% CI [1.00, 4.65]). SOFA score was associated with increased mortality at 30 days for mild (HR=1.19, 95% CI [1.04, 1.37]), moderate (HR=1.21, 95% CI [1.04, 1.40]), and severe (HR=0.72, 95% CI [0.54, 0.97]) thrombocytopenia. Serum lactate levels were associated with an increased risk of mortality at 30 days for severe thrombocytopenia (HR=1.56, 95% CI [1.21, 2.01]) compared to controls. Increased serum procalcitonin levels, increased BMI, older age, and more comorbidities were weakly associated with increased mortality. While lower BMI and fewer comorbidities were weakly associated with higher survival rates. A detailed description of the risk and protective factors at 30-, 60-, and 90-days for mild, moderate, and severe thrombocytopenia are presented in Table 4.

Discussion

We report an observational, prospective study that included 440 patients with septic shock at a university teaching hospital in a period of 21 months. We compared patients with and without thrombocytopenia at 30-, 60-, and 90-day time points. To the best of our knowledge, this is the first prospective longitudinal study that evaluates the association between thrombocytopenia, septic shock, and mortality. While previous work has shown that thrombocytopenia is a risk factor for mortality in patients with sepsis and septic shock, this study provides insight into the association between septic shock and thrombocytopenia. Confounders such as sepsis, hepatic cirrhosis, autoimmune diseases, and cancer diagnosis were excluded from this study to understand better the risk factors associated between septic shock and mortality. Overall, the case-fatality rates increased with time. First, we determined if the survival distribution were different between the compared groups. Along with the degree of thrombocytopenia (i.e. mild, moderate, and severe versus no thrombocytopenia), disease severity, organ failure, demographical factors (i.e. sex, age, BMI, and comorbidities), lactate and procalcitonin serum levels, were assessed as risk factors for increased mortality at 1, 2 and 3-months. For the first time, severe thrombocytopenia was associated as an independent risk factor for increased mortality at 90 days. Thus, the results presented here emphasize the need for longer follow-up windows for patients with severe thrombocytopenia and septic shock. Furthermore, although no randomized controlled clinical trials

Table 3
Survival pairwise comparisons analysis.

	Thrombocytopenia	No thrombocytopenia		Mild thrombocytopenia		Moderate thrombocytopenia		Severe thrombocytopenia	
		χ^2	Sig.	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.
Mortality at 30 days									
Breslow (Generalized Wilcoxon)	No thrombocytopenia			0.216	0.642	0.016	0.900	**26.497**	**≤0.001**
	Mild thrombocytopenia	0.216	0.642			0.066	0.797	**18.332**	**≤0.001**
	Moderate thrombocytopenia	0.016	0.900	0.066	0.797			**20.482**	**≤0.001**
	Severe thrombocytopenia	**26.497**	**≤0.001**	**18.332**	**≤0.001**	**20.482**	**≤0.001**		
Mortality at 60 days									
Breslow (Generalized Wilcoxon)	No thrombocytopenia			0.098	0.754	1.175	0.278	3.028	0.082
	Mild thrombocytopenia	0.098	0.754			0.412	0.521	1.791	0.181
	Moderate thrombocytopenia	1.175	0.278	0.412	0.521			0.776	0.379
	Severe thrombocytopenia	3.028	0.082	1.791	0.181	0.776	0.379		
Mortality at 90 days									
Breslow (Generalized Wilcoxon)	No thrombocytopenia			2.638	0.104	**7.001**	**0.008**	**21.565**	**≤0.001**
	Mild thrombocytopenia	2.638	0.104			0.900	0.343	**7.505**	**0.006**
	Moderate thrombocytopenia	**7.001**	**0.008**	0.900	0.343			3.599	0.058
	Severe thrombocytopenia	**21.565**	**≤0.001**	**7.505**	**0.006**	3.599	0.058		

Statistically significant figures in **bold**.

assessing platelet transfusion as an intervention to correct for thrombocytopenia in patients with septic shock has been performed, the International Guidelines for Management of Sepsis and Septic Shock recommends (i.e. weak recommendation due to very low quality of evidence) a prophylactic platelet transfusion when "counts are $<$10,000/mm^3 (10×10^9/L) in the absence of apparent bleeding and when counts are $<$20,000/mm^3 (20×10^9/L) if the patient has a significant risk of bleeding. Higher platelet counts [$\geq$50,000/mm^3 (50×10^9/L)] are advised for active bleeding, surgery, or invasive procedures".[2]

Survival

Mortality associated with septic shock has been reported between 40% and 60%.[28] Patients without thrombocytopenia had lower case-fatality rates (i.e. 22.29% at 30 days, 35.03% at 60 days, and 38.85% at 90 days); conversely, patients with severe thrombocytopenia and had higher case-fatality rates throughout the study, 53.97% at 30 days, 68.25% at 60 days, and 80.95% at 90 days follow-up. The survival time distributions were significantly different at 30 days between severe thrombocytopenia and the other three groups (i.e. mild, moderate, and no thrombocytopenia). Meanwhile, at 90 days the survival time distributions were significantly different between the control group and both the moderate and severe thrombocytopenia groups, as well as, between the mild and severe thrombocytopenia groups. Among the risk factors associated with a higher case-fatality rate at 30-, 60-, and 90-days, severe thrombocytopenia was associated with increased mortality at 30 days, while moderate thrombocytopenia and severe thrombocytopenia were associated with increased mortality at 90 days. Previous work assessing the role of thrombocytopenia in septic shock reported similar trends to those found in this study. Higher platelet counts have been associated with lower case-fatality rates and the opposite is also true with lower platelet totals and higher case-fatality rates. Previous work by Claushuis and colleagues (2016) and by Thiery-Antier and colleagues (2016) report a case-fatality rate between 22.8% and 35.6% for septic shock with no thrombocytopenia, between 25.1% and 42.1% for septic shock and mild thrombocytopenia, between 37.2% and 46.0% for septic shock and moderate thrombocytopenia, and between 54.1% and 60.0% for septic shock and severe thrombocytopenia at a month cutoff after admission to the ICU.[23,24] These rates at 30 days are comparable to those observed in this study (i.e. 22.29%, 24.78%, 23.36%, and 53.97% for without, mild, moderate, and severe thrombocytopenia) and can be are displayed in Fig. 3. Although the case-fatality rate in septic shock and moderate thrombocytopenia is lower than expected at 30 days, a positive linear trend is observed across time (Fig. 4).

Risk factors

Thrombocytopenia has been linked to higher mortality rates; moreover, the risk of death has been previously reported to increase with the severity of thrombocytopenia. A higher case mortality rate at 28 to 30 days has been associated with moderate and severe thrombocytopenia.[23,24] We did not find an association between any degree of thrombocytopenia and higher mortality rates at 30- and 60-days. However, severe thrombocytopenia was independently associated with increased mortality at 90 days. In our study, mild thrombocytopenia was not a risk factor for a higher case-fatality rate at 30-, 60-, and 90-days, which is compatible with previous findings. Other factors associated with increased case-fatality rates in septic shock are the site of infection, advanced age, comorbidities (e.g. diabetes mellitus and neoplastic process), and organ failure (i.e. greater SOFA score upon hospital admission).[29]

Organ failure was also associated with an increased risk of death. A higher SOFA score was associated with a higher case fatality rate at 30 days when comparing mild and moderate thrombocytopenia patients to controls. Conversely, a lower SOFA score was associated with an increased survival rate in severe thrombocytopenia (at 30 days. The severity of illness upon ICU admission (i.e. APACHE IV score) was also associated with a higher risk of death at 30 days when comparing septic shock and mild thrombocytopenia to the control group; meanwhile, a lower APACHE IV score was associated with increased survival at 30 days for severe thrombocytopenia. The interaction between the SOFA and APACHE IV scores at 30 days for severe thrombocytopenia was positively associated with higher mortality. This could imply that increased PaO_2 and FiO_2, the need for mechanical ventilation, increased bilirubin levels, increased mean arterial pressure, and a lower Glasgow coma scale score play an important role in disease severity and organ failure as it pertains to higher mortality in severe thrombocytopenia at 30 days. Furthermore, increased comorbidities (i.e. increased CCI) were associated with an increased risk of death at 30 days for severe thrombocytopenia; as well as, lower comorbidities (i.e. lower CCI) were also linked with higher survival rates at 60 days for moderate thrombocytopenia.

Procalcitonin is a biomarker for bacterial infection and lactate levels have been previously associated with increased mortality in septic shock.[30] Higher procalcitonin levels were associated with an increased risk of death for mild thrombocytopenia at 30 days and

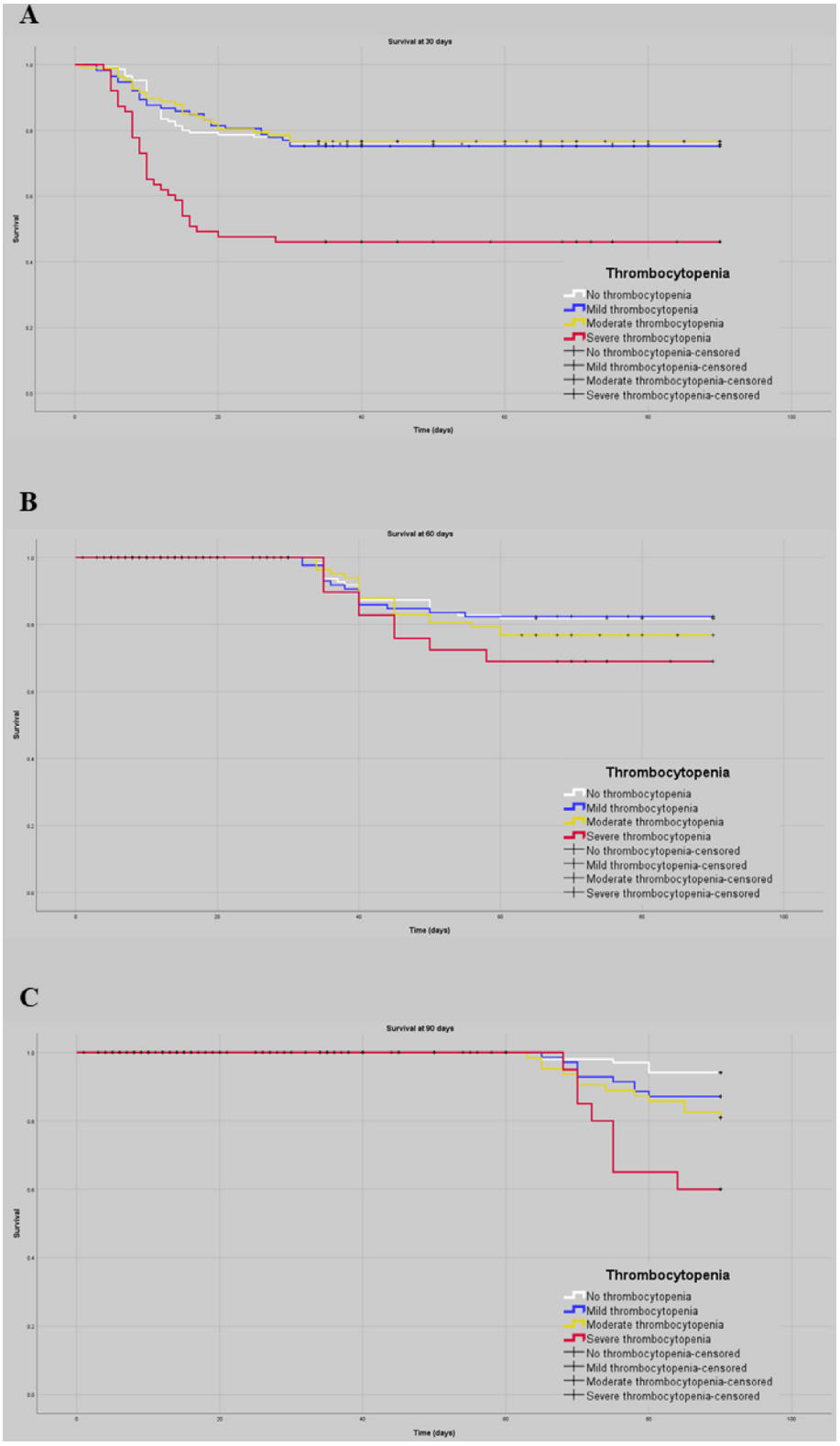

Fig. 2. Survival at 30, 60, and 90 days. Kaplan-Meier survival distribution curves displaying patient survival at 30, 60, and 90 days. A) Survival at 30 days. B) Survival at 60 days. C) Survival at 90 days. White line: no thrombocytopenia. Blue line: mild thrombocytopenia. Yellow line: moderate thrombocytopenia. Red line: severe thrombocytopenia.

90 days, for moderate thrombocytopenia at 90 days, and severe thrombocytopenia at 60 days. Furthermore, survival in septic shock has been associated with prompt and specific treatment of the infectious site.[7] Higher survival rates have been associated with septic shock secondary to urinary tract infections compared to unknown, pulmonary, or gastrointestinal sites.[31,32] When the site of infection is unknown, the case-fatality rate can reach 55%, compared to 30% in patients with a septic shock secondary to a urinary tract infection.[32] When compared to community-acquired infections, hospital-acquired infections have higher case-fatality rates (i.e. 10–24.6% for community-acquired infections to 14.9–33.9% for nosocomial infections).[33,34] Increased serum lactate levels have also been associated

Table 4
Cox proportional hazard coefficients and effect sizes for covariates.

Mild thrombocytopenia					95% CI for Hazard Ratio		
At 30 days		χ^2	p	b	Lower	HR	Upper
1	Body mass index	7.631	0.006	−0.121	0.813	0.886	0.965
2	APACHE IV Score	3.917	0.048	0.025	1.000	1.025	1.051
3	SOFA Score	6.110	0.013	0.177	1.037	1.194	1.374
4	Procalcitonin	15.395	≤0.001	0.022	1.011	1.023	1.034
At 60 days		χ^2	p	b	Lower	HR	Upper
1	Age	6.228	0.013	0.042	1.009	1.043	1.078
At 90 days		χ^2	p	b	Lower	HR	Upper
1	Procalcitonin	8.632	0.003	0.034	1.011	1.035	1.059
Moderate thrombocytopenia					**95% CI for Hazard Ratio**		
At 30 days		χ^2	p	b	Lower	HR	Upper
1	SOFA score	5.934	0.015	0.188	1.037	1.206	1.403
At 60 days		χ^2	p	b	Lower	HR	Upper
1	Males	6.390	0.011	0.850	1.211	2.341	4.526
2	Age	8.161	0.004	0.047	1.015	1.048	1.083
3	Charlson comorbidity index	8.464	0.004	−0.172	0.750	0.842	0.946
At 90 days		χ^2	p	b	Lower	HR	Upper
1	Procalcitonin	4.941	0.026	0.026	1.003	1.026	1.050
Severe thrombocytopenia					**95% CI for Hazard Ratio**		
At 30 days		χ^2	p	b	Lower	HR	Upper
1	Body mass index	4.334	0.037	−0.085	0.848	0.919	0.995
2	APACHE IV Score	7.161	0.007	−0.068	0.889	0.934	0.982
3	SOFA Score	4.698	0.030	−0.326	0.538	0.722	0.969
4	Charlson comorbidity index	4.080	0.043	0.095	1.003	1.100	1.206
5	Lactate	11.769	0.001	0.444	1.209	1.558	2.008
6	APACHE IV*SOFA Score	9.534	0.002	0.008	1.003	1.008	1.013
At 60 days		χ^2	p	b	Lower	HR	Upper
1	Males	3.836	0.05	0.769	1.000	2.157	4.653
2	Age	6.179	0.013	0.048	1.010	1.049	1.089
3	Procalcitonin	4.540	0.033	0.019	1.002	1.019	1.037
At 90 days		χ^2	p	b	Lower	HR	Upper
1	Severe thrombocytopenia	4.101	0.043	1.320	1.043	3.742	13.419

with a septic process. Lactate has been previously associated as an independent prognostic predictor of mortality for patients with sepsis and had a similar discriminating ability than the SOFA score.[35] We report that higher lactate levels were associated with a higher risk of death at 30 days for severe thrombocytopenia compared to controls.

Demographical characteristics were also risk and protective factors for death associated with the degrees of thrombocytopenia. Older age and comorbidities have been previously associated as an independent risk factor for death in septic shock.[29,36] Age was a risk factor at 60 days for all degrees of thrombocytopenia. As age increased, the case-fatality rate increased in mild, moderate, and

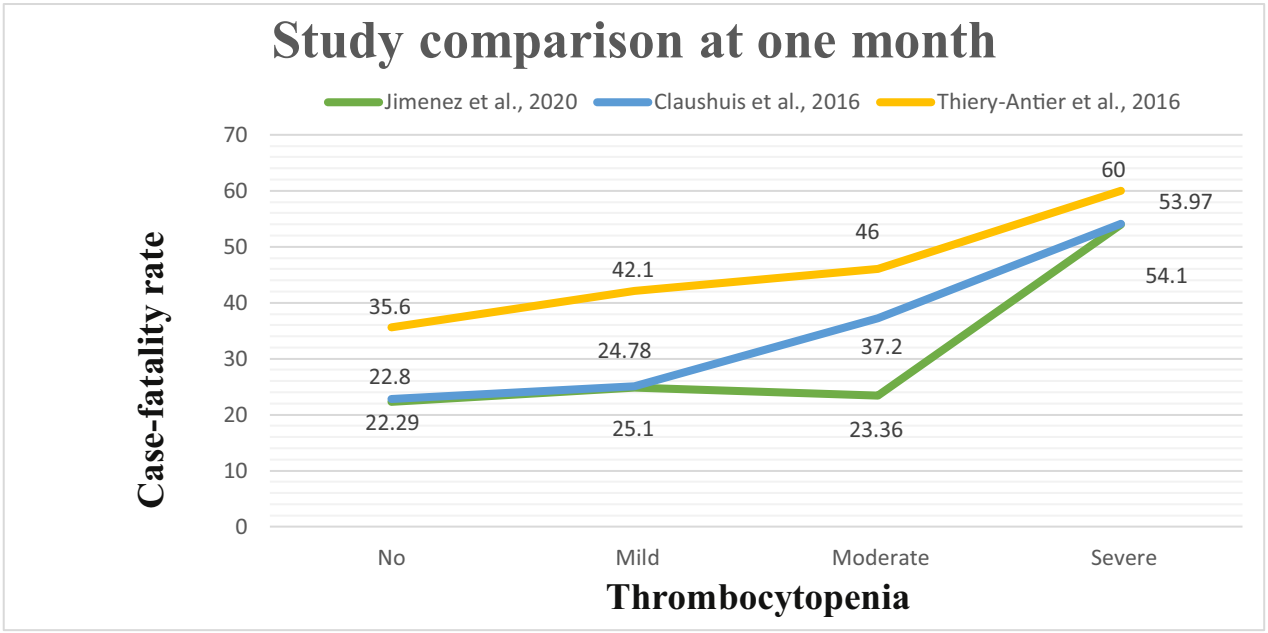

Fig. 3. Case-fatality rate between-study comparison. Case-fatality rate comparisons at 30 days between the present study and Claushuis and colleagues (2016) and by Thiery-Antier and colleagues (2016). Green line: present study results. Blue line: Claushuis et al., 2016 results. Yellow line: Thiery-Antier et al., 2016 results.

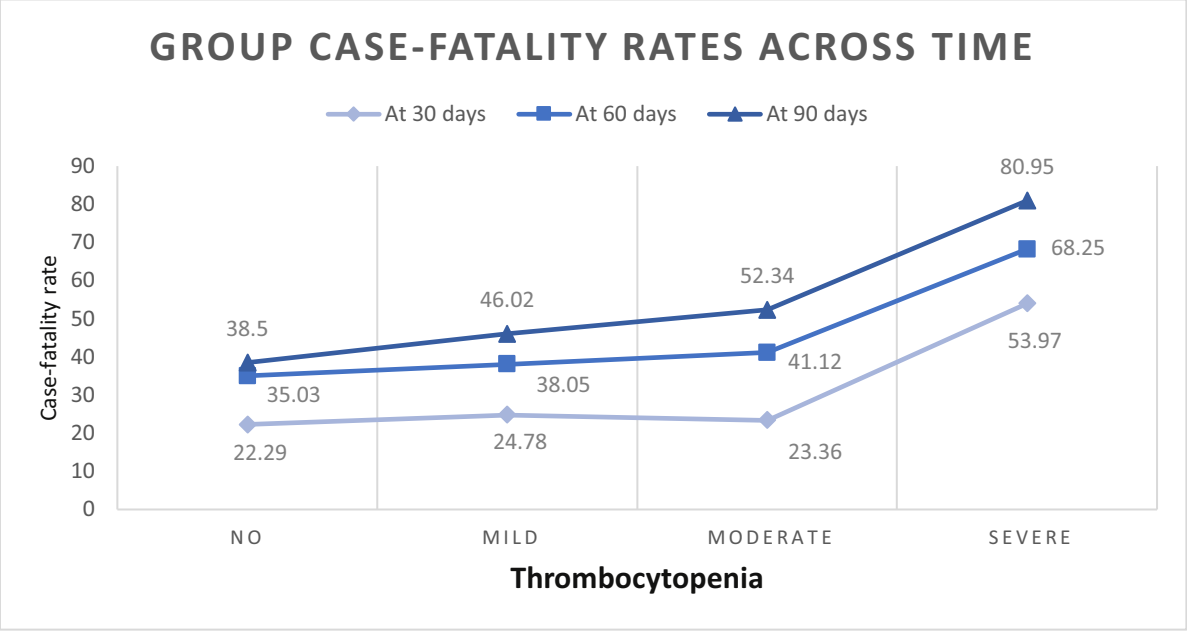

Fig. 4. Case-fatality rates across time. Case-fatality rates across the different outcome points (30, 60, and 90 days) for each group included in this study.

severe thrombocytopenia when compared to controls. More comorbidities were weakly associated with a higher risk of death at 30 days for severe thrombocytopenia (i.e. 10%); furthermore, fewer comorbidities were associated with a higher survival at 30 days (i.e. 15.8%) when comparing patients with septic shock and mild thrombocytopenia compared to controls. Similarly, male patients had an increased risk for mortality at 60 days compared to female patients with septic shock and moderate or severe thrombocytopenia. Meanwhile, lower body mass index was a protective factor in septic shock patients at 30 days, when comparing mild and severe thrombocytopenia with controls.

Limitations

Although autoimmune, neoplastic, and some pharmacologic causes of thrombocytopenia were excluded, all patients included in this study were administered an anticoagulation prophylactic dose of heparin. Heparin-induced thrombocytopenia was not explored (i.e. no platelet factor 4 complex antibody was performed) and cannot be excluded. Heparin-induced thrombocytopenia is observed in 1.7% of ICU patients.[37] Another limitation of this study was that we did not perform a survival analysis with platelet levels obtained during the stay in the ICU or before the patient being discharged. Our study design did not consider multiple sampling points as standardizing the sampling rate for all patients is a logistical labor-intensive task. Therefore, we can only attest to the predictive nature of platelet levels at or close to the onset of septic shock (i.e. admission to the ICU) and not throughout the clinical evolution of the septic process. Future studies should address the question associated with the role of platelet levels throughout the evolution of septic shock. Furthermore, as the aim of this study to assess the mortality distribution differences between three thrombocytopenia groups with septic shock, we opted to perform regression analyses to assess the predictive value of each variable at 30, 60, and 90 days. The authors consider that further analyses are necessary to assess the interaction between sepsis biomarkers in organ failure or sepsis severity; thus, the relationship between serum lactate, thrombocytopenia, and organ failure (e.g. liver failure) should be further investigated.

Conclusion

The risk of death increased with the severity of thrombocytopenia. Among septic shock patients, the case-fatality rate at 30 days was 22.29% for no thrombocytopenia, while 24.78%, 23.36%, and 53.97% for mild, moderate, and severe thrombocytopenia, respectively. Patients with severe thrombocytopenia and had higher case-fatality rates throughout the study, 53.97% at 30 days, 68.25% at 60 days, and 80.95% at 90 days follow-up; this last finding suggests, that there is a need for longer follow-up windows for patients with severe thrombocytopenia and septic shock. The overall survival distributions were different for all four groups at 30 days ($p \leq 0.001$) and 90 days ($p \leq 0.001$). While the between-group survival distributions were different between severe thrombocytopenia and three other groups (i.e. all had a $p \leq 0.001$) at 30 days; furthermore, differences were observed at 90 days between controls and moderate thrombocytopenia ($p = 0.008$), as well as, with severe thrombocytopenia ($p \leq 0.001$), and between mild and severe thrombocytopenia ($p = 0.006$).

Severe thrombocytopenia was independently associated with increased mortality at 90 days by tripling the risk of death than patients with a normal platelet count upon ICU admission. Organ failure was also discretely associated with an increased risk of death at 30 days independently for mild (i.e. 19.4%), and moderate (i.e. 20.6%) thrombocytopenia; while in septic shock patients with severe thrombocytopenia, a lower degree of organ failure increased the survival by 27.8% compared to controls. While the severity of illness upon ICU admission was also weakly associated with a higher risk of death at 30 days in patients with mild thrombocytopenia (i.e. 2.5%); meanwhile, for severe thrombocytopenia, a lower APACHE IV score was associated with a higher survival rate (i.e. 6.6%) at 30 days. Increased lactate serum levels were associated with a 55.8% increased risk of death for patients with severe thrombocytopenia at 30 days compared with patients with only septic shock and normal platelet count upon admission to the ICU. Meanwhile, males had twice the risk of death than females at 60 days in moderate and severe thrombocytopenia. Increased serum procalcitonin levels, increased BMI, older age, and more comorbidities were weakly associated with increased mortality. While lower

BMI and fewer comorbidities were weakly associated with higher survival rates.

Declaration of Competing Interest

This research did not receive any specific grant from funding agencies in the commercial sector, the authors report no conflict of interest.

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