

Methylene blue dosing strategies in critically ill adults with shock – a retrospective cohort study

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Abstract

Background: Shock increases mortality in the critically ill and the mainstay of therapy is the administration of vasopressor agents to achieve hemodynamic targets. In the past, studies have found that the NO-pathway antagonist methylene blue improves hemodynamics. However, the optimal dosing strategy remains elusive. Therefore, we investigated the hemodynamic and ICU outcome parameters of three different dosing strategies for methylene blue.

Methods: We performed retrospective cohort study of patients in shock treated with methylene blue. Shock was defined as norepinephrine dose $> 0.1 \mu\text{g}/\text{kg}/\text{min}$ and serum lactate level $> 2 \text{ mmol}/\text{l}$ at the start of methylene blue administration. Different demographic variables, ICU treatment, and outcome parameters were evaluated. To compare the differences in the administration of vasopressors or inotropes, the vasoactive inotropic score (VIS) was calculated at different time points after starting the administration of methylene blue. Response to methylene blue or mortality at 28 days were assessed.

Results: 262 patients from July 2014 to October 2019 received methylene blue. 209 patients met the inclusion criteria. Three different dosing strategies were identified: bolus injection followed by continuous infusion ($n=111$), bolus injection only (no continuous infusion; $n=59$) or continuous infusion only (no bolus prior; $n=39$). The groups did not differ in demographics, ICU scoring system, or comorbidities. In all groups, VIS decreased over time, indicating improved hemodynamics. Cardiogenic shock and higher doses of norepinephrine increased the chance of responding to methylene blue, while bolus only decreased the chance of responding to methylene blue treatment. 28-day mortality increased with higher SAPSII scores and higher serum lactate levels, while bolus injection followed by continuous infusion decreased 28-day mortality. No severe side effects were noted

Conclusions: Methylene blue as a bolus injection followed by continuous infusion decreases 28-day mortality in patients with shock. Clinicians should consider the administration of methylene blue in patients with refractory shock, regardless of the cause.

Background

Shock results from a multitude of pathophysiological processes and leads to hemodynamic collapse and death if left untreated. Clinicians differentiate forms of shock such as cardiogenic, hypovolemic, obstructive, or distribution shock (including septic shock) [1]. They all share the same features, leading to hypotension and impaired oxygen delivery or utilization in the peripheral circulation [2], either due to loss of vascular tone or decreased cardiac output. This jumpstarts a vicious circle consisting of an altered microcirculation leading to the production and release of pro-inflammatory mediators such as tumor necrosis factor alpha (TNF α) or interleukin-6 (IL-6), which in turn induce the expression of the inducible NO-synthase (iNOS) with subsequent production of the vasodilator nitric oxide (NO). NO acts as a vasodilator that directly counteracts the endogenous or exogenous vasoconstrictors and thus decreases the vascular tone [3]. Interestingly, increased endothelial NO production contributes substantially to the

pathophysiology of shock [4, 5] and leads to a decrease in mean arterial pressure resulting in impaired organ perfusion. If volume resuscitation alone cannot reestablish target values, patients require vasopressors. In recent decades, different vasopressors, such as dopamine, epinephrine, or norepinephrine, have been clinically tested in shock patients [6–8]. Norepinephrine has emerged as the first-line vasopressor agent in most forms of shock. In patients with severe circulatory failure, additional vasoactive substances are required to stabilize the blood pressure. Several clinical studies have evaluated NO-synthase inhibitors in experimental and clinical shock treatment [9], but the use of these substances never took hold in clinical therapy.

In 1992, patients with septic shock (a form of distributive shock) received the first fully synthetic drug ever used in medicine invented in 1876 [10]: methylene blue. Methylene blue was clinically tested as a shock treatment in several clinical trials [11, 12] evaluating different dosing strategies for methylene blue dosing regimens. They range from bolus administration regimens to bolus administration followed by continuous infusion, or continuous infusion only without additional bolus [13]. To date, no studies have investigated the optimal dose strategy for methylene blue in critically ill patients with shock, mainly due to the complex pharmacological properties of methylene blue. It has a large volume of distribution (up to 250 l) and a long half-life of up to 24 hours [13], making the prediction of clinical effects and hemodynamic responses difficult.

At our institution, the administration of methylene blue belongs to the standard operating procedure in the treatment of refractory shock. Three different dosing strategies have been used: bolus of methylene blue followed by continuous infusion, bolus injection only, or continuous infusion only. In the present retrospective cohort analysis, we performed an unbiased analysis of these three different regimens and investigated whether one dosing strategy leads to improved hemodynamic stability over the other. Next, we searched for variables that correlate with a clinical response to methylene blue, defined as a reduction in vasoactive or inotropic substances. Finally, we assessed which clinical variables correlate with reduced mortality at 28 days in critically ill adults.

Methods

Study population and ethics. All data were retrospectively collected from medical records from the University Hospital of Tuebingen. The study was approved by the Ethics Committee of the University Hospital Tuebingen (IRB# 768/2018BO2), which waived the need for informed consent because patient anonymity was maintained. All methods were approved by the local IRB and performed in accordance with the Declaration of Helsinki and the relevant guidelines.

Methylene blue therapy. Patients received methylene blue therapy according to the standard operating procedure in our intensive care unit (ICU). At our institution, when norepinephrine administration does not establish sufficient hemodynamics as defined by norepinephrine $> 0.3 \mu\text{g}/\text{kg}/\text{min}$, vasopressin at a dose of $0.06 \text{ IU}/\text{kg}/\text{min}$ is added for vasopressor therapy. If the mean arterial pressure cannot be maintained at more than 65 mmHg, methylene blue is regularly added to therapy. Three different dosing strategies have

been used in our institution in the past: bolus injection alone, continuous infusion without bolus, injection, or bolus injection followed by continuous infusion (bolus 2 mg/kg; continuous infusion with 0.25 mg/kg/min).

We define a patient as a "methylene blue responder" according to the following criteria: VIS decreases by > 10% within 3 hours after administration and mean arterial pressure > 65 mmHg.

Data collection. All patient records who were treated at the Department of Anesthesiology and Intensive Care Medicine between July 2014 and October 2019 were retrospectively screened for inclusion in this observational cohort study. We selected all patients who received methylene blue during their stay in the ICU. We included patients with the following inclusion criteria: Norepinephrine dose $\geq 0.1 \mu\text{g/kg/min}$, serum lactate $\geq 2 \text{ mmol/l}$ and methylene blue treatment.

Based on data from the clinical information system, a database was generated that contains relevant patient information, including age, sex, date of admission and discharge from the ICU, and Sequential Organ Failure Assessment (SOFA), Simplified Acute Physiology Score II (SAPS II) and Acute Physiology and Chronic Health Evaluation (APACHE) scores. We determined comorbidities from the patient records as well as the dosage of vasopressor or inotropic therapy.

Classification of shock. Based on the discharge note from the ICU, we retrospectively classified the patients into a category based on available parameters. Patients with documented infection and shock were classified as septic shock. Patients with extended hemodynamic monitoring and a cardiac index $< 2.5 \text{ l/min/m}^2$ were classified as cardiogenic shock. Patients with a shock criterion as described above but could not be categorized into one of the other two categories were classified as vasoplegic syndrome.

Vasoactive inotropic score. Because vasoactive and inotropic doses differ greatly between institutions, we calculated the vasoactive inotropic score (VIS) as described [14]. In short, vasoactive and inotropic doses differ greatly between institutions. VIS objectively quantifies the degree of hemodynamic support.

Statistical analysis. Data for continuous variables are expressed as the mean \pm standard deviation in the case of a normal distribution and as the median (interquartile range) in the case of a nonnormal distribution. Variables from the different groups were compared using the ANOVA test. Categorical variables expressed as proportions were compared using the chi-square test. To assess the association of different variables with the response status of methylene blue or mortality at 28 days, univariate logistic regression was used. Variables with biologic plausibility to influence the response status to methylene blue or mortality with a significant association in univariate regression (defined as $p < 0.05$) were considered for inclusion in a multivariate logistic regression model and collinearity was tested using variance inflation factors and condition indices. If two or more variables showed collinearity, the variable with the lowest p-value in the univariate logistic regression was included in the multivariate model. Residuals were tested for normal distribution. Goodness-of-fit was tested using the Hosmer-Lemeshow test. All statistical analyzes for this study were performed in Prism 9 (GraphPad Software Inc.) and JMP

16.0 (SAS Institute Inc., Cary, US). The p values are two-tailed and values < 0.05 are considered statistically significant.

Results

Patient selection and characteristics. The selection process is depicted in Fig. 1. In total, we identified 209 patients for analysis.

Demographic data. In our dataset, we identified three different dosing strategies: 53% ($n = 111$) of the patients received a bolus injection followed by continuous infusion. 28% of the patients received only a bolus injection ($n = 59$) and 19% received only a continuous infusion of methylene blue without prior bolus ($n = 39$). Baseline demographic characteristics, such as age, sex, height, weight, and body mass index, were similar between these groups (Table 1). The distribution across types of shock did not differ between the three different dosing strategies.

Table 1
Demographic data and comorbidities in shock patients

	Total (n = 209)	Bolus + infusion (n = 111)	Bolus only (n = 59)	Continuous infusion only (n = 39)	p- values
Percentage of total		53	28	19	
Age - year (mean ± SD)	65.0 ± 13.7	64.5 ± 13.9	67.9 ± 12.6	62.5 ± 14.4	0.1279
Male sex – no. (%)	74.1%	75.7%	72.9%	71.8%	0.8623
Height – cm (mean ± SD)	173.2 ± 9.5	174.3 ± 9.2	172.8 ± 10.0	170.9 ± 9.3	0.1566
Weight – kg (mean ± SD)	84 ± 19	86 ± 21	85 ± 18	79.4 ± 11.8	0.1962
Body Mass Index (kg/m ²)	28.1 ± 5.7	28.2 ± 6.4	28.4 ± 5.2	27.1 ± 3.5	0.5232
Etiology of shock n (% in group)					
Sepsis	82 (39.2)	40 (36.0)	24 (40.7)	18 (46.1)	0.5192
Cardiogenic	64 (30.6)	31 (27.9)	20 (33.9)	13 (33.3)	0.6662
Vasoplegia	63 (30.1)	40 (36.0)	15 (25.4)	8 (20.5)	0.1242
Comorbidities					
Lung disease (%)	29 (13.8)	11 (09.0)	13 (24.5)	5 (14.3)	0.0153
Coronary artery disease	171 (81.8)	104 (85.9)	41 (77.3)	26 (74.3)	0.1677
Peripheral artery disease	23 (11.0)	17 (14.0)	4 (7.5)	2 (5.7)	0.1025
Chronic kidney disease	33 (15.8)	14 (11.5)	12 (22.6)	7 (20.0)	0.3949
Arterial hypertension	168 (80.3)	103 (85.1)	39 (73.5)	26 (74.3)	0.0213
Diabetes mellitus	57 (27.2)	36 (29.7)	10 (18.8)	11 (31.4)	0.3468
Autoimmune disease	31 (14.8)	15 (12.3)	8 (15.0)	8 (22.9)	0.6453

The most common comorbidity in all data sets was coronary artery disease (81.8% of total) and arterial hypertension (80.3% of total). Although there were significant differences in preexisting lung disease (e.g., chronic obstructive lung disease, emphysema), all groups had a fairly low number. In summary, the groups were comparable in their baseline demographic data.

ICU treatment variables. Next, we investigated whether the different dosing strategies differed in their disease severity. Interestingly, 28-day mortality was significantly lower in the cohort treated with bolus injection first with subsequent continuous infusion (53.1%), compared to the other dosing strategies (bolus only: 71.2%; continuous infusion only: 74.3%; Table 2). We also assessed short-term mortality, which we defined as death within 12 hours after administration of methylene blue. The differences in 28-day and short-term mortality were not due to differences in disease severity, as SAPSII, APACHE and SOFA scores were similar between the groups. The rate of renal replacement therapy was similar between these groups, indicating that all groups had a similar rate of end organ failure, as acute renal failure is a common risk factor for ICU mortality [15]. However, patients with bolus + continuous infusion support underwent longer mechanical ventilator support.

Table 2
ICU variables

	Total (n = 209)	Bolus + infusion (n = 111)	Bolus only (n = 59)	Continuous infusion only (n = 39)	p Values
28-day mortality n (%)	130 (62.2)	59 (53.1)	42 (71.2)	29 (74.3)	0.0154
SAPS II (mean ± SD)	59.5 ± 12.0	59.5 ± 12.5	60.4 ± 12.6	61.9 ± 9.4	0.2091
APACHE II (mean ± SD)	25.8 ± 6.4	24.8 ± 6.1	26.9 ± 7.4	27.0 ± 5.4	0.0529
SOFA (mean ± SD)	11.3 ± 3.0	11.04 ± 3.1	11.3 ± 3.3	12.0 ± 2.5	0.2313
Renal replacement therapy in the ICU n (%)	183 (87.5)	98 (88.2)	50 (84.7)	35 (89.7)	0.7212
Median LOS-ICU of survivors (interquartile range)	22 (10–43)	11 (4–25)	7 (1–16)	4 (2–14)	0.0067
Median hours of ventilator support (interquartile range)	114 (37– 283)	160 (50–334)	113 (24– 279)	66 (24–220)	0.0304#
Cumulative dose of methylene blue					
Methylen blue (mg/kg)	5.04 ± 3.2	6.7 ± 3.2	2.7 ± 1.9	4.03 ± 2.5	< 0.0001
Initial doses of vasoactive substances at methylene blue administration					
VIS (interquartile range) [µg/kg/min]	59.8 (39.2– 82.4)	58.0 (37.3– 80.7)	60.3 (39.8– 91.4)	59.6 (41.8–80.5)	0.4243
Norepinephrine treatment n (%)	209 (100)	111 (100)	59 (100)	39 (100)	1.0000
Norepinephrine dose (interquartile range) [µg/kg/min]	0.47 (0.30– 0.73)	0.46 (0.28– 0.71)	0.53 (0.32– 0.77)	0.45 (0.32–0.68)	0.2662

Compared using Wilcoxon rank sum test.

	Total (n = 209)	Bolus + infusion (n = 111)	Bolus only (n = 59)	Continuous infusion only (n = 39)	p Values
Epinephrine treatment n (%)	56 (27)	32 (29)	13 (22)	11 (28)	0.6202
Epinephrine dose (interquartile range) [µg/kg/min]	0.043 (0.02–0.10)	0.037 (0.0–0.07)	0.088 (0.02–0.11)	0.10 (0.033–0.129)	0.8809
Vasopressin treatment n (%)	191 (91)	105 (94)	49 (83)	37 (94)	0.0265
Vasopressin dose (interquartile range) [U/kg/min]	3 (2–4)	3 (2–4)	3 (2–3)	3 (2–4)	0.5437
Dobutamine treatment n (%)	40 (19)	21 (18)	9 (15)	10 (25)	0.4395
Dobutamine dose (interquartile range) [µg/kg/min]	5.0 (3.1–6.21)	4.9 (2.8–6.0)	5.59 (2.9–8.2)	5.0 (4.0–6.2)	0.9533
Milrinon treatment n (%)	118 (56)	66 (59)	32 (54)	20 (51)	0.6218
Milrinon dose (interquartile range) [µg/kg/min]	0.44 (0.3–0.5)	0.41 (0.3–0.5)	0.42 (0.3–0.5)	0.5 (0.4–0.6)	0.4052
Point of care diagnostics					
Lactate (interquartile range) [mmol/L]	5.9 (3.3–11.2)	5.5 (3.1–9.8)	6.1 (4–10.7)	9.1 (3.5–12.9)	0.7743
pH (interquartile range)	7.32 (7.27–7.38)	7.33 (7.27–7.38)	7.31 (7.27–7.37)	7.32 (7.27–7.39)	0.6371
Laboratory values on the day methylene blue treatment – median (interquartile range)					

Compared using Wilcoxon rank sum test.

	Total (n = 209)	Bolus + infusion (n = 111)	Bolus only (n = 59)	Continuous infusion only (n = 39)	p Values
White blood cell count (x10 ³)	14.0 (8.4– 21.2)	13.8 (9.4–21.2)	14.9 (7.8– 20.8)	13.9 (7.6–20.8)	0.6081
Platelets (x10 ³)	141 (92– 211)	143 (100–215)	132 (76– 180)	157 (102–257)	0.1123
Creatinin (mg/dl)	1.6 (1.2– 2.2)	1.5 (1.1–2.2)	1.7 (1.2– 2.4)	1.7 (1.2–2.1)	0.1447
Bilirubin total (mg/dl))	2.2 (1.1– 4.3)	2.2 (0.9–4.2)	1.8 (1.1– 4.5)	3.0 (1.3–4.7)	0.3885
C-reactive protein (mg/dl)	8.2 (4.9– 14.4)	8.7 (5.6–15.4)	6.0 (1.6– 14.4)	7.8 (4.6–14.0)	0.4849
Procalcitonin (ng/ml)	5.85 (1.8– 20.4)	7.02 (1.8–21.6)	4.3 (1.7– 15.9)	5.85 (1.7–24.5)	0.6255
# Compared using Wilcoxon rank sum test.					

Next, we analyzed the differences in the support of vasoactive substances. As expected, patients with bolus + continuous infusion received the highest cumulative dose of methylene blue. All patients received more than one vasoactive substance. In our institution, we regularly use norepinephrine, epinephrine, vasopressin, milrinone and dobutamine for the treatment of hemodynamic instability. We found that at the beginning of methylene blue therapy, all patients received a comparable degree of pharmacological hemodynamic support (Table 2). Indicators of decreased perfusion, such as lactate and blood pH, were not significantly different between the groups.

Methylene blue dosing strategy, basic hemodynamic parameters, and vasopressor requirements. To understand whether dosing strategy influences the hemodynamic response to methylene blue administration, we analyzed the basic hemodynamic parameters of mean arterial pressure and heart rate (0, 1, 2 and 5 hours), after methylene blue (Figs. 2A + B). The hemodynamic response was similar between the three different dosing strategies. In a subset of patients, systemic vascular resistance (SVR) was measured by pulmonary artery catheterization or transpulmonary thermodilution. The evaluation of

the SVR measurement closest to the administration of methylene blue and 12 hours later did not vary between the different dosing strategies.

Furthermore, we investigated whether the vasopressor requirements changed differently over time according to the dosing. As shown in Figs. 2C + D, the doses of norepinephrine (used in 100% of patients) or VIS decreased over time in response to the administration of methylene blue in all groups. We found the largest relative change from VIS 0h to VIS 3h (DVIS) in the patient cohort treated with a methylene blue bolus only (DVIS $29 \pm 62\%$); however, this did not differ significantly from the other cohorts (bolus + continuous infusion DVIS $29 \pm 33\%$; continuous infusion only $11 \pm 37\%$; one-way ANOVA $p = 0.1184$). In summary, all methylene blue treatment regimens are equally effective in promoting hemodynamic stabilization.

Response rate to Methylene Blue. Methylene blue improves the mean arterial pressure and decreases the requirement for vasopressors in critically ill patients [11]. However, the response to methylene blue remains unpredictable and a proportion of patients remain unresponsive to administration. We investigated whether the response to methylene blue varies between different treatment strategies. To do this, we analyzed the proportion of clinical responders within the respective group (Fig. 3A). In the total cohort, 59.2% of the patients responded to methylene blue, with comparable percentages found in the bolus + continuous infusion and bolus groups (Fig. 3B-D). On the contrary, continuous infusion yielded a lower percentage of responders (44.7%, Fig. 3E). In a nominal logistic regression analysis, the cumulative dose of methylene blue was not correlated with response status (Odds ratio 1.03, 95% CI 0.94 to 1.13; $p = 0.4568$).

Next, we investigated whether clinical variables assessed at the bedside are associated with a response to methylene blue. As shown in Table 3, cardiogenic shock and norepinephrine were associated with a higher chance of responding to methylene blue administration, while the bolus treatment regimen was not significantly associated with response.

Table 3
Factors associated with Methylene Blue Response in the Logistic Regression

	Univariate logistic regression		Multivariate logistic regression	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
<i>Demographic/ICU variables</i>				
Age	0.97 (0.22 to 4.26)	0.9686		
Sex	1.65 (0.84 to 3.26)	0.1378		
SAPSII	0.99 (0.97 to 1.01)	0.7118		
Septic shock	0.67 (0.37 to 1.12)	0.1903		
Cardiogenic shock	2.10 (1.09 to 4.05)	0.0230	2.21 (1.12 to 4.36)	0.0214
Vasoplegia of other causes	0.77 (0.41 to 1.41)	0.4010		
<i>Point-of-care</i>				
Lactate	1.00 (0.95 to 1.06)	0.7722		
pH	0.38 (0.009 to 15.27)	0.6122		
Laboratory Values				
White blood cell count	1.00 (0.9 to 1.0)	0.0777		
Platelets	0.99 (0.99 to 1.00)	0.3442		
Serum creatinine	1.00 (0.96 to 1.04)	0.8313		
Total bilirubin	1.01 (0.97 to 1.06)	0.4398		
Procalcitonin	1.00 (0.99 to 1.00)	0.5199		
C-reactive protein	1.00 (0.95 to 1.05)	0.8216		
Hematocrit	0.96 (0.91 to 1.02)	0.2637		
Vasopressors / inotropes				
Vasoactive Inotropic Score	1.01 (1.00 to 1.02)	0.029		
Norepinephrine	3.75 (1.46 to 9.62)	0.0023	3.71 (1.42 to 9.66)	0.0073
Dobutamine	1.00 (0.95 to 1.05)	0.0317		
Vasopressin	1.21 (0.95 to 1.54)	0.0938		
Milrinon	0.17 (1.28 to 5.77)	0.0794		
Hemodynamic Variables				

	Univariate logistic regression	Multivariate logistic regression		
Mean arterial pressure	1.00 (0.97 to 1.02)	0.8052		
Heart rate	0.99 (0.98 to 1.01)	0.8175		
Heart index	1.22 (0.88 to 1.74)	0.1939		
Systemic Vascular Resistance	0.99 (0.99 to 1.00)	0.2189		
Dosing strategy				
Bolus + continuous infusion	1.52 (0.85 to 2.7)	0.1517		
Bolus only	0.48 (0.23 to 0.98)	0.0452	0.46 (0.22 to 0.98)	0.0438
Continuous infusion only	1.07 (0.55 to 2.08)	0.8413		

In the multivariate model, cardiogenic shock, noradrenalin dose at the time of methylene blue administration, and treatment with bolus alone (negative association) remained independently associated with response to methylene blue (Table 3). ROC analysis displayed a discrimination with an AUC of 0.68551. The goodness-of-fit was appropriate (Hosmer-Lemeshow; $p = 0.8851$).

Variables associated with mortality at 28 days. The association of variables with mortality at 28 days in the logistic regression analysis is shown in Table 4. In the univariate analysis, we found that SAPSII, lactate at the start of methylene blue administration, blood pH, and higher VIS (translated as higher doses of norepinephrine and vasopressin doses) were associated with mortality at 28 days. Interestingly, the administration of methylene blue as bolus + continuous infusion was associated with decreased mortality at 28 days. In a multivariate model, SAPSII, lactate, and methylene blue dosing strategies remained independently associated with mortality 28 days after methylene blue administration (AUC 0.8054). The Hosmer-Lemeshow test showed appropriate goodness-of-fit ($p = 0.8180$).

Table 4
Factors associated with mortality at 28 days in logistic regression

	Univariate logistic regression		Multivariate logistic regression	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
<i>ICU variables</i>				
Age	2.91 (0.69 to 12.3)	0.1450		
Sex	1.45 (0.75 to 2.80)	0.2622		
SAPSII	1.07 (1.04 to 1.10)	< 0.001	1.08 (1.04 to 1.12)	< 0.0001
Septic shock	1.54 (0.86 to 2.76)	0.1424		
Cardiogenic shock	1.12 (0.61 to 2.06)	0.7118		
Vasoplegia of other causes	0.55 (0.30 to 1.01)	0.0561		
<i>Point-of-care</i>				
Lactate	1.18 (1.09 to 1.26)	< 0.001	1.16 (1.08 to 1.25)	< 0.0001
pH	0.0014 (3,123E-5 to 0.071)	0.0006	0.07 (0.0006 to 10.05)	0.3054
Laboratory Values				
White blood cell count	1.00 (0.99 to 1.00)	0.5299		
Platelets	0.99 (0.99 to 1.00)	0.1230		
Serum creatinine	0.99 (0.95 to 1.02)	0.5790		
Total bilirubin	1.00 (0.96 to 1.03)	0.8612		
Procalcitonin	1.00 (0.99 to 1.00)	0.2156		
C-reactive protein	0.98 (0.93 to 1.02)	0.4310		
Hematocrit	0.98 (0.93 to 1.04)	0.6060		
Vasopressors / inotropes				
Vasoactive Inotropic Score	1.01 (1.00 to 1.02)	0.0004		
Norepinephrine	4.56 (1.75 to 11.88)	0.0004	3.06 (0.97 to 9.64)	0.0559
Dobutamine	1.03 (0.88 to 1.20)	0.6778		
Vasopressin	1.28 (1.00 to 1.63)	0.0314		

	Univariate logistic regression	Multivariate logistic regression		
Milrinon	1.12 (0.15 to 8.07)	0.9102		
Hemodynamic Variables				
Mean arterial pressure	0.99 (0.97 to 1.02)	0.9077		
Heart rate	1.00 (0.98 to 1.01)	0.6100		
Heart index	0.97 (0.71 to 1.32)	0.8704		
Systemic Vascular Resistance	1.00 (0.99 to 1.00)	0.6818		
Dosing strategy				
Bolus + continuous infusion	0.43 (0.24 to 0.76)	0.0039	0.45 (0.23 to 0.90)	0.0230
Bolus only	1.98 (0.90 to 4.32)	0.0759		
Continuous infusion only	1.74 (0.90 to 3.33)	0.8889		

In summary, with respect to the methylene blue treatment regimen, only bolus administration with continuous infusion was associated with a reduced mortality at 28 days.

Discussion

In this retrospective cohort study, we found that methylene blue reduces the requirement for vasoactive substances, regardless of the dosing strategy. When analyzing the factors associated with the response to methylene blue, we found that the response rate to methylene blue increased in patients with cardiogenic shock and with higher doses of norepinephrine. Administration of a methylene blue bolus without subsequent continuous infusion reduced the response to methylene blue. The administration of methylene blue as a bolus followed by continuous infusion decreased 28-day mortality. Importantly, no serious undesired effects were observed in our cohort. We only noticed minor undesired drug effects such as skin or urine discoloration.

Shock remains a significant problem in critically ill patients, with high inherent mortality. An international consensus conference defined shock as a *“life-threatening generalized maldistribution of blood flow that results in the inability to deliver and/or use an adequate amount of oxygen, leading to tissue dysoxia”* [16]. Numerous basic science and clinical studies investigated the underlying dysregulation of endogenous pathways that sustain defective hemodynamics in shock. Nitric oxide has gained substantial research interest because it influences multiple pathways associated with the development of shock. In homeostasis, NO regulates blood flow in resistance vessels [17], influences coagulation, and modifies inflammatory pathways. During shock, NO production increases dramatically up to 1000 times [1, 18].

In 1992, a case series reported that hemodynamics improved in patients with shock after patients received the NO pathway inhibitor methylene blue [19]. Since then, several prospective randomized controlled trials have investigated whether methylene blue reduces vasopressor requirements [11, 12, 19, 20]. One trial reported that in patients with vasoplegia after cardiac surgery, mortality in the methylene blue treated group decreased to 0% versus 21.4% in the placebo treated group [12]. However, the study included only 28 patients per group, limiting the generalizability of the trial. In our cohort, the ICU scoring systems on the day of methylene blue treatment implied a mortality of 50–70% in all groups based on the mean of SAPSII, APACHE III and SOFA. Interestingly, the measured mortality at 28 days in patients receiving a bolus + continuous infusion was at the lower end of the predicted mortality compared to the other dosing regimens. In a univariate and multivariate logistic regression analysis, bolus administration followed by continuous infusion was associated with a lower mortality at 28 days.

Interestingly, the reduction in mortality in our cohort appeared to be not related to vasopressor requirements, because the three groups experienced similar hemodynamic stabilization. Methylene blue as a bolus and subsequent continuous infusion could cause a sustained inhibition of the NO pathway, as methylene blue blocks the NO pathway in two stages: it inhibits both NO synthase [21] and the downstream effector enzyme, soluble guanylate cyclase (sGC) [22]. However, methylene blue also interferes with numerous other pathways [23], and therefore the beneficial properties observed could induce effects in addition to hemodynamics or NO-path inhibition alone. In fact, inhibition of NO-synthase leaves shock mortality unchanged [24]. The methylene blue concentration in whole blood exceeds the plasma concentration, indicating that the substance accumulates in blood cells. In acute inflammation, immune cells, such as macrophages, release NO [25], thus enhancing the inflammatory response [26]. The accumulation of methylene blue in these cells could dampen an overshooting inflammatory response. In particular, in septic shock, NO overproduction contributes to hypotension, cardiodepression, and vascular hyporeactivity [27]. Therefore, the observed effects of methylene blue could modulate inflammatory responses and subsequently reduce mortality.

In our analysis, the response to methylene blue administration was correlated with the diagnosis of cardiogenic shock. This seems counterintuitive at first because, due to the mechanism of the proposed action, most of the studies investigated methylene blue in vasoplegia. However, there is some evidence that increased NO release enhances the detrimental spiral of events in cardiogenic shock. As such, NO blocks the adrenergic response of the myocardium and reduces myocardial contractility [28]. Similarly, NO concentration increases in the peripheral blood of patients with acute myocardial ischemia [29]. Furthermore, 20% of patients with underlying cardiogenic shock experience a systemic inflammation response syndrome, and the majority show a positive blood culture with low systemic vascular resistance [30]. This provides an explanation why patients with the primary diagnosis of cardiogenic shock could benefit from methylene blue treatment.

Finally, our study has several limitations. Our results should be seen in the context of the study design. First, we performed our study under the assumption that methylene blue decreases the vasopressor requirements. We have not included a control group with shock and without methylene blue treatment.

Being retrospective in design, our study has the inherent limitation that we cannot account for all variables that impacted the prescription and timing of methylene blue administration. As such, blood transfusion or volume resuscitation could have changed the findings of our study. In addition, we did not assess the length of stay in the ICU prior to methylene blue treatment. Another important limitation is the size of the individual cohorts. Even though we did not detect a significant difference in demographic parameters or in ICU scoring systems, the numeric differences between the groups are notable. Moreover, we did not perform retrospective matching (such as propensity score matching) as this technique does not account for unmeasured characteristics and cofounders [31].

Despite these limitations, our study adds to the body of evidence on the effect of methylene blue on shock. To our knowledge, our study presents the largest cohort of methylene blue treatment in ICU patients to date. Thus, we have concluded that methylene blue is safe in this population and increases survival in some patients.

Conclusion

In summary, we report the results of the largest cohort of methylene blue-treated ICU patients to date. Methylene blue reduces mortality at 28 days in critically ill patients with shock when administered as a bolus followed by a continuous infusion. No immediate side effects were observed and improved hemodynamics regardless of the underlying cause of the shock. Future research needs to clarify when clinicians should use methylene blue and whether it should become part of standard shock therapy, regardless of the underlying cause.

Declarations

Ethics approval. The approval for this study was obtained from the local institutional review board and the ethics committee of the Tübingen University Hospital Tübingen (549/2019BO2).

Consent for publication. Not applicable.

Availability of data and materials. All data included in this study are available from the corresponding author on reasonable request.

Competing interests. No competing interests.

Funding. Not applicable.

Authors' contribution. SS-Y, PW and MiKo designed the study. MiKo Y-SF and performed data analysis and interpretation, and statistical data analysis. HM and MaKe performed statistical sub-analysis and helped to write the manuscript. HAH, CS and PR contributed to the manuscript preparation, drafting, critique, and review. All authors approved the final version of the manuscript submitted.

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Figures

Figure 1

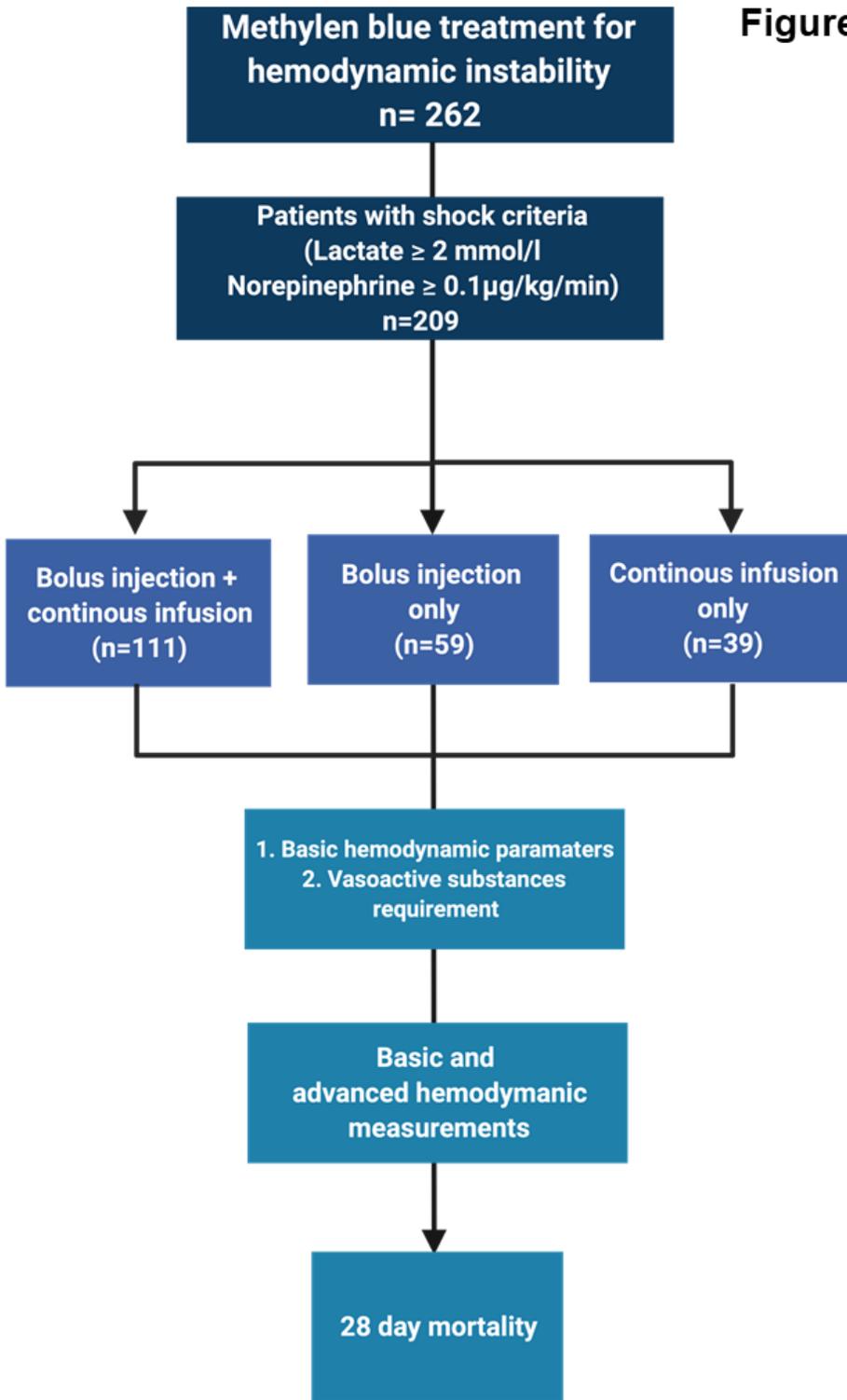


Figure 1

Patient selection strategy

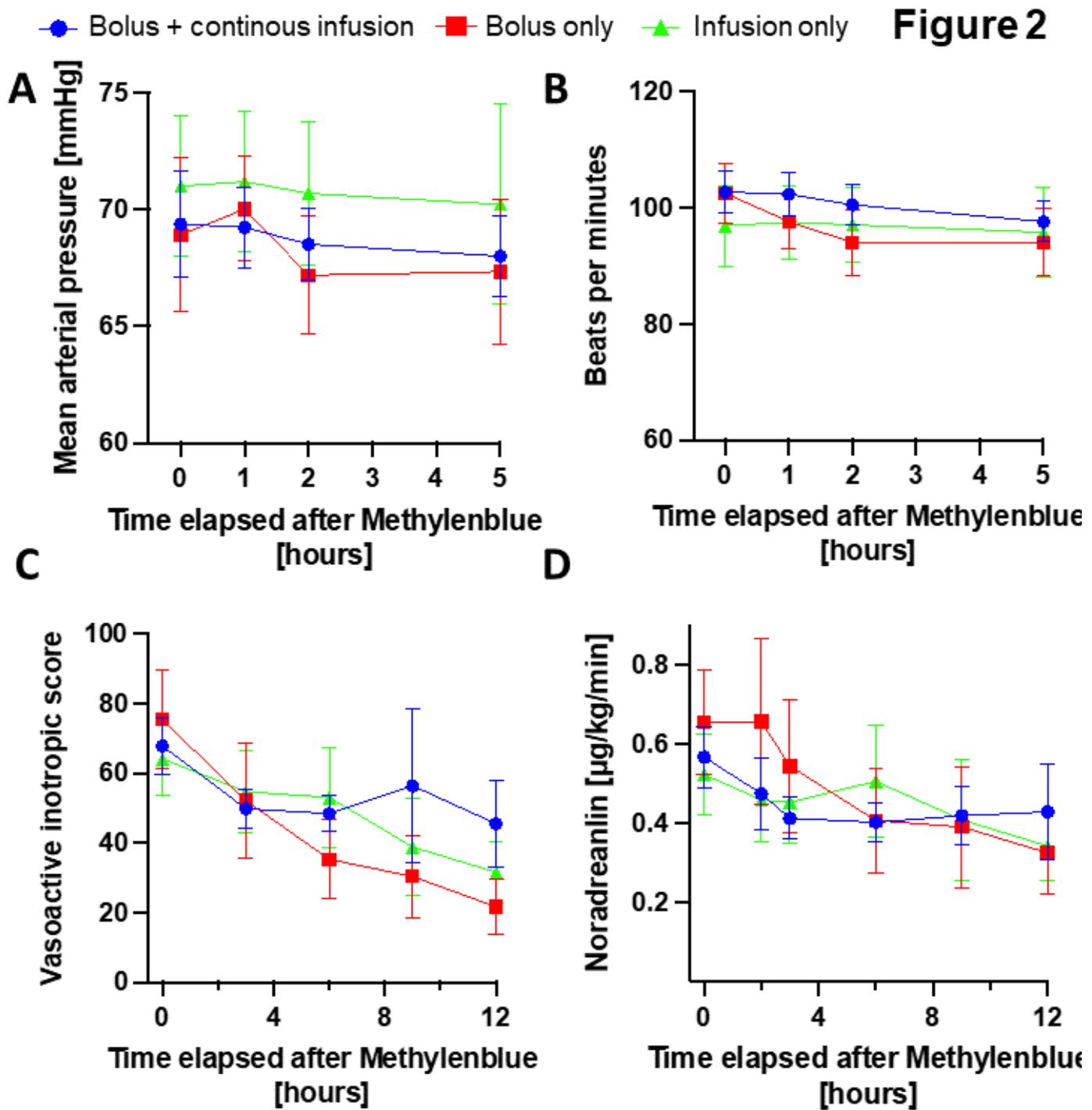


Figure 2

Hemodynamic changes after methylene blue administration

The injection time points of the methylene blue bolus were defined as “0 hours”; all subsequent time indicators were defined in relation to the administration of methylene blue administration (A) Mean arterial pressure over time in the different methylene blue treatment groups over time (B) Heart rate over

time after methylene blue administration (A+B: bolus+ continuous infusion: n=111-110; bolus only: n=47-59; continuous infusion only n=35-39) **(C)** Noradrenaline infusion rate in $\mu\text{g}/\text{kg}/\text{min}$ over time after methylene blue administration **(D)** Development of vasoactive inotropic score after methylene blue administration **(C+D)**: bolus + continuous infusion: n=111-110; bolus only: n=47-59; continuous infusion only n=35 to 39);

All data are shown as mean \pm 95% confidence interval

Figure 3

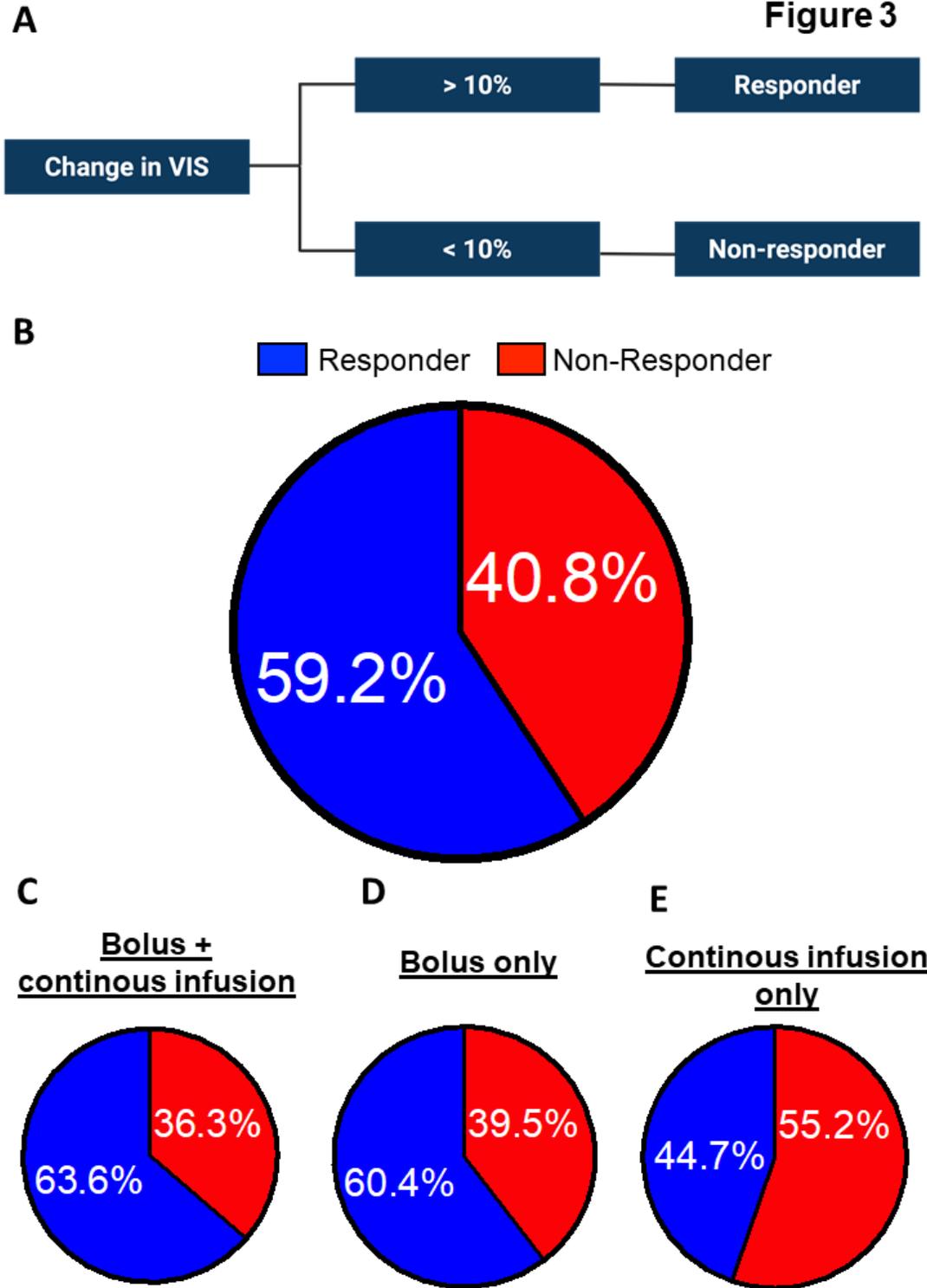


Figure 3

Percentage of methylene blue responders per administration group

(A) Schematic of the definition of responder or non-responder status to methylene blue treatment (B) Proportion of responders and non-responders in the study cohort (n=196 in total) (C) bolus + continuous infusion cohort (n=110 in total) (D) bolus only infusion group (n=48) or (E) continuous only cohort (n=38)

