Methylene Blue in Septic Shock – A Systematic Review and Meta-Analysis

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Supplemental Figure 1: Search Strategy

MEDLINE Search

- 1. Exp Methylene blue/
- 2. Methylene blue.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 3. 1 or 2
- 4. Sepsis or septic.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 5. Multiple organ failure/
- 6. Critical illness/
- 7. Respiratory distress syndrome/
- 8. ((severe or serious or critical or intensive) adj3 (illness or infection* or shock or care)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 9. Organ adj3 failure.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
- 10. 4 or 5 or 6 or 7 or 8 or 9
- 11. 3 and 10

Supplemental Table 1: Standardized Data Extraction Sheet.

Data to be Extracted	Notes to Reviewer
Basic Study	Information
Study Title	
Journal	
Year of Publication	
Language	
Author	List first author only
Correspondence Email	
Study Design	
Number of Sites	
Country/Countries of Study	
Eligibility .	Assessment
Does the study include adult patients (i.e.	If "No" – Exclude
\geq 16 years of age)?	
Does the study enroll or present a	If "No" – Exclude
subgroup of patients with sepsis or septic	
shock?	
Definition of "Sepsis/Septic Shock" (e.g.,	
Sepsis-3 criteria; SIRS criteria, etc.).	
Does the study evaluate the use of	If "No" – Exclude
intravenous methylene blue (at any dose,	
duration of therapy, and with any timing)?	
Does the study compare patients receiving	If "No" – Exclude
methylene blue with those not receiving	
MB, or those administered placebo?	
	racteristics
Was population was included?	E.g. mixed, surgical, etc.
What was the mean duration of	
hospitalization?	
Were elderly patients included?	
Were pregnant patients included?	
Were patients with any other co-morbidity	
included/excluded?	
Methylene Blue Dosing	
Control (if applicable)	
	Assessment
How were patients randomized?	
In either the intervention or the control	
group, were there any deviations from the	
protocol?	
Was there any missing outcome data?	
Were the outcome measures objective?	

Were all important pre-specified outcomes	
presented?	
Any other bias noted?	
Outco	me #1
Outcome being evaluated	e.g. Short-term mortality
Dichotomous or continuous outcome?	
Methylene Blue: N analyzed	
Methylene Blue: Number of Events/Mean	
Control: N analyzed	
Control: Number of Events/Mean	
Comments	
Outco	me #2
Outcome being evaluated	e.g. Duration of vasopressors
Outcome being evaluated Dichotomous or continuous outcome?	e.g. Duration of vasopressors
	e.g. Duration of vasopressors
Dichotomous or continuous outcome?	e.g. Duration of vasopressors
Dichotomous or continuous outcome? Methylene Blue: N analyzed	e.g. Duration of vasopressors
Dichotomous or continuous outcome? Methylene Blue: N analyzed Methylene Blue: Number of Events/Mean	e.g. Duration of vasopressors
Dichotomous or continuous outcome? Methylene Blue: N analyzed Methylene Blue: Number of Events/Mean Control: N analyzed	e.g. Duration of vasopressors
Dichotomous or continuous outcome? Methylene Blue: N analyzed Methylene Blue: Number of Events/Mean Control: N analyzed Control: Number of Events/Mean Comments	e.g. Duration of vasopressors Contact
Dichotomous or continuous outcome? Methylene Blue: N analyzed Methylene Blue: Number of Events/Mean Control: N analyzed Control: Number of Events/Mean Comments	

<u>Supplemental Table 2:</u> Characteristics of the 6 randomized trials. *Abbreviations:* MB = methylene blue; SIRS = systemic inflammatory response syndrome; SSRI = selective serotonin reuptake inhibitor

First Author, Year	Journal	Sites	Country	Population	Inclusion Criteria	Exclusion Criteria	Dose of Methylene Blue	Timing of Methylene Blue Administration	Total Sample Size	Mean Age (Years)	% Male	% Mortality (Short-term)
Ibarra- Estrada, 2023	Crit Care	1	Mexico	Intensive Care Unit	Age ≥ 18 years, with septic shock (Sepsis-3 criteria)	>24h of norepinephrine infusion; Pregnancy; High probability of death; Concurrent hemorrhage; Obstructive or hypovolemic shock; Pending surgery; Major burn injury; Personal or family history of G6PD deficiency; Allergy to MB, phenothiazides, or food dyes; Recent SSRI intake	Intravenous infusion of 100mg MB in 500mL of 0.9% normal saline over 6 hours daily; 3 days total	Within 24 hours of initiation of norepinephrine	92	46.5	60.9	39.6
Kirov, 2001	Crit Care Med	1	Norway	Intensive Care Unit	Age ≥ 18 years, with septic shock (≥ 2 SIRS criteria and a mean arterial pressure of <70mmHg at 30 mins despite fluid resuscitation or receiving vasopressors)	Pregnancy; Receiving corticosteroids; Immunosuppressants; Chemotherapy; Known irreversible underlying disease (such as end-stage neoplasms)	Intravenous bolus of 2mg/kg for 15 mins) followed by infusion of escalating doses of 0.25-2 mg/kg/hr for 1 each	Within 2 hours of randomization	20	57.4	55	40.0
Li, 2021	Diet Health	1	China	Intensive Care Unit	Septic shock (Unclear definition)	Abnormal liver and kidney function; Patients with incomplete data	Intravenous bolus of 2mg/kg for 15 mins	Unclear	66	52.7	59.1	22.7
Lu, 2019	Nat Med J China	1	China	Intensive Care Unit	Age ≥ 18 years, with septic shock (Sepsis-3 criteria)	History of previous myocardial infarction or stroke in the preceding 3 months; Pregnancy; Known anaphylaxis to MB; Nitrate use in previous 3 days	Intravenous bolus of 2mg/kg with or without 2mg/kg infusion over 24 hours	Unclear	54	64	53.7	18.5
Memis, 2002	Anaesth Intensive Care	1	Turkey	Operating Room and Intensive Care Unit	Age ≥ 18 years, with septic shock (≥ 2 SIRS criteria and a mean arterial pressure of <70mmHg at 30 mins despite fluid resuscitation or receiving vasopressors)	Pregnancy; Receiving corticosteroids; Immunosuppressants; Chemotherapy; Known irreversible underlying disease (such as end-stage neoplasms)	Intravenous infusion of 0.5 mg/kg/hr over 6 hours	Unclear	30	52.4	60	26.7
Xiong, 2010	China J Anesthesiol	1	China	Operating Room and Intensive Care Unit	Septic shock (Unclear definition) undergoing emergency surgery	Abnormal liver or kidney function; Previous methemoglobinemia; Previous carbon monoxide or cyanide poisoning	Intravenous infusion of 0.5- 1.0 mg/kg/hr during surgery	Intraoperative	40	47.5	52.5	Not Recorded

Supplemental Table 3: Quality Assessment for Risk of Bias of the 6 studies. N.B. "Yes" = low risk of bias.

Author, Year	Allocation Sequencing?	Allocation Concealment?	Blinding?	Infrequent Loss to Follow-up?	Free of Selective Outcome	No Other Risk of Bias?	Overall Risk of Bias
					Reporting?		
Ibarra-Estrada, 2023	Probably Yes	Definitely Yes	Probably Yes	Definitely Yes	Definitely Yes	Definitely Yes	Low
Kirov, 2001	Definitely Yes	Definitely Yes	Probably No	Definitely Yes	Probably Yes	Probably Yes	High
Li, 2021	Definitely No	Definitely No	Probably No	Definitely Yes	Probably Yes	Probably Yes	High
Lu, 2019	Probably No	Probably No	Probably No	Definitely Yes	Probably Yes	Probably Yes	High
Memis, 2002	Probably Yes	Definitely Yes	Probably Yes	Definitely Yes	Probably Yes	Probably Yes	Low
Xiong, 2010	Probably Yes	Probably Yes	Probably No	Definitely Yes	Probably Yes	Probably Yes	High

Supplemental Figure 2: Forest plots depicting efficacy and safety of methylene blue versus placebo or usual care.

Short-term Mortality

	Favours Methylen	e Blue	Conti	rol		Risk Ratio	Risk Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	M-H, Random, 95% CI
Ibarra-Estrada 2023	15	45	21	46	46.4%	0.73 [0.43, 1.23]	
Kirov 2001	5	10	7	10	22.8%	0.71 [0.34, 1.50]	
Li 2021	4	33	11	33	11.6%	0.36 [0.13, 1.03]	
Lu 2019	5	36	5	18	10.3%	0.50 [0.17, 1.51]	
Memis 2002	4	15	4	15	8.9%	1.00 [0.31, 3.28]	
Total (95% CI)		139		122	100.0%	0.66 [0.47, 0.94]	•
Total events	33		48				
Heterogeneity: Tau ² =	0.00; Chi ² = 2.22, df:	4 (P = 0	0.70); $I^2 =$	0%			
Test for overall effect:	Z = 2.28 (P = 0.02)						0.1 0.2 0.5 1 2 5 10 Favours Methylene Blue Favours Control

Mean Arterial Pressure at 6 Hours (mmHg)

	Co	ontro	I	C	ontrol			Mean Difference		Me	an Difference		
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV, R	andom, 95% (i .	
Kirov 2001	91.4	11	10	71.8	20.3	10	8.1%	19.60 [5.29, 33.91]			 -	-	
Memis 2002	85	14	11	74	10.3	11	15.7%	11.00 [0.73, 21.27]			├-		
Xiong 2010	68	8	20	59	7	20	76.2%	9.00 [4.34, 13.66]					
Total (95% CI)			41			41	100.0%	10.17 [6.10, 14.24]			•		
Heterogeneity: Tau ²			-		= 0.38); I² = 0	%		-100	-50	-	50	100
Test for overall effect	1. ∠= 4.90) (F <	0.000	טון)						Favours Co	ntrol Favours	Methylene	Blue

Duration of Vasopressors (Hours)

	Methy	/lene B	llue	C	ontrol			Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ibarra-Estrada 2023	70.3	18.4	45	103	51.3	46	84.9%	-32.70 [-48.47, -16.93]	-
Kirov 2001	71.4	34.2	10	93.3	49.7	10	15.1%	-21.90 [-59.29, 15.49]	-
Total (95% CI)	0.00, 06	:= 0 O	55	1 (D = 0	COV 13		100.0%	-31.07 [-45.60, -16.54]	
Heterogeneity: Tau² = Test for overall effect: I				1 (P = U	.bu); 11	= 0%			-100 -50 0 50 100' Favours Methylene Blue Favours Control

Duration of Invasive Mechanical Ventilation (Days)

	Methyl	lene B	lue	Co	ontro	I		Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ibarra-Estrada 2023	4.2	1.4	45	5	1.5	46	82.7%	-0.80 [-1.40, -0.20]	
Kirov 2001	3.5	1.8	10	3.1	2.4	10	8.5%	0.40 [-1.46, 2.26]	
Memis 2002	7	2	15	8	3	15	8.8%	-1.00 [-2.82, 0.82]	
Total (95% CI)			70			71	100.0%	-0.72 [-1.26, -0.17]	•
Heterogeneity: Tau² =				2 (P = 0	.46);	I² = 0%			-10 -5 0 5 10
Test for overall effect:	Z = 2.59 (1	P = 0.0	010)						Favours Methylene Blue Favours Control

Intensive Care Unit Length of Stay (Days)

	Methyl	ene B	lue	Co	ontro	I		Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ibarra-Estrada 2023	6.3	2.1	45	6.9	2.5	46	61.3%	-0.60 [-1.55, 0.35]	- ■+
Kirov 2001	6.4	4	10	6.1	4.5	10	14.4%	0.30 [-3.43, 4.03]	
Memis 2002	13	2	11	16	4	11	24.3%	-3.00 [-5.64, -0.36]	
Total (95% CI)			66			67	100.0%	-1.05 [-2.62, 0.51]	•
Heterogeneity: Tau² = 1 Test for overall effect: 2			•	2 (P = 0	.21);	I² = 379	6		-10 -5 0 5 10 Favours Methylene Blue Favours Control

Hospital Length of Stay (Days)

1	0		•	•						
	Methy	lene E	llue	C	ontrol			Mean Difference	Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI	
Ibarra-Estrada 2023	8.2	2.3	45	10.2	6	46	13.6%	-2.00 [-3.86, -0.14]		
Kirov 2001	17.4	15.5	10	16.1	15.6	10	0.3%	1.30 [-12.33, 14.93]	·	\longrightarrow
Li 2021	6.6	1.2	33	8.7	1.8	33	86.2%	-2.10 [-2.84, -1.36]	-	
Total (95% CI)			88			89	100.0%	-2.08 [-2.76, -1.39]	•	
Heterogeneity: Tau ² =				*	.88); I²	= 0%			-10 -5 0	5 10
Test for overall effect:	Z= 5.94	(P < 0.1	00001)						Favours Methylene Blue Favours Co	ontrol

Methemoglobin Concentration (%)

	Methy	lene B	llue	C	ontrol			Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
lbarra-Estrada 2023	2.8	0.84	45	0.53	0.23	46	33.1%	2.27 [2.02, 2.52]	•
Kirov 2001	1.06	0.22	10	0.9	0.05	10	33.5%	0.16 [0.02, 0.30]	•
Memis 2002	1.03	0.26	11	0.73	0.18	11	33.4%	0.30 [0.11, 0.49]	•
Total (95% CI)			66			67	100.0%	0.91 [-0.21, 2.02]	•
Heterogeneity: Tau ² = Test for overall effect:				= 2 (P •	0.000)01); l²:	= 99%		-10 -5 0 5 10 Higher in Control Higher in Methylene Blue

Supplemental Table 4: GRADE Evidence Profile.

Author(s):
Question: Methylene blue compared to usual care for distributive shock
Setting:
Bibliography:

Bibliograph	y. 		Certainty a	ccccment			Nº of p	ationto	Effec			
			Certainty a	ssessment			M≅ OI Þ	auents	Ellec		Certainty	Narrative
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	methylene blue	usual care	Relative (95% CI)	Absolute (95% CI)	Certainty	Narrative
Mortality												
5	randomised trials	serious ^a	not serious	not serious	serious ^b	none	33/139 (23.7%)	48/122 (39.3%)	RR 0.66 (0.47 to 0.94)	134 fewer per 1,000 (from 209 fewer to 24 fewer)	ФФОО	Methylene blue may decrease mortality.
MAP at 6 h	ours (assessed	with: mmHg)										
3	randomised trials	serious ^a	not serious	not serious	serious ^b	none	41	41	-	MD 10.17 mmHg higher (6.1 higher to 14.24 higher)	ФФОО	Methylene blue may increase MAP at 6 hours.
ICU Length	n of Stay (assess	sed with: days)								<u>, </u>		
3	randomised trials	serious ^a	not serious	not serious	serious ^d	none	66	67	-	MD 1.05 days fewer (2.62 fewer to 0.51 more)	⊕⊕⊖⊖ _{Low}	Methylene blue may reduce ICU length of stay.
Hospital Le	ength of Stay (as	ssessed with: day	s)									
3	randomised trials	seriousa	not serious	not serious	seriousb	none	88	89	-	MD 2.08 days fewer (1.39 fewer to 2.76 fewer)	⊕⊕⊖⊖ _{Low}	Methylene blue may decrease hospital length of stay.
Duration o	f IMV (assessed	with: days)										
3	randomised trials	serious ^a	not serious	not serious	serious ^e	none	70	71	-	MD 0.72 days fewer (1.26 fewer to 0.17 fewer)	ФФСО	Methylene blue may have no effect on duration of IMV.

			Certainty a	ssessment			Nº of p	atients	Effec	t		
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	methylene blue	usual care	Relative (95% CI)	Absolute (95% CI)	Certainty	Narrative
Duration o	f vasopressors	(assessed with: d	ays)									
2	randomised trials	serious ^a	not serious	not serious	serious ^b	none	55	56	,	MD 31.07 hours fewer (16.54 fewer to 45.6 fewer)	ФФОО Low	Methylene blue may decrease the duration of vasopressors.
MetHgb Co	oncentration (as	sessed with: perd	entage)									
3	randomised trials	serious ^a	serious	not serious	serious ^d	none	66	67	-	MD 0.91 percentage higher (0.21 lower to 2.02 higher)	⊕⊖⊖⊖ Very low	Methylene blue has an uncertain effect on MetHgB concentration.

CI: confidence interval; MD: mean difference; RR: risk ratio

Explanations

- a. Majority of evidence comes from studies at high risk of bias
 b. small number of patients and events contributes to imprecision
 c. Visual inspection of the forest plot suggests important variability amongst included studies
 d. Wide confidence intervals that don't exclude harm or benefit
 e. Small numbers, and confidence interval doesn't exclude benefit

Supplemental Figure 3: Sensitivity Analyses – Forest Plots.

Excluding "High Risk of Bias" Studies

Short-term Mortality

	Favours Methylen	e Blue	Cont	rol		Risk Ratio	Risk Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	M-H, Random, 95% CI
Ibarra-Estrada 2023	15	45	21	46	83.9%	0.73 [0.43, 1.23]	
Memis 2002	4	15	4	15	16.1%	1.00 [0.31, 3.28]	
Total (95% CI)		60		61	100.0%	0.77 [0.48, 1.24]	-
Total events	19		25				
Heterogeneity: Tau ² =	0.00; Chi ² = 0.23 , df	= 1 (P = 0)	0.63); I ^z =	0%			01 02 05 1 2 5 10
Test for overall effect:	Z = 1.09 (P = 0.28)						Favours Methylene Blue Favours Control

Mean Arterial Pressure at 6 hours (mmHg)

	Co	ontro	l	С	ontrol	`	Ο,	Mean Difference		Mea	n Difference		
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV, Ra	andom, 95% (CI	
Memis 2002	85	14	11	74	10.3	11	100.0%	11.00 [0.73, 21.27]			-		
Total (95% CI)			11			11	100.0%	11.00 [0.73, 21.27]			•		
Heterogeneity: Not a Test for overall effect	•		0.04)						-100	-50 Favours Cor	0 ntrol Favour	50 s Methylene	100 Blue

Intensive Care Unit Length of Stay (Days)

	Methyl	ene B	lue	Co	ontro	i	•	Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ibarra-Estrada 2023	6.3	2.1	45	6.9	2.5	46	63.8%	-0.60 [-1.55, 0.35]	
Memis 2002	13	2	11	16	4	11	36.2%	-3.00 [-5.64, -0.36]	
Total (95% CI)			56			57	100.0%	-1.47 [-3.73, 0.79]	-
Heterogeneity: Tau² = Test for overall effect: 2				1 (P = 0	.09);	P= 649	%		-10 -5 0 5 10 Favours Methylene Blue Favours Control

Hospital Length of Stay (Days)

	Methyl	ene B	lue	Co	ntro	I		Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ibarra-Estrada 2023	8.2	2.3	45	10.2	6	46	100.0%	-2.00 [-3.86, -0.14]	
Total (95% CI)			45			46	100.0%	-2.00 [-3.86, -0.14]	•
Heterogeneity: Not app Test for overall effect: 2		P = 0.0	04)						-10 -5 0 5 10 Favours Methylene Blue Favours Control

Duration of Invasive Mechanical Ventilation (Days)

	Methy	lene B	Blue	Co	ontro	I		Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ibarra-Estrada 2023	4.2	1.4	45	5	1.5	46	90.4%	-0.80 [-1.40, -0.20]	-
Memis 2002	7	2	15	8	3	15	9.6%	-1.00 [-2.82, 0.82]	
Total (95% CI)			60			61	100.0%	-0.82 [-1.39, -0.25]	◆
Heterogeneity: Tau² = Test for overall effect:				1 (P = 0	.84);	l² = 0%			-10 -5 0 5 10 Favours Methylene Blue Favours Control

Duration of Vasopressors (Hours)

	Methy	lene B	lue	C	ontrol			Mean Difference	Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI	
Ibarra-Estrada 2023	70.3	18.4	45	103	51.3	46	100.0%	-32.70 [-48.47, -16.93]	-	
Total (95% CI)	- l' l- l -		45			46	100.0%	-32.70 [-48.47, -16.93]	•	
Heterogeneity: Not ap Test for overall effect: 2		(P < 0.0	0001)						-100 -50 0 50 Favours Methylene Blue Favours Contro	100 ol

Excluding Studies with MB Bolus Only (i.e. without infusion)

Short-term Mortality

	Favours Methyler	ne Blue	Conti	rol		Risk Ratio	Risk Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	M-H, Random, 95% CI
Ibarra-Estrada 2023	15	45	21	46	52.5%	0.73 [0.43, 1.23]	
Kirov 2001	5	10	7	10	25.8%	0.71 [0.34, 1.50]	
Lu 2019	5	36	5	18	11.7%	0.50 [0.17, 1.51]	
Memis 2002	4	15	4	15	10.1%	1.00 [0.31, 3.28]	
Total (95% CI)		106		89	100.0%	0.72 [0.49, 1.04]	•
Total events	29		37				
Heterogeneity: Tau ² =	0.00; Chi ² = 0.72 , di	f = 3 (P = 0)	0.87); I ^z =	0%			01 02 05 1 2 5 10
Test for overall effect:	Z = 1.73 (P = 0.08)						0.1 0.2 0.5 1 2 5 10 Eavours Methylene Blue Favours Control

Mean Arterial Pressure (6 Hours)

	Co	ntro	I	C	ontrol			Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	I IV, Random, 95% CI
Kirov 2001	91.4	11	10	71.8	20.3	10	8.1%	19.60 [5.29, 33.91]]
Memis 2002	85	14	11	74	10.3	11	15.7%	11.00 [0.73, 21.27]]
Xiong 2010	68	8	20	59	7	20	76.2%	9.00 [4.34, 13.66]] -
Total (95% CI)			41			41	100.0%	10.17 [6.10, 14.24]	1 ◆
Heterogeneity: Tau² : Test for overall effect			-		= 0.38); l² = 0°	%		-100 -50 0 50 100 Favours Control Favours Methylene Blue

Intensive Care Unit Length of Stay (Days)

	Methyl	lene B	lue	Co	ontro	i	•	Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ibarra-Estrada 2023	6.3	2.1	45	6.9	2.5	46	61.3%	-0.60 [-1.55, 0.35]	-≣ +
Kirov 2001	6.4	4	10	6.1	4.5	10	14.4%	0.30 [-3.43, 4.03]	
Memis 2002	13	2	11	16	4	11	24.3%	-3.00 [-5.64, -0.36]	
Total (95% CI)			66			67	100.0%	-1.05 [-2.62, 0.51]	•
Heterogeneity: Tau ^z = Test for overall effect:				2 (P = 0	.21);	l² = 379	%		-10 -5 0 5 10 Favours Methylene Blue Favours Control

Hospital Length of Stay (Days)

	Methy	lene B	Blue	C	ontrol			Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ibarra-Estrada 2023	8.2	2.3	45	10.2	6	46	98.2%	-2.00 [-3.86, -0.14]	—
Kirov 2001	17.4	15.5	10	16.1	15.6	10	1.8%	1.30 [-12.33, 14.93]	· ·
Total (95% CI) Heterogeneity: Tau² = Test for overall effect:				1 (P = 0	.64); l²		100.0%	-1.94 [-3.78, -0.10]	-10 -5 0 5 10 Favours Methylene Blue Favours Control

Duration of Invasive Mechanical Ventilation (Days)

	Methylene Blue			Control			Mean Difference		Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Ibarra-Estrada 2023	4.2	1.4	45	5	1.5	46	82.7%	-0.80 [-1.40, -0.20]	
Kirov 2001	3.5	1.8	10	3.1	2.4	10	8.5%	0.40 [-1.46, 2.26]	- - -
Memis 2002	7	2	15	8	3	15	8.8%	-1.00 [-2.82, 0.82]	
Total (95% CI)			70			71	100.0%	-0.72 [-1.26, -0.17]	•
Heterogeneity: Tau² = Test for overall effect: 2				2 (P = 0		-10 -5 0 5 10 Favours Methylene Blue Favours Control			

Duration of Vasopressors (Hours)

	Methylene Blue Control						Mean Difference	Mean Difference		
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI	
Ibarra-Estrada 2023	70.3	18.4	45	103	51.3	46	84.9%	-32.70 [-48.47, -16.93]	-	
Kirov 2001	71.4	34.2	10	93.3	49.7	10	15.1%	-21.90 [-59.29, 15.49]	-	
Total (95% CI)			55			56	100.0%	-31.07 [-45.60, -16.54]	•	
Heterogeneity: Tau² = Test for overall effect: :				1 (P = 0	.60); l²		-100 -50 0 50 Favours Methylene Blue Favours Control	100		

Methemoglobin Concentration (%)

_	Methylene Blue			Control				Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	I IV, Random, 95% CI
Ibarra-Estrada 2023	2.8	0.84	45	0.53	0.23	46	33.1%	2.27 [2.02, 2.52]]
Kirov 2001	1.06	0.22	10	0.9	0.05	10	33.5%	0.16 [0.02, 0.30]]
Memis 2002	1.03	0.26	11	0.73	0.18	11	33.4%	0.30 [0.11, 0.49]	-
Total (95% CI)			66			67	100.0%	0.91 [-0.21, 2.02]	•
Heterogeneity: Tau² = Test for overall effect: 2				= 2 (P •	-10 -5 0 5 10 Higher in Control Higher in Methylene Blue				