Efficacy and safety of heat-killed Mycobacterium w in Gram-negative sepsis: Prospective study of intravenous administration

Dear Editor,

In India, sepsis and septic shock account for mortality in 30%–80% in an intensive care unit (ICU).[1] Up to 75.5% of sepsis cases are due to gram-negative bacteria %.[2] Mycobacterium w (Mw), also known as *Mycobacterium indicus pranii*, is a non-pathogenic, rapidly growing atypical mycobacterium. Mw, administered intradermally, is a potent toll-like receptor (TLR)-2 agonist,[3] inhibits TLR-9,[4] and augments the Th1 immune response.[5] Mw has been studied for its immune-modulating properties in patients with pulmonary tuberculosis, tuberculous pericarditis, sepsis, lung cancer, and leprosy.[6-11] Intradermal (ID) is the traditional route of administration for Mw in gram-negative bacterial sepsis. However, additional trainings, skills, and a maximum dose limit of up to 0.1 ml are limitations of the ID route. Intravenous (IV) administration of Mw can bypass this hurdle. Previous studies of Mw have explored IV administration up to 5 ml which was found safe and effective.[12,13] However, limited data exists on the safety and effectiveness of Mw via IV route in sepsis. This is the first prospective study evaluating the efficacy and safety of Mw administration via the IV route in patients with gram-negative sepsis. We enrolled prospectively 20 patients (>18 years of

Table 1: Laboratory investigations

Parameters	Mean values (X̄±SD)							
	n	Baseline	*n*	Day 4	*n*	Day 7	*n*	Day 14
Neutrophils (%)	20	83.46±10.20	20	83.58±7.47	16	75.62±14.77	09	77.41±14.30
TLC	20	13.46±07.48	20	09.73±04.12* (-03.73±07.37 (0.035)	17	16.54±18.06	09	13.80±07.87
Lymphocytes (%)	20	12.61±16.12	20	10.49±7.13	17	14.01±10.20	09	12.13±9.27
Eosinophils (%)	20	0.59±16.12	20	0.12±0.21	17	0.34±0.66	09	1.13±1.46
Monocytes (%)	20	5.72±3.46	20	5.40±2.96	17	6.79±3.46	09	9.07±4.38
Basophils (%)	19	0.18±0.12	20	0.25±0.21	17	0.25±0.18	09	0.24±0.16
Platelet Count (/cmm)	20	185.70±104.23	20	174.11±101.47	17	195.08±121.94	09	216.56±147.43
Serum total bilirubin	11	1.03±0.53	09	0.80±0.55	09	0.80±0.27	02	0.75±0.64
Alkaline Phosphatase	10	93.61±59.34	10	330.83±749.19	09	349.78±427.31	02	115.00±104.65
AST (SGOT)	11	46.36±33.65	09	100.67±87.68	09	150.78±325.17	02	38.00±4.24
ALT (SGPT)	11	52.09±50.27	09	120.11±123.33	09	88.89±129.58	20	9.30±1.31
Serum Creatinine	20	1.83±1.57	20	1.60±1.35	18	1.81±1.77	09	2.44±2.14
Blood Urea	20	99.30±64.73	20	99.05±76.53	18	104.94±75.66	09	112.78±67.08
C reactive protein	06	173.88±160.01	01	62.10±0.00	04	86.05±55.10	00	-

*By Student *t*-test: Not Significant for all except TLC count at day 4. All laboratory parameters did not show any significant change after therapy till the end of the study except * TLC count at day 4

Table 2: The cause of mortality

Patient no	Reason for death
1	Death due to septic hhock with multiple organ dysfunction syndromes
2	Death due to electrocution injury, sepsis with septic shock, AKI on hemodialysis
3	Death due to subarchanoid hemorrhage, sepsis with multiple organ dysfunction syndromes
4	Death due to sepsis with septic shock, pneumonitis, right pneumothorax
5	Sepsis with septic shock and multiple organ dysfunction syndromes, Post-COVID-19 DPLD
6	ARDS, DM, Old CVA
7	Death due to morbid obesity with septic shock, LRTI, DM, HTN

AKI: acute kidney injury; ARDS: acute respiratory distress syndrome; CVA: cerebrovascular accident; COVID-19: coronavirus disease; DPLD: Diffuse parenchymal lung diseases; DM: diabetes mellitus; HTN: hypertension; LRTI: lower respiratory tract infection

Table 3: Comparing SOFA score between live vs mortality group

SOFA score	Alive	Death	*P*
Enrollment	4 (2.5, 0)	8 (6, 8)	0.01*
Day 1	4 (2.5, 0)	8 (6, 8)	0.01*
Day 2	4 (2.5, 0)	8 (5, 9)	0.01*
Day 3	5 (3, 0)	8 (4, 9)	0.06
Day 4	3 (3, 0)	8 (4, 8)	0.03*
Day 7	3.5 (1.25, 0)	8 (3.75, 10.25)	0.05*
Day 14	6 (4, 0)	9 (4, 0)	0.29

Data are expressed as median (interquartile range). $P<0.05$* is significant. Mann Whitney test applied. SOFA scores were significantly higher at baseline in the patients who had mortality vs those who survived

age), presumed to have gram-negative sepsis as per sepsis 3 criteria. Patients who had a history of allergic reactions attributed to Inj. Sepsivac® or any of its excipients, pregnant and lactating women, and those with generalized septic skin conditions were excluded. Institutional Ethics Committee approval (MICR 12001/2020) was obtained and so was informed consent from the patient/family. This trial was registered at The Clinical Trials Registry-India (CTRI) as CTRI No: CTRI/2021/02/030882. All patients received Inj. Sepsivac® [an autoclaved suspension in physiological saline of Mw (heat-killed; 0.5×10^9)]; 0.3 ml diluted in 100 ml normal saline was administered as a slow IV infusion over at least 15 minutes, along with standard of care. Inj. Sepsivac was supplied by Cadila Pharmaceuticals Ltd. (Bhat, Sarkhej-Dholka Road, Ahmedabad, Gujarat). All patients were admitted to the hospital until the investigator deemed discharge from the hospital appropriate. Standard therapy for severe gram-ve sepsis was given to all the patients as per institution protocol. Each patient was followed up for 14 days from the day of enrolment. Baseline, Day 1, Day 2, Day 3, Day 4, Day 7, and Day 14 were the time points on which details on efficacy endpoints such as vital signs (change in respiratory rate, heart rate, systolic blood pressure, diastolic blood pressure, and temperature), SOFA score and laboratory investigations [total leukocyte count (TLC)], and [peripheral capillary oxygen saturation (Spo2), CRP, ALT, and AST] were recorded. Patients were also followed up for the safety of intervention assessed by allergic reactions, including anaphylaxis and mortality till Day 14. Patients were also grouped based on mortality (alive or dead), and SOFA score was compared on Day 1, 2, 3, 4, 7, and 14. All the data were analyzed using SAS software version 9.4 or higher. Descriptive analysis was performed to record the characteristics of the study population. Quantitative data were expressed as mean and standard deviation whereas categorical data were expressed as numbers and percentages. Means and differences in means at different time points compared to baseline were compared using the student *t*-test. A *P* value of <0.05 was considered significant. The majority of the 20 patients with gram-negative sepsis were males [11 (55%)], mean age was 56.50 ± 10.85 years (range 30–71 years). All vitals

Table 4: Changes in SOFA score: Correlation with Mw

Variable	Correlation coefficient (r)	SOFA score (Day 2) (*n*=20)	SOFA score (Day 3) (*n*=20)	SOFA score (Day 4) (*n*=20)	SOFA score (Day 7) (*n*=18)	SOFA score (Day 14) (*n*=8)
SOFA score (Day 1)	*r*	0.951**	0.793*	0.800*	0.857*	0.778*
(*n*=20)	*P*	0.001	0.034	0.031	0.029	0.033
SOFA score (Day 2)	*r*	1	0.893**	0.891**	0.907*	0.573
(*n*=20)	*P*		0.007	0.007	0.012	0.612
SOFA score (Day 3)	*r*		1	0.967**	0.943**	0.359
(*n*=20)	*P*			0.000	0.005	0.766
SOFA score (Day 4)	*r*			1	0.907*	0.176
(*n*=20)	*P*				0.013	0.887
SOFA score (Day 7)	*r*				1	0.233
(*n*=18)	*P*					0.850

**.Correlation is significant at the 0.01 level (2-tailed). *.Correlation is significant at the 0.05 level (2-tailed)

and laboratory parameters showed no significant change post-treatment till Day 14 compared to baseline [Table 1]. On Day 4, mean TLC showed a significant change from baseline; however, at the end of Days 7 and 14, mean TLC did not show any significant change from baseline. Out of 20 patients, 7 (35%) died during the study period. The reasons for mortality are mentioned in Table 2. This is the first prospective study that evaluated the IV administration of Mw in gram-negative sepsis. IV administration of Mw and standard care of treatment were found to be well tolerated. None of the patients developed any major adverse event due to IV use of Mw. The overall mortality in the present study was 35%. A recent study from India also observed an ICU mortality of 34% in severe sepsis.[2] A significant difference in the baseline in median SOFA score [Table 3] was reported in the present study in alive patients compared to those who died [8 vs 4; $P < 0.01$]. We found a significant correlation with Mw with respect to change in SOFA score at baseline vs Day 7 and at Day 14 [Table 4]. A recent multicentric randomized controlled trial of Mw in severe presumed gram-negative sepsis has reported a significant change in SOFA score.[7] Another randomized controlled trial of Mw found significantly lesser odds (OR, 0.37 [95% CI, 0.15–0.9]) of mortality.[14] None of the patients had a mortality due to side effects of the drug. A recent observational study from India also found that the IV route of Mw is safe even in elderly patients (mean age of 62 years).[15] However, our study is prospective as compared to the previous one. The present study's findings provide more strength to observations of the previous series. Limitations of our study include a small sample size, lack of randomization, and absence of a comparator arm (ID route). Based on our preliminary experience, we believe that adjunctive Mw via IV route is safe in patients with severe sepsis. However, the efficacy needs to be evaluated in a future randomized controlled trial.

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Conflicts of interest
There are no conflicts of interest.

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