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ORIGINAL ARTICLE

Serum Mannose Receptor as a Diagnostic Bio-Marker for Sepsis in ICU Patients in Ain Shams University Hospitals

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ABSTRACT

Background: *Sepsis is one of commonest complications that is associated with extended hospital stays. Making an early diagnosis is critical for improving its prognosis. Serum Mannose Receptor (sMR) is a biological biomarker which can be used in early diagnosis of sepsis.* ***Objective:*** *To investigate the value of sMR as a diagnostic biomarker for sepsis.* ***Methodology:*** *This study included 65 patients and 25 healthy individuals as control group. The patients' group was divided into two groups according to SIRS criteria. Blood samples from patients were collected for blood cultures. sMR levels were measured in patients and control groups.* ***Results:*** *The commonest isolated organism by blood culture was S. aureus. SMR levels among cases in the both groups were higher than controls with statistically significant difference. SMR could be used to discriminate cases in both groups with 97.5% sensitivity, 80.0% specificity.* ***Conclusion:*** *sMR is a promising biomarker in early predictor of sepsis.*

INTRODUCTION

Sepsis, a life-threatening organ failure caused by an abnormal host response to infection, is a leading cause of death worldwide. Despite the declining trend of sepsis in high-income countries, it is still the main cause of non-cardiac death in critically ill patients 1. Early detection of sepsis and treatment with suitable antibiotics has been shown to enhance outcomes in this population. Prompt laboratory identification of pathogens causing blood stream infections (BSIs) is therefore critical for the optimal treatment of patients with septic shock 2.

The gold standard for diagnosing bacteremia is blood culture. However, this method can take several days to reveal diagnosis, during which time the patient might deteriorate. ICU physician can use biomarkers in identifying patients, who may develop bacteremia in advance of the blood culture diagnosis 3.

Microorganisms can be detected in 30% of blood cultures and it usually takes 48 to 72 hours. Biomarkers have a significant role in the diagnosis of sepsis. Although several biomarkers have been evaluated for the diagnosis and prognosis of sepsis, the gold standard biomarker has not yet been found 4.

The innate immune system recognizes BSIs by pathogen recognition receptors, such as dectin-1 and the mannose receptor (MR or CD206). The serum mannose receptor (sMR) is an endocytic receptor present in macrophages, dendritic cells, and endothelial cells. During inflammation sMR is delivered to the cell surface. It contains several extracellular domains, each having their own binding capacities to sulfated glycans, carbohydrates, collagens, allergens, and pathogens. Metalloproteinase is activated when a ligand binding with the receptor. This ligand activates the cleavage the cell-bound receptor into a soluble form, which is shed into the circulation as soluble MR (sMR). High level of sMR has been recorded in patients with sepsis, liver disease, malignancies and invasive pneumococcal disease 5.

As the sMR is shed from macrophage cell surfaces after exposure to infections, we evaluate whether its sMR can serve as a biomarker of sepsis or not.

METHODOLOGY

Study design:

This study was conducted on 65 patients from ASUHs in the period from October 2019 till October 2020, where blood samples were obtained from patients in the Surgery and Internal Medicine Intensive Care Units. Patients were divided into two groups. Group I consisted of 40 blood samples from patients suspected clinically as septic patients with systemic inflammatory response syndrome (SIRS) criteria 6**.** Patients included had two or more of the **following** SIRS Criteria: (a) Fever > 38°C or hypothermia less than 36°C, (b) heart rate of more than 90b/m, (c) respiratory rate of more than 20 beats /m or partial pressure of carbon dioxide in arterial blood (Paco2) of less than 32mmHg and (d) leukocytosis or leukopenia or more than 10% immature forms of WBCS. Group II included 25 blood samples from critically ill patients with no SIRS criteria. Control group of 25 samples from apparently healthy people with matched age and sex for the patient group, were

included in this study for measuring sMR level. Exclusion criteria: Patients who had received antibiotics before the blood sample collection were excluded from the research.

Ethical consideration:

- The ethical committee approval was obtained from Faculty of Medicine, Ain Shams University.
- Informed consent for sample collection was taken directly from both control and the patients or their relatives.

Sample collection:

Two blood samples were collected under complete aseptic condition for performing duplicate blood cultures. The samples were taken from two different veins. Serum from patients and control was collected and kept frozen at -80 C until analysis for measuring sMR by ELISA technique.

Blood culture specimens:

Blood culture bottles were incubated at 37°C for 14 days. Subculture was done every 24 h on blood agar, MacConky's agar and Sabaroud's dextrose agars, for isolation. Colonies were inspected for their size, shape, colour, consistency and effect on the inoculated culture media, Gram-stained films of the recovered isolates were examined, further identification was done according to **Wilson ML and his *colleague*** 7.

Serum Mannose receptor:

SMR levels were analysed in the serum of patients and control groups by Enzyme Linked ImmunoSorbant Assay using Human Mannose receptor ELISA Kit (Bioassay Technology Laboratory, Cat.No E0337Hu, China).

Statistical analysis:

Statistical analysis was performed with IBM SPSS Statistics, Version 20.0. Armonk, NY: IBM Corp. Qualitative data were presented as Frequencies (n) and percentage (%). The statistical significance of the difference between two study group means was determined using the Student T Test. Chi-Square test was used to study correlation between two qualitative variables. The ROC Curve (receiver operating characteristic) to evaluate the sensitivity and specificity of Mannose receptor. The significance level was set as $P \leq 0.05$.

RESULTS

This study was performed on 65 blood samples obtained from ICU patients from ASUHs and 25 blood samples as control group. The mean age of patient group I was 47.50 (± 15.10) and the mean age of patient group II was 49.88 (± 20.10), while the mean age of control group was 41.88 (± 16.87). No statistically significant difference regarding age and sex in all groups as shown in table (1).

Table 1: Demographic data of the patient groups & the control group:

		Group I	Group II	Control group	Test value	P-value	Sig.
		No. = 40	No. = 25	No. = 25			
Age	Mean ± SD Range	47.50 ± 15.10 12 – 80	49.88 ± 20.10 12 – 82	41.88 ± 16.87 12 – 70	2.187*	0.118	NS
Sex	Female Male	16 (40.0%) 24 (60.0%)	9 (36.0%) 16 (64.0%)	8 (32.0%) 17 (68.0%)	0.431•	0.806	NS

Laboratory parameters were analysed for patient groups. CRP and ESR, showed no statistical difference, while total leukocytic count (TLC) showed statistically significant difference, as shown in table (2).

Table 2: Laboratory parameters in patient groups:

		Group I	Group II	Test value	P-value	Sig.
		No. = 40	No. = 25			
CRP	Median (IQR) Range	144.5 (85.5 - 245.5) 45 – 480	195 (110 - 245) 45 – 380	-0.958	0.338	NS
ESR	Mean ± SD Range	95.93 ± 24.49 55 – 154	100.84 ± 29.24 55 – 154	-0.730	0.468	NS
TLC	Median (IQR) Range	16.5 (9.7 - 18.8) 1.9 – 30.9	8.5 (4.5 - 14.5) 1.9 – 28	-2.415	0.016	S

Blood culture:

As for group I, 30 samples (75%) were confirmed as positive blood culture the most frequently isolated organisms were *Staphylococcus aureus (S. aureus) (27.5%), Klebsiella pnemoniae (K.pnemoniae)* (15.0 %), and *Escherichia coli (E.coli)* (12.5 %). In group II, 6 samples (24%) were positive for blood culture, the isolated organisms were *S.areus (8.0%), Micrococcus* (8.0%), *E.coli* (4.0%) *and Proteus* (4.0%) as shown in figures (1&2).

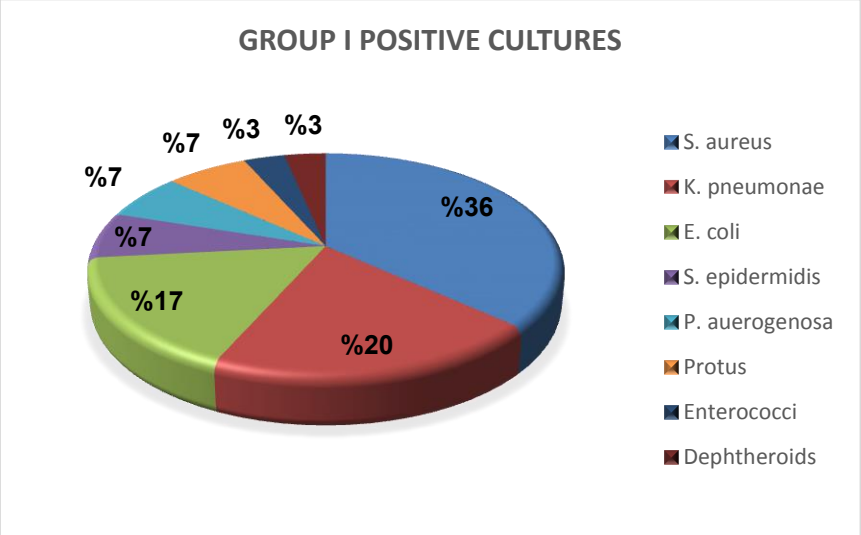


Fig. 1: Results of positive blood cultures in Patients' group I.

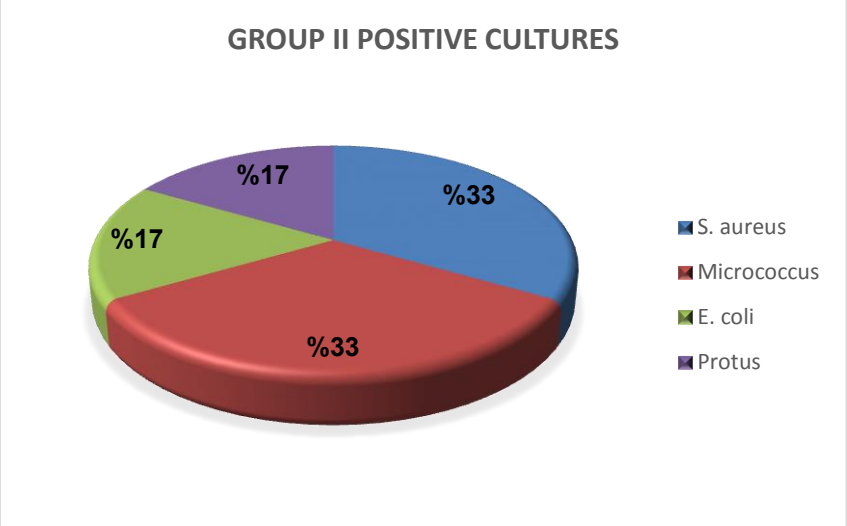


Fig. 2: Results of positive blood cultures in Patients' group II.

Level of sMR:

Level of sMR was measured in both patient groups and control group by ELISA. The range of sMR in patient groups was 0.4 – 16 ng/ml and in control group was 0.25 – 0.7 ng/ml. There was statistically significant increase in the level of sMR in the patient groups in comparison with control group as shown in table (3).

Table 3: Comparison between the patient groups & the control group regarding the serum level of mannose receptor:

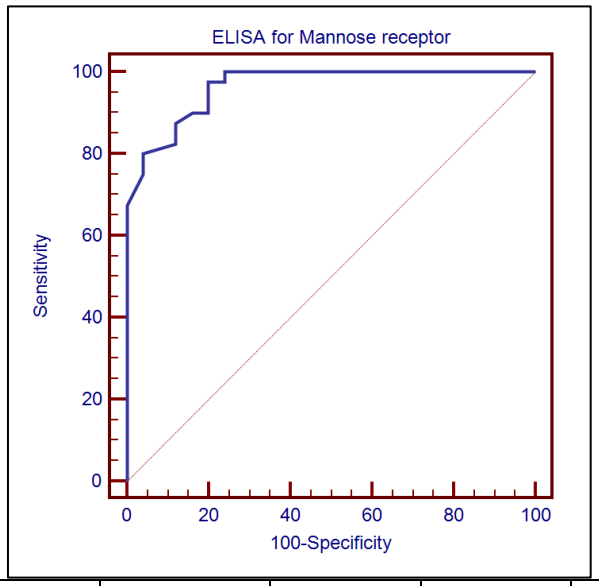
S. Level of mannose receptor	Patients group	Control group	Test value	P-value	Sig.
	No. = 65	No. = 25			
Median (IQR)	5 (2 - 10)	0.4 (0.25 - 0.6)	-7.023	0.000	HS
Range	0.4 – 16	0.25 – 0.7			

The range of sMR level was 2.6 -16 ng/l in patients' group I, 0.4 – 5.5 ng/ml in patients' group II and 0.25 – 0.7 ng/ml in control group. The median has been reported as 7.65 ng/L, 1.5 mg/L, 0.4 ng/L, for group I, II and controls respectively as shown in table (4).

Table 4: Comparison between the patient groups I, II & the control group regarding the level of sMR:

level of sMR	Group I	Group II	Control group	Test value ǂ	P-value	Sig.
	No. = 40	No. = 25	No. = 25			
Median (IQR) Range	7.65 (5.25 - 13) 2.6 – 16	1.5 (0.7 - 2.3) 0.4 – 5.5	0.4 (0.25 - 0.6) 0.25 – 0.7	71.782	0.000	HS

Using ROC curve analysis, it was shown that sMR can be used to discriminate cases in group I&II at a cut off level of ≥ 2.7ng/ml with 97.5% sensitivity, 80.0% specificity. Area under the curve (AUC) was 0.964 as shown in fig (3)



Parameter	AUC	Cut of Point	Sensitivity	Specificity	PPV	NPV
ELISA for Mannose receptor	**0.964**	**>2.7**	**97.5**	**80.0**	**88.6**	**95.2**

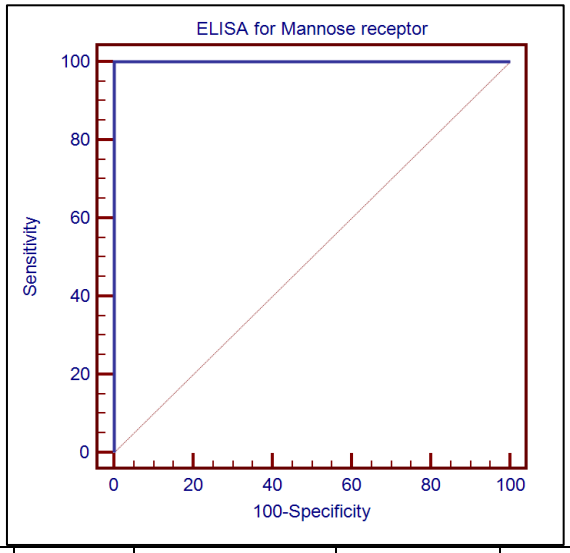
Fig 3: ROC curve using the level of sMR to differentiate group I from group II.

Levels of sMR were high in group I patients, in both positive and negative blood culture results, with no statistically significant difference. In group II, however, sMR levels were considerably greater in patients with positive blood cultures compared to those with negative blood cultures as showen in table (5).

Table 5: Correlation between the level of sMR with the result of blood culture in the patient groups (I&II):

level of sMR		Culture		Test value	P-value	Sig.
		Negative	Positive			
Group I	Median(IQR) Range	5.5 (5 - 10) 3 – 16	8.25 (6 - 13.5) 2.6 – 16	-1.019	0.308	NS
Group II	Median(IQR) Range	1 (0.7 - 2) 0.4 – 3.5	3.95 (2.7 - 4.5) 1.8 – 5.5	-3.159	0.002	HS

Using ROC curve analysis, it was shown that sMR could be used to discriminate cases in group I (septic patients) from controls at a cut off level of ≥0.7ng/l with 100% sensitivity, specificity, positive predictive value and negative predictive value. AUC was 1.000 as shown in fig (4).



Parameter	AUC	Cut of Point	Sensitivity	Specificity	PPV	NPV
ELISA for Mannose receptor	**1.000**	**>0.7**	**100.0**	**100.0**	**100.0**	**100.0**

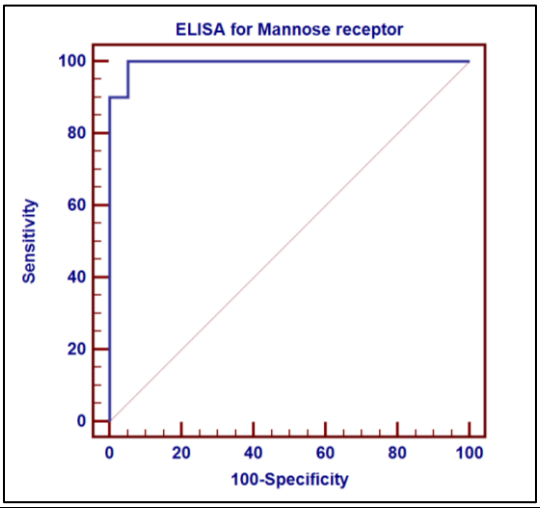
Fig. 4: ROC curve using the level of sMR to differentiate group I (septic patients) from controls.

Levels of sMR in group I patients, who had negative blood culture reults were higher than levels of sMR in group II patients, who had negative blood culture with highly significant difference as showen in table (6).

Table 6: comparison between levels of sMR in group I &II patients, with negative blood culture results:

ELISA for Mannose receptor	Group I No. = 10	Group II No. = 19	Test value	P-value	Sig.
Median(IQR) Range	5.5 (5 - 10) 3 – 16	1 (0.7 - 2) 0.4 – 3.5	-4.322	0.000	HS

Using ROC curve analysis, it was shown that sMR could be used to discriminate negative blood culture cases in group I (septic patients) from negative blood culture cases in group II (critical ill patients) at a cut off level of >2.3ng/l with 100% sensitivity, 94.74% specificity, 90.9% positive predictive value and 100% negative predictive value. AUC was 0.995 as shown in fig (5).



Parameter	AUC	Cut of Point	Sensitivity	Specificity	PPV	NPV
ELISA for Mannose receptor	**0.995**	**>2.3**	**100.0**	**94.74**	**90.9**	**100.0**

Fig. 5: ROC curve using the level of sMR to differentiate group I patients with negative blood cultures from group II patients with negative blood cultures.

Laboratory parameters were correlated between sMR level in patients' groups, only temperature and total leucocytic count were found to have statistically significant positive correlation while no statistically significant correlation found with the other studied parameters as shown in table (7).

Table 7: Correlation between the level of serum mannose receptor with temperature and other laboratory parameters in patient groups (I&II)

	Level of S. Mannose receptor	
	R	P-value
Temperature	0.796**	0.000
CRP	-0.032	0.799
ESR	-0.085	0.502
TLC	0.555**	0.000

DISCUSSION

In the current study 65 blood samples were divided into group I (40 samples), that contained patients with SIRS criteria and group II (25 samples), that consisted of critical ill patients without SIRS criteria.

Our findings showed that 52.7% of all isolated organisms from blood cultures were Gram +ve bacteria and 47.2% were Gram –ve bacteria. Regarding Group I, among 30 positive cultures, the most commonly isolated organisms were *S. aureus* (11) 36% and *K. pnemoniae* (6) 20%, followed by *E. coli* (5) 16.6%, *Proteus* (2) 6.6%, *Pseudomonaus auerоginosa* (2) 6.6%, *Staph epidermidis* (2) 6.6%, *Enterococci* (1) 3.3% and *Diphteroids* (1) 3.3%.

As for Group II, the most commonly isolated organisms in total of 6 positive cultures were *S. aureus* (2) 33% and *Micrococcus* (2) 33% followed by *E. coli* (1)16.6%, *Proteus* (1) 16.6%.

Our study agreed with the study done by Curtoni and his colleague[8], who found that 74.7% of the isolated microorganisms were Gram-positive bacteria, 19.8% Gram-negative bacteria, and 5.6% yeasts.

Our findings are close to the study performed by *Caskurlu and his colleague* [9] who found that, (57.9%) of the positive blood cultures were for Gram-positive bacteria, (31.4%) of them were for Gram-negative bacteria, and (10.6%) of them were yeasts. It was determined that Gram-positive bacteria consisted of *coagulase-negative staphylococci* (CONS) (78.9%), *Enterococcus spp*. (10.6%) and *S. aureus* (10.4%).

Our results agreed with the study which was done in South African by Ramasawmy and his colleague[3] who found that *S. aureus* (38.9%) was the bacterium which was most frequently isolated from blood cultures, followed by *K.pnemoniae* (11.1%) and *Enterobacter cloacae* (11.1%).

Nearly similar results were obtained from the study performed by Rule and his colleague[11] who found that, the most frequently detected organisms were *Staphylococcus* 28 (35.9%) followed by *Enterobacterales* 27 (34.6%), *K.pneumoniae* 16 (20.5%) and *A.baumannii* 10 (12.8%).

These results disagreed with the study done by Umemura and his colleague[12] who found that, 42.2% Gram-positive bacteria, 52.4% Gram-negative bacteria, and 2.5% fungi. They also found that, the leading pathogens associated with septic patients in ICU was *E. coli* (21.5%), followed by *K. pneumoniae* (9.0%), Methicillin sensitive *S. areus* (6.5%), and *Streptococcus pneumoniae* (5.0%). and also disagreed with the results by Mulatu et al. [13] who found that: Among the available 29 culture-positive results, gram-negative bacilli were observed in 19 (65.5%), and gram-positive bacteria were seen in 9 (31%) patients. The rest one isolate was fungal species. The most common isolates were *Pseudomonas aeroginosa* in 10 (34.4%), *K. pneumoniae* in 7 (24%), *S. aureus* in 5 (17.2%) and *E. coli* in 4 (16%) patients.

Koichi and Sophia[14], found that 62.2% of patients had positive blood cultures harboring Gram-negative bacteria and 46.8% were infected with Gram-positive bacteria. *E. coli* can be found in approximately 1 in 6 culture-positive septic patients, and other predominant Gram-negative bacteria species in sepsis include *Pseudomonas*, *Klebsiella* and *Enterobacter* species.

The differences in percentages of pathogens isolated in cases of sepsis among different studies may be due to different patient groups with different cause of admission to ICU, difference of level of infection control measures applied in health care facilities, different dominant or resistant pathogens in ICU, different antibiotics used according to per guidelines and different comorbidities.

In this study the sMR values (median) have been reported for group I, II and controls as 7.65 mg/L, 1.5 mg/L, 0.4 mg/L, respectively. The difference between patients' group I and group II was highly significant. Similar results were obtained by Relster et al. [15], who found that, the Median sMR was significantly higher in patients with bacteremia compared with patients without bacteremia (0.57 mg/L vs. 0.41 mg/L).

Another study done by De Vlieger et al.[16], reported that sMR concentrations were significantly different in healthy controls, patients with noninfectious inflammation, in patients with bacterial infection and in patients with invasive fungal infection. Nearly similar results were obtained from a study which was done by Rødgaard-Hansen et al. [17] who found that, the mean concentration in healthy individuals is 0.28 mg/L while it was 1.0 mg/L in ICU patients.

Our findings were closed to the study performed by Rødgaard-Hansen et al. [18] who found that, the median

S.MR concentration in the entire group of patients was 0.77 mg/L, and in healthy individuals was (0.1 – 0.43 mg/L).

In our study sMR level at a cut-off value of ≥0.7ng/l was able to discriminate between septic (group I) and healthy (controls) with100% sensitivity, specificity. This agreed with the study done by De Vlieger and his colleague[16] who found that the optimal cut-off to differentiate infection from no infection for sMR was 0.71 mg/L with a corresponding sensitivity of 64.4% and specificity of 68.8%. Another study performed by Kjærgaard et al. [19], stated that a cut-off value of 0.61 mg/ml was able to discriminate between septic and non-septic patients with 100% sensitivity and 100% specificity.

CONCLUSION

Blood steam infections are one of the leading causes of increase of ICU admission and increase rate of morbidity and mortality between them. In this study gram positive organisms were detected more than gram negative organisms in septic patients and critical ill patients.

sMR could be used as an early predictor of sepsis in critically ill patients, further studies are needed to evaluate this marker on larger scales.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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