Research Article

The Role of sTREM-1 as a predictor of Septic Shock and Death in Neonatal sepsis

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Abstract
Introduction: Neonatal sepsis is the third leading cause of neonatal mortality, only behind rematurity and intrapartum related complication. **Objectives:** We aimed to determine the role of sTREM-1 as predictor of septic shock and death in septic neonates and to determine cut off value for prognosis of sepsis. **Patients and methods:** The study was conducted over the period from August 2016 to July 2017 and included 60 neonates with neonatal sepsis who were collected from NICU at Minia Children & Maternity University Hospital and were grouped according to outcome into surviviors group and death group. All neonates were subjected to full history taking , clinical examination and laboratory investigations including CBC including differential white blood cell count and platelet count, CRP, Plasma sTREM-1 level quantified with sTREM-1 human ELISA kit and blood culture)
Results: Higher Percentage of death related to female sex and even within surviving group the percentage of males was higher than females The gestational age was statistical significantly lower in death group than in the survivor group. From clinical signs mean arterial blood pressure level was significantly lower in the death group than in survivors group but the heart rate and respiratory rate were highly significant in death group than in survivor group. TLC, neutrophil percentage and STREM-1 were statistical significantly higher in the death group than in survivors group. sTREM-1 cut-off value of 448.2% pg/ml would exhibit a sensitivity of 86.67% and specificity of 95.56% PPV would be 86.7% and NPV 95.6% for prognosis of neonatal sepsis   There was no statistical significance difference between the death and survivors group as regarding the organisms detected in both blood cultures. **Conclusion:** sTREM-1 has the potential to provide excellent predictive value. For outcome of neonatal sepsis Further trials with larger sample sizes are needed to identify the optimal cut-off value and to establish a diagnostic accuracy.
Key words: Neonatal sepsis septic sTREM.1

Introduction

More than 40% of under-five deaths globally occur in the neonatal period with 3.1 million newborn deaths each year (UNICEF et al., 2011)

Neonatal sepsis remains one of the leading causes of morbidity and mortality both among term and preterm infants.(Camacho et al., 2013)

Although advances in neonatal care have improved survival and reduced complications in preterm infants, sepsis still contributes significantly to mortality and morbidity among very low birth weight (VLBW,<1500g) infants in Neonatal intensive care units (NICU). (Hornik et al., 2012)

On the other hand, the survivors of neonatal sepsis are vulnerable to short- and long term neurodevelopmental morbidity (Ferreira et al., 2014)

Diagnosis and management of sepsis is a great challenge facing neonatologists in NICUs. Clinical diagnosis of presentation is difficult due to nonspecific signs and symptoms. In addition, laboratory diagnosis is time consuming. The transmembrane glycoprotein TREM-1, a 30 kDa member of the immune-globulin superfamily, triggers an inflammatory response by releasing cytokines, increasing cell surface receptors, and activating neutrophil degranulation and oxidative burst (Bouchon et al.,2000).Initial interpretations of many studies led to the proposal of developing sTREM-1 as a biomarker for the diagnosis of acute inflam-matory diseases, such as septic shock However, this observation was not supported by subsequent studies and is now considered as a

possible indicator of increased severity of the disease (Bucova et al., 2012).We aimed at this study is to determine the role of sTREM-1 as a predictor of septic shock and death in septic neonates and to determine the cut off value of sTREM-1 for prognosis of sepsis.

Patients and Methods

This randomized study included 60 neonates with sepsis (39males,21females) who were collected from NICU of Minia Children and Maternity University hospital during the period from August 2016 to July 2017.

The studied neonates who developed sepsis in NICU were either:

1- Early onset neonatal sepsis during the first 3 days (This group included 33neonate 55% (22 males,11 females).

2- Late onset neonatal sepsis after 3 days of life. (This group included 27 neonate 45% (17 males,10 females).

According to outcome of these neonates our studied groups were sud divpded into:

Group (1): included survived neonates from neonatel sipsis they were 45 members (33 males, 12 females)

Group (2): included neonates who died as a result of septic shock they were 15 members (6 males, 9 females)

Inclusion criteria:

Neonates with clinical signs suggestive of neonatal sepsis

1-Body temperature changes

2-Breathing problems

3-Diarrhea

4-Low blood sugar

5-Reduced movements

6-Reduced suckling

7-Seizures

8-Bradycardia

9-Swollen belly area

10-Vomitting

11-Jaundice (conjugated hyperbillirubinemia)

Laboratory evidence of infection was a CRP >14 mg/L, neutrophilia >50% and thrombo-cytopenia <150000, fever >38C and total parenteral nutrition for ≥14 days (Stoll et al., 2002).

Exclusion criteria:

Neonate with any of the following :

- Born from mothers with clinical chorioamnionitis.

- Born from HIV positive mothers.
- Congenital infection.
- Congenital anomalies.
- Endocrine disorders.
- Dehydration.
- Asphyxia or birth trauma-

All septic neonates included in our study were followed up for 7 days after admission to observe for the development of septic shock or death.

Septic shock was considered to have occurred when a septic neonates had hemodynamic deterioration defined as

1. Tachycardia (heart rate above 180 beats/ min)

2. Mean arterial blood pressure < 30 mmHg in two consecutive measurements two hours apart that require ed fluid and inotropic therapy.

3. Two or more of the following criteria:

Capillary refill time > 4 seconds; oliguria, with urine output < 0.5 mL/h; and metabolic acidosis with a lactic acid level above 2 mmol/L (Sarafidis, et al., 2010).

Methods

Studied groups were subjected to:

1) Full history for mother and her neoborn

A) Maternal:

1. Mother age
2. Parity
3. Complication during pregnancy.
4. Medications.
5. Premature rupture of membrane
6. Mode of delivery
7. Meconium
8. History of streptococcal prophylaxis

B) Neonatal data were recorded from patient's files in NICU

1- Apgar score at 5 minutes

2- Baby sex

3- Infant's complications at birth

4- Maturity rate scale by Ballerd score

2) Thorough clinical examination to all neonates

1- Vital signs

2- Measurements (weight,length,head circum-ference and abdominal circumference)

3- Chest, heart, abdomen and neurological examination including neonatal reflexes (Moro and suckling)

3) Laboratoy investigations:

Under complete aseptic technique 5 ml of venous blood were collected from every

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neonate participated in this study left to be clotted in the incubator and centrifuged

The samples taken for:

a)   CBC (Diferential white blood cell count - platelet count) which were assayed by Celltaces chemical analyzer/ German

b) CRP was assayed by biomed CRP latex reagent

c)   Blood culture that was assayed by conventional technique (trepticase soya broth)

d) Plasma sTREM-1 level (quantified with sTREM human ELISA kit) was assayed by Cat. ab100659, Abcan/USA

Statistical analysis:

The collected data were coded, tabulated, and statistically using SPSS program (Statistical Package for Social Science)software version 20. Descriptive statistics were done for numerical data by mean, standard deviation and minimum & maximum of the range. While they were done for categorical data by number and percentage. Analytical analysis were done for quantitative variables using t-test in cases of two groups with parametric data and Mann Whitney U in cases of two groups with non parametric, while correlations were done using Spearman Correlation for non-parametric data.

Analytical analysis were done for qualitative data using Chi square test for cases more than 5 in the variable, and Fisher's Exact test for cases less than 5 in the variable.

-The level of significance was taken at P value < 0.050.

-The correlation grade :
• Weak 0-0.24   * Fair 0.25-0.45
• Moderate 0.5-0.74   * Strong 0.75-1

Results

Higher Percentage of death related to female sex and even within surviving group the percentage of males was higher than females The gestational age was statistical significantly lower in death group than in the survivor group. From clinical signs mean arterial blood pressure level was significantly lower in the death group than in survivors group but the heart rate and respiratory rate were highly significant in death group than in survivor group.

TLC, neutrophil percentage and STREM-1 were statistical significantly higher in the death group than in survivors group. sTREM-1 cut-off value of 448.2% pg/ml would exhibit a sensitivity of 86.67% and specificity of 95.56% PPV would be 86.7% and NPV 95.6% for prognosis of neonatal sepsis. There was no statistical significance difference between the death and survivors group as regarding the organisms detected in both blood cultures.

Table (1): Comparison between the death group and survivors group as regarding some demographic data

	Prognosis		P value
	Survivors group (n=45)	Death group (n=15)	
($)Age of onset of sepsis (days) Median IQR	2 (1-5)	3 (1-6)	0.889
Sex n (%) Male Female	33(73.3%) 12(26.7%)	6(40%) 9(60%)	**0.019***
	0.002*	0.439	
Weight (kg) Range Mean ± SD	(1.3-3.5) 2.1±0.4	(1.1-3) 1.9±0.6	0.247
Gestational Age (weeks) Range Mean ± SD	(30-40) 35.1±2.7	(29-40) 33.3±2.8	**0.030***
Mode of delivery SVD CS	19(42.2%) 26(57.8%)	5(33.3%) 10(66.7%)	0.543
	0.297	0.197	

Table (2): Comparison between death group and survivors group as regarding some clinical data

	Survivors group	Death group	P value
Onset of sepsis Early Late	24 (53.3%) 21 (46.7%)	9 (60%) 6(40%)	0.653
HR(beat/min)	143.3±16.8	189.1±5.7	<0.001*
RR(per min)	51.3±6.2	75.7±8.9	<0.001*
Mean arterial blood pressure (mmhg)	47.2±8.6	25.1±2.3	<0.001*

Table (3): Comparison between the death group and survivors group as regarding some laboratory data

	Prognosis		P value
	Survivors group (n=45)	Death group (n=15)	
TLC (x10^3/ul) Range Mean ± SD	(15-20) 17.3±1.3	(16-20) 18.8±1.1	**<0.001***
Neutrophils (%) Range Mean ± SD	(45-88) 59.5±9.1	(63-87) 78±7.6	**<0.001***
($)CRP(mg/l) Median IQR	48 (24-48)	48 (24-48)	0.799
($)Platelets(x$10^3$/ul) Median IQR	68 (40.5-120)	50 (28-100)	0.245
($)sTREM-1 (pg/ml) Median IQR	412.2 (377.5-428.3)	571.7 (469.1-1170)	**< 0.001***

Table (4): ROC curve analysis of sTREM-I for prediction of mortality

	Optimal cutoff	AUC	P value	Sensitivity	Specificity	PPV	NPV	Accuracy
STREMI	>448.2	0.875	<0.001*	86.67	95.56	86.7	95.6	93.33
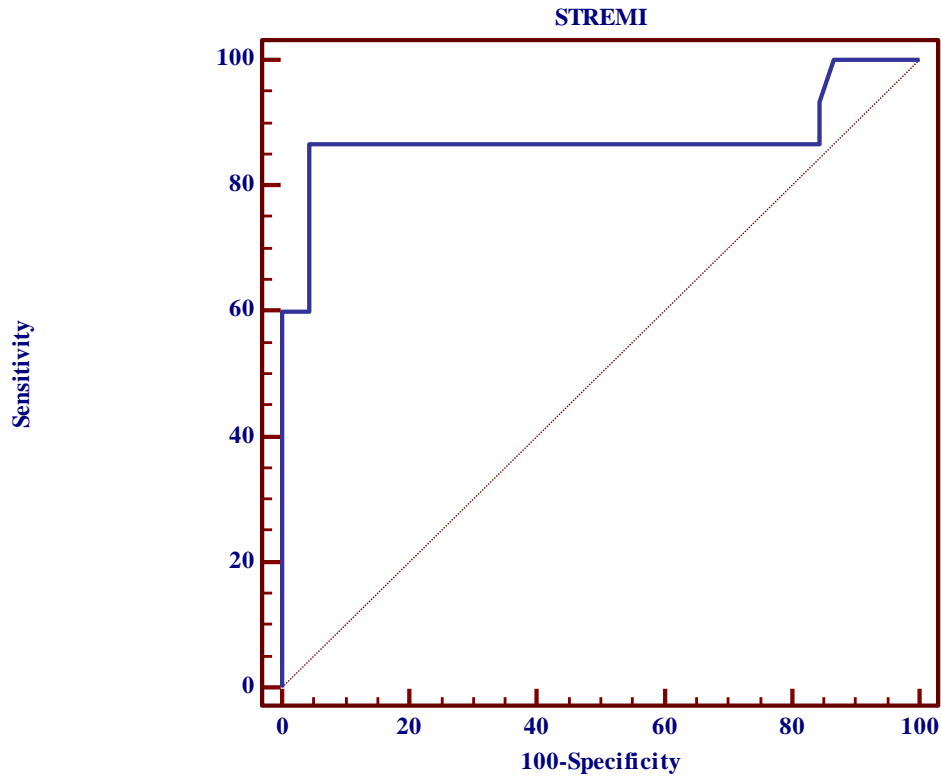
Table (5): Simple logistic regression analysis to determine the factors that predicting mortality

	OR	95% CI	P value
Sex			
Male	1		
Female	4.13	1.21-14.06	**0.024***
Gestational age	0.78	0.61-0.99	**0.037***
Neutrophils	1.22	1.11-1.35	**<0.001***
STREMI	1.03	1.01-1.05	**0.003***
Age of onset	1.03	0.9-1.1	0.492
Sepsis	1.9	0.6-6.2	0.300
TLC	2.7	1.5-5	**0.001***
Platelets	0.99	0.98-1.01	0.282
CRP	1.001	0.98-1.03	0.903

Discussion

Neonatal sepsis is a systemic condition of bacterial, viral or fungal(yeast)infection that is associated with haemodynamic changes and other clinical manifestations and results in substantial morbidity and mortality (Shane et al., 2014)

Neonatal sepsis is classified according to the timing of infection to early onset sepsis (in the

first 3 days of life) and most commonly associated with group *B streptococci, E.coli, H influenza and listeria monocytogenes* (Klinger et al., 2009)

Late onset sepis occurs at 4-90 days of life and is acquired from the giving environment and most commonly associated with the following organisms: *coagulase negative staphylococcus, staphylococcus aureus, E.coli, klebsiella, pseudomonas, Enteriobacter, candida,GBS, serratia and anaerobes* (Anderson et al., 2015)
It was estimated that the incidence of suspected neonatal sepsis in NICUs of three Egyptian Neonatal Network participants in Mansora Hospitals is 45.9% among the admitted neonates at the NICU with a mortality rate of 51% for proven EOS and 42.9% for proven LOS (Eman et al., 2015), In contrast very low rates were reported in the developed countries (Xiao et al., 2013) only about 2-4 per 1000 live births developed to neonatal sepsis (Baltimore et al., 2003)

Septic shock occurs in subset of patients with sepsis and comprises an underlying circulatory and cellular or metabolic abnormalities that is associated with increased mortality (Andre et al., 2017)

Septic shock defined as persistent hypotension that require vasopressors to maintain a mean arterial pressure of normal value and serum lactate level greater than 2mmol/l (18 mg/dl) despite adequate volume resuscitation (Singer et al., 2016)

The transmembrane glycoprotein sTREM-1, a 30kda member of the immunoglobulin super family triggers an inflammatory response by releasing cytokines, increasing cell surface receptors and activating neutrophil degranu-lation and oxidative burst. (Bouchon et al., 2000)

This study was done in NICU of Minia Children and Materity University hospital over the period from August 2016 to July 2017 and included 60 neonate (39males 65% & 21 females 35%) who were developed neonatal sepsis (early onset 55% and late onset 45%)

The aim of the present study was to determine the role of sTREM-1 as a predictor of septic shock and death in septic neonates and to determine the cut off value of Strem-1 for prognosis of sepsis.
Our study revealed that death was related more to low gestational age of studied neonates (P=0.03) (Table 1)

This result was in agreement with Kermorvant-Duchemin et al., 2008 who studied the outcome and prognostic factors in neonates with septic shock and found that infants with adverse outcome had significantly lower gestational age.
This means that prematurity is a risk factor for sepsis and septic shock which explained that the innate immune response are key to the protection against infection early in life and emerging data suggest that such responses are deficient in the newborn especially in preterm infants (Andrew et al., 2010)

We detected higher percentage of death related to female sex (P=0.019) (Table 1) and even within survivor group the percentage of males is higher than females(P=0.002),like our result was Pietropaoli et al., 2010 who found that females with severe sepsis or septic shock had a high risk of dying in the hospital than did males.
The mean arterial blood pressure level was significantly lower in death group than in survivors group(P<0.001) but heart rate and respiratory rate were significantly higher in death group than in survivors group (P<0.001) (Table 2). These findings were in agreement with Goldstein et al., 2005 who defined hemodynamic deterioration as tachycardia (heart rate above 180 beats/min) and mean arterial blood pressure< 30 mmhg in two consecutive measurements two hours apart that required fluids and inotropic therapy.

It was found that in death group 60% were of early onset sepsis and 40% of late onset sepsis and in survivor group 53.3% of early onset sepsis and 46.7% of late onset sepsis .

TLC may increase or decrease depending on the severity of sepsis or shock , the patient's immunologic status and the etiology of infection. Concurrent corticosteroid use may elevate WBC count and thus mask WBC changes due to trends in the illness (Paul et al., 2016).

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We have found that the TLC was significantly higher in death group than in survivors group (P<0.001) (Table 3)

This result was not in harmony with Tanaka et al., 2015 who studied white blood cell counts impact on septic patient outcome followed by polymyxin-b immobilized fiber with direct hemoperfusion and found that the mortality rate of the group with low TLC tended to be higher than that of the group with high TLC.
Also we have found that neutrophil count was significantly higher in death group than in survivors group (P < 0.001) (Table 3) and this was also in agreement with Xuan liu et al., 2016 who studied the prognostic significance of neutrophil to lymphocyte ratio in patient with sepsis and found that patients with severe sepsis or septic shock turned to have higher levels of neutrophil count compared with patient with sepsis.

The cause responsible for high neutrophil count correlating poor outcome in patient with sepsis remains unclear, although there are a variety of possible explanations .one of those explanation is based primarily on the physiological link between neutrophilia with systemic inflammation and stress Zahorec, 2001 neutrophils is the key cellular component of host defense in the innate immune system against infection.

In contrast Salciccioli et al., 2015 found that there is no statistically significant relationship between neutrophil lymphocyte ratio and mortality in patient with sepsis. Another recent research indicated that NLR on admission was significantly lower in patients who died before day 5 of septic shock onset than in survivors, and an increased NLR from day1 to day 5 was associated with late death (Riche et al., 2015)
The CRP level was found to be increased in all studied septic neonates (Table 3) either survivors or dead and this was in agreement with povoa et al., 2011.

The platelet count was significantly decreased in all studied neonates (Table 3) survivors and death. This was in agreement with Guclu et al., 2013 who studied the effect of severe sepsis on platelet count and their indices and found that platelet count was lower in septic patients.

The transmembrane glycoprotein TREM-1 triggers an inflammatory response.Its soluble fraction (sTREM-1) has been shown to have diagnostic accurancy for neonatal sepsis (Arizaga et al., 2014).

It was detected that STREM-1 level is significantly higher in death group than in survivors group (P<0.001) (Table 3)

This result was in agreement with Arizaga et al., 2014 who studied the STREM-1 as a predictor of septic shock and death in late onset neonatal sepsis and found that there is a greatly significant difference for STREM-1 values between the non- shock and shock / death group.

Our results were in agreement with many studies as Adly et al., 2014 who found much higher values of sTREM-1 in those individuals who dead than those who survived.

Also Gibot et al., 2004 and Zhang et al., 2011 who studied dynamic changes of sTREM-1 reflect sepsis severity and can predict prognosis and found that sTREM-1 levels accurately reflect the severity of sepsis and are a sensitive prognostic tool.

Sarafidis 2010 puplished the results of their prospective,observational study that aimed to compare the diagnostic accuracy for LONS of sTREM-l value to those of interleukin 6 ,however STREM-1 was not found to be more accurate than interleukin 6 in differentiating infected from non infected neonates.

Our study revealed potential sTREM1 cut off value of 448.2 pg/ml (Figure 1) which would result in the best relation of sensitivity to specifity. Therefore from data of the whole study population a proposed sTREM1cut off value of 448.2 pg/ml would exhibit sensitivity of 86.67 and specifity of 95.56

As per routine work up for all septic neonates , blood culture was done and revealed that there was no statistical significance difference between the survivors group and death group as regarding the organisms detected in both groups. But *Staph saprophyticus* was the most common organism detected in the survivors

group and *Klebsiella* was the most common organism detected in the death group.

No growth (culture negative) found in 55.6% of cases included in the survivors group and 40% of cases included in dead group.

Nicolas et al., 2013 who studied unrevealing culture negative severe sepsis and found that only 40 to 60% of patients with severe sepsis or shock have microbiologically documented infection but the remaining percentage have no growth.

By using the simple logistic regression analysis (Table 6) to determine the factors that predict septic shock and death we found that female sex,low gestational age neutrophilia ,high sTREM-1 level and high TLC were predictors of mortaliy and this was in agreement with Gibot et al., 2004,Kermorvant- Duchemin et al., 2008, Pietropaoli et al., 2010, Zhang et al., 2011, Arizaga et al., 2014, Adly et al., 2014, Paul et al., 2016 ,and Xuanliu et al., 2016 .

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