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ORIGINAL ARTICLE

Diagnostic and Prognostic Value of Pro-Adrenomedulim in Neonatal Sepsis

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ABSTRACT

Background: *Neonatal sepsis (NS) is one of the leading causes of morbidity and mortality both among term and pre term infants. Early diagnosis of NS is difficult because of non specific signs and symptoms and non infectious disease may mimic NS. Rapid diagnosis of bacterial infections is crucial for early initiation of adequate antibiotic treatment. Systemic inflammation and sepsis lead to an increased release of Pro-Adrenomedullin (pro- ADM) into circulation thus it could be helpful in the early diagnosis of sepsis and in monitoring such conditions.* ***Objective:*** *To determine serum levels of pro-ADM in newborns with sepsis and its relation to diagnosis and prognosis.* ***Methodology:*** *Our study included fifty neonates fulfilled the criteria of sepsis (group1), they were subclassified into 2 subgroups; 29 cases with proven sepsis who had positive blood cultures (group1a) and 21 cases with clinical sepsis who had negative blood cultures (group1b), forty healthy gestational age, birth weight and sex matched neonates served as a control (group 2). Serum levels of Pro-ADM were measured by ELISA in all neonates and blood cultures were done in septic ones.* ***Results:*** *Serum level of Pro-ADM was significantly higher in group1as compared with group 2 (P=0.000) and in group1a as compared with group1b (P < 0.001). There was highly significant increase in serum level of pro-ADM with increased severity of NS (P = 0.000) and in non- survived neonates compared to those who survived (P = 0.000). Serum levels of Pro-ADM were positively correlated with WBCs count, I/T ratio (immature-to-total neutrophil ratio) and CRP serum level (r = 0.361, P = 0.010, r = 0.320, P = 0.024, r = 0.343, P = 0.015) respectively.* ***Conclusions:*** *Pro-ADM can be considered as valuable biomarker for diagnosis and prognosis of NS.*

INTRODUCTION

Neonatal sepsis is one of the life threatening clinical condition that requires early intervention. NS is associated with high morbidity and mortality if not treated promptly[1]. In these patients it is essential to initiate early specific treatments and support measures, since, a clear relationship has been found between the time antibiotics are started and prognosis[2].The current gold standard for the diagnosis of NS is blood culture for isolation of the causal pathogen. In addition to the time required to confirm blood culture results (48-72 h), the test itself can be unreliable, as sepsis may give rise to positive blood culture in only about 30% of patients. This test, combined with a lack of specific clinical signs for sepsis in neonates, often makes diagnosis and determining an appropriate duration of antimicrobial therapy for NS difficult [3].

The rapid diagnosis of bacterial infection and early assessment of bad prognosis are fundamental for septic patients so, a more sensitive, specific and reliable diagnostic marker for bloodstream infection is therefore needed[4]. HumanAdrenomedullin (ADM), is a 52 amino acids peptide with immune modulating, metabolic, and vasodilator activity. It has an important role in regulating blood volume as it helps to maintain blood supply in each organ. Its level was found to be elevated as a response to disrupted blood circulation and it seems to have an important role in the initiation and continuation of the hyper-dynamic circulatory response in sepsis[5].

It also has potent microbicidal action against both Gram-positive and Gram-negative bacteria and *Candida albicans*. This activity is performed by opening the hydrophilic channels, altering the membrane permeability. Thismicrobicidal activity produce a protective effect against organ damage, particularly in sepsis[6]. Its serum levels show rapid elevations during sepsis, followed by rapid clearance from the circulation as it has a half-life of 22min which imped its clinical use. However, another fragment termed Pro-ADM, a more stable precursor molecule to ADM, seems to be a promising sepsis biomarker. When blood samples are stored at 20C°, pro-ADM is stable up to 24 h. Thus, it is more suitable for daily routine use in clinical practice[7, 8].

Its level was found to be elevated during viral and bacterial infections and it was reported to correlate

well with other markers such as IL-6 and CRP as a predictor of prognosis in patients with sepsis[6, 9]. Elevation of pro-ADM levels have been reported in systemic inflammatory response syndrome (SIRS), sepsis, and septic shock[10]. Pro-ADM level was found to be more effective than procalcitonin and CRP levels to determine an unfavorable outcome and the risk of mortality in patients with sepsis. It has been identified as a prognostic marker, stratifying the mortality risk in patients with sepsis[4, 11]. In the light of these data, the aim of the present study was to determine the serum level of Pro-ADM in newborns with sepsis, and to evaluate its relation to diagnosis and prognosis.

METHODOLOGY

Patients

This prospective comparative study was carried out on neonates admitted to the neonatal intensive care unit (NICU) of AL-Zahraa University Hospital and Abo-Elreish University Hospital, during the period from January 2013 to February 2014. Ninety neonates, with gestational age between 32 and 40 weeks were enrolled in this study. All of neonates were subjected to complete history taking (to detect risk factors of sepsis), through clinical examination (general, local, neurological). They were divided into two groups: group 1 which included fifty sick neonates with clinical picture suggestive of NS, who have two or more of the following clinical criteria:

- Respiratory manifestation: tachypnea, increased apnea, ventilator support, or desaturation.
- Cardiovascular manifestations: bradycardia, pallor, decreased perfusion, or hypotension.
- Metabolic manifestations: hypothermia, hyperthermia, feeding intolerance, glucose instability, or metabolic acidosis.
- Neurological manifestations: lethargy, hypotonia or decreased activity[12].

Group1 was subclassified into 2 subgroups:

- *Group1a* (proven sepsis) which included 29 neonates with clinical diagnosis of sepsis and had positive blood cultures.
- *Group1b* (clinical sepsis) which includes 21neonates with clinical diagnosis of sepsis and had negative blood cultures. Blood samples were extracted from neonates on admission to NICU to determine their serum level of pro-ADM. Those septic neonates who had higher levels of pro-ADM were followed up clinically and evaluated by laboratory tests to determine their clinical outcome. They were divided according to progression of sepsisas follows: SIRS (neonates; having two or more of these criteria: temperature instability, tachycardia, tachypnea, WBCs count >12.000/mm^3 or < 4.000/mm^3 or > 10% immature forms), sepsis (SIRS + source of infection), severe sepsis was defined as sepsis complicated by organ dysfunction; and septic shock was defined as sepsis-induced acute circulatory failure characterized by persistent arterial hypotension despite adequate volume resuscitation and not being explained by other causes[13].

Group 2:

- Forty gestational age, birth weight and sex matched healthy neonates served as a control.

Exclusion Criteria:

Newborn infants with asphyxia, heart failure, severe intracranial bleeding, congenital abnormalities, inborn errors of metabolism and history of maternal preeclampsia were excluded from this study.Informed written consents were obtained from parents of the infants before inclusion in this study.

Laboratory investigations

Four ml of venous blood was collected from each neonate under complete aseptic condition, divided into three portions and processed as the following:

- ***Blood culture:*** under complete aseptic condition, 1 ml of venous blood which was obtained from 50 neonates suspected clinically to have NS, inoculated into 2 neonatal culture bottles (Oxoid, England) for, aerobic and anaerobic culture of organisms. The culture bottles were incubated at 37 C° for7 days and inspected visually every morning. Subcultures were performed 14-18h after inoculation, and every 48h under both aerobic and anaerobic conditions on blood agar, and MacConkey agar and incubated overnight at 37c°. Gram stained films were made from positive growths and complete identification of the growing colonies were carried out on Walk away-40 system (Siemens, Memphis, TN). All procedures were performed according to the manufacturer's instructions.
- ***CBC:*** 1 ml of whole blood was collected in evacuated tube containing EDTA as anticoagulant for CBC, which was carried out by Sysmex KX-21N (Sysmex, kobe, Japan). Manual differential leukocytic count was done and, the I/T ratio was calculated as the total number of immature neutrophils (promyelocytes, myelocytes, metamyelocytes and bands) divided by the total number of cells in the neutrophilic cell line (immature plus segmented neutrophils)[14].
- ***CRP and Pro- ADM measurements:*** 2 ml of venous blood was taken into serum separator tube, left to clot and then centrifuged and a part of serum was obtained for quantitative measurement of CRP using automatic auto-analyzer INTEGRA 400 plus (Roche Diagnostics, Mannheim, Germany), and the remaining part of serum was stored at -20 C° for Pro-ADM level assay.Its measurement was performed by using commercially available human ELISA kits, which supplied by CUSABIO Biotech USA, catalog number (CSB-E14356h).This assay

employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for pro-ADM has been pre-coated onto a microplate and any pro-ADM present is bound by the immobilized antibody. The lower detection limit of pro-ADM was less than 0.08 pmol/L, Intra-assay Precision (CV% <8%), Inter-assay Precision (CV% <10%). Measurement of pro-ADM was done on ELISA system (reader; A31851 and washer; 909 from DAS (Italy), according to manufacturer's instructions.

Statistical analysis

Data were collected, revised, coded and entered to SPSS version 17. Qualitative data were presented as number and percentages, while the quantitative data were presented as mean, standard deviations. The comparison between two groups with qualitative data were done by using Chi-square test, while the comparison between two groups with quantitative data and parametric distribution were done by using independent t-test. The comparison between more than two independent groups with quantitative data and parametric distribution was done by using one way analysis of variance (ANOVA) test. Spearman correlation coefficients test was used to assess the significant relation between two quantitative parameters in the same group. Receiver operating characteristic curve (ROC) was used to assess the best cut of point, area under the curve (AUC), sensitivity, specificity, positive and negative predictive values. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the P-value was considered non significant at the level of > 0.05, significant at the level of < 0.05 and highly significant at the level of < 0.01.

RESULTS

The results of this study showed no significant differences between group1and group 2 in terms of maternal age, gestational age, birth weight, gender and mode of delivery, (table 1). While there was highly significant increase in serum Pro-ADM , CRP levels, WBCs count and I/T ratio in group1 as compared with group 2 (P = 0.000, P = 0.000, P = 0.004, P = 0.000) respectively, (table 2). Also highly significant increase in serum Pro-ADM, CRP levels and I/T ratio in group1a as compared with group1b (P < 0.001, P < 0.001, P < 0.001) respectively, (table 3). There was highly significant increase in serum Pro-ADM, CRP levels (P = 0.000, P = 0.000) and significant increase in I/T ratio (P=0.014) in non survived neonates compared to those who survived, (table 4). There was highly significant increase in serum Pro-ADM level with increased severity of NS (P=0.000). Highly significant increase in serum Pro-ADM level was found between sepsis versus SIRS, severe sepsis versus SIRS, septic shock versus SIRS, severe sepsis versus sepsis, and septic shock versus sepsis, and septic shock versus severe sepsis, (table 5). The commonest organisms that were isolated from blood cultures of group1a patients were Coagulase negative staph (CONS) in 10 cases (34.4%), followed by Klebseilla (24.1%), Acintobacter (13.8%) and Staph. Aureus (13.8%), (table 6). Significant positive correlations were found between Pro-ADM level and WBCs count, I/T ratio and CRP level (r = 0.361, P = 0.010, r = 0.320, P = 0.024, and r = 0.343, P = 0.015) respectively, (table 7), figure (1, 2, 3). ROC curve analysis of pro-ADM serum level, I/T ratio and CRP level to define their optimal diagnostic accuracy of NS showed that, at a cutoff point >15 pmol/l for pro-ADM, AUC was 0.89 with 82% sensitivity and 100% specificity. At a cutoff point > 0.18 % for I/T ratio, AUC was 1.00, with 98.0 % sensitivity and 100%specificity. At a cutoff point >37mg/L for CRP, AUC was 0.64with 41.38% and 85.71% sensitivity and specificity respectively, table (8), figure (4).

Table 1:Demographic characteristics of the studied subjects

Parameter	Group 1 (n=50)	Group 2(n=40)	P value
Maternal age* (year)	25.6±4.9	26.6±5.7	0.06
Gestational age* (week)	33.1±5.2	35.4±3.8	0.32
Birth weight* (kg)	2.016±1.070	2.903±0.768	0.48
Gender°(female) Gender°(male)	23(46%) 27 (54%)	27(67.5%) 13 (32.5%)	0.73
Mode of delivery ˚(cesarean) ˚ (normal)	39 (78%) 11(22%)	29 (72.5%) 11(27.5%)	0.25

*Values are given as mean ± SD ° Values are given as percentage.

Table 2: Comparison between group1and group2 regarding different laboratory parameters

Investigations	Group1 (Mean±SD)	Group 2 (Mean±SD)	Independent t-test	
			t	p-value
Hemoglobin (gm/dl)	14.23±2.91	16.8±1.33	5.159	0.466
WBCs (10^3/mm^3)	16.79±2.58	9.09±1.65	-9.998	0.004
Platelets (10^3/mm^3)	198.18±59.21	240.92±74.88	3.025	0.213
I/T ratio	0.28±0.08	0.13±0.02	-11.286	0.000
CRP (mg/L)	30.06±20.24	1.29±1.11	-8.972	0.000
Pro-ADM (pmol/L)	25.03±10.01	8.81±4.62	-9.467	0.000

Table 3: Comparison between group1a and group1b regarding studied laboratory parameters

Parameter	(group 1a) (n=29)	(group 1b) (n=21)	P value
I/T ratio	0.3±0.09	0.24±0.05	<0.001
CRP(mg/L)	34.52±22.75	23.9±14.5	<0.001
Pro-ADM(pmol/L)	29.8±7.43	18.45±9.47	<0.001

Table 4: Comparison between survivor and non-survivor neonates regarding studied laboratory parameters

	Survivors (no=43)		Non-survivors (no=7)		Independent t-test	
	Mean	SD	Mean	SD	t	p-value
I/T ratio	0.27	0.07	0.35	0.12	-2.554	0.014
CRP (mg/L)	25.70	14.24	56.86	30.84	-4.442	0.000
pro-ADM (pmol/L)	22.81	8.40	38.66	8.53	-4.630	0.000

Table 5: Comparison between mean serum level of Pro-ADM in different stages of progression of sepsis

Groups	No.&%	pro-ADM		One Way ANOVA	
		Mean	SD	f	P-value
SIRS	9(18%)	9.11	2.63	44.927	0.000
Sepsis	31(62%)	25.50	3.86		
Severe sepsis	5(10%)	33.80	6.77		
Septic shock	5(10%)	42.04	5.18		
Sepsis vs. SIRS	severe sepsis vs. SIRS	septic shock vs. SIRS	severe sepsis vs. sepsis	septic shock vs. sepsis	Septic shock vs. severe sepsis
0.000	0.000	0.000	0.004	0.000	0.000

Table 6: Frequency of isolated organisms in group1a patients

	Blood Culture result	
	No.	%
CONS	10	34.4
Klebsiella spp.	7	24.1
Acintobacter spp.	4	13.8
Staph. Aureus	4	13.8
Ecoli	3	10.4
Enterobacter	1	3.5
Total	29	100

Table 7: Correlation between pro-ADM serum level and the studied laboratory parameters in group1

	Pro-ADM level	
	r	p-value
Hb	0.184	0.202
WBCs	0.361**	0.010
Plt	-0.043	0.768
I/T ratio	0.320**	0.024
CRP	0.343**	0.015

***Positive correlation*

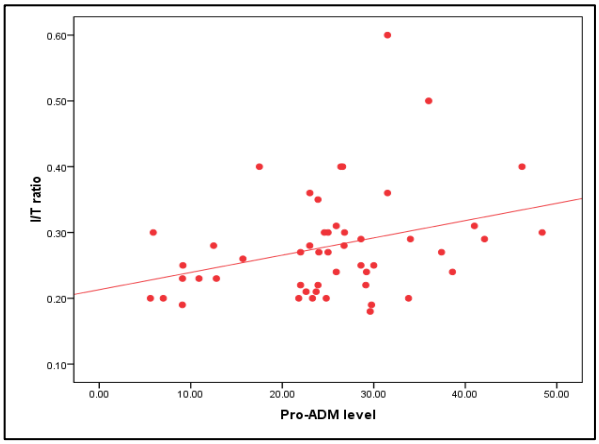

Fig. 1: Correlation between Pro-ADM serum level and I/T ratio.

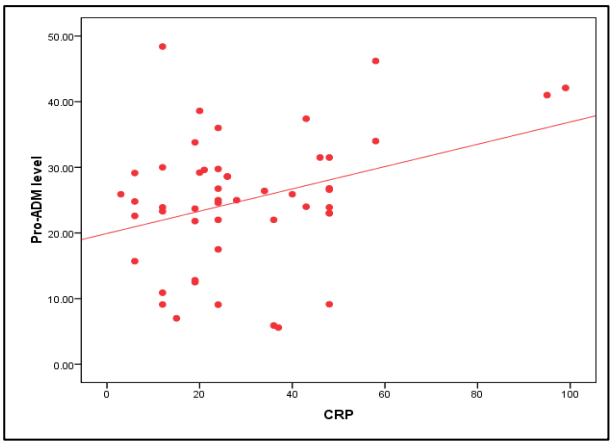

Fig. 2: Correlation between Pro-ADM serum level and CRP.

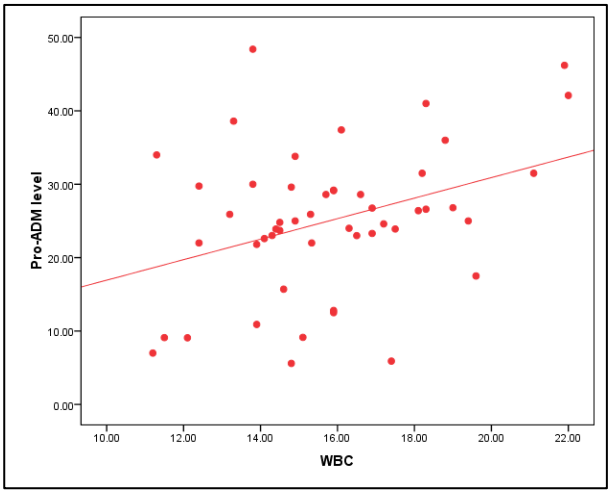

Fig. 3: Correlation between Pro-ADM serum level and WBCs count.

Table 8: ROC curve analysis for I/T ratio, Pro-ADM, and CRP between group1and group2

Parameters	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
I/T ratio	>0.18	1.00	98.00	100.00	100.0	97.6
Pro-ADM	> 15	0.89	82.0	100	100.0	81.6
CRP	>37	0.64	41.38	85.71	80.0	51.4

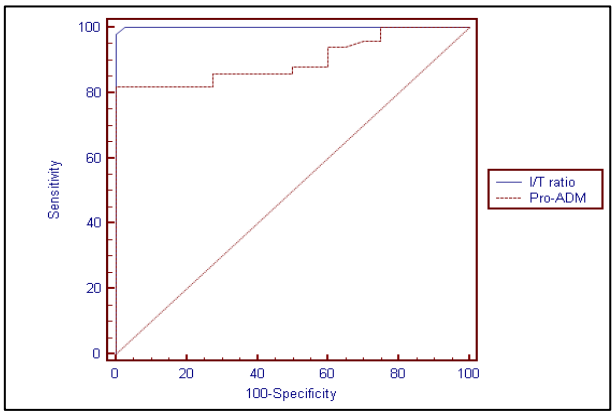

Fig. 4: ROC curve for I/T ratio and Pro-ADM between group1 and group2

DISCUSSION

Neonatal sepsis is a common and life threatening disorder, since its outcome and prognosis depend on early and efficient antibiotic therapy. Beside many efforts to improve outcome and new advances in the treatment, sepsis is still one of the most frequent causes of death in critically ill neonates. It is the most common condition with high mortality in intensive care units[15, 16].

Our study showed highly significant increase in serum level of CRP, WBCs count and I/T ratio in group 1 as compared with group 2 and in group1a as compared with group1b(except WBCs count), this was in accordance withEnguix-Armada et al.[17], Lorrot et al.[18]andCaldas et al. [19] but these inflammatory mediators are not specific and not reliable since they do not discriminate between infectious and non infectious conditions[18] so, there is a need for more sensitive and specific indicator for sepsis at the earliest stage of the disease, which could dramatically improve the condition.

In this study we found highly significant increase in Pro-ADM level in the serum of septic patients as compared with control. This was in agreement with previous studies done by Oncel et al. [9], Cao et al.[20]who found that Pro-ADM levels were significantly higher in newborns with sepsis as compared with healthy control. They reported that, Pro-ADM is a useful laboratory marker which indicate bacterial infection in neonates.

Elevation of Pro-ADM serum level in sepsis may be due to three mechanisms. First, Pro-ADM is widely

expressed and extensively synthesized during sepsis, similar to other calcitonin peptides including procalcitonin-gene related peptides because Pro-ADM is a member of CALC gene family[21]. Bacterial endotoxins and pro-inflammatory cytokines up regulate Pro-ADM gene expression in many tissues[22].In a second mechanism, decreased clearance by the kidney may be responsible in part for increased its level in sepsis[23]. Third, in sepsis, bacteria entering the blood stream may release their toxins, resulting in a host-derived mediator response activating an inflammatory cascade of various peptides. Pro-ADM, was found to be a mediator in changes of vascular tone as it contributes to vasodilatation and hypotension often seen in severe sepsis[24].

By comparing cases of proven and clinical sepsis as regard serum level of Pro-ADM, we found that its level was significantly higher in newborns with proven sepsis than those with clinical sepsis. This was matched with Oncel et al.[9] who revealed a significant increase in serum level of Pro-ADM in proven sepsis as compared with clinical sepsis neonates. They stated that, Pro-ADM can be used especially for early diagnosis of NS.AlsoValenzuela et al.[4]reported thatPro-ADM levels help to identify the infectious origin and organ dysfunction in patients with SIRS since in these patients the levels at admission were 10 times higher than those in non septic patients.

In addition, our study showed highly significant increase in Pro-ADM level in the serum of non-survivors neonates as compared with survivors. This was in accordance withAnglentini et al.[25] Valenzuela et al.[11]andAndaluz et al.[26]who found that Pro-ADM level was significantly higher in non- survivors as compared with survivors. They concluded that, higher Pro-ADM level was associated with poor outcome for severe infection. Similarly Hagag et al. [27] showed the same results.

Pro-ADM can be used as a predictor for prognosis in NS, this is because our study showed a stepwise statistically significant increase in serum Pro-ADM level between SIRS, sepsis, severe sepsis, and septic shock.

Similar results were obtained by Anglentiniet al.[25], andValenzuela et al. [4] who showed that patients with sepsis, severe sepsis and septic shock demonstrated progressively higher Pro-ADM levels, as endothelial damage is more extensive in such patients so Pro-ADM would be expected to be higher. They concluded that, Pro-ADM is a valuable marker for predicting sepsis severity and outcome.

In the present study, the most common microorganisms that were isolated from the blood cultures of patients with sepsis were CONS in 34.4% of the cases, Klebsiella in 24.1% of the cases, Acintobacter and Staphylococcus Aureus in 13.8% of the cases, and E-coli in 10.4% of the cases. This result was matched with Gheibi et al. [28]who found that CONS (54.6%) and KlebsiellaPneumoniae (14.1%) together with E.Coli (11.5%) are the leading causative agents of neonatal sepsis. However in another study done by Oncel et al.[9] showed that the microorganisms that were isolated from the blood cultures of patients with sepsis were Staphylococcus Epidermidis in 14 cases(45.1%), Escherichia coli in six cases(19.3%), Klebsiella pneumonia in six cases (19.3%), and Acintobacterbaumannii in two cases (6.5%). Another study by Shresthaet al.[29] found that E-coli was the pre-dominated organisms isolated in septic cases. These differences in type of isolated organisms depend upon the common organism prevalent in each community, supportive care practice between centers.

Our study showed significant positive correlation between pro-ADM level, I/T ratio, WBCs count and CRP level; denoting their pivotal roles in the development of NS, so use of pro-ADM with these conventional inflammatory indicators may be more useful in diagnosis and follow-up of patients with NS[30,9].

Upon applying the ROC curve to define the optimal diagnostic accuracy for Pro-ADM values in septic patients, using a cut off value of 15 pmol/l, we obtained a sensitivity of 82% and a specificity of 100%, positive predictive value (PPV) of 100% and negative predictive value (NPV) of 81.6%, which were higher than CRP and lower than I/T ratio. This result was matched with previous study of Oncelet al.[9] who demonstrated a specificity and PPVof 100% for pro-ADM. The sensitivity and NPVof pro-ADM were 86.8% and 83.9%, respectively.

CONCLUSIONS

Pro-ADM is a valuable marker for diagnosis of NS and can be used as a predictor for prognosis and outcome in these patients. We advise to incorporate pro-ADM to the panel of biomarkers necessary for the diagnosis and monitoring of NS as, the use of this molecule could lead to several advantages, such as faster diagnosis, more accurate risk stratification, and optimization of treatment, with consequent benefit to the patient.

REFERENCES

1. Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal infectious disease: evaluation of neonatal sepsis. PediatrClin North Am. 2013; 60:367-389.
2. Garnacho-Montero J, García-Garmendía JL, Barrero-Almodóvar AE, et al. Impact of adequate empirical antibiotic therapy on the outcome in patients admitted to the ICU with sepsis. Crit Care Med 2003;31:2742-51.

3. Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. ClinPerinatol. 2010; 37:421-438.
4. Valenzuela Sanchez F, Valenzuela Méndez B, Bohollo de Austria R, et al. Diagnostic and prognostic usefulness of mid-regional pro-adrenomedullin levels in patients with severe sepsis. Intensive Care Med Exp 2015; 3:A306.
5. Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. Endocr Rev. 2000; 21:138–167.
6. Allaker RP, Grosvenor PW, McAnerney DC, et al. Mechanisms of adrenomedullin antimicrobial action.Peptides 2006;27:661-6.
7. Struck J, Tao C, Morgenthaler NG, Bergmann A. Identification of an Adrenomedullin precursor fragment in plasma of sepsis patients.Peptides. 2004;25:1369–1372.
8. Goode KM. Nicholls R. Pellicori P, Clark AL, Cleland JG. The in vitro stability of novel cardiovascular and sepsis biomarker at ambient temperature. ClinChem Lab Med 2014; 52: 911-918.
9. Oncel MY, Dilmen U, Erdeve O, et al**.**Proadrenomedullin as a prognostic marker in neonatalsepsis.Pediatric Research J 2012;72, 507–512.
10. Wang RL and kang FX. Prediction about severity and outcome of sepsis by pro-atrial natriuretic peptide and pro-adrenomedullin.Chin J Traumatol. 2010;13(3):152-7.
11. Valenzuela Sanchez F, Valenzuela Méndez B, Bohollo de Austria R, et al. pro-Adrenomedullin, procalcitonin, C-reactive protein as prognostic markers of mortality in patients with severe sepsis. Intensive Care Med 2014; 40: S43.
12. Haque KN. Defining infections in children and neonates. J Hosp Infect. 2010; 65:110-114.
13. Dellinger RP, Levy MM, Rhodes A, et al., Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock,2012,". Crit Care Med 2013; 41:580-637.
14. Manroe BL. Weinberg AG, Rosenfeld CR, et al. The neonatal blood count in health and disease ,1: reference values for neutrophilic cells. J Pediatr. 1979;95:89-98.
15. Vincent JL, Martinez EO, Silva E. Evolving concepts in sepsis definitions. Crit Care Clin J. 2009; 25: 665-675.
16. KojicD,Siegler BH, Uhle F, et al. Are there new approaches for diagnosis,therapy guidance and outcome prediction of sepsis? World J Exp Med. 2015; 20; 5 (2):50-63.
17. Enguix-Armada A, Escobar- Conesa R, Garcia- De La Torre A et al. Usefulness of several biomarkers in the management of septic patients: C reactive protein, procalcitonin, presepsin and mid-regional pro-adrenomedullin. ClinChem Lab Med. 2016;54(1):163-8.
18. Lorrot M, Moulin F, Cost J, et al. Procalcitonin on pediatric emergency: comparison with C- reactive protein, interleukin 6 and interferon alpha in differentiation between bacterial and viral infections : press-med.2008; 29:128-134.
19. Caldas JP, Marba ST, Blotta MH, et al. Accuracy of white blood cell count,C- reactive protein, interleukin-6 and tumor necrosis factor alpha for diagnosing late neonatal sepsis.JPediatr (Rio J). 2008;84(6): 536-42.
20. Cao Y, Xia Q, Chen C, Yang Y. Precursors of adrenomedullin, endothelin and atrial natriuretic peptide as diagnostic markers of neonatal infection. ActaPaediatr (Norway). 2012; 101(3):242–246**.**
21. Becker KL, Nylen ES, White JC, et al. Clinical review 167: procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precusors. J ClinEndocrinolMetab. 2004; 89:1512-1525**.**
22. Linscheid P, Seboek D, Zulewski H, et al. Autocrine/ paracrine role of inflammation-mediated calcitonin gene related peptide and adrenomedullin expression in human adipose tissue. Endocrinology J. 2005; 146:2699-2708.
23. Hirata Y, Mitaka C, Sato K, et al. Increased circulating adrenomedullin, a novel vasodilatory peptide, in sepsis. J ClinESndocrinolMetab.1996 ; 81:1449-1453.
24. Benito J, Luaces-Cubells C, Mintegi S et al. Lack of value of midregional pro-adrenomedullin and C-terminal pro-endothelin-1 for prediction of severe bacterial infections in infants with fever without a source. European Journal of Pediatrics. 2013; 172(11):1441-1449.

25. Angeletti S, Spoto S, Fogolari M, et al. Diagnostic and prognostic role of procalcitonin (PCT) and MR-pro-Adrenomedullin (MR-proADM) in bacterial infections. APMIS 2015; 123:740-8
26. Andaluz-Ojeda D, Cicuéndez R, Calvo D, et al. Sustained value of proadrenomedullin as mortality predictor in severe sepsis. J Infect 2015; 71:136-9.
27. Hagag AA, Elmahdy HS, Ezzat AA, et al. Prognostic value of plasma proadrenomedullin and antithrombin III levels in neonatal sepsis. Indian Pediatr J 2011; 48 (6):471-473.
28. Gheibi S, Karamyyar M, Mahmoodzadeh H.Coagulase negative Staphylococcus; the most common cause of neonatal septicemia in Urmia, Iran. Iran J Pediatr. 2008; 18(3):237-243.
29. Shrestha P, Das BK, Bhatta NK, et al. Clinical and bacteriological profile of blood culture positive sepsis in newborns. J Nepal Pediatr. 2011; 27:64-67.
30. Debiane L, Hachem RY, Al Wohoush I, et al. The Utility of Proadrenomedullin and Procalcitoninin Comparison to C-Reactive Protein as Predictors of Sepsis and Bloodstream Infections in Critically Ill Patients With Cancer. Crit Care Med. 2014; 42:2500-07.