Original article

T cell activation markers "CD45RO and CD45RA"; neutrophil CD11b expression and soluble tumor necrosis factor receptor (p55) in early diagnosis of neonatal sepsis.

Background: *Neonatal sepsis is a common and life threatening disorder whose outcome and prognosis depend on early and efficient therapy. A sensitive and specific indicator at an early stage of the disease would certainly aid the early diagnosis of neonatal sepsis.*

Objective: *We sought to investigate the diagnostic value of measuring the up-regulation of T-lymphocyte activation markers CD45RA/CD45RO, CD45RO, neutrophil CD11b expression, and soluble tumor necrosis factor receptor (P55) as tests for early detection of neonatal sepsis; as well as comparing them with the conventional methods of diagnosis.*

Methods: *The subjects of this study comprised 39 neonates: 25 males (64%) and 14 females (36%). In addition to the clinical assessment, different laboratory investigations for the diagnosis of sepsis were done. These included a complete blood picture, quantitative measurement of C-reactive protein and blood and or CSF culture and sensitivity. Assays for T cell activation markers CD45RA/CD45RO and neutrophil CD11b by flow cytometry and soluble tumor necrosis factor receptor sTNFR1 by ELISA were carried out as well as chest plain x-raying.*

Results: *The up-regulation of CD45RA/ CD45RO dual expression and CD45RO expression on T-lymphocytes was observed in the septic group. The percent expression of CD45RA/CD45RO and CD45RO was significantly elevated in the high-risk group when compared to the control group. However, CD45RA/CD45RO and CD45RO showed no significant difference in percent expression between the septic group and the high-risk group. CD11b expression was also significantly higher in the septic (72.8± 17.8) than the control group (17.2 ±9.9%, p<0.05) and also in the high-risk group (42.2 ± 25.9%, p<0.05) as compared to the control group. sTNFR55 was significantly high in septic group (18.14 ± 9.38 ng/ml) as well as in the high risk group (26.90 ± 16.89 ng/ml) when compared to the control group (6.44 ± 1.49 ng/ml, p<0.05) with no significant difference between the former two groups (p>0.05). In addition and according to the follow up sample taken after one week, a significant increase in the expression of CD45RO was realized in the septic group.*

Conclusion: *The surface activation markers of T-lymphocytes (CD45RA/ CD45RO, CD45RO), neutrophil activation marker (CD11b) and soluble TNF receptor 1 are useful early indicators of neonatal sepsis, and are superior to the hematological scoring system and CRP in the early detection of the disease.*

Key words: neonatal sepsis, surface activation markers, neutrophil activation marker, CD45RA/CD45RO, CD45RO, CD11b, soluble TNF receptor 1.

**Magda A. Khazbak,
Safaa S. Imam,
Hala Abd El-Khalik*,
Maha H. Mohamed**

*From the Departments of Pediatrics
and Clinical Pathology*,
Faculty of Medicine,
Ain Shams University,
Cairo, Egypt.*

Correspondence:
*Dr. Safaa Shafik
22 Morad El-Shereiy
Street, Cairo 11361,
Egypt.
Fax: +20 26376720.
E-mail: safy@softhome.net*

INTRODUCTION

Infection is still an important cause of neonatal morbidity and mortality despite the development of broad spectrum antibiotics and technical advances in life support therapy[1]. Since outcome and prognosis depend on early and efficient antibiotic therapy, there is a need for sensitive and specific indicators of sepsis at the earliest stage of the disease[2]. Early signs and symptoms are often non specific, subtle, and can be easily confused with

110

non-infective causes such as apnea of prematurity, variation in environmental temperature, and acute exacerbation of chronic lung disease[3]. Laboratory indicators such as complete blood cell count, ratio of immature to total neutrophils and C-reactive protein (CRP) do not have high sensitivity especially if measured early in the course of sepsis[2].

A more reliable method for detection of infection may be the demonstration of activated T lymphocytes, which can be done rapidly and before isolation of the offending organism. Human T lymphocytes can be divided into various subsets by the expression of the isoforms of the leukocyte common antigen, CD45. The CD45RA isoform is brightly expressed on the newborn naive T cells. The CD45RO isoforms marks memory T cells, which respond to recall antigenic stimulation by proliferation. It also provides helper activity for antigen-specific antibody synthesis. It is probable that isoform switching form CD45RA to CD45RO occurs in vivo in the newborn following antigenic stimulus by an infective organism[6]. Also the expression of surface functional antigen on neutrophils and their up-regulation in infection may be of value. Neutrophil CD11b is a promising test for exclusion of early onset neonatal infection[4].

Cell surface receptors for tumour necrosis factor (TNF) and their soluble forms, which are released into the circulation, are important in mediating and regulating the divergent TNF effects. Two TNF receptors with molecular masses of 55 and 75 KDa have been cloned. The 55-KDa receptor mediates cytotoxic effects, up regulates adhesion molecules, and appears to be important for resisting infection by the intracellular organisms[5].

This study is aimed to evaluate the value of up-regulation of T lymphocyte activation markers CD45RA/CD45RO, CD45RO, neutrophil CD11b expression, and soluble tumour necrosis factor receptor (P55) as tests for early detection of neonatal sepsis in comparison to some conventional methods of diagnosis.

METHODS
Study design:
The current study was conducted on 39 neonates, 25 males (64 %) and 14 females (36 %), of whom 15 were preterms (38 %) and 24 were full term babies (62 %).They were enrolled selected from the NICU and general nursery of the Departments of Pediatrics and Gynecology and Obstetrics, Ain Shams University Hospitals. The studied neonates were subclassified into 3 groups:

Group I:
This group included 9 neonates at high risk for neonatal sepsis, but not clinically septic. However, they acquired clinical signs of sepsis after a few days from enrollment. They were 5 males and 4 females; 5 preterms and 4 full terms. Their ages ranged between 1 and 7 days (mean = 3 ± 2 days) . Their weight values ranged between 1.8 and 3.5 kg with a mean weight of 2.3 ± 2.5 kg.
Inclusion criteria:
1- Maternal intrapartum risk factors as unexplained prematurity, prolonged premature rupture of membranes, intrapartum fever, documented UTI.
2- Cases that underwent exchange transfusion.
3- Cases that were mechanically ventilated.
4- Cases that underwent surgery.

Group II:
This group included 21 cases of neonatal sepsis (15 males and 6 females); 7 preterms and 14 full terms. Their postnatal ages ranged between 3 and 40 days (mean = 15.28 ± 12.06 days). Their weight values ranged between 1.3 and 3.8 kg (mean = 2.7 ± 0.73 kg). The neonates were diagnosed on basis of their history, physical examination, and laboratory investigations.
Inclusion criteria:
1- Symptoms and signs suggestive of sepsis (Tollner score > 10.5)[4].
2- Positive blood, urine or CSF culture.
3- Elevated CRP.
4- Positive Hematological sepsis score.

Group III - Control Group:
This group included 9 healthy neonates, 5 males and 4 females; 3 preterm and 6 full term babies. Their post-natal ages ranged between 1 and 24 days (mean = 3.8 ± 7.6 days). Their weights ranged between 1.5 and 3.4 kg with mean value of 2.44 ± 0.64 kg.

Study measurements:
All neonates (cases and controls) were subjected to the following:
1- Medical history taking from the parents, laying stress on gestational age, birth weight, mode of delivery, premature rupture of membranes, maternal perinatal fever, maternal infection e.g. UTI, fetal distress, isoimmunization or exchange transfusion.
2- Clinical examination, for assessment of gestational age, weight, manifestations of neonatal sepsis as lethargy, sluggish Moro reflex, poor suckling, hypothermia, mottling, sclerema, umbilical sepsis, jaundice, respiratory distress or abdominal distension.

3- Laboratory investigations:

Blood samples were collected on admission or the day after. A second sample was collected 7 days later from neonates still present in the NICU.

a. Complete blood picture using coulter T660 (Coulter corporation, Hialeah, Florida, USA): two ml of venous blood were taken from each neonate on EDTA solution and differential count was done on Leishman stained peripheral blood smear. This included total leukocytic count (TLC), hemoglobin (Hb), absolute neutrophil count, band cell count, immature to total neutrophil ratio, immature to mature neutrophil ratio, degenerative changes in neutrophils and platelet count. Hematological scoring system of Rodwell et al[6] was used together with sepsis scoring system of Tollner [4].

b. Quantitative C-reactive protein (CRP) by latex agglutination slide test[7].

c. Blood culture and sensitivity test for aerobic and anaerobic cultures using sheep blood agar.

d. Examination of CSF and culture and sensitivity for cases with neurological manifestations.

e. Assay for T cell activation markers CD45RA/CD45RO and neutrophil CD11b by flow cytometry (FCM) using Coulter Epics XL FCM (Coulter electronics, Hielaeh FL, USA).

f. Soluble tumour necrosis factor receptor sTNFR1 by ELISA (sTNF-R1 EASIA, Biosource, Europe SA).

4- Plain chest x-ray postero-anterior view.

Techniques:

I- Detection of CD45RA/CD45RO and CD45RO by flow cytometry:

Surface marker analysis was performed on peripheral blood by whole blood lysis method using CD4 per CP, CD45RO PE and CD45RA-FITC (*Becton Dickinson BD San Jose, CA, USA*).

Negative isotope-matched controls were used to determine the non-specific binding. Stained samples were evaluated on the Coulter XL flow cytometry and were considered positive for a marker (if > 20% of the cells were positive). Gating of CD4+ lymphocytes was achieved by collecting a minimum of 10,000 low side scatter events in fluorescence channel 3. Analysis was performed on a FAC Scan flow cytometer and lysis II software, using dot plot, contour plot, and histograms outputs[1]

II- Detection of neutrophil CD11b by flow-cytometry:

Samples were drawn into prechilled tubes; all subsequent cell handling steps were carried out on ice at 4°C to minimize spontaneous up-regulation in vitro. Whole blood 0.1 ml anticoagulated with EDTA was diluted with 2.5 ml of cold staining medium composed of deficient RPMI 1640 medium, 1% fetal calf serum with 1 mol/1 N-2 hydroxy ethylpiperazine-N-2-ethane sulphonic acid pH 7.2 (Sigma, USA). Sodium azide was added to a concentration of 0.02 %. Samples were washed once with staining medium and then re-suspended in 0.5 ml of staining medium. For each sample, 100 μl of suspension was stained for 15 minutes with fluorescein isothiocyanete (FITC) conjugated CD15 (10 μl) to identify neutrophils, and with 10 μl of phycoerythrin conjugated CD11b mouse antihuman monoclonal antibodies (Becton-Dickinson, California, USA). Before staining FITC-conjugated, anti CD15 was diluted with unconjugated anti-CD15 antibody to bring CD15 staining into mid range on the flow cytometry instrument (FAC scan, Becton-Dickinson). The instrument was adjusted so that fluorescence of standard leads (3.2 μm sphere Rainbow Fluorescent Particles; Sphertech, Inc. Libertyville, III) in each fluorescence channel would be exactly the same in each run.

Samples were analyzed on the FAC scan with a threshold set in a way that only cells with the correct range of CD15 fluorescence for neutrophils were accepted as events. This threshold excludes erythrocytes and other leukocytes. The median fluorescence of neutrophil CD11b was determined for each sample on the basis of distribution of CD11b fluorescence per 30,000 cells counted and ranged from 1 to 475 fluorescence units[7].

III - Detection of sTNFR1 (p55):

Soluble TNFR1 was detected by means of immunoenzymo-metric assay (sTNF-R1 EASIA, Biosource, Europe SA). 50 μl of each standard, control, or sample were pipetted into appropriate wells. 200 μl of anti-sTNF-R conjugate solution were pipetted into each well. They were incubated for 1 hour at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm. The liquid from each well was aspirated. The plate was washed 3 times by dispensing 0.4 ml of biosource wash solution into each well, aspirating the content of each well. 50 μl of chromogenic solution was put into each well within 15 min following the washing step. The plate was incubated for 15 min at room

temperature on a horizontal shaker set at 700 ± 100 rpm, avoiding sunlight (one word). 200 µl of stop solution were put in each well. Absorbance was read at 450 nm and 490 nm (reference filter: 630 or 650 nm) within 3 hours, and the results were calculated using a standard curve[8].

Statistical analysis:

Data were analyzed with a computer statistical package V.5 (StatSoft, Tulsa, OK, USA). Numeric data were expressed as mean ± standard deviation. The significance of the difference between means was determined by student t test for independent samples and paired t test for dependent samples. The Mann Whittney U test was used for non-parametric data. The Pearson r correlation coefficient test was used to determine the relationship between different quantitative variables. For all tests, a probability of less than 0.05 was considered significant.

RESULTS

On performing blood culture, 76 % of the septic group had positive pathogens, while 24 % had negative blood culture. None of the high-risk group yielded positive blood culture. Concerning the causative organism, 75 % of the patients with positive blood culture showed gram negative pathogens, while 25% of them showed gram positive pathogens.

The up-regulation of CD45RA/CD45RO dual expression and CD45RO expression on T-lymphocytes had occurred in the septic group (table 1). Also, the % expression of CD45RA/CD45RO and CD45RO was significantly high in the high-risk group when compared to the control group. On the other hand, there was no significant difference between CD45RA/CD45RO and CD45RO % expression between the studied septic group and the high-risk group. Also, there was no difference between those who died or survived in their baseline results (table 3). The follow up sample taken after 1 week showed a significant increase in the expression of CD45RO compared to the initial sample in the septic group. In the high-risk group, there was no significant difference between the initial samples and the follow up samples (table 2). There was no significant difference in the expression of CD45RA/CD45RO or CD45RO in the preterm septic neonates when compared to the full term septic neonates and there was no gender difference.

CD11b expression was significantly high in septic group ($72.8 \pm 17.3\%$) when compared to the control group ($17.2 \pm 9.9\%$, $p<0.05$), and was significantly elevated in the high- risk group ($42.2 \pm$

25.9 %, $p<0.05$) as compared to the control group (table 1). Furthermore, there was no significant effect of gestational age or gender on the level CD11b ($p>0.05$). Those with positive blood culture have significantly higher CD11b level than those with negative blood culture ($p< 0.05$). No difference could be elicited between those who died or survived in the initial samples, or between them in the follow up samples. The optimal cutoff point obtained from ROC method was >25% expression with 93% sensitivity and 89% specificity (table 4).

Table (1): Comparison between the patients and controls in some clinical and laboratory variables.

	Control (group III) n = 9	High risk neonates (group I) n = 9	Septic neonates (group II) n = 21
Septic score	2.89 ± 1.27	8.22 ± 2.82	12.93 ± 1.85
p		**0.0004**	**< 0.001**
TLC (× 10⁹/L)	7.68 ± 2.74	7.94 ± 2.29	14.06 ± 8.18
p		0.7239	0.0186
PLC (× 10⁹/L)	337 ± 181.5	149.33 ± 116.4	142.00 ± 173.7
p		**0.0092**	**0.0033**
Hb (gm / dL)	14.41 ± 1.46	12.58 ± 3.66	11.80 ± 2.51
p		0.1820	**0.0071**
PNL (× 10⁹/L)	4.64 ± 2.14	5.32 ± 2.26	9.92 ± 6.89
p		0.2697	**0.0223**
Imm. (× 10⁹/L)	0.04 ± 0.06	0.16 ± 0.21	0.95 ± 1.12
p		0.2332	**0.0012**
CRP (mg/dL)	2.88 ± 1.30	2.22 ± 1.72	67.6 ± 39.8
p		0.29	**< 0.001**
CD11b (%)	17.2 ± 9.9	42.4 ± 25.9	72.8 ± 17.3
p		**0.0036**	**< 0.0001**
Dual exp (%)	1.67 ± 0.46	8.36 ± 3.28	11.62 ± 6.01
p		**0.0006**	**< 0.0001**
CD45RO (%)	3.43 ± 0.93	11.40 ± 4.43	13.35 ± 7.81
p		**0.0005**	**< 0.0001**
sTNFR1 (ng/ml)	6.44 ± 1.49	26.90±16.89	18.14 ± 9.38
p		**0.0092**	**0.0008**

CRP= C- reactive protein , Hb= hemoglobin, Imm.= Immature cells, PLC = Platelet count, PNL= polymorphonuclear leukocytes, sTNFR1= Soluble tumour necrosis factor receptor 1, TLC= Total leukocytic count. p≤ 0.05 = significant

sTNFR55 was significantly high in the septic (18.14 ± 9.38 ng/ml) as well as in the high risk

groups (26.90 ±16.89 ng/ml) when compared to the control group (6.44 ±1.49 ng/ml) (p<0.05) with no significant difference between the former two groups when compared together (p>0.05) (table 1). No effect of gestational age or gender on the level of sTNFR55 in all studied neonates was observed. Also, there was no significant difference between the initial samples and the follow up samples (p>0.05) (table 3), and between survivors and non - survivors whether initially or in the follow up samples (p>0.05) as far as was concerned (table 2). The cutoff point obtained by ROC method was 8.4 ng/ml with sensitivity of 80% and specificity of 100% (table 4).

CRP was significantly high in the septic group when compared to the control group (p<0.05). In the high risk group, CRP was negative initially, which turned positive with the appearance of signs and symptoms of infection. It had a sensitivity of 70% and a specificity of 100% (table 4).

CD11b was positively correlated to both CD45RA/CD45RO and CD45RO in neonatal sepsis. At the same time, it was found to hold a positive correlation with sTNFR1 in the same group. On attempting to correlate CD45RA/CD45RO to CD45RO, a positive association could be elicited as well as in the septic neonates.

Table (2): Comparison between the initial samples and the follow up samples of the septic neonates in the studied variables.

	Initial n = 9 Mean ± SD	Follow-up n = 9 Mean ± SD	p
Septic score	10.94 ± 4.25	12.00 ± 2.32	0.4965
TLC (×10⁹/L)	13.90 ± 6.40	12.41 ± 8.56	0.8572
Plts (×10⁹/L)	125.67 ± 141.81	91.22 ± 172.16	0.2585
Hb (gm/dL)	**12.93 ± 3.69**	**9.69 ± 2.80**	**0.0138**
PNL (×10⁹/L)	9.83 ± 6.40	9.48 ± 7.92	0.7642
Imm. (×10⁹/L)	0.92 ± 1.14	0.48 ± 0.25	0.4413
CRP (mg/dL)	86.40 ± 21.47	60.00 ± 36.00	0.5961
CD11b (%)	61.22 ± 29.52	71.44 ± 16.64	0.4065
CD45RO (%)	**14.93 ± 11.66**	**18.19 ± 12.43**	**0.0151**
Dual (%)	10.30 ± 8.11	3.89 ± 3.26	0.0854

sNFR1(ng/ml)	21.90 ± 12.22	18.36 ± 9.86	0.4777

CRP= C- reactive protein , Hb= hemoglobin, Imm.= Immature cells, PLC = Platelet count, PNL= polymorphonuclear leukocytes, sTNFR1= Soluble tumour necrosis factor receptors, TLC= Total leukocytic count. p≤ 0.05 = significant

Table (3): Comparison between survivors and non survivors of the neonates in the studied variables.

	Non survivors n = 15	Survivors n = 15	p
TLC (×10⁹/L)	11.3 ± 8.78	13.2 ± 6.03	0.495
Plts (×10⁹/L)	121.5 ± 114.3	166.9 ± 191.6	0.437
Hb (gm/dL)	11.5 ± 2.82	12.6 ± 2.87	0.279
PNL (×10⁹/L)	7.89 ± 7.21	9.19 ± 5.22	0.578
Imm. (×10⁹/L)	0.75 ± 1.19	0.68 ± 0.82	0.839
CD11b (%)	64.07 ± 27.61	63.33 ± 21.56	0.7851
Dual (%)	9.93 ± 2.94	11.35 ± 7.27	0.9835
CD45RO (%)	13.88 ± 4.47	14.45 ± 9.23	0.5614
sTNFR1(ng/ml)	25.83 ± 14.53	15.71 ± 7.56	0.1620

CRP= C- reactive protein , Hb= hemoglobin, Imm.= Immature cells, PLC = Platelet count, PNL= polymorphonuclear leukocytes, sTNFR1= Soluble tumour necrosis factor receptors, TLC= Total leukocytic count. p≤ 0.05 = significant

Table (4): Results of ROC analysis for discrimination between cases and control.

Variable	AUC	± SE	95 % CI	p	Optimal cut-off	Sensitivity (%)	Specificity (%)
CD11b	0.972	0.024	0.861 - 0.996	*	>25	93	89
CD45RO	0.996	0.008	0.902 - 1.000	*	>5	97	100
Dual	0.994	0.010	0.898 - 1.000	*	>2.2	97	100
sTNFR1	0.883	0.055	0.740 - 0.963	*	>8.4	80	100

	Cases		Control				
	n	%	n	%		Sensitivity (%)	Specificity (%)
CRP	+	21	70	0	0	7	100

						0	
	-	9	30	9	100		

AUG = Area under the ROC curve; CI = Confidence interval, sTNFR1= Soluble tumour necrosis factor receptors SE = Standard error; * Significant Optimal cut-oft (highest sensitivity & specificity) is obtained from the ROC (receiver-operator characteristic) curve.

DISCUSSION

On comparing the laboratory data between the septic and control neonates, it was found that the hemoglobin (Hb) level was significantly lower in the septic group and that 71% of that septic group showed thrombocytopenia (PLT $<150 \times 10^9$/L) compared to 55% of the high-risk group. There was a significant increase in the total leukocytic counts (TLC) of the septic neonates as compared to the control group while no such difference could be elicited when comparing the high-risk group to the controls. In the mean time, 48 % of the septic group showed absolute neutrophilia ($>7.5 \times 10^9$/L) with 57% absolute immature forms ($>0.4 \times 10^9$/L) compared to values of 11% and 11% respectively in the high-risk group. Thus, on applying the hematological scoring system (HSS) of Rodwell et al [6] to cases and controls, 61% of the septic group had scores ≥ 3 (positive) compared to 11% of the high-risk group. Manucha et al. [9] reported that a hematological score ≥ 3 had a sensitivity of 86% and negative predictive value of 96%, thus permitting an objective assessment of hematological changes that occur in a neonate suspected to have sepsis.

C-reactive protein (CRP) was found to be significantly high in the septic as compared to the control group. The high-risk group had negative CRP initially, which turned positive on appearance of signs and symptoms of infection. CRP was found to have a sensitivity of 70% and specificity of 100%. This was in agreement with Kuster et al [2] who stated that CRP is of a low sensitivity especially if measured early in the course of sepsis, but CRP concentrations increase later in the course of sepsis. Nuntnarumit et al [10] reported that serial CRP gave very high predictive values for diagnosis of neonatal sepsis.

The current study revealed that up-regulation of CD45RA/CD45RO dual expression and CD45RO expression on T-lymphocytes had occurred in the septic group. This was in agreement with Hodge et al[1] who found that in all cases of infection, confirmed by bacterial culture or viral serology, up-regulation of either CD45RA/ CD45RO or CD45RO on CD4+ T-lymphocytes had occurred. Concurrently, the % expression of CD45RA/CD45RO and CD45RO was significantly high in the high-risk group when compared to the control group with no significant difference

115

between their expression among the septic and high-risk groups. Hodge et al [1] stated that CD45RA/CD45RO co-expression is a very early activation marker, whose enhanced expression can be detected in early stages of infection, before up-regulation of the CD45RO isoform. On attempting to correlate CD45RA/CD45RO to CD45RO, a positive association was found. This can be explained by the dual positive expression of CD45RA/CD45RO after stimulation of lymphocytes before conversion to single CD45RO isoform[1].

There was no difference in the base line levels of T-lymphocyte activation markers between those who died or survived. However, the follow up samples taken 1 week later showed a significant increase in the expression of CD45RO than the initial samples of the septic group. This was in contrast to what was reported by Field et al [11] who found that non-survivors had lower CD45RO than survivors of sepsis. They explained their finding by immunological immaturity of infants as a result of low numbers of CD45RO+ (memory/antigen-primed) T-cells. The activated T-cells in infants with confirmed bacterial infection in their series persisted for several weeks and returned to normal ranges within 4-6 weeks.

There was no variation in the expression of CD45RA/CD45RO or CD45RO with gestational age or gender. This was in agreement with Hodge et al [1] who stated that prematurity had no effect on the up-regulation of CD45RA/ CD45RO or CD45RO.There was no significant difference between cases with positive and those with negative hematological sepsis scores in the expression of CD45RA / CD45RO and CD45RO. On the other hand, their co-expression was found to be significantly higher in those with sepsis confirmed by blood culture. Weirich et al [12] found no significant relationship between white blood cell sub-population counts (e.g., segmented neutrophils, immature neutrophils, lymphocytes) and positive cultures, clinical signs of infection or CRP status. In our series, CD45RA/CD45RO had a 97% sensitivity and 100% specificity with a cut-off value >2.2% expression. Similarly, CD45RO had a 97% sensitivity and 100% specificity with a cut-off value >5% expression. This confirms or matches the results given by Hodge et al [1] who stated that the predictive value of the test was upheld by results which showed that 100% of infants with confirmed infection demonstrated increased expression of CD45RA/CD45RO or CD45RO.

CD11b expression was found significantly higher in the high-risk than the control group and

was significantly higher in the septic when compared to the high-risk group. The same finding was previously reported by El-Kerdani et al [7] who added that CD11b was elevated in neonates with proven infection and in nearly all neonates in whom clinical signs suggested sepsis and its expression increased rapidly after the onset of sepsis, before rise in serum concentration of CRP, the most commonly used marker for infection.

No significant influence of gestational age or gender could be noted on the level of CD11b elevation. The same observation was made by Nupponen et al [13] who reported that there was no association between birth weight or gestational age and the level of CD11b expression. The same finding was also demonstrated by El-Kerdani et al [7], denoting that even the premature infant can express CD11b. Moreover, the neonates with positive hematological septic scores did not differ from those with negative scores in the expression of CD11b. On the other hand, septic cases with positive blood culture had significantly higher CD11b levels than culture negative cases. Weirich et al [12] similarly reported that there was no significant relationship between the white blood cell sub-population counts (segmented neutrophils, immature neutrophils, lymphocytes) and levels of CD11b. Nupponen et al [13] also found a two to four fold increase in neutrophil CD11b expression in infants with blood culture positive sepsis.

There was no difference between those who died or survived in the initial samples, or between them in the follow up samples as far as the expression of neutrophil CD11b was concerned. Also, there was no significant difference between the initial and follow up sample taken after 1 week in each group. Muller et al [14] reported that poor prognosis is associated with a lower expression of activation markers on neutrophils, suggesting that poor outcome may be due to compensatory anti-inflammatory response. Neutrophil CD11b had a sensitivity of 93% and a specificity of 89% with a cut off value >25% expression. Weirich et al [12] reported negative and positive predictive values, sensitivity and specificity for CD11b of 100%, 99%, 96% and 100% respectively, whereas Nupponen et al [13] reported a sensitivity of 100% and a specificity of 100%.

Soluble TNF-α receptors (sTNFRs) are soluble proteins shed from target cells during experimental endotoxemia and clinical sepsis [15]. The soluble receptor (p55) was detected in serum from all term and preterm neonates in this study whether infected or not. However, its level was significantly higher in the septic as well as in the high-risk groups when compared to control group. On the other hand, no significant difference between the levels of sTNFR1 in the former two groups was detected. Maymon et al [16] reported that levels of sTNFRs increase in maternal serum and in amniotic fluid during pregnancy and labour, and increased shedding of sTNFRs may be a regulatory mechanism of cytokine activity during pregnancy and labour. Similar results were obtained by El-Kerdani et al [7] who found that sTNFR1 level was significantly high in septic neonates. No effect of gestational age or gender on the level of sTNFR1 in the septic and high-risk groups was encountered in our study. Similar findings were reached by Doellner et al [17] on comparing preterm and full term infected neonates.

In this study, no significant difference was found between the mean level of sTNFR1 measured initially and one week later or between its level in survivors and non survivors, whether initially or by follow up. Doellner et al [18] stated that the levels of sTNFR p55 seemed to decrease as time passed after delivery (whether infected or not). These time-related changes in sTNFRs levels may be due to a physiological elimination or may be due to the effect of antibiotics. The cases with positive hematological scores did not differ significantly from those with negative scores in the level of sTNFR p55 neither did those with positive or negative blood cultures. sTNFR1 was found to have a sensitivity of 80% and a specificity of 100% for a cut-off value > 8.4 ng/ml. This was in discordance with Doellner et al [18] who reported a sensitivity of 70% and a specificity of only 65% for a cut off level of 8 ng/ml while Messer et al [19] reported a sensitivity of 75% and specificity of 69% for a cut off level of 6 ng/ml.

CD11b was positively correlated in our series to both CD45RA/CD45RO and CD45RO. When "naive" CD45RA+ T-lymphocytes are activated by inflammatory processes such as infection, CD45RO is up-regulated and CD45RA is down-regulated [1] and neutrophil CD11b up-regulation occurs on stimulation by pathogens or their products. At the same time, CD11b was found to have a positive correlation with sTNFR1 in the septic group. Neutrophils are known to increase their expression of CD11b on stimulation with TNF-α in vivo and in vitro [12]. CD11b was found to have a positive correlation with CRP levels in the septic group. Mehr et al [20] stated that TNF-α together with IL-6 induce CRP synthesis in the liver.

To conclude, the surface activation markers of T-lymphocytes (CD45RA/CD45RO and CD45RO), the neutrophil activation marker (CD11b) and the

soluble TNF receptor 1 can be useful early indicators of neonatal sepsis, and might be superior to the hematological scoring system and CRP in the detection of neonatal sepsis at its early stages.

REFERENCES

1. HODGE S, HODGE G, FLOWER R, HAN P. Surface activation markers of T lymphocytes : role in the detection of infection in neonates. Clin Exp Immunol 1998; 113: 33-8.

2. KUSTER H, WEISS M, WILLEITNER AE, DETLEFSEN S, JEREMIAS I, ZBOJA J, ET AL. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. Lancet 1998; 352: 1271-7.

3. NG PC, CHENG SH, CHUI KM, FOK TF, WONG MY. Diagnosis of late-onset neonatal sepsis with cytokines adhesion molecule, and C-reactive protein in preterm very low birth weight infants. Arch Dis Child 1997; 77: F221-7.

4. TOLLNER U. Early diagnosis of septicaemia in the newborn. Clinical studies and sepsis score. Eur J Pediatr 1982; 138(10): 331-7.

5. KERN WV, ORISTRELL J, SEGURA-PORTA F, KERN P. Release of soluble tumour necrosis factor receptors in Mediterranean Spotted Fever Rickettsiosis. Clin Diagn Lab Immunol 1996; 3(2): 233-5.

6. RODWELL RL, LESLIE AL, TUDEHOPE DI. Early diagnosis of neonatal sepsis using a haematologic scoring system. J Pediatr 1988; 112(5): 761-7.

7. EL-KERDANI TA, ABDEL-WAHED MA, ALY GS, ABDEL-BASSET FZ, EL-BARBARY MN. The value of neutrophil CD11b expression, interleukin-6 and soluble receptors of tumour necrosis factor in early diagnosis of neonatal sepsis. Egypt J Neonatol 2001; 2 (2): 115-25.

8. GODFRIED MH, VANDERPOLL T, JONSEN J, ROMIJN JA, EEFTINEK-SCHATTENKERK JKM , ENDERT E. Soluble receptors for tumour necrosis factor : a putative marker of disease progression in HIV infection. AIDS 1993; 7: 33-6.

9. MANUCHA V, RUSIA U, SIKKA M, FARIDI MM, MADAN N. Utility of haematological parameters and C-reactive protein in the detection of neonatal sepsis. J Pediatr Child Health 2002; 38(5): 459-64.

10. NUNTNARUMIT P, PINKAW O, KITIWANWANICH S. Predictive values of serial C-reactive protein in neonatal sepsis. J Med Assoc Thai 2002; 85 Suppl4: S1151-8 [Abstract].

11. FIELD CJ, CLANDININ MT, VAN AERDE JE. Polyunsaturated fatty acids and T cell function: implications for the neonate. Lipids 2001; 36(9): 1025-32.

12. WEIRICH E, RABIN RL, MALDONADO Y, BENITZ W, MOLDER S, HERZENBERG LA , ET AL. Neutrophil CD11b expression as a diagnostic marker for early-onset neonatal sepsis. J Pediatr 1998; 132(3): 445-51.

13. NUPPONEN I, ANDRESSON S, JARVENPAA AL, KAUTIAINEN H, REPO H. Neutrophil CD11b expression and circulating interleukin-8 as diagnostic markers for early onset neonatal sepsis. Pediatrics 2001; 108(1): E12.

14. MULLER KAC, TULLEKEN JE, ZIJLSTRA JG, SLUITER W, HERMANS J, KALLENBERG CG, ET AL. Leukocyte activation in sepsis: correlations with disease state and mortality. Intensive Care Med 2000; 26(7): 883-92 [Abstract].

15. O'BRIEN DP, BRILES DE, SZALAI AJ, TU AH, SANZ I, NAHM MH. Tumor necrosis factor alpha receptor 1 is important for survival from streptococcus pneumoniae infections. Infect Immunol 1999; 67(2): 595-601.

16. MAYMON E, GHEZZI F, EDWIN SS, MAZOR M, YOON BH, GOMEZ R, ET AL. The tumour necrosis factor α and its soluble receptor profile in term and preterm parturition. Am J Obstet Gynecol 1999; 181(5): 1142-8.

17. DOELLNER H, VATTEN L, AUSTGULEN R. Early diagnostic markers for neonatal sepsis : comparing C-reactive protein, interleukin-6, soluble tumour necrosis factor receptors and soluble adhesion molecules. J Clin Epidemiol 2001; 54(12): 1251-7.

18. DOELLNER H, ARNTZEN KJ, HAEREID PE, AAG S, BRUBAKK AM, AUSTGULEN R. Increased serum concentrations of soluble tumor necrosis factor receptors p 55 and p 75 in early onset neonatal sepsis. Early Hum Dev 1998; 52: 251-61.

19. MESSER J, EYER D, DONATO L, GALLATI H, MATIS J, SIMEONI U. Evaluation of interleukin-6 and soluble receptors of tumour necrosis factor for early diagnosis of neonatal infection. J Pediatr 1996; 129(4): 574-80.

20. MEHR S, DOYLE LW. Cytokines as markers of bacterial sepsis in newborn infants : a review. Pediatr Infect Dis J 2000; 19(9): 879-87.