

DETERMINATION OF nCD64, mHLA-DR, AND SI IN FULL-TERM NEWBORNS WITH SEPSIS AT VIETNAM NATIONAL CHILDREN'S HOSPITAL (2019 - 2021)

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Abstract: The study was conducted to determine the role of nCD64, mHLA-DR and SI in the diagnosis of neonatal sepsis. The study was designed using experimental descriptive research method at the laboratory. Mean values of nCD64, mHLA-DR and SI in full-term newborns with sepsis were 10167.1 ± 6136.9 molecules/cell, 9898.4 ± 14173.9 molecules/cell, and 274.6 ± 287.5 respectively. SI and nCD64 had high values in the diagnosis of sepsis in full-term newborns (AUC = 0.8, $p < 0.01$), higher than that of CRP, platelets and white blood cells. The sensitivity and specificity of SI at the SI cut-off point > 29.1 were 95.1% and 41.2%, respectively. The sensitivity and specificity of nCD64 at the nCD64 cut-off point > 5004 were 88.2% and 44.5%, respectively. SI and nCD64 are highly valuable in the diagnosis of sepsis in full-term newborns (AUC = 0.8, $p < 0.01$).

Keywords: sepsis, newborns

1. Introduction

Sepsis is a serious life-threatening infection that occurs when the body's responses to infectious agents cause damage to tissues and organs [1]. Premature infants are more susceptible to infection with infectious agents, but sepsis also causes very high mortality, sequelae and disability rates in full-term newborns [2]. More and more modern tests are being applied to contribute to early and accurate diagnosis of diseases. In the world, flow cytometry quantifying cell surface marker 64 on neutrophils (nCD64) and human leukocyte antigen DR type on monocytes (mHLA-DR) have been shown to be very significant in the diagnosis of neonatal sepsis [3], [4]. In Vietnam, the first data confirming

the value of these tests have also been published, but the studies have only been conducted on adults [5], [6]. The research in infants has not yet been published, in order to supplement the new research field in Vietnam, we carry out the study "Determination of nCD64, mHLA-DR and sepsis index (SI) in full-term newborns at Vietnam National Children's Hospital" to determine the roles of nCD64, mHLA-DR and SI in the diagnosis of neonatal sepsis.

2. Study subjects and Methods

2.1. Subject, location, and time of study

Full-term newborns meet diagnostic criteria for sepsis

Site of the study: The study was conducted at the National Children's Hospital

Time of the study: The study was carried out from January 1, 2020 to

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2.2. Study methods

2.2.1. Study design

The study was designed as experimental descriptive research method at the laboratory.

2.2. Sample size and sample selection

Apply the estimation formula to a ratio:

$$n = Z^{2_{1-\alpha/2}} \frac{(1-p)}{p\varepsilon^2}$$

n: Minimum sample size

p: Rate of arrhythmia symptoms in newborns with sepsis, we choice p = 0,55 (according to the study of Nguyen Nhu Tan) [7].

$Z_{1-\alpha/2}$: Confidence coefficient, for 95% confidence, then $Z_{1-\alpha/2} = 1.96$

ε : Desired relative error, we choose $\varepsilon = 0.2$

With the selected values, the calculated sample size is 79 newborns. In fact, we selected 85 neonates with positive blood cultures, which met the study criteria.

2.2.2. Study contents

In the framework of this article, we do in-depth analysis on nCD64, mHLA-DR, SI in full-term newborns with sepsis.

2.2.3. Techniques used in the study

The study used techniques to determine CD64 and HLA-DR expressions on neutral BC cells and monocytes by flow cytometry.

- Chemicals: BD FACS Lysing solution; BD PBS sample washing solution; Antibodies: CD14 FITC, CD64/CD45 (PE/PerCP), HLA-DR/Mono (PE/PerCP), CD45 APC-H7

- Equipment: BD FACS Canto II systems (6 colors) and Canto (10 colors); Centrifuge; Pipettes and pipette tips of 20 μ l, 200 μ l and 1000 μ l types; Shaker: Vortex mixer; 5 ml BD tube; Gloves and other necessary tools.

- Principles of the process

+ *In order to determine CD64 and HLA-DR expressions on neutral BC cells and monocytes by flow cytometry, blood samples were incubated with fluorescently bound monoclonal antibodies. These antibodies would specifically bind to specific antigens on the surface of each type of white blood cell. Fluorescently attached white blood cells were then passed through laser beams on the Flow-Cytometry BD FACS Canto system. the impression of the impression of the imprint was identified based on the size, density, and fluorescent color.*

+ *Quanti BRITE PE reagent is packaged in lyophilized granules attached to 4 different concentrations of fluorescence to measure the fluorescence signal on the PE channel to calculate the number of PE molecules. When using PE molecules bound to antibodies in a known ratio, the number of PE molecules could be converted to the number of antibodies bound per cell.*

- The steps of the process

+ Sample staining process: including sample staining process: (Stain-Lyse-Wash); sample staining process: QuantiBRITE PE beads

+ Run samples:

+ Analyze results on tubes:

PMN CD64 Sample Tube: record the

mean fluorescence intensity (MFI) of CD64 on neutrophils (nCD64), lymphocytes (LyCD64) and monocytes (mCD64). Calculate ABC values for nCD64, LyCD64 and mCD64

Mono HLA DR: Analysis (QuantiBRITE PE beads): record the mean fluorescence intensity (MFI) of HLA-DR on monocytes (mHLA-DR) and neutrophils (nHLA-DR). Calculate ABC values for nHLA-DR and mHLA-DR. Record the mean fluorescence intensity (MFI) shown on QuantiBRITE PE beads. Calculate ABC values for nHLA-DR and mHLA-DR. Enter nCD64 and mHLA-DR values into the software to calculate the SI.

2.3. Biomedical statistic data analysis and processing methods

- Data after being collected according to the research medical record form will be coded, entered and analyzed using SPSS 22.0 software.

- Based on the ROC (Receiver Operating

Characteristic) curve to determine the cut-off point and the sensitivity and specificity of nCD64, mHLA-DR, SI test indicators.

2.4. Study ethics

The child's parents or legal guardians are informed about their participation in the study and those who give consent will be selected for the study. The study results help to evaluate the clinical and paraclinical characteristics of the newborns to better diagnose the disease. The conduct of the study was approved by the Ethics Council in Medical Research at the National Children's Hospital according to Decision No. 332/BVNTW-VNCSKTE dated March 18, 2020.

3. Results

The table below shows the values of immunological indices nCD64, mHLA-DR and SI in newborns. There were 85 newborns with positive blood culture, 50 newborns with negative blood culture and the following results were recorded:

Table 3.19: Values of nCD64, mHLA-DR, SI of newborns with sepsis and non-infection (n=135)

Index	Positive blood culture (n=85)	Negative blood culture (n=50)	P value
nCD64 (molecules/cell)	10.167,1 ± 6.136,9 (1198 - 32965)	1900,9 ± 1.589,1 (238 - 7569)	< 0,01
mHLA-DR (molecules/cell)	9.898,4 ± 14.173,9 (434 - 96881)	30.476,8 ± 20.205,1 (3.052 - 93.049)	< 0,01
SI	274,6 ± 287,5 (18,7 - 1376,8)	7,9 ± 5,5 (1 - 22)	< 0,01

There was a statistically significant difference in nCD64, mHLA-DR and SI values between the group with positive

blood culture and the group with negative blood culture, with $p < 0.01$.

Table 3.20: Values of nCD64, mHLA-DR, SI of full-term newborns with sepsis with positive blood and negative blood culture (n=257)

Index	Positive blood culture (n=85)	Negative blood culture (n=172)	P value
nCD64 (molecules/cell)	10.167,1 ± 6.136,9 (1.198 - 32.965)	5.985,1 ± 4.916,3 (783 - 47.953)	< 0,01
mHLA-DR (molecules/cell)	9.898,4 ± 14.173,9 (434 - 96881)	1.3897,1 ± 27.223,2 (7 - 31.1904)	> 0,05
SI	274,6 ± 287,5 (18,7 - 1376,8)	(153,3 - 570,0) 3,5 - 7313	> 0,05

nCD64 value of group with positive blood culture was significantly higher than the value of group with negative blood culture, p < 0,01. There was no difference in mHLA – DR and SI values between the two groups, p > 0,05.

Table 3.21: nCD64, mHLA-DR và SI quantification according to the group cause of the disease (n = 85)

Index	Gram (-) (n = 44)	Gram (+) (n = 33)	Fungi (n = 8)
nCD64 (molecules/cell)	9.360,5 ± 5.819,8 (1198 - 32965)	11.024,8 ± 6.147,1 (2648 - 29954)	11.064,6 ± 7915,5 (4511 - 27876)
mHLA-DR (molecules/cell)	8.137,6 ± 10.040,2 (434 - 51.783)	11.935,9 ± 17.109,1 (1.532 - 96.881)	11.177,8 ± 20.309,2 (973 - 60207)
SI	287,3 ± 303,6 (30,2 - 1.376,8)	243,6 ± 265,3 (18,7 - 1.126,0)	332,5 ± 307,1 (28,1 - 876,0)

The nCD64 and SI values were higher, and the mHLA-DR values were lower in all three groups compared with the indicators of the non-infectious group. However, the difference in values of nCD64, mHLA-DR, SI indicators between groups of children infected with different causes were not statistically significant.

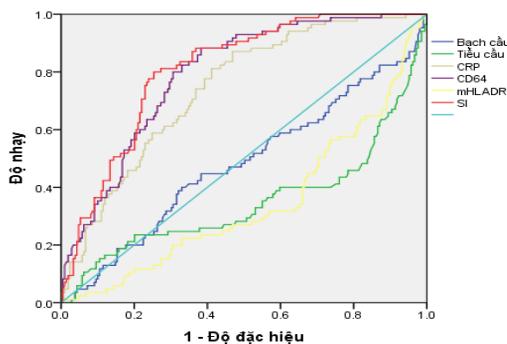


Figure 3.6. Area under the ROC curve examines the values of laboratory indicators in the diagnosis of neonatal sepsis

More specifically about the value of the ROC curve, we have the following data table:

Table 3.22: Area under the ROC curve examines the value of laboratory indicators in the diagnosis of neonatal sepsis

Index	Area under the ROC curve (AUC)	P value
Leukocytes	0,48	0,60
Platelet Count	0,34	0,00
CRP	0,74	0,00
nCD64	0,80	0,00
mHLA-DR	0,34	0,00
SI	0,80	0,00

SI and nCD64 had the highest (AUC=0,34, p=0,00); CRP, platelet count diagnostic values (AUC = 0,8, p = 0,00); had average diagnostic values. mHLADR had low diagnostic value

Table 3.23. Sensitivity and specificity of tests at the cut-off points

Index	Chỉ số	Sensitivity (%)	Specificity (%)
Leukocytes > 20x10 ⁹ /L		41,3	64,4
Platelet Count < 100x10 ⁹ /L		51,3	16,5
CRP > 15mg/L		88,1	44,5
nCD64 > 5004 molecules/cell		88,2	62,1
mHLA-DR < 6886 phân tử/tế bào		33,0	35,0
mHLA-DR < 6886 molecules/cell			
SI > 29,1		95,1	41,2

At cut-off values, SI > 29.1 and nCD64 > 5004 molecules/cell had the highest sensitivity (95.1% and 88.2%), nCD64 > 5004 molecules/cell had the highest specificity (0.62).

4. Discussion

This is the first study on the quantification of nCD64, mHLA-DR and SI indicators.

4.1. Quantification of nCD64:

In this study, the group of newborns with sepsis had an average nCD64 of 10167.1 ± 6136.9 molecules/cell, that was 5.3 times higher than the mean value of the non-infection children group (nCD64

= 1900.9 ± 1589.1 molecules/cell) and 2 times higher than the group of newborns with sepsis with negative blood cultures (nCD64 = 5985.1 ± 4916.3 molecules/cell) (p < 0.01).

The study of Pak C Ng reported that the mean values of nCD64 on the 1st and 24th hours of infection group were 8320 molecules/cell and 9704 molecules/cell respectively, which were higher than those of the non-infection group (1st hour and 24th hour values were 3915 molecules/cell and 4491 molecules/cell respectively). In the control group (healthy children), the mean value of nCD64 was 3426 molecules/cell [3].

The values of nCD64 in premature

infants were also very different in the groups of infants with sepsis, infants without sepsis and healthy infants in Jikun Du's study. The group of infection had the mean value of nCD64 of 2869.67 molecules/cell while the healthy group had the mean value of nCD64 of 1610.80 molecules/cell ($p=0.0001$) [8].

4.2. Quantification of mHLA-DR

We found that the group with positive blood culture had the mean mHAL-DR value of 9898.4 ± 14173.9 molecules/cell, equivalent to 32.4% of the healthy group's value (30476.8 ± 20205.1 molecules/cell). Talita Freitas Manzoli showed that the reduction of <30% in mHLA-DR value compared with that of the control group was a predictor of mortality in the first week of hospital admission [9].

Christian Meisel showed that all patients with severe sepsis had mHLA-DR < 8000 molecules/cell [10]. Sandra Tamulyte's study identified three mHLA-DR thresholds including ≤ 8000 molecules/cell, ≤ 5000 molecules/cell and ≤ 2000 molecules/cell as the mHLA-DR levels predicting the patient's severity condition, regardless of the causes of disease. mHLA-DR values of 2000 molecules/cell and 5000 molecules/cell predicted longer stay in the resuscitation department, ventilator time and antibiotic treatment time, as well as higher concentration of pathogenic microorganisms. The value of mHLA-DR in our study was higher because we quantified on the group of infection while Sandra Tamulyte's study performed on all children entered the resuscitation department with various causes [11].

4.3. Quantification of SI

The SI is calculated according to the values of nCD64 and mHLA-DR. In our group of infants with positive blood

cultures, SI had an average value of $274,6 \pm 287,5$ (18,7 – 1376,8), which was much higher than the value of infants without infection (SI = 7,9).

Sneha Goswami's study measuring nCD64 and mHLA-DR using the mean fluorescence concentration (MFI) also showed that nCD64 value was significantly increased in the group with infection compared with the value of the healthy group ($p < 0.05$), but mHLA-DR value insignificantly decreased in the group with infection compared with the value of the group with negative blood culture. Thus, the SI value was also different in the groups of infection, group with negative blood culture and group of healthy children when converted [12].

The mean value of SI in the group of adult patients with sepsis and trauma of Pham Thi Ngoc Thao was 112.95 (46.16 – 270,66) [6]. The result of this study was higher because the study subjects were full-term infants.

4.4. Diagnostic values of nCD64, mHLA-DR and SI in the diagnosis of sepsis at full-term newborns

When examining the diagnostic value of immunological indicators in diagnosing sepsis in full-term neonates, we found that nCD64 and SI were the highest values with the area under the curve 0.80 ($p < 0.01$). While commonly used indicators to diagnose infections such as CRP, platelets, and leukocytes had lower values (AUC < 0.75).

The study by Umlauf VN on neonatal patients showed that in the Early diagnosis of neonatal sepsis, nCD64 had sensitivity value, specificity value, positive diagnostic value, negative diagnostic value of 96%, 71%, 71%, 97% respectively. In the late diagnosis

of neonatal sepsis, the values were 75%, 83%, 83%, 96% respectively. These values were both higher than nCD11 and CRP in the diagnosis of neonatal sepsis. In particular, when nCD64 and CRP are combined, sensitivity and specificity could reach 100% [13].

We recorded at cut-off points of nCD64 > 5004 molecules/cell with sensitivity value up to 88.2%, specificity value of 62.1%; cut-off points of SI value > 29.1 had sensitivity value of 95.1%, specificity value of 41.2%. Compared with the cut-off points of leukocytes > 20x10⁹/L, platelets < 100x10⁹/L, CRP > 15mg/L to assess neonatal sepsis according to 2010 EMA criteria, it was clear that nCD64 and SI had higher sensitivity and specificity. Our results, however, also confirmed that mHLA-DR value had low sensitivity and specificity in the diagnosis of neonatal sepsis.

nCD64 is a marker that has been evaluated to be valuable in the diagnosis of sepsis with high specificity and sensitivity. However, the cut-off point varies depending on each study, study object, study method. The study of Hugh Simon Lam on the group of premature neonates with necrotizing enterocolitis showed that the cut-off point nCD64 = 23777 molecules/cell [14].

Our cut-off point nCD64 = 5004 molecules/cell is higher than the study of Pham Thi Ngoc Thao, but lower than the study of Hugh Simon Lamdo due to differences in study subjects.

Pham Thi Ngoc Thao also showed that SI > 39.69 had higher sensitivity and specificity than leukocytes, CRP, procalcitonin in diagnosis of sepsis in traumatized adults [6]. With the SI cut-off point > 29.1, we also proved that the diagnostic value for neonatal sepsis of SI was higher than the commonly used test indicators.

5. Conclusion and Recommendation

The mean value of nCD64 was 10167.1 ± 6136.9 molecules/cell, the mean value of mHLA-DR was 9898.4 ± 14173.9 molecules/cell, the mean value of SI was 274.6 ± 287.5.

SI and nCD64 had high values in the diagnosis of sepsis at full-term newborns (AUC = 0.8, p < 0.01), which were higher than the diagnosis values of CRP, platelets and leukocytes.

It is recommended inclusion of nCD64, nCD64, and SI in the diagnosis of sepsis in full-term newborns.

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