Diagnostic Performance of CD64, CD11b, CD14 and Presepsin in Neonatal Sepsis

Thesis Submitted for Partial Fulfillment of MD
In Clinical Pathology
By

Heba Ezzat Hashem Osman
MB BCh, M.Sc.
Assistant Lecturer of Clinical Pathology
Faculty of Medicine - Ain Sham University

Supervised by

Professor / Amira Mohammed Mokhtar
Professor of Clinical Pathology
Faculty of Medicine - Ain Shams University

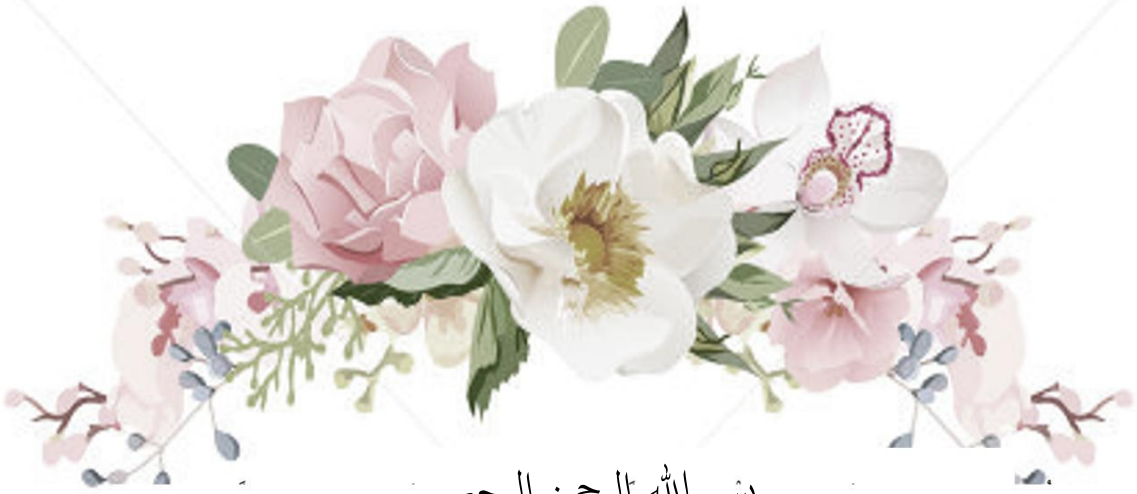
Professor / Eman Mohammed Kamel
Professor of Clinical Pathology
Faculty of Medicine - Ain Shams University

Doctor / Sherine Ahmed El Masry
Assistant Professor of Clinical Pathology
Faculty of Medicine - Ain Shams University

Doctor/ Rania Mohammed Abdel Halim
Lecturer of Clinical Pathology
Faculty of Medicine - Ain Shams University

Doctor / Nour El Deen Mohammed Abd El-Aal
Lecturer of pediatric and neonatology
Faculty of Medicine - Ain Shams University

Faculty of Medicine
Ain Shams University
2018



بسم الله الرحمن الرحيم

" إِنَّ الَّذِينَ آمَنُوا وَعَمِلُوا الصَّالِحَاتِ كَانَتْ لَهُمْ جَنَّاتُ الْفِرْدَوْسِ نُزُلًا (107) خَالِدِينَ فِيهَا لَا يَبْغُونَ عَنْهَا حِوَلًا (108)
قُل لَّوْ كَانَ الْبَحْرُ مِدَادًا لِّكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَن تَنفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا (109)
قُلْ إِنَّمَا أَنَا بَشَرٌ مِّثْلُكُمْ يُوحَىٰ إِلَيَّ أَنَّمَا إِلَٰهُكُمْ إِلَٰهٌ وَاحِدٌ ۖ
فَمَن كَانَ يَرْجُو لِقَاءَ رَبِّهِ فَلْيَعْمَلْ عَمَلًا صَالِحًا وَلَا يُشْرِكْ بِعِبَادَةِ رَبِّهِ أَحَدًا (110)"

سورة الكهف

First and Foremost, thanks to **"ALLAH"** the kindest and the most merciful.

I want to express my deep thanks and great gratitude to ***Prof. Dr. Amira Mohammed Mokhtar*** *and* ***Prof. Dr. Eman Mohammed Kamel*** Professors of the clinical pathology department, Faculty of Medicine, Ain Shams University who provided me with their precious time & valuable comments to submit this work scientifically. It was a great honor for me to work under their supervision.

And I would like to express my grateful thanks and appreciation to ***Dr. Shereen Ahmed El Masry,*** Assistant professor of the clinical pathology, Faculty of Medicine, Ain Shams University, and ***Dr. Rania Mohammed Abdel Halim*** lecturer of the clinical pathology, Faculty of Medicine, Ain Shams University for their supervision, continuous guidance, cooperation and helpful instructions.

And I'm deeply indebted to ***Dr. Nour EL Din Mohammed Abdel Aal,*** Lecturer of pediatrics and neonatology, Faculty of Medicine, Ain Shams University for his continuous guidance, help, and encouragement throughout this work.

Heba Ezzat Hashem

This work is dedicated to …

My parents for always being for me and to whom I owe everything I ever did in my life and will achieve.

List of contents

List of Abbreviations

Abbreviations	*Full term*
AAP	American Academy of Pediatric
ADC	Analogue-to-Digital Conversion
ADCC	Antibody Dependent Cellular Cytotoxicity
ALC	Absolute lymphocyte Count
AMC	Absolute Monocyte Count
ANC	Absolute Neutrophil Count
AUC	Area Under the Curve
CBC	Complete Blood Count
CD	Cluster of Differentiation
CoNS	Coagulase-Negative *Staphylococci*
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
CSFs	Colony Stimulating Factors
CVP	Central Venous Pressure
dC	Delta Change
DNA	Deoxyribonucleic Acid
DOH	Duration of Hospitalization
E. coli	*Escherichia coli*
ELISA	Enzyme-Linked Immuno-Sorbant Assay
EDTA	Ethyl- Enediamine Tetraacetic Acid
EFF.	Efficacy
EOS	Early Onset Sepsis
ETT	Endotracheal Tube
FcγRI	Fc-Gamma Receptor 1
FITC	Fluorescein Isothio-Cyanate
FN	False Negative
FP	False Positive
FSC	Forward Side Scatter
GA	Gestational Age
GBS	Group B *Streptococcus*
GC-MS	Gas Chromatography–Mass Spectrometry
G-CSF	Granulocyte Colony Stimulating Factor
Hb	Hemoglobin

HDL	High Density Lipoprotein
hs-CRP	Highly Sensitive C-Reactive Protein
HSS	Hematological Scoring System
I/T Ratio	Immature: Total Neutrophil Ratio
ICAM-1	Circulating Intracellular Adhesion Molecule-1
IL-1	Interlukin-1
IL-3	Interlukin-3
IL-6	Interleukin-6
IL-8	Interleukin-8
ILO	International Labor Organization
LBW	Low Birth Weight
LDL	Low Density Lipoprotein
LOS	Late Onset Sepsis
LPS	Lipopolysaccharide
mCD14	Monocyte CD14
MFI	Mean Fluorescent Intensity
nCD11b	Neutrophil CD11b
nCD64	Neutrophil CD64
nCD64 MFI	nCD64% Mean Fluorescent Intensity
nCD64%	nCD64 Percent
NICU	Neonatal Intensive Care Unit
NPV	Negative Predictive Value
P Value	Probability Value
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PCT	Procalcitonin
PLT	Platelet Count
PMNL	Total Polymorph Nuclear Leukocyte
POC	Point of Care
PPV	Positive Predictive Value
PROM	Premature Rupture of Membrane
P-SEP	Presepsin
ROC	Receiver of Curve
sCD14-ST	Soluble CD14 Sub-Type
SIRS	Systemic Inflammatory Response Syndrome.
Spp.	Species
SPS	Sodium-Polyanetholesulphonate
SSC	Side Scatter
sTREM-1	Soluble Triggering Receptor Expressed on Myeloid Cells-1

TAT	Turnaround Time
TC	Total Cholesterol
TG	Triglycerides
TLC	Total Leukocyte Count
TN	True Negative
TNF-α	Tumor Necrosis Factor-α
TP	True Positive
UN	United Nations
UNICEF	United Nations International Children's Emergency Fund
USA	United States of America
UTI	Urinary Tract Infection
VLBW	Very Low Birth Weight
WBC	White Blood Cells
WHO	World Health Organization

List of Tables

List of figures

Introduction

Neonatal sepsis is a very critical medical situation. Despite the extensive researches for understanding and managing neonatal septicemia, it is still a major source of the morbidities and mortalities specially among the developing countries ***(Cohen et al., 2015).***

Neonatal septicemia passes into various clinical stages; systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, septic shock and multi-organ failure ending by death ***(Mearelli et al., 2015).***

The minimal initial non-specific symptoms and signs of the disease besides to many obstacles encountered in the diagnostic modalities, makes the early diagnosis is very challenging for the clinicans. Furthermore, the clinical course can be fulminate and fatal if the proper management couldn`t initiated at the proper time and with the proper dosage ***(Zambon et al., 2008 & Umlauf et al., 2013).***

The traditional sepsis diagnostic modalities still suffer from many disadvantages; the blood culture remains the gold standard for the diagnosis despite the fact that its results are usually delayed for more than 48 hours besides to the many false positives due to the impossibility of excluding contamination and many false negatives encountered in case of prior antibiotic administration and with special consideration in the neonatal setting where the withdrawn blood volume may be insufficient in some circumstances ***(Shozushima et al., 2011 & Camacho-Gonzalez et al., 2013)*.**

As a result, during the last decades, many studies were directed toward a new diagnostic and prognostic modality not only for early accurate diagnosis; but also, for the rational antibiotic use ***(Laxminarayan et al., 2013& Mahmoud et al., 2014).***

Those Effective biomarkers included; cell surface markers [e.g; cluster of differentiation 64 (CD64), Soluble CD14 subtype (sCD14-ST), CD14, CD163, CD11b], bacterial surface antigens, genetic biomarkers, protein biomarkers [e.g procalcitonin (PCT), Neopterin], cytokines and chemokines ***(Chauhan et al., 2017).***

Regarding the C-Reactive Protein (CRP) which is the most extensively studied sepsis marker, it represents the preferred index in many neonatal intensive care units (NICUs) despite the ongoing rise and fall of the new infection biomarkers ***(Hofer et al., 2013).***

The sensitivities and specificities of CRP widely differ between the studies, ranging from 29% to 100% and from 6% to 100%, respectively. In addition, the sensitivity of CRP is well known to be the lowest during the initial stages of the infection ***(Hofer et al., 2013).***

Among the new sepsis markers, neutrophil CD64 (nCD64) represents a one of the most researchable and valuable early diagnostic biomarker ***(Mahmoud et al., 2014 & Mearelli et al., 2015)***.

Neutrophil CD64 is a membrane glycoprotein that mediates endocytosis, phagocytosis, antibody dependent cellular cytotoxicity (ADCC), cytokine release, and superoxide generation.

It is constitutively expressed on monocytes and the macrophages ***(Delanghe and Speeckaert., 2015)***.

It is well known that nCD64 is expressed at low concentration on the surface of the non-activated neutrophils but can be markedly up-regulated at the onset of the sepsis process ***(Ten Oever et al., 2016).***

Neutrophil CD11b (nCD11b) is another sepsis biomarker. It acts as Fc-receptor which expressed in huge quantities on the surface of the activated inflammatory cells upon encountering bacteria or their cellular products by the same mechanism as CD64 acts. nCD11b appears to be promising for neonatal sepsis diagnosis ***(Hofer et al., 2012).***

Monocyte CD14(mCD14) has also been investigated as a valuable sepsis diagnostic tool, it represents a specific high-affinity receptor for the complexes of lipopolysaccharide (LPS) and LPS binding protein (LBP) which activates a specific proinflammatory signaling cascade, and thereby starting the inflammatory reaction of the host against the different infectious agents ***(Mussap et al., 2013)*.**

The soluble CD14 subtype (sCD14-ST) has been extensively researched as another biomarker which named (Presepsin), it originates from the cleavage of CD14 on the cell membrane by the cathepsin and the other lysosomal enzymes ***(Mussap et al., 2012)***.

Several studies suggest a promising role for Presepsin as an early diagnostic and prognostic sepsis marker ***(Ulla et al., 2012, Ali et al., 2016, Jacobs and Wong., 2016 & Tabl and Abed., 2016)***.