

# **Monocyte CD 40 expression in sepsis and the effect of interferon gamma administration on outcome**

Thesis submitted for partial fulfilment of doctorate degree in Critical Care Medicine

By  
**Aly makram abdrabo Aly Habib**

M.B.B.Ch, M.Sc.

Supervisors

**Prof. Hosam A. Mowafy**

Professor of internal & Critical Care Medicine

Faculty of medicine, Cairo University

**Prof. Sanaa S. AbdelShafy**

Professor of Clinical pathology

Faculty of medicine, Bany swef University

**Dr. Hassan S. Effat**

Lecturer of Critical Care Medicine

Faculty of medicine, Cairo University

Faculty of medicine

Cairo University

2006

# Acknowledgement

*I thank God that give me the strength and will to complete this work.*

*I'd like to thank Prof. Sherief Mokhtar: the previous head of the department of critical care medicine, faculty of medicine, Cairo University and the past director of the critical care center for the moral and the financial support given to this work*

*I'd like also to express my sincere gratitude and deepest appreciation to Prof. Hossam Mowafy: professor of internal and critical care medicine, the past director of department of critical care medicine, faculty of medicine, Cairo University. He was the God father for me and my colleagues during our career and guided us with his knowledge and vision.*

*I'd like to thank Professor Hasan Khaled: professor of critical care medicine, faculty of medicine, Cairo University for his unforgettable role in facilitating this collaboration with the Belgian team to bring this work into a reality.*

*I'm deeply grateful to Prof. Sanaa Abdelshafy: Professor of clinical pathology, faculty of medicine, Cairo University, for her patience, guidance, and meticulous comments. She was the hidden solider who gave much of her time and effort to bring this work to the final picture.*

*I'd like also to thank Dr Hasan Effat: Lecturer of critical care medicine, faculty of medicine, Cairo University for his continuous support and enthusiasm*

*I'd like to thank Prefessor Jean-Louis Vincent: Head of the department of intensive care medicine, Erasme University hospital, Free university of Brussels. Professor Vincent gave much of support, endless cooperation and invaluable advices to this work. His guidance and dynamicity created this work and saved it in many other times. I learnt a lot through my stay in his department.*

*I'd like to thank also Dr Olivier Pradier: The Adjoint chief of the Laboratory of Immunology and hematology department, Erasme University hospital, Free university of Brussels. He taught me everything about the flow cytometry and dedicated himself for the production of his work. Besides, he was a very good friend over the work time.*

*I'd like to thank all the residents, and working stuff of the department of intensive care medicine, Erasme University hospital, Free university of Brussels especially: Michael Piagnerilli, Marc Van nufflen, Omar Abed., Michael Shroeder. They accepted and helped me during my work.*

*I'd like also to thank the research fellows at the department of intensive care medicine, Erasme University hospital, Free university of Brussels who existed at the same time with me: Ahmed Zakaria, Alejandro Brun, Colin Verdant, Su Fhong, Carla-Maria Clausi, Arino Yagouchi and Zhen Wong. They were like my family and helped me with their ideas during the work.*

*Finally, I'd like to thank my colleagues in Cairo who replaced me in shifts and work for two years.*

## Abstract

**Authors:** Aly M Habib, MSc. Hasan S Effat, MD. Hossam A Mowafy, MD and Sanaa S Abdel Shafy, MD. Critical Care department, Faculty of medicine, Cairo University

**Introduction:** Sepsis induces an early inflammatory cascade initiated by the innate immune response. This often results in the development of multisystem organ failure. CD40 is a cell surface protein belonging to the tumor necrosis factor (TNF) receptor family. Ligation of monocyte CD40 by the T cell-derived CD40 ligand can trigger the production of various mediators, the transcription and activation of enzymes, and the upregulation of costimulatory molecules involved in the pathogenesis of sepsis. Interferon gamma (INF- $\gamma$ ) is a major activator of monocytes that increase their antigen presenting capacity. Administration of (INF- $\gamma$ ) has been shown to decrease infection. The aim of this work was to test the CD40 expression on monocyte as an objective tool to detect sepsis in ICU patients and the effect of (INF- $\gamma$ ) administration on improving survival.

**Methods:** The study included 60 patients who were admitted to the ICU with the diagnosis of sepsis (Dec 2003 - March 2004 and Sept - Nov 2004) and 15 normal healthy volunteers.. Blood was collected every day to perform flow cytometry analysis of the circulating monocytes in a lysis, no wash direct staining technique with quantification of fluorescence using the median fluorescence index (MFI). The patients were blindly randomized into 2 groups: one receiving conventional treatment of sepsis and the second receiving (INF- $\gamma$ ) 100 $\mu$ g daily by subcutaneous injection together with the conventional treatment of sepsis.

**Results:** The study included 60 patients (23 medical, 37 surgical), of whom 21 were female (36%), with a mean age of  $63 \pm 17$  years. The overall 28-day mortality was 30 % (18/60). The mean of CD40 expression on surface of monocytes of septic patients was  $7.3 \pm 0.6$ . The ROC curve for CD40 showed a specificity of 70%, a sensitivity of 75%, with an area under the curve (AUC) of 0.82, positive predictive value of 57% and negative predictive value of 81% at the level of 6.4. Administration of (INF- $\gamma$ ) in the second group of patients did not improve the survival (8/30, 26%) compared to placebo group (10/30, 33%),  $p = 0.3$ .

**Conclusion:** CD40 can be used to detect septic patients among the critically ill patients. Interferon-gamma administration was not successful in decreasing mortality among septic patients.

**Key words:** CD40, monocytes, sepsis, interferon gamma

## List of Figures

Figure	Title	Page
<b>Figure 1</b>	The Lectin Pathway of Complement Activation.	9
<b>Figure 2</b>	Signaling Pathway of Toll-like Receptors.	11
<b>Figure 3</b>	Function of Interdigitating Dendritic Cells.	15
<b>Figure 4</b>	A System Used by Natural Killer Cells to Recognize Normal Cells and Cells That Lack Major-Histocompatibility-Complex Class I Surface Molecules.	17
<b>Figure 5</b>	The Acute Inflammatory Response.	20
<b>Figure 6</b>	Structure of Immature and Mature B-Cell and T-Cell Antigen Receptors.	23
<b>Figure 7</b>	Recognition of Epitopes by B Cells.	26
<b>Figure 8</b>	Positive and Negative Selection in the Thymus.	30
<b>Figure 9</b>	The Germinal Center.	36
<b>Figure 10</b>	Activation of T Cells.	42
<b>Figure 11</b>	An Overview of Lymphocyte Responses.	45
<b>Figure 12</b>	Role of Antibodies.	47
<b>Figure 13</b>	The Receptors Involved in the Interplay of the Innate and Adaptive Immune Systems.	51
<b>Figure 14</b>	Septic shock represents the end of the spectrum of increasing inflammation and host response to a toxic insult (i.e. infection).	56

<b>Figure 15</b>	The toxic stimulus activates macrophages, and neutrophils.	59
<b>Figure 16</b>	Inflammatory insult causes PMN-PMN aggregation, resulting in microvascular occlusion and tissue ischemia.	61
<b>Figure 17</b>	Alternative pathways of TLR4 activation.	79
<b>Figure 18</b>	The Response to Pathogens, Involving "Cross-Talk" among Many Immune Cells, Including Macrophages, Dendritic Cells, and CD4 T Cells.	84
<b>Figure 19</b>	Immunologic Response of Three Hypothetical Patients with Sepsis.	88
<b>Figure 20</b>	A simplified illustration of Flow cytometry.	91
<b>Figure 21</b>	A diagram showing excitation and emission of a fluorochrome molecule.	94
<b>Figure 22</b>	The optical system schematic for the XL analyser as well as the optical configuration for the four FL PMT sensors.	96
<b>Figure 23</b>	One parameter histogram.	97
<b>Figure 24</b>	Two parameter histogram Dot Plot displaying FL1-FITC on the x axis and FL2-PE on the y axis.	98
<b>Figure 25</b>	Picture of FACScan flow cytometry.	115
<b>Figure 26</b>	Diagram showing patients included in the study.	121
<b>Figure 27</b>	Number and percentage of patients in the three groups of the study.	122
<b>Figure 28</b>	Mean $\pm$ SD age for the patients in the 3 groups included in the study.	122
<b>Figure 29</b>	Overall male and female number and percentage.	123

<b>Figure 30</b>	Males and females number in the 3 study groups.	124
<b>Figure 31</b>	Overall medical and surgical numbers and percentages.	124
<b>Figure 32</b>	Numbers of medical and surgical diagnosis in the 3 study groups.	125
<b>Figure 33</b>	Percentages of different systems affection on admission.	126
<b>Figure 34</b>	Values of MAP, and heart rate (lowest and highest).	127
<b>Figure 35</b>	Values of temperature (highest and lowest) (°C), and maximum respiratory rate (brpm).	128
<b>Figure 36</b>	Median amount of daily urine output in patients of 3 study groups.	128
<b>Figure 37</b>	Maximum PEEP for mechanically ventilated patients (cmH <sub>2</sub> O).	129
<b>Figure 38</b>	APACHE II, SOFA and GCS for patients in 3 study groups.	131
<b>Figure 39</b>	Maximum dobutamine and norepinephrine doses for 3 study groups (µgm/Kg/min).	132
<b>Figure 40</b>	Median serum lactate levels in the 3 study groups.	132
<b>Figure 41</b>	Total WBC count and lymphocyte % in the patients of the 3 study groups.	133
<b>Figure 42</b>	Median CRP levels for the patients of the 3 study groups.	134
<b>Figure 43</b>	Overall 28 days mortality for all patients.	135
<b>Figure 44</b>	Number of survivals and non survivals in 3 study groups.	135
<b>Figure 45</b>	The mean ICU length of stay in the 3 study groups	136

<b>Figure 46</b>	Panels A to P showing different markers for non septic and septic patients on day of admission to the ICU.	138-140
<b>Figure 47</b>	ROC curve on admission day for 4 markers which showed significantly different levels between septic and non septic patients.	144
<b>Figure 48</b>	Panels A to P showing different markers for NICUS patients during their stay in the ICU.	146-148
<b>Figure 49</b>	ROC curve for CD14% and Quantibrite CD64 for sepsis prediction in the NICUS patients.	150
<b>Figure 50</b>	Panel A to P showing the median values for the survivors and non survivors for the different markers tested in the first 5 days of ICU admission.	152-154
<b>Figure 51</b>	ROC curve of Quantibrite CD64 predicting 28 day mortality on the first day (panel A) and on the second day (panel B) of admission to the ICU.	156
<b>Figure 52</b>	ROC Curve of CD16b MFI predicting 28 day mortality on second day (panel A) and on third day (panel B) and fourth day (panel C) after admission.	157
<b>Figure 53</b>	ROC Curve of CD16b % predicting 28 day mortality on second day (panel A) and on third day (panel B) after admission	158
<b>Figure 54</b>	ROC Curve of quantibrite HLA-DR predicting 28 day mortality on second day (panel A) and on fifth day (panel B) after admission.	160
<b>Figure 55</b>	ROC Curve of CD14% predicting 28 day mortality on fourth day after admission.	160
<b>Figure 56</b>	ROC Curve of lymphocyte CD57 % predicting 28 day mortality on third day after admission.	161

## List of Abbreviations

TNF- $\alpha$	: Tumor necrosis factor-alpha
IL	: Interleukin.
TGF- $\beta$	: Tumor growth factor-beta.
SIRS	: Systemic inflammatory response syndrome
CARS	: Compensatory anti-inflammatory response syndrome
HLA	: Human leukocyte antigen.
APCs	: Antigen presenting cells.
CD	: Cluster of differentiation.
PMNs	: Polymorph-nuclear leukocytes.
MHC	: Major-histocompatibility-complex
NF- $\kappa$ B	: Nuclear factor- kappa beta
TLRs	: Toll like receptors.
MASP1	: Mannan-binding lectin-associated proteases 1
IRAK	: Interleukin-1 receptor-associated kinase
TRAF-6	: Tumor necrosis factor-associated factor 6
MAP3K	: Mitogen-activated protein kinase kinase kinase.
PAMPs	: Pathogen-associated molecular patterns
PRR	: Pattern-recognition receptors.
Fc $\gamma$ R	: Fc gamma receptor.
Fab	: Antigen-binding fragments.
LFA-1	: Lymphocyte-function-associated antigen 1
Ig	: Immunoglobulin
Th1	: T helper cell type 1
MOF	: Multi-organ failure
MODS	: Multi-organ dysfunction syndrome.
PAF	: Platelet-activating factor.
ROS	: Reactive oxygen species
NO	: Nitric oxide
PLA2	: Phospholipase A2

AA	: Arachidonic acid
PG	: Prostaglandin
TXA2	: Thromboxane A2
RBCs	: Red blood corpuscles
DIC	: Disseminated intravascular coagulation
TFPI	: Tissue factor pathway inhibitor
LPS	: Lipopolysaccharide
GH	: Growth hormone.
CIP	: Critical illness polyneuropathy
PMT	: Photomultiplier tube
ADC's	: Analog to digital converters
PE	: Phyco-erythrin
FLS	: Forward Light Scatter
ICU	: Intensive care unit
HIV	: Human immunodeficiency virus
NICUS	: Nosocomial ICU sepsis
CDC	: Center of disease control
SCCM	: Society of critical care medicine
ESICM	: European society of intensive care medicine
ATS	: American thoracic society
APACHE II	: Acute Physiology And Chronic Health Evaluation II
SOFA	: Sequential organ assessment score.
FITC	: Fluorescein iso-thio-cyanate
PerCP	: Peridinin chlorophyll
TCR	: T cell receptor
WBCs	: White blood cells
MPO	: Methyl peroxidase
GM-CSF	: Granulocyte- monocyte colony stimulating factor.
NIF	: Neutrophil inhibitory factor
MIF	: Monocyte inhibitory factor

## List of Tables

<b>Table</b>	<b>Title</b>	<b>Page</b>
<b>Table 1</b>	Metabolic alterations in sepsis.	74
<b>Table 2</b>	Fluorescence spectra of commonly used fluorochromes.	92
<b>Table 3</b>	Demographic baseline characteristics.	123
<b>Table 4</b>	Baseline detailed system diagnosis of patients.	125
<b>Table 5</b>	Baseline hemodynamics and laboratory findings for 3 groups	130
<b>Table 6</b>	Outcome measurements for the 3 study groups.	134
<b>Table 7</b>	Values of different markers for patients with sepsis on admission to the ICU (group1) and those without sepsis (group 2 and 3) at the same time	137
<b>Table 8</b>	The difference between the control patients (group 2) and the NICUS patients (group3).	141
<b>Table 9</b>	The predictive parameters of different markers for sepsis on admission	143
<b>Table 10</b>	The values of different markers 4 days before and on the day of clinical identification of sepsis for the NICUS patients	145
<b>Table 11</b>	The predictive parameters of CD14% and Quantibrite CD64 to sepsis development	149
<b>Table 12</b>	The median values for the markers that show significant difference between the survivors and the non survivors in the first 5 days after admission to ICU.	151
<b>Table 13</b>	The Receiving Operation Characteristic (ROC) figures for the markers that show significant difference between the survivors and the non survivors.	159
<b>Table 14</b>	Values of the different markers for the 15 normal healthy volunteers	162

## Aim of The Work

**T**he aim of this work is to study selected immune markers (which represent both innate and adaptive immune systems) on the surface of white blood cells of critically ill patients who are expected to stay for a relatively long period in the ICU

With the primary endpoints are trials to identify:

1. A marker that can predict the development of nosocomial sepsis in the ICU (NICUS) patients while their stay in the ICU.
2. A marker that can separate septic from non septic patients immediately after admission to the ICU.

The secondary end point is: Trying to identify a marker that can predict the development of mortality among the ICU patients.

# Introduction

**D**espite important advances in critical care medicine during the last decades, the mortality rate of sepsis has remained high. Recent studies have shown that sepsis is a bimodal entity. The first phase is characterized by the systemic release of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 and IL-8, and by activation of the complement and coagulation cascades. In the second phase, anti-inflammatory mediators such as TGF- $\beta$ , IL-10, Prostaglandin E<sub>2</sub> may be released in an effort to counteract ongoing inflammation. Depending whether pro or anti-inflammatory responses predominates, some people referred to a systemic inflammatory response syndrome (SIRS) and a compensatory anti-inflammatory response syndrome (CARS).<sup>(1)</sup>

The development of an adequate immune response to bacterial challenge relies on the complex interplay between the innate and specific (adaptative) immune systems. The innate immune system is dedicated to recognition of pathogens using invariant markers. Monocyte & macrophage functions include the recognition, uptake & killing of invading organisms in order to initiate an immune response then to present antigen to specific T lymphocytes to initiate the adaptative immune response. This initiation may be accomplished primarily by the Major Histo-compatibility Complex of class II comprising HLA-DR, HLA-DP, HLA-DQ, which are constitutively expressed on antigen presenting cells (APCs), with the help of costimulatory molecules such as CD40, CD80/86 and the secretion of pro-inflammatory mediators, such as TNF, IL-1, IL-12.<sup>(2, 3)</sup>

Studies have shown a decreased expression of HLA-DR on monocytes in patients with sepsis constitutes a marker of immune-paralysis. These patients might benefit from immune-stimulants, while patients with severe sepsis and normal or high monocyte HLA-DR expression might benefit from anti-inflammatory strategies. <sup>(1)</sup> Several groups have reported an increase in serum Soluble CD14 concentrations in human gram-negative and gram-positive septic shock with an associated increased mortality. <sup>(4)</sup>

On the other hand, neutrophils (PMNs) functions have been shown to follow a bimodal response during severe sepsis or septic shock with an increased expression of the functional molecule, eventually followed by a depression. During sepsis, stimulation of PMNs enhances interaction with endothelial cells <sup>(5)</sup> and PMNs appear to possess an activated phenotype as judged by an increased expression of adhesion molecule CD11b <sup>(6)</sup> and decreased expression of L-selectin. <sup>(7)</sup> Recently, it was noted that most PMNs that bind to endothelial monolayer express CD64.

Regarding the lymphocytes, many groups <sup>(8, 9)</sup> have described increase in the expression of CD 69 and DR during sepsis, which are considered activation markers of the T lymphocytes.

## Chapter I

### The Immune System

**T**he immune system is an organization of cells and molecules with specialized roles in defending against infection. There are two fundamentally different types of responses to invading microbes. Innate (natural) responses occur to the same extent however many times the infectious agent is encountered, whereas acquired (adaptive) responses improve on repeated exposure to a given infection. The innate responses use phagocytic cells (neutrophils, monocytes, and macrophages), cells that release inflammatory mediators (basophils, mast cells, and eosinophils), and natural killer cells. The molecular components of innate responses include complement, acute-phase proteins, and cytokines such as the interferons. Acquired responses involve the proliferation of antigen-specific B and T cells, which occurs when the surface receptors of these cells bind to antigen. Specialized cells, called antigen-presenting cells, display the antigen to lymphocytes and collaborate with them in the response to the antigen. B cells secrete immunoglobulins, the antigen-specific antibodies responsible for eliminating extracellular microorganisms. T cells help B cells to make antibody and can also eradicate intracellular pathogens by activating macrophages and by killing virally infected cells. Innate and acquired responses usually work together to eliminate pathogens. <sup>(10)</sup>

All these cells develop from pluripotent stem cells in the fetal liver and in bone marrow and then circulate throughout the extracellular fluid. B