



**Early prediction of Gram negative
bacteremia in febrile cancer patients:
Correlation between some inflammatory
mediators, exposure to gamma radiation
and severity of infection**

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree

In Pharmaceutical Sciences
(Microbiology and Immunology)

By

Amira Abuzeid Abdelbaset

Bachelor of Pharmaceutical sciences, 2007
Pharmacist at National Centre for Radiation Research and
Technology (NCRRT)

2016



Early prediction of Gram negative bacteremia in febrile cancer patients: Correlation between some inflammatory mediators, exposure to gamma radiation and severity of infection

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

Master degree

In Pharmaceutical Sciences

(Microbiology and Immunology)

By

Amira Abuzeid Abdelbaset

Pharmacist at National Centre for Radiation Research and Technology
(NCRRT)

Under Supervision of

Dr. Mohammad Mabrouk Aboulwafa, PhD

Professor of Microbiology and Immunology,
Faculty of Pharmacy, Ain Shams University

Dr. Hala Abdallah Farrag, PhD

Professor of Medical Microbiology,
National Centre for Radiation Research and Technology (NCRRT)
Atomic Energy Authority

Dr. Khaled Mohamed Anwar Aboshanab, Ph.D

Associate Professor of Microbiology and Immunology,
Faculty of Pharmacy, Ain-Shams University

2016

ACKNOWLEDGEMENT

No words can ever express my sincere gratitude to ALLAH who guide, aid and bless me in everything and everywhere in my life.

I offer my sincere thanks and appreciations to **Prof. Dr. Mohammad Mabrouk Aboulwafa**, Professor of Microbiology and Immunology, Chairman of National organization for research and control of biological products, Former head of Microbiology and Immunology department and Former Vice dean for community service and environmental development affairs, Faculty of Pharmacy, Ain Shams University. Professor Aboulwafa structured the study protocol to comply its specific requirement format and meticulously refined the experimental data in a knowledgeable form as appeared in their presented form. His efforts in revising the thesis can not be forgotten.

I wish to express my sincere appreciation, deepest thanks and gratitude to **Prof. Dr. Hala Abdallah Farrag** Professor of Medical Microbiology and Head of Biotechnology sector, National Center for Radiation Research and Technology (NCRRT) for her valuable time in the supervision during all stages of this study, continuous facilities offered to accomplish this work. Professor H. Farrag suggested the idea, designed the study, wrote the protocol, managed the analysis of the study and revised the thesis. I have the honor and pleasure to be under her supervision.

I am greatly indebted to **Prof. Dr. Khaled Mohamed Aboshanab**, Associate Professor of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, for his keen supervision, beneficial discussion, great effort and continuous encouragement and effective support in all stages of the work.

I record my cordial thanks and gratefulness to my father, my mother, my brothers and my husband for their praying, assistance, patience, understanding and their effective support.

My hearty thanks **Dr. Asrar Mourad** for her great help in the section of animal infection model.

Finally, I am thankful also, to my colleagues and staff members in Drug Radiation Research Department, National Center for Radiation Research and Technology for their friendly cooperation and encouragement.

Amira Abuzeid

Table of Contents

ACKNOWLEDGEMENT.....	
TABLE OF CONTENTS.....	I
LIST OF ABBREVIATIONS	IV
LIST OF FIGURES.....	VI
LIST OF TABLES.....	VIII
ABSTRACT	1
INTRODUCTION	6
LITERATURE REVIEW	10
1. Bloodstream infection.....	10
2. Overall picture of Gram-negative bacteremia	14
3. What happens when microorganism enters body?.....	16
4. Cytokines.....	20
5. The role of chemokines in infectious diseases (Bacterial infections).....	23
6. The complement system.....	26
7. Fever as a sign of infection.....	27
8. Blood cultures for identifying bacteremia.....	28
9. Antimicrobial resistance	30
10. Ionizing Radiation.....	30
11. Radiotherapy and inflammatory mediators.....	38
12. The ROC curve analysis.....	39
MATERIALS AND METHODS.....	42
MATERIALS.....	42
1. Microorganisms	42
2. Blood specimens	42
3. Media	42
4. Kits	42
5. Blood culture bottles.....	43
6. Tubes used for plasma and sera separation	43
7. Diffu-plate.....	43
8. Antimicrobial discs	43
9. Laboratory animals	43
10. Devices.....	44

METHODS.....	45
11. Collection and manipulation of specimens	45
12. Plasma and serum samples collection.....	45
13. Isolation of pathogenic bacteria	45
14. Identification of isolated organisms from blood specimens	46
15. Total leukocytic count assay	47
16. Assay of serum C-Reactive Protein (CRP).....	47
17. Assay of serum Interleukin-6 (IL-6)	48
18. Assay of serum Interleukin-8 (IL-8)	49
19. Assay of serum complement C3.....	51
20. Antimicrobial susceptibility of pathogenic Gram negative bacterial isolates.....	51
21. Effect of <i>in-vitro</i> gamma radiation on some selected isolates.....	53
22. Detection of Lipase and Protease enzymes production.....	54
23. Effect of gamma radiation at dose levels of 2 and 7.5 Gy on IL-6 serum levels in rats with induced fever of bacterial and non-bacterial origin	54
24. Assay of IL-6 serum concentration in rats.....	56
25. Statistical methods	57
RESULTS	58
1. Recovery, Collection and identification of pathogenic bacteria recovered from blood cultures.....	58
2. Age, sex, white blood cells count and serum levels of CRP levels for cancer and non-cancer patients.....	58
3. Interleukin-6 serum levels (sIL-6) in cancer and non-cancer patients	64
4. Interleukin-8 serum levels (sIL-8) in cancer and non-cancer patients.....	66
5. Relationship between CRP, sIL-6 and sIL-8 in some selected cancer and non-cancer patients with positive and negative blood cultures	68
6. Relationship between white blood cells count and each of CRP, sIL-6 and sIL-8 in some selected cancer and non-cancer patients with positive blood cultures	71
7. Statistical analysis of CRP and IL-6 serum markers of some selected cancer and non-cancer patients with positive blood cultures by Receiver Operating Characteristic Curves (ROC).....	74
8. Complement C3 serum levels (C3) in cancer and non-cancer patients	82
9. <i>In-vitro</i> effect of gamma irradiation on antimicrobial susceptibility of some bacterial isolates recovered from cancer and non-cancer patients.....	83

10.Activity profiles of lipase and protease enzymes as virulence factors of some selected bacterial species recovered from cancer and non-cancer patients.....	95
11.Effect of gamma irradiation on IL-6 serum levels and WBCs in rats with induced fever of bacterial and non-bacterial origin.....	97
12.Statistical analysis of IL-6 serum levels in bacteremic groups both with and without exposure to gamma radiation by Receiver Operating Characteristic Curve (ROC).....	101
DISCUSSION	103
SUMMARY.....	119
REFERENCES.....	124
الملخص العربي	

List of Abbreviations

AK	Amikacin
AMC	Amoxicillin/Clavulanic Acid
AUC	Area under the curve
BSI	Bloodstream infection
C	Chloramphenicol
C3	Complement 3
CAZ	Ceftazidime
CN	Gentamicin
CRD	Carbohydrate recognition domain
CRP	C- reactive protein
CTX	Cefotaxime
DNA	Deoxyribonucleic acid
Eff	Efficacy
ELISA	Enzyme linked immunosorbent assay
FEP	Cefepime
FN	Febrile neutropenia
FUO	Fever of unknown origin
Gy	Gray
HRP	Horseradish peroxidase
i.p	Intraperitoneal
ICU	Intensive care unit
IFN	Interferon
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-8	Interleukin-8
IPM	Imipenem
K₂EDTA	Potassium salt of ethylene diamine tetraacetic acid
LEV	Levofloxacin
LOS	Lipooligosaccharide
LPB	Lipopolysaccharide binding protein
LPS	Lipopolysaccharides
LTA	Lipoteichoic acid

MBL	Mannose binding lectin
MCP-1	monocyte chemoattractant protein -1
NDL	Non diagnostic level
NPV	Negative predictive value
OFX	Ofloxacin
PAMP	pathogen-associated molecular patterns
PBS	phosphate-buffered saline
PMNs	Polymorphnuclear leukocytes
PPV	Positive predictive value
ROC	Receiver Operating Characteristic
SAM	Ampicillin/ Salbactam
SIRS	Systemic inflammatory response syndrome
Sn	Sensitivity
Sp	Specificity
SXT	Sulphamethoxazole/ trimethoprim
TLR	Toll like receptor
TMB	3,3',5,5'-Tetramethylbenzidine
TNF-α	Tumor necrosis factor- α
TOB	Tobramycin
TR	Rectal temperature
TZP	Tazobactam/Piperacillin
WBCs	White blood cells

List of Figures

Figure 1	Distribution of overlapped test results.	40
Figure 2	The ROC curve distribution between Sensitivity and Specificity.	41
Figure 3	BD BACTEC 9050 Blood culture System.	44
Figure 4a	Standard calibration curve of Human IL-6 serum concentration as determined by ELISA technique.	65
Figure 4b	Standard calibration curve of Human IL-8 serum concentration as determined by ELISA technique	67
Figure 5	Relationship between IL-8 and CRP serum concentrations in (a) cancer patients and (b) non-cancer patients both with Gram negative bacteremia.	70
Figure 6	Relationship between IL-8 and IL-6 serum concentrations in (a) cancer patients and (b) non-cancer patients both with Gram negative bacteremia.	70
Figure 7	Relationship between IL-6 and CRP serum concentrations in (a) cancer patients and (b) non-cancer patients both with Gram negative bacteremia.	71
Figure 8	Relationship between WBCs count and serum Levels of IL-6 both simultaneously measured in (a) cancer patients and (b) non-cancer patients both with positive blood cultures.	73
Figure 9	Relationship between WBCs count and serum Levels of IL-8 both measured simultaneously in (a) cancer patients and (b) non-cancer patients both with positive blood cultures.	73
Figure 10	Relationship between WBCs count and serum Levels of CRP both measured simultaneously in (a) cancer patients and (b) non-cancer patients both with positive blood cultures.	73
Figure 11	ROC curve analysis showing the diagnostic performance of CRP and IL6 for discriminating patients with positive culture from those negative among cancer patients	76
Figure 12	ROC curve analysis showing the diagnostic performance of CRP and IL6 for discriminating patients with positive culture from those with negative culture among non-cancer patients	78

Figure 13	ROC curve analysis showing the diagnostic performance of CRP and IL6 and their combinations for discriminating patients with positive culture from those negative culture among cancer and non-cancer patients.	82
Figure 14	Relative percentages of different susceptibility profiles (sensitive, intermediate resistant and resistant) of some selected bacterial species recovered from cancer patients against some antimicrobial agents inhibiting bacterial cell wall synthesis before and after gamma irradiation.	88
Figure 15	Relative percentages of different susceptibility profiles (sensitive, intermediate resistant and resistant) of some selected bacterial species recovered from cancer patients against some antimicrobial agents inhibiting protein synthesis before and after gamma irradiation.	89
Figure 16	Relative percentages of different susceptibility profiles (sensitive, intermediate resistant and resistant) of some selected bacterial species recovered from cancer patients against some antimicrobial agents inhibiting nucleic and folic acids syntheses before and after gamma irradiation.	90
Figure 17	Relative percentages of different susceptibility profiles (sensitive, intermediate resistant and resistant) of some selected bacterial species recovered from non-cancer patients against some antimicrobial agents inhibiting bacterial cell wall synthesis before and after gamma irradiation	92
Figure 18	Relative percentages of different susceptibility profiles (sensitive, intermediate resistant and resistant) of some selected bacterial species recovered from non-cancer patients against some antimicrobial agents inhibiting protein synthesis in bacteria before and after gamma irradiation	93
Figure 19	Relative percentages of different susceptibility profiles (sensitive, intermediate resistant and resistant) of some selected bacterial species recovered from non-cancer patients against some antimicrobial agents inhibiting nucleic and folic acids syntheses before and after gamma irradiation	94
Figure 20	ROC curve analysis showing the diagnostic performance of serum IL-6 for discriminating rats with bacteremia (bacteremic fever) with exposure to gamma radiation from those without exposure to gamma radiation	102

List of Tables

Table 1	Characters of antibiotic discs used for antimicrobial susceptibility test	43
Table 2	The interpretive standards breakpoints in mm for the selected antibiotics	52
Table 3	Rat groups used for testing the effect of gamma irradiation on serum level of IL-6	55
Table 4	Number and frequency of positive and negative cases for microbial growth of blood culture samples from cancer and non-cancer patients	58
Table 5	Age, sex, white blood cells count, serum CRP measurements and bacterial species isolated from cancer patients with bacterial infection (positive blood cultures)	59
Table 6	Age, sex, white blood cells count and serum CRP measurements of cancer patients without bacterial infection (negative blood cultures)	60
Table 7	Age, sex, white blood cells count serum CRP measurements and bacterial species isolated from non-cancer patients with bacterial infection (positive blood cultures)	61
Table 8	Age, sex, white blood cells count and serum CRP measurements of non-cancer patients without bacterial infection (negative blood cultures)	62
Table 9	Numbers and percentages of different bacterial species recovered from cancer and non-cancer patients	63
Table 10	Levels of CRP of some cases of cancer and non-cancer patients with positive and negative blood cultures	64
Table 11	Levels of serum IL-6 in some cases of cancer and non-cancer patients with positive and negative blood cultures	66
Table 12	Levels of serum IL-8 in some cases of cancer and non-cancer patients with positive and negative blood cultures	68
Table 13	Summarization of Serum levels of CRP, IL-6 and IL-8 in cancer and non-cancer patients with positive and negative blood cultures	69
Table 14	Mean values of tested serum markers (CRP, IL-6 and IL-8) in some selected cancer and non-cancer patients	70
Table 15	Relationship between WBCs count and serum Levels CRP, IL-6 and IL-8 measured at the same time in some selected cancer and non-cancer patients with positive blood cultures	72
Table 16	Diagnostic validity test results for serum CRP to discriminate positive and negative blood cultures in cancer patients	75

Table 17	Diagnostic validity test results for serum IL-6 to discriminate positive and negative blood cultures in cancer patients	76
Table 18	Diagnostic validity test results for serum CRP to discriminate positive and negative blood cultures in non-cancer patients	77
Table 19	Diagnostic validity test results for serum IL-6 to discriminate positive and negative blood cultures in non-cancer patients	78
Table 20	Diagnostic validity test results for serum CRP to discriminate positive and negative blood cultures (cancer and non-cancer patients)	79
Table 21	Diagnostic validity test results for serum IL-6 to discriminate positive and negative blood cultures among cancer and non-cancer patients	80
Table 22	Multi-ROC test results of CRP with IL6 at cut-off value of 120.9 pg/ml to discriminate positive and negative blood cultures among cancer and non-cancer patients	81
Table 23	Levels of serum C3 in some selected cancer and non-cancer patients with positive and negative blood cultures	83
Table 24	Susceptibilities of some selected bacterial species recovered from cancer patients against different antimicrobial agents before and after gamma radiation exposure	85
Table 25	Susceptibilities of some selected bacterial species recovered from non-cancer patients against different antimicrobial agents before and after gamma radiation exposure	86
Table 26	Profiles of lipase and protease enzymatic activities, number of resistant antimicrobial agents, serum levels of IL-6 and IL-8 of some selected bacterial species recovered from cancer and non-cancer patients	96
Table 27	White blood cells count and IL-6 serum concentration in control group (saline only)	97
Table 28	White blood cells count and IL-6 serum concentration in radiation control group	98
Table 29	Rectal temperatures, white blood cells count and IL-6 serum concentrations in fever of non-bacterial origin (yeast fever) group	98
Table 30	Rectal temperatures, white blood cells count and IL-6 serum concentrations in fever of non-bacterial origin (yeast fever) group with gamma radiation exposure	98
Table 31	Rectal temperatures, white blood cells count and IL-6 serum concentrations in fever of bacterial origin (<i>Pseudomonas aeruginosa</i>) group	99
Table 32	Rectal temperatures, white blood cells count and IL-6 serum concentrations in fever of bacterial origin (<i>Pseudomonas aeruginosa</i>) group with gamma radiation exposure	99

Table 33	Rectal temperatures, white blood cells count and IL-6 serum concentrations in fever of bacterial origin (<i>Klebsiella pneumoniae</i>) group	99
Table 34	Rectal temperatures, white blood cells count and IL-6 serum concentrations in fever of bacterial origin (<i>Klebsiella pneumoniae</i>) group with gamma radiation exposure	100
Table 35	Rectal temperatures, white blood cells count and IL-6 serum concentration in different groups of rats	100
Table 36	ROC analysis data of sIL-6 for bacteremic groups with and without exposure to gamma radiation	102

Abstract

Bloodstream infections (BSI) have a significant impact on morbidity and mortality in the general population and in critically ill patients, it has worse outcomes as immunocompromised patients. The host response to such infection varies from clinical signs and release of certain inflammatory mediators, including Interleukin-6 (IL-6), Interleukin-8 (IL-8). Suggesting that inflammatory cytokines are already released in febrile patients before positive reports of microbiological cultures.

The usefulness of these mediators, is to be used as early predictors of bacteremia caused by Gram negative bacilli during onset of fever in cancer and non-cancer patients, in comparison to traditionally used markers as C-reactive protein (CRP) and complement C3 (C3) was reported in our study.

One hundred twenty four feverish (cancer and non-cancer) in-patients were enrolled in the study. Serum samples were separated from collected blood samples at onset of fever for assay of inflammatory biomarkers IL-6 and IL-8 (using (Enzyme linked immunosorbent assay) ELISA technique), CRP (by Turbiquickreader) and C3 (by Diffu-plate). Plasma samples were separated for assay of total leukocytic count (using Beckman/Coulter semi automated). Blood samples were collected in blood culture bottles (BACTEC Peds Plus™/F) and cultured on MacConkey's agar No.3 for isolation of Gram negative bacilli which were identified by API 20E technique. Antimicrobial susceptibility test was performed (by disc diffusion method) using 14 antimicrobial agents with different mode of actions with and without exposure to gamma radiation at dose level of 24.4 Gy which is biologically equivalent to the fractionated multiple therapeutic dose used in the protocol of cancer therapy as well as lipase and protease enzymatic activities as virulence factors were performed (via tween and gelatin agar plates, respectively). Assay of serum IL-6 in rats was also done using ELISA technique. Cesium 137 (¹³⁷Cs) Gamma cell 40 located at National Center for Radiation Research and Technology (Cairo, Egypt) was the irradiation source used in the study.

Seventy cancer patients were representing 56% of all cases and 54 non-cancer patients were representing 44% were non-cancer patients. Positive blood culture samples

for Gram negative bacilli in cancer and non-cancer patients were 49% and 54%, respectively.

The most predominant Gram negative isolates in cancer patients were *E. coli* representing 41%, followed by *Pseudomonas* species as 27%, then *K. pneumoniae* as 23%. While, in non-cancer patients the most predominant isolates were *K. pneumoniae* representing 41%, followed by *E. coli* as 38%, then 4% of *Pseudomonas* sp.

IL-6 and IL-8 serum levels were higher in feverish patients with Gram negative bacteremia comparing to those with non-microbial fever for both groups (cancer and non-cancer) of patients. For cancer patients with Gram negative bacteremia and those without there was significant difference in IL-6 and IL-8 serum levels (**P=0.0001** and **0.0059**, respectively). Similar results were also obtained for non-cancer patients (**P=0.0288** and **0.0059**). Moreover, serum levels of both mediators were higher in cancer patients with Gram negative bacteremia than in non-cancer patients with Gram negative bacteremia.

The Cut-off levels to distinguish between bacteremic (positive blood cultures) and non-bacteremic (negative blood cultures) cases were determined using receiver operating characteristic curves (ROC): for CRP it was 29 mg/l for cancer patients, 119 mg/l for non-cancer patients and with 60% and 100% specificity and NPV 60% and 62.5%, respectively.

While, the cut-off level of serum IL-6 was 398.6 pg/ml for cancer patients, 120.9 pg/ml for non-cancer patients and with 100% specificity and NPV 100% for both groups. So the efficacy of IL-6 as marker to discriminate between positive and negative blood cultures was higher than that of CRP.

The ROC curve analysis showing diagnostic performance of CRP and IL-6 and their combination (via multi-ROC) could be used for discriminating patients with positive cultures from those with negative cultures (all cancer and non-cancer tested cases). We found that the best cut-off value of IL-6 was 120.9 pg/ml with 60% specificity, 100% sensitivity, NPV 100%, PPV 85.7%, Efficacy 88.2%, while for CRP the cut-off value was 85.9 mg/l with 50% specificity. Using the multi-ROC for both markers to improve the results for CRP, we found that CRP best cut-off value was 220 mg/l at IL-6 of 120.9 pg/ml with improved Sp, Sn, NPV, PPV and Efficacy of values 90%, 100%, 100%, 96%