Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

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)

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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

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Amira Abuzeid

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List of Abbreviations

AK	Amikacin
AMC	Amoxacillin/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
K_2EDTA	Potassium salt of ethylene diamine tetraacetic acid
LEV	Levofloxacin
LOS	Lipooligosaccharide
LPB	Lipoplopysaccharide 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

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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 bottels (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 ratss was also done using ELISA technique. Cesium 137 (^{137}Cs) 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 4 4% 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. pneumonia*e as 23%. While, in non-cancer patients the most predominant isolates were *K. pneumonia*e 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 Gam 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 resultswere 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-cancertested 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%