FUNGAL AND BACTERIAL INFECTIONS IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

Thesis

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*First and foremost, thanks to **Allah**, the most beneficent, the most merciful.*

I dedicate this thesis to the soul of my mother

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Abstract

Infectious complications are an important cause of morbidity and mortality in febrile neutropenic (FN) patients with hematological malignancies.

Aim of the study: to detect bacterial and fungal pathogens associated with infections in FN patients, assessing the current treatment protocol followed and determining outcome of febrile neutropenic episodes.

Methods: The present study included 31 patients who experienced 65 FN episodes. All patients were subjected to full detailed history, complete physical examination, radiological investigations, complete blood picture, blood culture, other cultures according to the presenting symptoms and signs, galactomannan test for selected cases and PCR for sepsis screen for selected cases.

Results: Blood stream infections were diagnosed in 23.1% of episodes by either blood cultures or PCR. Other cultures were positive in 4.6% of episodes. Bacterial infections were detected alone in 15% of episodes; fungal infections alone in 14% of episodes, while both bacterial and fungal infections were detected in the same episode in 8% of episodes. Gram positive bacteria (57.9%) were more prevalent than gram negative bacteria (42.1%). CoNS were the most frequent microorganisms isolated. High resistance to ceftazidime was reported among gram-negative isolates. There was one proven fungal infection with *Candida* non-albican, 7 probable and 6 possible fungal infections. Mortality rate among patients was 7.7%.

Conclusion: Blood culture is a very important tool to diagnose bacteremia in FN patients. CoNS are the most common microorganisms isolated from FN patients. Antibiotic susceptibility is essential to guide the treatment in FN patients.

Key words:

Febrile neutropenia, Hematological malignancy, fungal infections, Blood culture.

Contents

List of Abbreviations

ALL	**Acute lymphoid leukemia**
AMB	**amphotericin B**
AML	**Acute Myeloid Leukemia**
ANC	**Absolute Neutrophil Count**
BAL	**Broncho-alveolar lavage**
BG	**Beta D- Glucan**
CD	**Cluster of Differentiation**
CHOP	**(C)yclophosphamide, (H)ydroxydaunorubicin, (O)ncovin (vincristine) and (P)rednisone or (P)rednisolone**
CHOP- R	**CHOP- Rituximab**
CLSI	**Clinical Laboratory Standard Institute**
CMV	***Cytomegalovirus***
CoNS	**Coagulase negative *Staphylococci***
CSF	**Colony Stimulating Factor**
DOM	**Detection Oligo-Mix**
DPO	**Dual priming oligonucleotide**
DR	**Drug resistance**
ELISA	**Enzyme Linked Immunosorbent Assay**
EORTC	**European Organization for Research and Treatment Center**
ESBL	**Extended-spectrum beta-lactamase**
FN	**Febrile neutropenia**
FUO	**Fever of Unknown Origin**
G- CSF	**Granulocyte Colony Stimulating Factor**
GM	**Galactomannan**
GNB	**Gram negative bacteria**
GPB	**Gram positive bacteria**

HSCT	**hematopoietic stem cell transplantation**
HSV	***Herpes simplex* Virus**
HZV	***Herpes Zoster* Virus**
IA	**Invasive aspergillosis**
IDSA	**Infectious Diseases Society of America**
IFI	**Invasive Fungal Infection**
IV	**Intravenous**
KPC	***Klebsiella pneumoniae* carbapenemase**
MASCC	**Multinational Association for Supportive Care in Cancer**
MDR	**Multi- drug resistant**
MGUS	**Monoclonal Gammopathy of Unknown Significance**
MM	**Multiple Myeloma**
MRSA	**Methicillin-resistant *Staphylococcus aureus***
MSG	**Mycoses Study Group**
NADPH	**Reduced form of Nicotinamide Adenine Dinucleotide Phosphate**
NCCN	**National comprehensive cancer network**
NET	**Neutrophil Extraction Trap**
NHL	**Non- Hodgkin Lymphoma**
PCP	***Pneumocystis Carinii* Pneumonia**
RBC	**Red Blood Cell**
ROS	**Reactive Oxygen Species**
RS	**Reed Steinberg**
TMB	**tetramethylbenzidine**
TMP/SMX	**trimethoprim/sulfamethoxazole**
VRE	**Vancomycin-resistant *Enterococci***
WHO	**World Health Organization**
WPC	**Whole Process Control**

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INTRODUCTION

Neutropenia is defined as a decrease in the absolute number of neutrophils in the blood to less than 1500cell/µl. It is a common side effect of cancer chemotherapy. Apart from being associated with considerable morbidity and mortality, neutropenia could also result in the dose reduction or a delay in chemotherapy courses that could have a detrimental effect on cancer patients' quality of lives and could cause a considerable economic burden **(Dale, 2005).** Neutropenia, resulting from cytotoxic chemotherapy is the most common risk factor for severe infections in hematological malignancies **(Sharma et al, 2005).** Fever is the principle sign of infection in neutropenic patient and frequently may be the only evidence of infection. The pattern of fever in neutropenia is non-specific and not pathognomonic of any type of infections or non-infectious process non-infectious etiologies of pyrexia include: the underlying malignancy, chemotherapy, transfusion of blood products, antimicrobial agents, colony stimulating factor (CSF) or allergic reaction (**László Rókusz, 2005).**

Infections in febrile neutropenia might be bacterial, fungal or viral in etiology. The spectrum of bacterial isolates in febrile neutropenia has changed considerably over the past four decades. European Organization for Research and Treatment of Cancer (EORTC) has shown that the pattern of microorganisms isolated changes almost every 2-3 years. Therefore, it is suggested to study the pattern of infections and causative organisms at an interval of 2-3 years. The prevalence of gram-negative organisms decreased, whereas the prevalence of gram-positive organisms increased **(Saeidpour et al, 2008).**

Invasive fungal infections are a primary cause of morbidity and mortality in patients with hematological malignancies. Establishing a definite diagnosis of invasive fungal infection in febrile neutropenic patients is particularly challenging and time-consuming, but a delay of antifungal treatment leads to higher mortality. This situation has led to the strategy of initiation "empirical" antifungal therapy prior to the detection of fungi. Meanwhile, improvements in diagnostic procedures are achieved, especially with imaging techniques and nonculture based methods which include antigen-based assays and molecular detection of fungal DNA from body fluid samples **(Ruhnke et al, 2003).**

AIM OF THE WORK

1- To determine the bacterial and fungal etiology of infections in febrile neutropenic patients with hematological malignancies.
2- To determine the antibiotic susceptibility pattern of bacterial isolates.
3- To assess the current treatment protocol of febrile neutropenia followed in the Oncology Department in Kasr al Ainy School of Medicine.
4- To determine outcome of febrile neutropenic episodes.

NEUTROPHILIC GRANULOCYTES

1-1 - Formation of Neutrophilic Granulocytes.

All blood cells are derived from hematopoietic stem cells in bone marrow. After stem cell division, daughter cells commit to either the lymphoid or myeloid lineage. Through division, differentiation and maturation, the myeloid progenitor cells give rise to red cells, thrombocytes, monocytes and granulocytes **(Yoder, 2009).** At an early stage in granulopoiesis, the stem cells convert into unipotent myeloblasts, which differentiate and maturate subsequently to promyelocytes, myelocytes and metamyelocytes, (figure 1). The transition to the myelocyte stage is associated with the acquisition of secondary or "specific" granules, which give the characteristic staining that differentiates neutrophils from eosinophils and basophils. Neutrophlic metamyelocytes differentiate into band forms and segmented neutrophils, which constitute more than 50 % of the total granulocyte mass, primarily as a mobilizable pool of cells, in the bone marrow **(Khanna-Gupta and Berliner, 2009).**

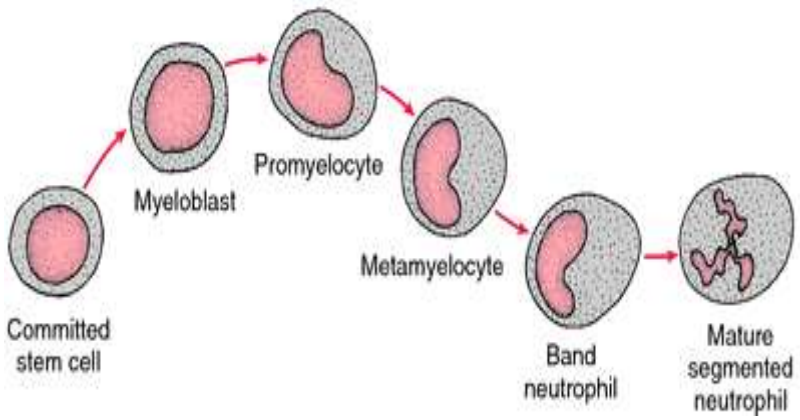

Figure 1: Neutrophil maturation (Ignatavicius and Workman, 2002)

The circulating neutrophil pool represents only 5% of the body's total neutrophil number. Maturation of neutrophil precursors occur over 6 to 10 days. The majority of mature neutrophils constitute a storage pool that remains in bone marrow poised for release as needed. The proliferating pool and storage pool together make up about 95% of total granulocyte mass. Of the remaining 5% of neutrophils that enter peripheral circulation about 60% are "a marginating pool" that adheres to the vascular endothelium and easily mobilized into circulation in response to stress. **(Greer et al, 2013)**

Circulating neutrophil survive only for 6 to 12 hours. When mobilized to sites of infection or inflammation, they can migrate into tissues where they survive for 1 to 4 days. **(Ronald et al, 2013)**

1-2- Role of Neutrophilic Granulocytes In Host Defense Against Bacteria And Fungi.

Neutrophils are essential for host defense; they play a crucial role in the immune defense against bacterial and fungal pathogens, and participate in the development of the inflammatory reaction **(Nathan, 2006).**

Recruitment of neutrophils from the bloodstream into the sites of infection is initiated by chemokines and cytokines for example secreted by infected epithelia. Bacteria also produce molecules that directly recruit neutrophils. Before their transmigration from the bloodstream through the endothelium to tissues, neutrophils begin to roll along vessel walls. This "rolling" is mediated by glycoproteins called selectins. Adhesion to the vessel wall is mediated by leukocyte adhesion molecules such as integrins. Once firmly bound, several neutrophil surface CD-molecules

facilitate transmigration into tissues. At the infection site, microorganisms and microbial compounds activate the neutrophils via transmembrane receptors **(Kobayashi et al. 2005 and Ermert et al, 2008).** At the site of infection, neutrophils bind and ingest the invading microorganisms by phagocytosis, forming bacteria-containing phagosomes **(Kobayashi et al, 2005)** (figure 2). Once phagosome is formed, neutrophils begin a professional antimicrobial killing program **(Nordenfelt and Tapper, 2011)**. Various noxious agents, including reactive oxygen species (ROS) generated through the activity of the NADPH oxidase **(Leto and Geiszt, 2006),** and the contents of intracellular granules are then released into the phagosome, resulting in killing and digestion of the microorganisms **(Borregaard et al., 2007 and Soehnlein, 2009)**.

Activated neutrophils can also eliminate microbes in the extracellular space by releasing chromatin decorated granular antimicrobials **(Brinkmann et al. 2004),** the so called neutrophil extracellular traps (NETs), which capture and kill microorganisms such as *Shigella flexneri*, *Staphylococcus aureus* (*S. aureus*) and *Candida albicans* **(Ermert et al. 2008).** However improper activation may also lead to tissue damage during an autoimmune or exaggerated inflammatory reaction **(Nathan, 2006 and Nemeth et al, 2012).**

The phagocytosis of bacteria triggers the synthesis of neutrophil genes encoding immunomodulatory agents. These cytokines and chemokines recruit more neutrophils, modulate subsequent neutrophil functions and are involved in coordinating early responses of monocytes, macrophages, dendritic cells and lymphocytes in the inflammatory states. The production of these agents by neutrophils serves as a link between the innate and acquired immune responses **(Scapini et al, 2000).**

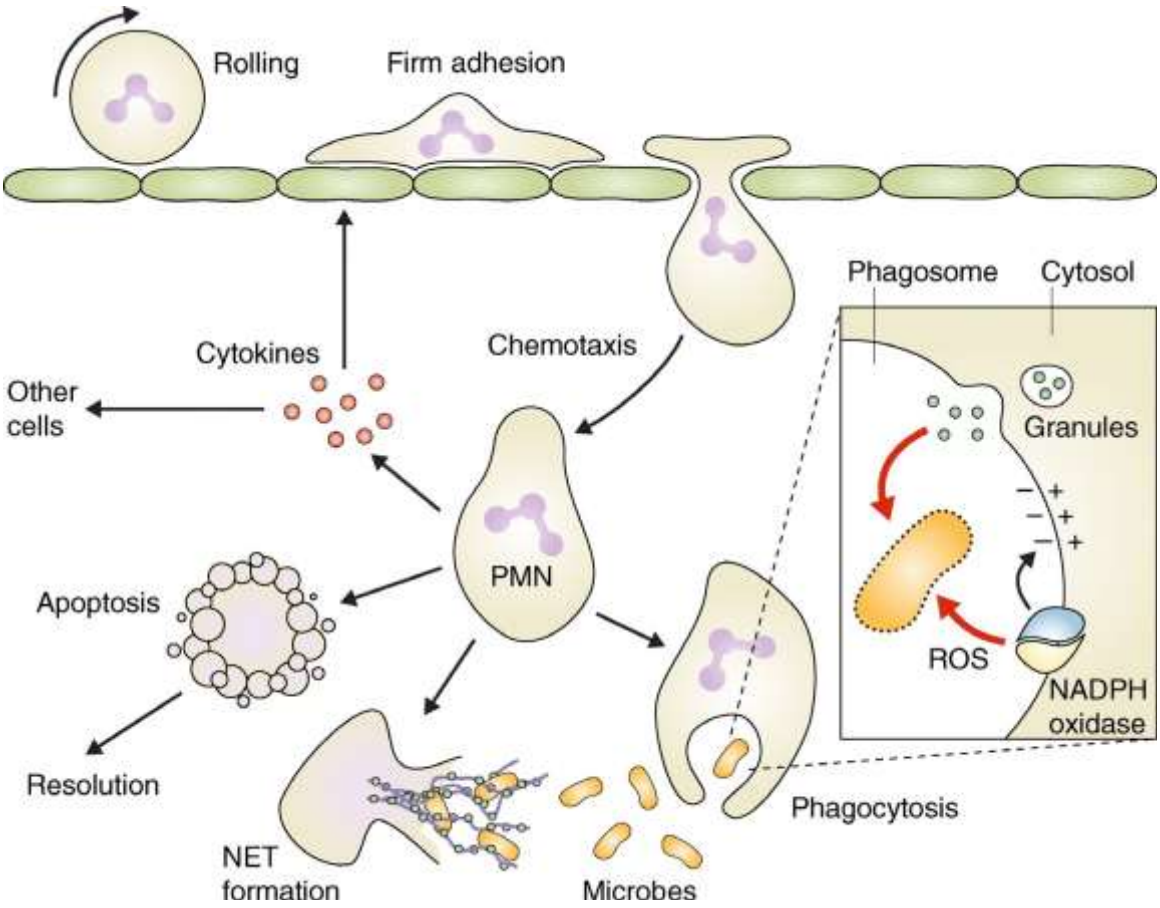

Figure 2: Neutrophil functions: After migrating to the site of inflammation, neutrophils (PMN) phagocytose and digest the invading microbes; release NETs, which likely trap bacteria; and produce cytokines, which contribute to the inflammatory reaction. Once infection is cleared, neutrophils die by apoptosis and trigger an active program to resolve inflammation. Inset, pathogen killing inside the phagosome occurs by ROS generated by the NADPH oxidase, as well as by granule enzymes released from intracellular granules. The NADPH oxidase also induces depolarization of the phagosomal membrane, which may be required for providing optimal environment inside the phagosome. (Mocasi, 2013).

NETs: Neutrophil extracellular traps

ROS: Reactive Oxygen species

NADPH: reduced form of NADP" Nicotinamide adenine dinucleotide phosphate".