Detection of Galactomannan Antigen in Diagnosis of Invasive Aspergillosis

Thesis

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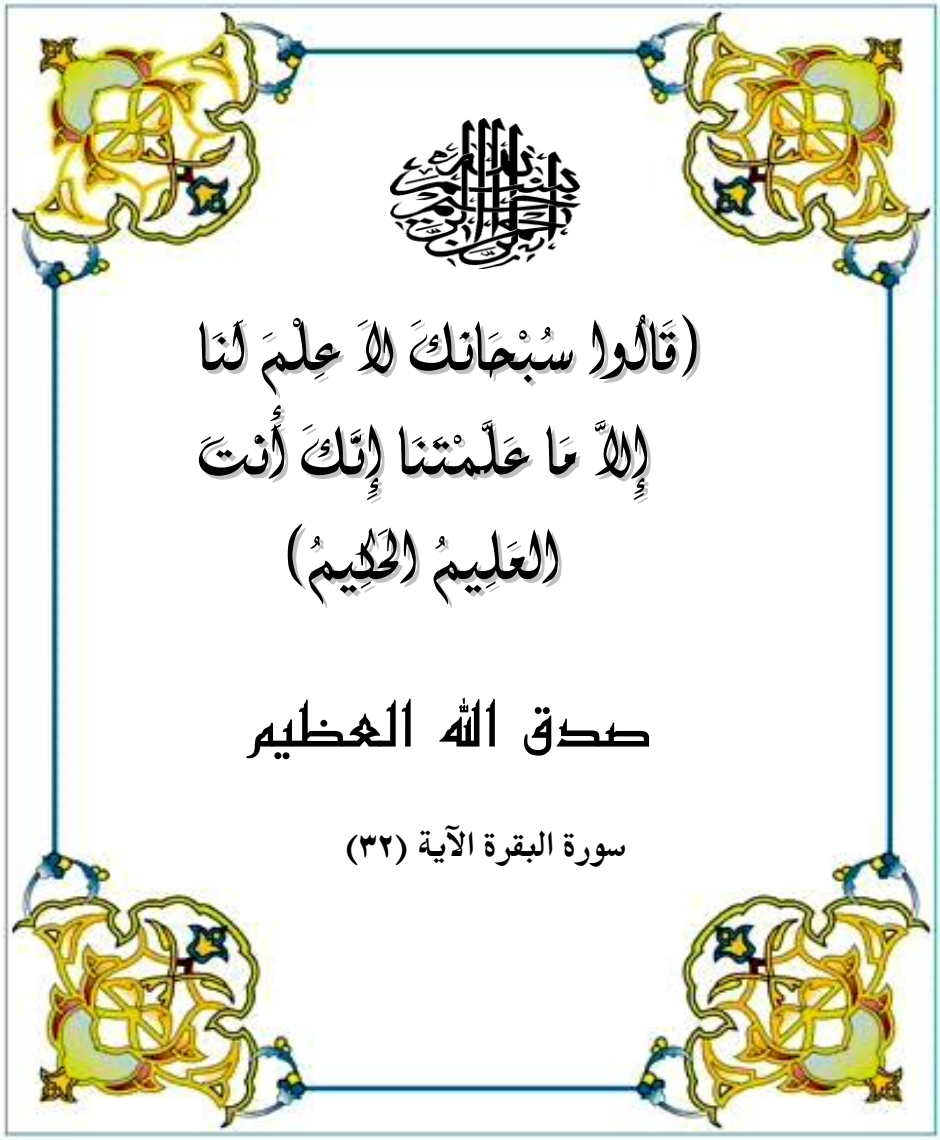

(قَالُوا سُبْحَانَكَ لاَ عِلْمَ لَنَا
إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ
العَلِيمُ الحَكِيمُ)

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List of Abbreviations

AFD	*Aspergillus fumigatus* diffusible product
AIDS	Acquired immunodeficiency syndrome
AMs	Alveolar macrophages
BAL	Bronchoalveolar lavage
BG	1,3-β-D- glucan
BHIA	Brain heart infusion agar
CNS	Central nervous system
CSF	Cerebrospinal fluid
CZA	Czapeck agar
DC-SIGN	Dendritic cell-specific ICAM-3-grabbing nonintegrin
ELISA	Enzyme-linked immunosorbent assay
EORTC/MSG	European Organization of the Research and Treatment of Cancer/Mycoses Study Group
FDA	Food and Drug Administration
FI	Fungal infection
Galf	Galactofuranose
GM	Galactomannan
GMS	Gomori's methenamine silver
GVHD	Graft-versus-host disease
HSCT	Hematopoietic stem cell transplantation
IA	Invasive aspergillosis
IDO	Indoleamine 2,3-dioxygenase

IL	Interleukin
LRTI	Lower respiratory tract infection
MEA	Malt extract agar
NETs	Neutrophil extracellular traps
OD	Optical density
PA ELISA	Platelia *Aspergillus* ELISA
PAMP	Pathogen associated molecular patterns
PAS	Periodic acid schiff
PMNLs	Polymorphonuclear leukocytes
PRR	Pathogen-recognition receptors
RIAs	Radioimmunoassays
ROI	Reactive oxygen intermediates
SDA	Sabouraud dextrose agar
SP-A	Surfactant protein A
SP-D	Surfactant protein D
Th1	T helper 1
Th2	T helper 2
TLR	Toll like receptors
TNF α	Tumor necrosis factor α

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INTRODUCTION

Invasive aspergillosis (IA) is the most common fungal infection in severe immunocompromised patients, such as bone marrow transplant recipients and patients who have received extensive chemotherapy for hematologic malignancies. Patients with solid tumors are also at risk for IA **(Kiertiburanakul et al., 2007).**

The presenting symptoms of invasive aspergillosis are nonspecific and the gold standard for establishing the diagnosis is invasive procedures relying on tissue biopsy or histopathological specimens. These lead to difficulty in making the diagnosis **(Hachem et al., 2009).**

Techniques to improve timely diagnosis have focused on the detection of circulating *Aspergillus* markers, including DNA, galactofuranose (galf) antigens {e.g., galactomannan}, and 1,3-β-D-glucan (BG). The galf antigen enzyme-linked immunosorbent assay (ELISA) is the most widely used assay because it is standardized and commercially available, unlike DNA detection assays. Furthermore, circulating *Aspergillus* antigen can be detected at an early stage of infection in patients with IA. On the other hand, the release of DNA might depend on the immune status of the host and is likely to be negligible during the early course of infection in severely

immunocompromised patients. The Fungitell BG test seems quite promising. However, the assay is not specific for *Aspergillus* species, and the value of the test for early diagnosis of IA has yet to be determined **(Mennink-Kersten et al., 2008).**

Galactomannan (GM) is a cell wall polysaccharide released by growing *Aspergillus* hyphae. Circulating galactomannan may be detected at a median of 5–8 days (range, 1–27 days) before clinical signs and symptoms of invasive aspergillosis become evident **(Klont et al., 2004).** Furthermore serum GM antigenemia correlates with tissue fungal burden, increasing with progressive disease and declining with effective antifungal therapy and may therefore be used to monitor the patient's response to treatment **(Koo et al., 2010).**

A commercially available double-sandwich enzyme-linked immunosorbent assay Platelia *Aspergillus* detects this polysaccharide cell wall component. This method detects the *Aspergillus* GM with a limit ranging from 0.5 to 1 ng of GM per ml of serum, making this assay 15 to 30 times more sensitive than the former latex agglutination assay **(Bart-Delabesse et al., 2005).**

The reported sensitivity of Platelia ELISA was variable, with a range from 30% to 100%, and the specificity ranges from 38% to 98% **(Hachem et al., 2009).**

Because galactomannan is a water-soluble carbohydrate, it can be detected in samples of other fluids obtained from patients with invasive aspergillosis, including urine, CSF, pleural fluid, and bronchoalveolar lavage (BAL) **(Klont et al., 2004).**

AIM OF THE WORK

The aim of this work is to evaluate *Aspergillus* galactomannan detection by ELISA as a rapid screening method for early diagnosis of invasive aspergillosis in febrile neutropenic patients both in serum and urine and also, to compare the sensitivity of its detection in urine to that of serum.