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Early Diagnosis of Invasive Aspergillosis in Neutropenic Patients using Galactomannan Antigen Detection Test

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ABSTRACT

Background/Aim: Fungal infections pose significant problems for patients with hematological malignancies undergoing chemotherapy and/or bone marrow transplantation. *Aspergillus fumigatus* is a pathogenic filamentous fungus associated with several illnesses, including pulmonary aspergillosis and invasive aspergillosis. Reports reveal an increased incidence of Aspergillus infections in cancer patients. Consequently, prompt diagnosis is crucial to diminish morbidity and death, particularly in neutropenic individuals. The present study aim to measure the serial levels of Galactomannan in the serum of adult patients with hematological malignancies to detect early preclinical fungal infections and relate these findings with clinical and radiological outcomes, as well as prognosis. **Patients and Methods**: We assessed serial galactomannan levels via ELISA in blood samples from 59 adult patients with hematological malignancies undergoing therapy at Ain Shams University Hospital. **Results:** This investigation analyzed 59 hematologic patients. Notable findings revealed that neutropenia was present in 20.3% of cases, whereas fungal infections affected 23.7% of patients and shown a significant correlation with neutropenia (75% against 10.6% in non-neutropenic patients, $p<0.001$) and increased mortality rates (66.7% compared to 12.8%, $p<0.001$). Diagnostic methods, including the galactomannan assay and radiographic findings, demonstrated significant agreement (Kappa=0.75-0.95) in identifying fungal infections. The overall mortality rate was 23.7%, primarily due to infections (57.1%), with neutropenic patients facing a 5.2-fold elevated mortality risk. **Conclusions:** These findings underscore the necessity for stringent antifungal prophylaxis and meticulous monitoring in neutropenic patients, particularly following bone marrow transplantation. Therefore, it is prudent to undertake routine screening for these individuals via the GM test to initiate early antifungal therapy, even before the appearance of any clinical or radiological signs.

Keywords: Invasive Aspergillosis, neutropenia, galactomannan antigen

Introduction

Aspergillus species are the main causes of invasive mold infections in patients with compromised immune systems, particularly among patients who received chemotherapy for hematologic malignancies (Badiee *et al.,* 2008). Pulmonary aspergillosis is mainly acquired by inhaling Asp. fumigatus conidia. Humans often have hypersensitivity disorders such as severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis. Patients with compromised immune systems are more vulnerable to invasive infections, which occur less frequently (Van Dijk *et al.,* 2024).

Early diagnosis is essential as invasive aspergillosis (IA) is a leading cause of morbidity and mortality in individuals with severe neutropenia. The predominant causes of IA and nasal sinus infection that can spread to brain are Aspergillus fumigatus and Aspergillus flavus, respectively (Da Silva *et al.,* 2010).

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Conidia from A. fumigatus can infiltrate the lungs owing to their diminutive size (3–5 μm), whereas conidia from A. flavus are obstructed in the sinuses due to their larger dimensions (Challa 2018).

The increased incidence of Aspergillus infections in cancer patients is attributable to several factors. More intense chemotherapy regimens are associated with mucosal barrier deterioration and Profound neutropenia (El-Masry *et al.,* 2014).

Early management of invasive aspergillosis (IA) and prompt early diagnosis are crucial for improving patient outcomes. The initiation timing of empirical therapy influences death rates from IA, which vary between 40% and 90% (Avcu *et al.,* 2017).

Early diagnosis of IA is still challenging since clinical and radiological signs are nonspecific, and can be similar to bacterial or viral infection. One of the diagnostic test is microbiological culture techniques which take too long for incubation plus have a poor sensitivity, and species identification is required. Histological diagnosis by tissue biopsy is the gold standard for IA, but it is invasive and can have potentially fatal adverse effects, especially in individuals with coagulopathy and thrombocytopenia (Dinand *et al.,* 2016).

Consequently, a diagnosis is generally established through a combination of clinical symptoms, radiologic anomalies, and clinical indicators (Herbrecht *et al.,* 2002). The definitive diagnostic test for invasive pulmonary aspergillosis (IPA) in neutropenic patients is serum galactomannan (GM) testing, a non-culture method; however, in non-neutropenic individuals, the sensitivity decreases to 30% or lower (Jenks *et al.,* 2019).

Cell walls of Aspergillus spp. have a polymer known as galactomannan (GM), which is discharged into the bloodstream when Spores or conidia germinate and progress into hyphae. GM can be detected in peripheral blood (serum and plasma), bronchoalveolar lavage fluid (BALF), urine, cerebrospinal fluid, or pleural fluid in individuals with IA (Eigl *et al.,* 2017).

Patients with acute leukemia were particularly susceptible to developing an invasive fungal infection. Especially, Lower Respiratory Tract Infection (LRTI) are the most common clinical manifestation of invasive infection. Also, the likelihood of developing fungemia is elevated in individuals with severe neutropenia and multiple infection sites (El-Masry *et al.,* 2014).

Invasive Aspergillosis is commonly diagnosed with the Platelia Aspergillus Ag assay (Bio-Rad), designed to detect Aspergillus galactomannan (GM) (Guigue *et al.,* 2015).

Patients and Methods

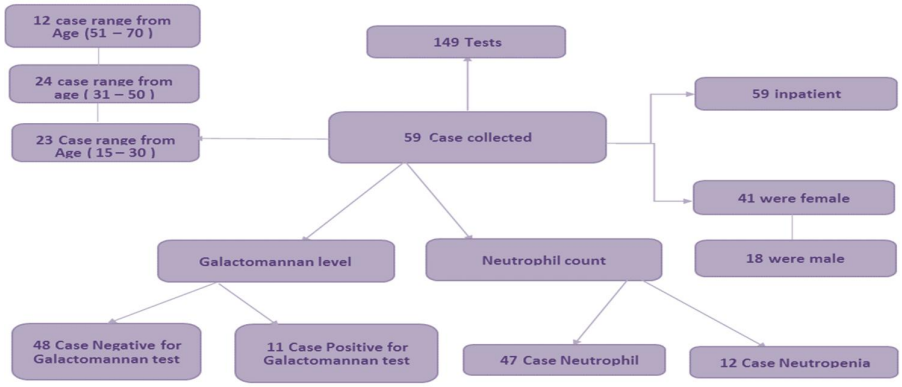
Subjects

This study involved 59 participants undergoing multiple frequent sampling, who were referred to the Clinical Hematology and Oncology Unit in the Internal Medicine Department at Ain Shams University from June 2021 to June 2022

All patients will undergo the following procedures at the time of recruitment:

- Comprehensive history acquisition and clinical assessment.
- Laboratory studies comprising: (a) Complete Blood Count (CBC) with differential and reticulocyte percentage.
- Routine metabolic profile: assessments of renal and hepatic function, lactate dehydrogenase levels.
- Coagulation profile: Prothrombin Time (PT) and Partial Thromboplastin Time (PTT).
- Disease-specific laboratories: bone marrow aspiration, flow cytometry, and cytogenetic analysis.
- Measurement of serum Galactomannan levels by enzyme-linked immunosorbent assay (ELISA)

2.2. Study Design



Materials and Methods

Study area and blood samples

The present study was conducted at Demerdash hospital venous blood sample was obtained from randomly selected patients attending hospital during the study period. Data of age and sex of patients were recorded. The 3 ml of blood from vein of selected subjects was collected in a tube containing (EDTA) ethylenediaminetetraacetate anticoagulant and Vacutainer tube without anticoagulant**.**

Materials for Neutrophil count

1. Purple vacutainer tube or capillary collector (EDTA) ethylenediaminetetraacetate
2. Slides and blue capillary tube
3. Needle or lancet
4. Vacutainer holder
5. Alcohol swab
6. Cotton balls
7. Absorbent materials
8. Slide case
9. cell counter

Measure Galactomannan level by Platellia Aspergillus EIA test kit (Bio-Rad)

Table 1: Contents of the kit

Component	Component	Contents	Quantity
R1	**Microwell strip plate**	**Microplate:** - 96 wells (12 strips of 8 wells each) coated with antigalactomannan - Monoclonal antibodies - Strip tabs labeled "85"	1 Plate / 12 x 8 Wells
R2	**Concentrated washing solution (20X)**	**Concentrated Washing Solution (20X):** - Tris NaCl buffer (pH 7.4) - 2% Tween® 20 - Preservative: 0.04 % ProClin™ 300	1 x 70 mL
R3	**Negative control serum**	**Negative Control Serum:** - Human negative serum - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs Ag - Preservative: 0.3% ProClin™ 300	2 x 1.7 mL
R4	**Cut-off control serum**	**Cut-off Control Serum:** - Human serum containing *galactomannan* - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs Ag - Preservative: 0.3% ProClin™ 300	2 x 1.7mL

R5	**Positive control serum**	**Positive Control Serum:** - Human serum containing *galactomannan* - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs Ag - Preservative: 0.3% ProClin™ 300	2 x 1.7 mL
R6	**Conjugate**	**Conjugate (ready to use):** - Anti- *galactomannan* monoclonal antibody / peroxidase labeled - Preservative: 0.3% ProClin™ 300	1 x 8 mL
R7	**Sample treatment solution**	**Sample Treatment Solution (ready to use):** - EDTA acid solution	1 x 13 mL
R9	R9 **Chromogen: TMB solution**	**Chromogen TMB Solution (ready to use):** - 3,3',5,5'-tetramethylbenzidine* (<0.1%) - H2O2 (<1.0 %)	1 x 28 mL
R10	**Stopping solution**	**Stopping Solution (ready to use):** - 1 N sulphuric acid solution (H2SO4)	1 x 28 mL

***Note**: TMB (3,3',5,5'-tetramethylbenzidine) is a non-carcinogenic and non-mutagenic chromogen for peroxidase.

Equipment and material required but not provided

1. Distilled or deionized water, for dilution of Concentrated Washing Solution.
2. Absorbent paper.
3. Disposable gloves.
4. Protective glasses.
5. Sodium hypochlorite (bleach) and sodium bicarbonate.
6. Pipettes or multipipettes, adjustable or fixed, to measure and dispense 50 µL, 100 µL, 300 µL, and 1000 µL.
7. 1.5 mL polypropylene microcentrifuge tubes with airtight stoppers, able to support heating to 120°C.
8. Laboratory bench centrifuge for 1.5 mL polypropylene tubes capable of obtaining 10,000g.
9. Heat block or 100°C (boiling water bath).

Specimen Collection and Storage

Collect blood samples according to standard laboratory procedures. Serum samples must be uncontaminated with fungal spores and/or bacteria. Transport and store samples in sealed tubes, unexposed to air. Unopened samples can be stored at 2-8°C for up to 5 days prior to testing. After initial opening, samples may be stored at 2-8°C for 48 hours prior to testing. For longer storage, store the serum at -70°C.

Serum samples can be subjected to a maximum of 4 freezing / thawing cycles. Previously frozen specimens should be thoroughly mixed after thawing prior to testing.

The results are not affected by samples containing 20 mg/L of bilirubin, lipemic samples containing the equivalent of 2 g/L of triolein (triglyceride) or hemolyzed samples containing 500 mg/dL of hemoglobin.

Interferences related to excess albumin have not been tested.

Treatment of the sera / controls

All control sera: negative (R3), cut-off (R4) and positive (R5) must be processed at the same time as serum samples.

1. Pipette 300 µL of each test serum and control into individual 1.5 mL polypropylene tube.
2. Add 100 µL of Sample Treatment Solution (R7) to each tube.
3. Mix tubes thoroughly by vigorous mixing to mix thoroughly. Tightly close the tube to prevent opening during heating,

Water bath option

If using a boiling water bath: heat tubes for 3 minutes at 100°C Tubes must be placed in the water

bath only when the prescribed temperature is reached or Heat block option:

Heat tubes for 6 minutes in a heat block at 120°C. Tubes must be placed in the block only when the prescribed temperature is reached

1. Carefully remove hot tube from the heat block or the boiling water bath and centrifuge tubes at 15000 gm for 10 minutes
2. The supernatant is used for analysis
3. After preparation, the supernatant may be removed and stored at 2_8 ºC for up to 48 hours prior to testing.

Methods

Neutrophil count Procedure

1. Specimen is collected into EDTA (purple) vacutainer.
2. Preparation of peripheral blood smear from prolong stored (> 6hr) sterile EDTA containing blood sample tube at room temperature (Figure 1).

Step 1: A small drop of venous blood is placed on a glass microscope slide, using a glass capillary pipette.
Step 2: A spreader slide is positioned at 450 angle and slowly drawn toward the drop of blood.
Step 3: The spreader slide is brought in contact with the drop of blood and is being drawn away.
Step 4: The spreader slide is further pulled out, leaving a thin layer of blood behind.
Step 5: The blood smear is nearly complete
Step 6: End result will be a glass slide with a wellformed blood film. After drying for about 10 minutes, the slide is fixed in methanol & stained with Leishman stains (Figure 2).

A well-made peripheral smear is thick at the frosted end and becomes progressively thinner toward the opposite end. The “zone of morphology” (area of optimal thickness for light microscopic examination) should be at least 2 cm in length. The smear should occupy the central area of the slide and be margin-free at the edges.

(Leishman stain is named after its inventor, the Scottish pathologist William Boog Leishman. It is a version of the Romanowsky stain, and is thus similar to and partially replaceable by Giemsa stain, Jenner's stain, and Wright's stain).

Fig. 1: Blood films before Leishman stain

Fig. 2: Blood films on Lesishman stain

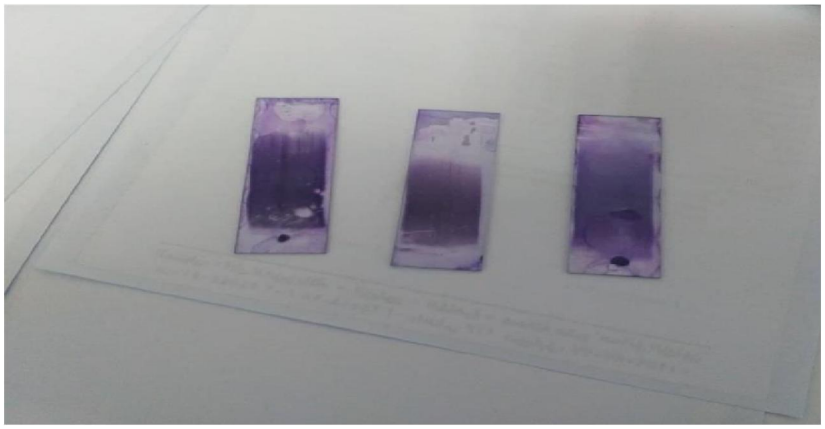


Fig. 3: Blood films after Leishman stain

3.8.2. Microscopy

Leukocytes can be classified as granulocytes and agranulocytes based on the presence and absence of microscopic granules in their cytoplasm when stained with Leishman stains.

Neutrophils are (12 to 15 µm) in diameter, have multi-lobed nuclei typically consisting of 3 to 5 segments joined by thin strands, or isthmuses. Thus, it is also called polymorphonuclear neutrophils.

Different types of WBCs in the provided dataset

Human blood consists of five types of white blood cells, namely (Fig. 4)

- Neutrophils (40-60%)
- Lymphocytes (20-40%)
- Monocytes (2-8%)
- Eosinophils (1-4%)
- Basophils (0.5-1%)

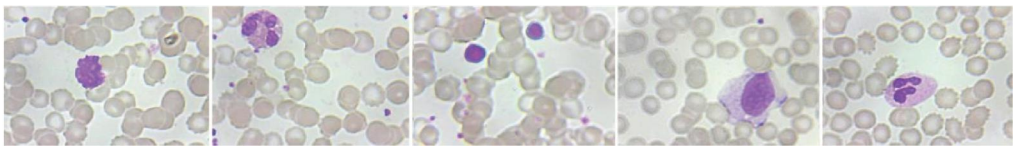


Fig. 4: Different types of WBCs in the provided dataset

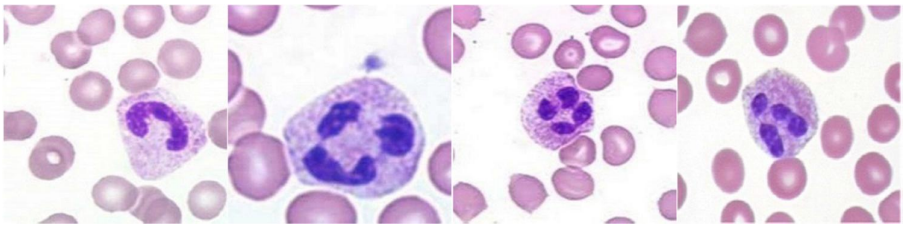


Fig. 5: Neutrophil cell at different maturity stages with different lobes. **A.** early stage called (band neutrophil); **B,C,D.** intermediate stage called (segmented neutrophil) **.**

Measure Galactomannan level by Platellia Aspergillus EIA test kit (Bio-Rad)
Assay Procedure

1. Bring reagents to room temperature (+18-25°C) for at least 30 minutes before use.
2. Prepare the Working Washing Solution.
3. Prepare a chart for identification of test sera Samples and controls in the microplate. Use one well for the Negative Control Serum (R3), two wells for the Cut-off Control Serum (R4), and one well for the Positive Control Serum (R5).
4. Remove the plateholder and microwell strips (R1) from the plate pouch. Return any strips that will not be used to the pouch, with the desiccant, and reseal the pouch.
5. Mix the contents of the Conjugate bottle (R6) by inverting before use. Add 50 µL of Conjugate (R6) to each well. Next, add 50 µL of treated serum supernatant to each well, as designated above.

Do not add serum samples to the wells before the conjugate.

6. Cover plate with plate sealer, or other means to prevent evaporation, ensuring that entire surface is covered and watertight.
7. Incubate the microplate in a dry microplate incubator for 90 ± 5 minutes at 37°C (± 1°C).
8. Remove the plate sealer. Aspirate the contents of all wells into a waste container (containing sodium hypochlorite). Wash the plate 5 times with a microplate washer (using 800 µL of Working Washing Solution). After the last wash, invert the microplate and gently tap on absorbent paper to remove remaining liquid.
9. Rapidly add 200 µL of the Chromogen TMB (R9) Solution to each well, avoiding exposure to bright light.
10. Incubate the microplate in the dark at room temperature (+18-25°C) for 30 ± 5 minutes. Do not use adhesive film during this incubation step.
11. Add 100 µL of Stopping Solution (R10) to each well, utilizing the same order for addition of Chromogen TMB Solution. Mix well.
12. Thoroughly wipe the bottoms of each plate.
13. Read the optical density of each well at 450 nm (reference filter of 620/630 nm). Microplates must be read within 30 minutes of addition of Stopping Solution.

Calculation of the mean Cut-off Control optical density

Add the optical densities of the two wells containing Cut-off Control Serum (R4) and divide the total by 2.

Calculation of an index (I) for each test sample

Calculate the following ratio for each test sample

I = mean OD (sample) / OD (CONTROL Cut–off)

Expected Values and Assay Limitations

Sera with an index < 0.50 are considered to be negative for galactomannan antigen.

Sera with an index ≥ 0.50 are considered to be positive for galactomannan antigen..

Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when

parametric and median with inter-quartile range (IQR) when non-parametric. Also qualitative variables were presented as number and percentages.

The comparison between groups regarding qualitative data was done by using Chi-square test and/or Fisher exact test when the expected count in any cell found less than 5.

The comparison between more than two independent groups with quantitative data and parametric distribution was done by using One Way ANOVA test while with non-parametric data were done by using Kruskall-Wallis test.

Kappa-agreement was used to assess the agreement between fungal infection, radiological evidence of fungal infection and galactomannan level with its 95% confidence interval.

Receiver operating characteristic curve (ROC) was used to assess the best cut off point with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve (AUC).

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

- P-value > 0.05: Non-significant (NS)
- P-value < 0.05: Significant (S)
- P-value < 0.01: Highly significant (HS)

Results

In this study, we enrolled 59 patients treated at Ain Shams University Hospitals. The patients' mean age was 36.6±14.5 years. A trimodal age distribution was observed in the cohort, with 69.5% of the participants being female.

Table 2: Demographic data of the studied patients

Age & Sex		No. = 59
Age	Mean ± SD	36.63 ± 14.46
	Range	16 – 60
	Age (15-30)	23 (39.0%)
	Age (31-50)	24 (40.7%)
	Age (51-70)	12 (20.3%)
Sex	Female	41 (69.5%)
	Male	18 (30.5%)

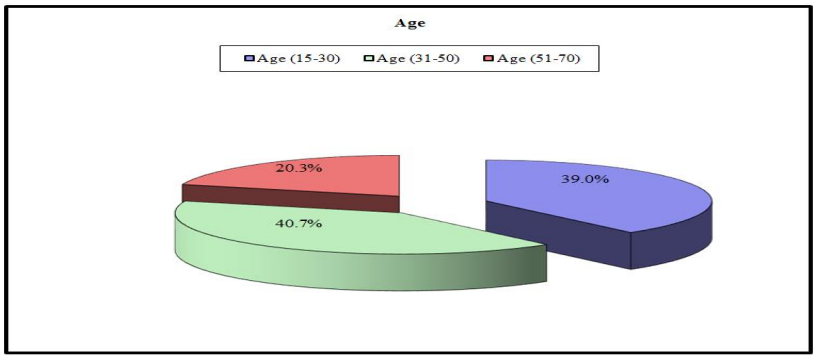


Fig. 6: Age distribution among the studied patients Using: Statistical Analysis (IBM SPSS) version 23.

Our patients samples was collected from adult patients for one year with underlying hematological diseases especially leukemia who were at risk for developing IA at Demerdash Hospital patients with an expected duration of neutropenia of less than 2 $\times 10^{9}$/L 59 patients (female 41 (69.5%) & (male 18 (30.5%) (median age 36.63 years) from 16 - 60 years with acute lymphoblastic leukemia (ALL) (8 Patients) 13.6% & acute myeloid leukemia (AML) (11 Patients) 18.6% & non Hodgkin lymphoma (NHL) (8 patients) 13.6% & Myloma (15 Patients) 25.4% & Hodgkin disease (HD) (9 patients) 15.3% & aplastic anemia (5 patients) 8.5% & Lymphoma (2 patients) 3.4% & chronic myeloid leukemia (CML) (1 patient) 1.7% and some of them undergoing bone marrow transplantation where 46 patients

(78.0%) with bone marrow transplant & 13 patients (22%) without bone marrow transplant. Diagnosis and bone marrow transplantation among the studied patients data are summarized in Table (3) & Fig. (7).

Table 3: Diagnosis and bone marrow transplantation among the studied patients

Diagnosis & Bone marrow transplant		No.	%
Diagnosis	ALL	8	13.6%
	AML	11	18.6%
	NHL	8	13.6%
	MYELOMA	15	25.4%
	HD	9	15.3%
	Aplastic anemia	5	8.5%
	Lymphoma	2	3.4%
	CML	1	1.7%
Bone marrow transplant	No	13	22.0%
	Yes	46	78.0%

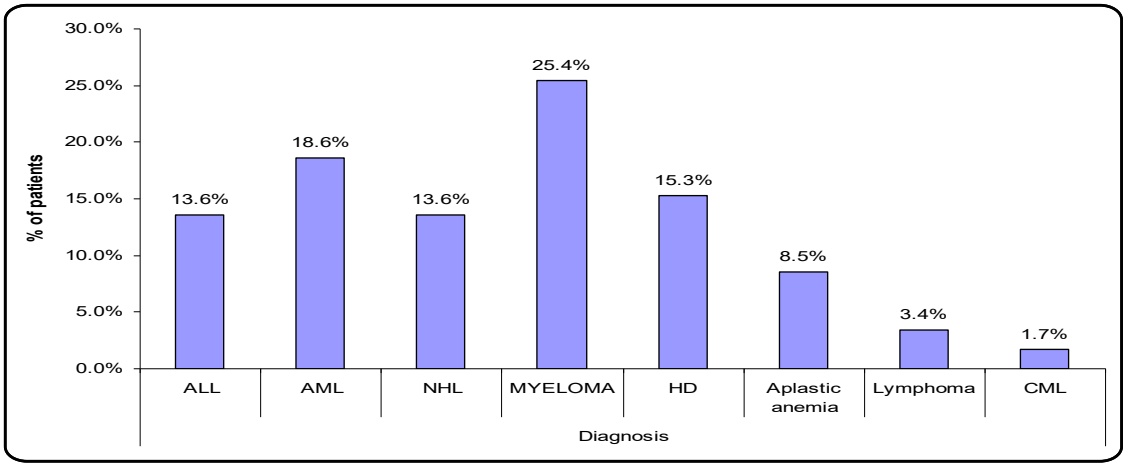


Fig. 7: Distribution of cases according to diagnosis using: statistical analysis (IBM SPSS) version 23

Table 4: Relation between neutrophil count groups and demographic data of the studied patients

Age & Sex		Neutropenia No. = 12	Neotrophil No. = 47	Test value	P-value	Sig.
Age	**Mean ± SD**	30.50 ± 14.76	38.19 ± 14.11	-1.670•	0.100	NS
	Range	16 – 60	17 – 57			
	Age (15-30)	7 (58.3%)	16 (34.0%)			
	Age (31-50)	3 (25.0%)	21 (44.7%)	2.457*	0.293	NS
	Age (51-70)	2 (16.7%)	10 (21.3%)			
Sex	**Female**	6 (50.0%)	35 (74.5%)	2.699*	0.100	NS
	Male	6 (50.0%)	12 (25.5%)			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

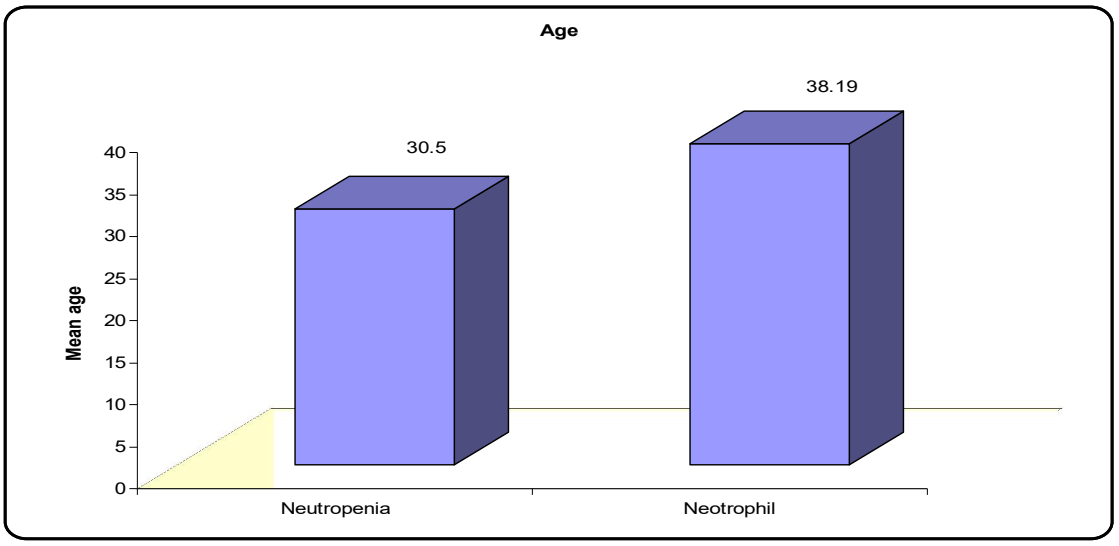


Fig. 8: Relation between neutrophil count groups and age of the studied patients

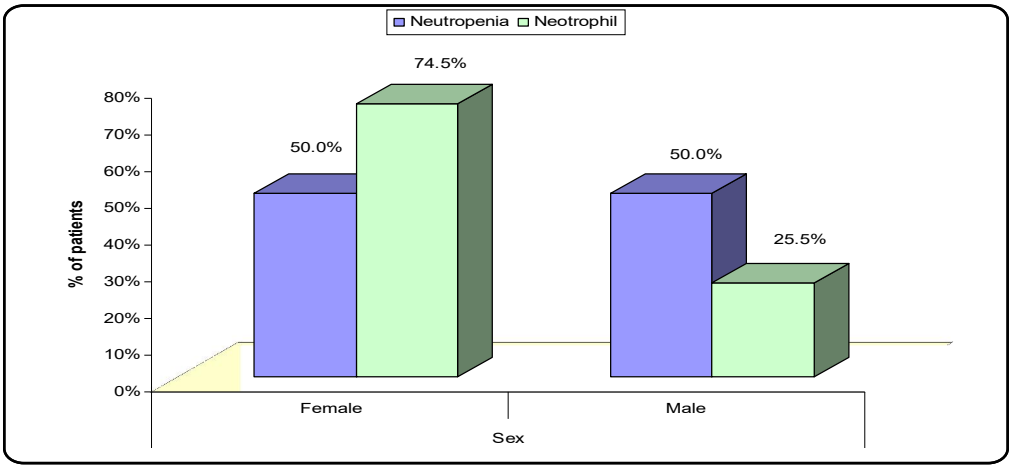


Fig. 9: Relation between neutrophil count groups and sex of the studied patients.
Using: Statistical Analysis (IBM SPSS) version

We found Positive results from GM antigen testing (Platelia Aspergillus EIA) were observed in 18.6% (11/59) of patients overall, with a significantly higher rate in neutropenic patients (58.3% versus 8.5% in non-neutropenics, p<0.0001) summarized in Table (5).

Table 5: GM results

Galactomannan level		Neutropenia		Neutrophil		Test value*	P-value	Sig.
		No.	%	No.	%			
Galactomannan Level	**Negative**	5	41.7%	43	91.5%	15.644	0.000	HS
	Positive	7	58.3%	4	8.5%			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
Using: Statistical Analysis (IBM SPSS) version 23

Table 6: Relation between fungal infection and neutrophil count

Fungal infection		Neutropenia		Neutrophil		Test value*	P-value	Sig.
		No.	%	No.	%			
Fungal infection	**No**	3	25.0%	42	89.4%	21.880	0.000	HS
	Yes	9	75.0%	5	10.6%			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
Using: Statistical Analysis (IBM SPSS) version 23.

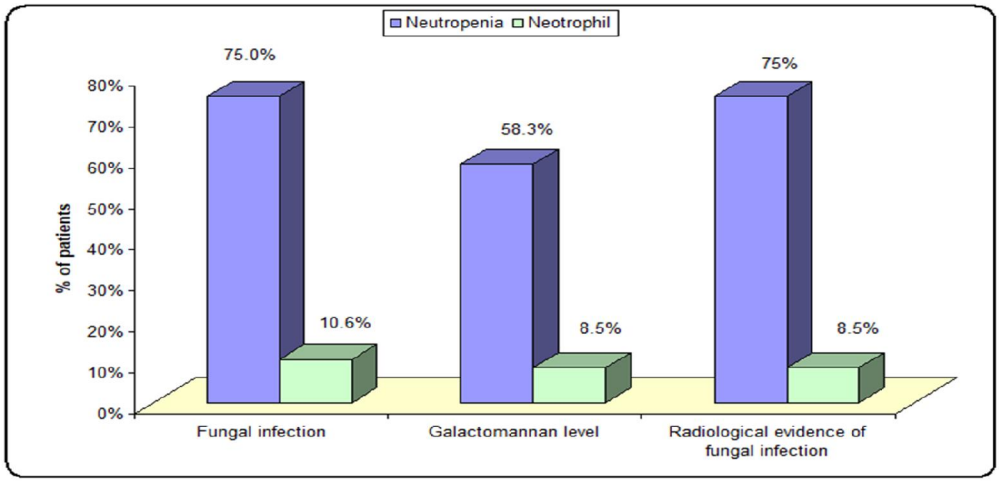


Fig. 10: Relation of neutrophil count groups with fungal infection, galactomannan level, radiological evidence of fungal infection among the studied patients. Using: Statistical Analysis (IBM SPSS) version 23.

Table 7: Relation of neutrophil count groups with mortality among the studied patients

Mortality		Neutropenia		Neutrophil		Test value*	P-value	Sig.
		No.	%	No.	%			
Mortality	**No**	4	33.3%	41	87.2%	15.345	0.000	HS
	Yes	8	66.7%	6	12.8%			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
Using: Statistical Analysis (IBM SPSS) version 23.

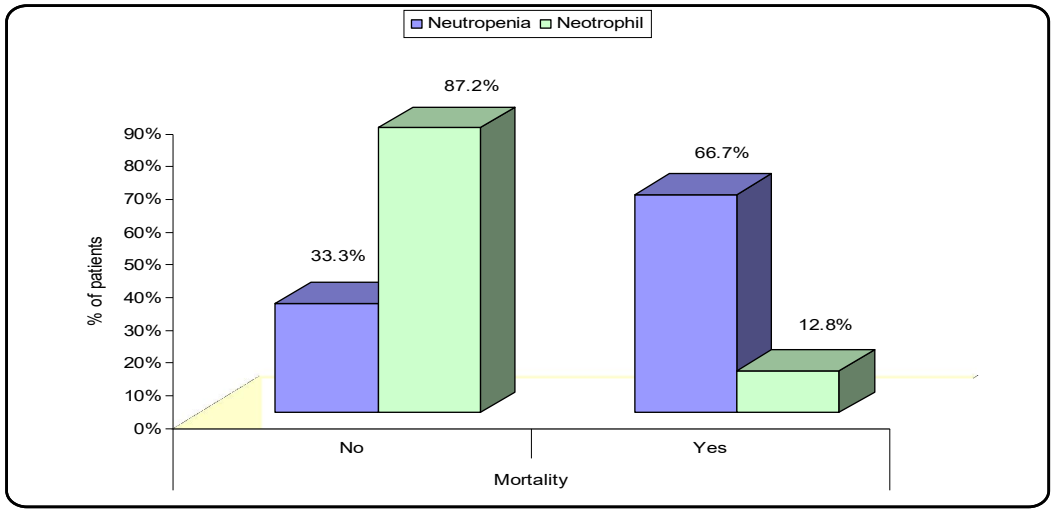


Fig. 11: Relation of neutrophil count groups with mortality among the studied patients. Using: Statistical Analysis (IBM SPSS) version 23.

Table 8: Etiology of mortality among the studied patients

Etiology of mortality		Neutrpenia		Neutrophil		Test value*	P-value	Sig.
		No.	%	No.	%			
Etiology of mortality	**Infection**	6	75.0%	2	33.3%	4.813	0.186	NS
	Relapse	1	12.5%	3	50.0%			
	GVHD	0	0.0%	1	16.7%			
	Renal failure	1	12.5%	0	0.0%			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

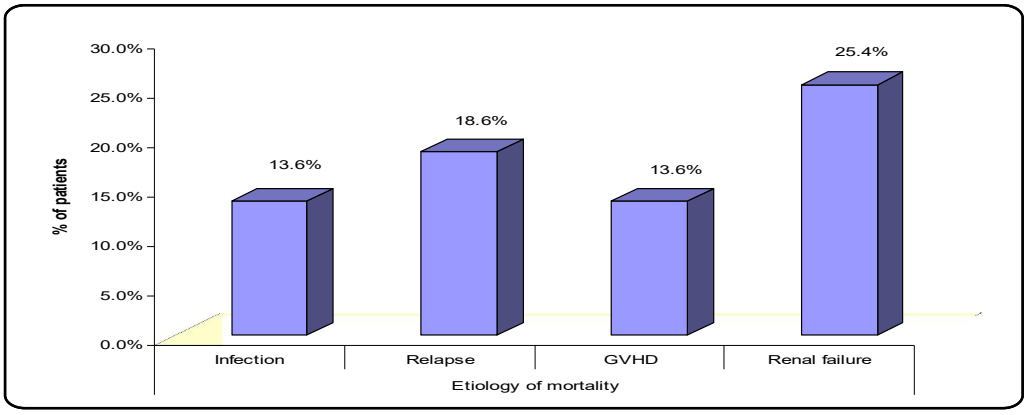


Fig. 12: Etiology of mortality among the studied patients. Using: Statistical Analysis (IBM SPSS) version 23

Table 9: Relation of neutrophil count groups with diagnosis and bone marrow transplantation of the studied patients

Diagnosis & Bone marrow transplantation		Neutrpenia		Neutrophil		Test value*	P-value	Sig.
		No.	%	No.	%			
Diagnosis	ALL	3	25.0%	5	10.6%	1.682	0.195	NS
	AML	1	8.3%	10	21.3%	1.056	0.304	NS
	NHL	1	8.3%	7	14.9%	0.351	0.554	NS
	MYELOMA	1	8.3%	14	29.8%	2.321	0.128	NS
	HD	1	8.3%	8	17.0%	0.558	0.455	NS
	Aplastic anemia	4	33.3%	1	2.1%	12.001	0.001	HS
	Lymphoma	1	8.3%	1	2.1%	1.124	0.289	NS
	CML	0	0.0%	1	2.1%	0.260	0.610	NS
Bone marrow transplant	No	5	41.7%	8 39	17.0%	3.380	0.066	NS
	Yes	7	58.3%		83.0%			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS).
Using: Statistical Analysis (IBM SPSS) version 23 - Relation of neutrophil count groups with laboratory data of the studied patients

Table 10: Relation of neutrophil count groups with ceratinine clearance, percentage of patients with HCV, HBV and HIV among the studied patients

Laboratory data		Neutropenia		Neutrophil		Test value*	P-value	Sig.
		No.	%	No.	%			
Creat clearance	Normal	7	58.3%	27	57.4%	1.630	0.803	NS
	Mild impairment	2	16.7%	8	17.0%			
	Moderate impairment	2	16.7%	9	19.1%			
	Severe impairment	1	8.3%	1	2.1%			
	Renal failure	0	0.0%	2	4.3%			
HCV	No	11	91.7%	36	76.6%	1.340	0.247	NS
	Yes	1	8.3%	11	23.4%			
HBV	No	11	91.7%	43	91.5%	0.000	0.984	NS
	Yes	1	8.3%	4	8.5%			
HIV	No	12	100.0%	47	100.0%	NA	NA	NA
	Yes	0	0.0%	0	0.0%			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

Table 11: Relation of fungal infection with mortality

Fungal infection	Mortality				Test value*	P-value	Sig.
	No		Yes				
	No.	%	No.	%			
No	39	86.7%	6	42.9%	11.324	0.001	HS
Yes	6	13.3%	8	57.1%			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
Using: Statistical Analysis (IBM SPSS) version 23

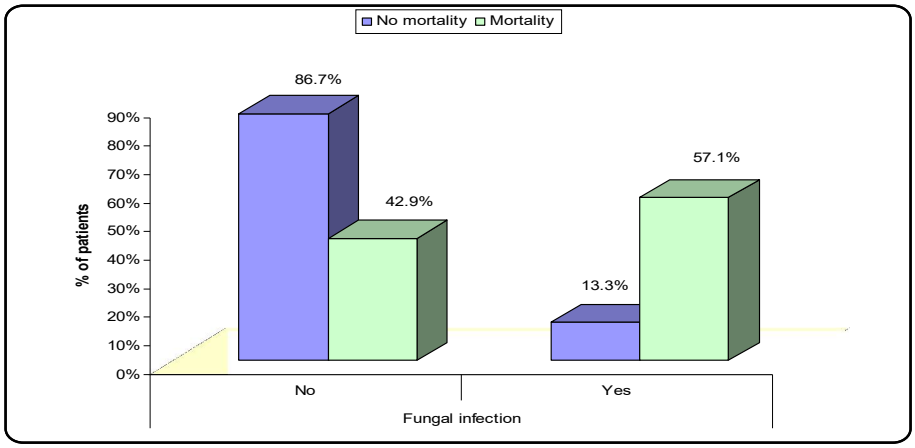


Fig. 13: Relation of Fungal infection with Mortality**.** Using: Statistical Analysis (IBM SPSS) version 23

Discussion

Hematological diseases exhibit a broad spectrum of variation between benign and malignant conditions that may lead to neutropenia, such as aplastic anemia and acute leukemia resulting from bone marrow infiltration. Neutrophils are crucial in the body's defense against infections, particularly those caused by bacteria and fungi. A decreased neutrophil count correlates with an increased risk of infection (Avcu and Karapinar, 2018)**.**

Fungal infections are prevalent among humans. Pathogenic fungi are opportunistic organisms that can induce fungal infections in individuals with compromised immune systems, including those with malignancies, undergoing chemotherapy, receiving transplants, suffering from acquired immunodeficiency syndrome, or using immunosuppressive medications, particularly in patients who have undergone hematopoietic stem cell or solid organ transplants. Invasive infections are predominantly attributed to Aspergillus species, Mucoromycetes, Cryptococcus species, and Candida species (Mei-Sheng, 2021)**.**

Despite the implementation of enhanced antifungal prophylactic protocols, these infections result in significant morbidity and mortality.

This study involved 59 individuals. The cohort included 12 patients with Myeloid Malignancies (AML and CML) and 42 patients with Lymphoid Malignancies (ALL and Lymphoma). Five patients with aplastic anemia and 18 male and 41 female patients with a mean age of approximately 36 years were treated at the Clinical Hematology and Oncology Unit, Internal Medicine Department, Ain Shams University. Notably, 78% of these patients underwent bone marrow transplantation in the BMT unit.

There is a necessity for novel approaches to detect invasive fungal infections at an early stage, characterized by high sensitivity and specificity. Galactomannan, a constituent of the cell wall of Aspergillus spp., is released during the initial growth phases, resulting in tests for its presence exhibiting sensitivity of 71% and specificity of 89% (Pfeiffer *et al.,* 2006)**.**

The detection of invasive fungal infection relies on both Galactomannan levels and chest CT imaging, particularly in patients with prolonged neutropenia or neutropenic fever without localization. Traditional microbiological culture-based testing for fungi yields low results and incurs time delays; however, the introduction of Galactomannan has facilitated the detection of Aspergillosis.

Chest computed tomography (CT) scans serve as essential diagnostic instruments for identifying pulmonary or hepatic invasive fungal infections in immunocompromised individuals. Fungal infections like invasive aspergillosis manifest as nodules encircled by haziness, commonly referred to as the halo sign. be linked to cavitary lesions. Early identification of these lesions through CT scans has reduced the time required for diagnosing invasive mold infections and facilitated the prompt initiation of appropriate antifungal therapy.

A total of 148 serum samples from 59 patients were analyzed at Demerdash Hospital, where the study was conducted over the course of one year.

Neutropenia was identified in 20.3% (12/59) of patients, as shown in Figures 31 and 32 and Table 7. The majority of these cases were associated with lymphoid malignancies (7/12) and aplastic anemia (4/12), with one patient diagnosed with a myeloid neoplasm. In accordance with the standard antifungal protocol at the Hematology unit of Ain Shams University Hospitals, all patients received primary antifungal prophylaxis with fluconazole or secondary prophylaxis with voriconazole.

In our study, we found 23.7% (14/59) of patients had invasive fungal infections. Galactomannan was positive in 18.6% (11/59) of patients, and CT chest imaging detected fungal infections in 23.7% (14/59) of patients.

GM results, fungal infection, and clinical and radiological findings were statistically significant in relation to neutrophil count.

The incidence of fungal infection among patients with neutropenia was 75% (9 out of 12), representing 15% of the total study population.

The patient exhibited a normal neutrophil count but subsequently developed a fungal infection, occurring in 25% of those with fungal infections and 8% of the overall study population. This phenomenon can be attributed to the use of immunosuppressive therapy. The mortality rate from fungal infection, as shown in Figure 40 and Table 16, was 13.5% (8 out of 59 patients) due to the progression of respiratory distress despite antifungal treatment.

The management of invasive aspergillosis primarily involves the initial administration of Voriconazole, followed by a reassessment of the patient's condition based on symptom improvement and biweekly monitoring of Galactomannan levels to evaluate changes in titer. Radiological assessment is conducted on day +7 post-therapy initiation. If symptoms improve, Voriconazole is continued for a minimum of three months; if symptoms deteriorate, a second-line treatment such as Amphotericin B, Posaconazole, or Caspofungin is introduced. The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology provide guidance for these lines of therapy (Avcu and Karapinar, 2018)**.**

The findings align with those reported by Saadia *et al.* (2021) regarding the epidemiology and control of nosocomial pathogens in select governmental hospitals in Cairo, Egypt.

Our results align with those obtained by Pagliarone *et al.* (2025)**.** The galactomannan serum test is an effective method for detecting invasive aspergillosis, especially in cases involving the sinuses or nose.

Huang *et al.* (2024) Indicated that a meta-analysis may encompass as many as sixteen studies. Serum GM and β-D-glucan (BG) yielded a pooled sensitivity of 0.53 and a pooled specificity of 0.94 across all studies for confirmed invasive aspergillosis (IA). The pooled sensitivity and specificity for the combined GM and BG assays (GM/BG) in diagnosing IA were 0.84 and 0.76, respectively. The combined GM/BG test demonstrated superior sensitivity for diagnosing IA when compared to the GM or BG test individually.

A related study conducted by Loschi *et al.* (2015) examined the incidence of invasive aspergillosis in a cohort of 438 neutropenic patients. The majority of hospitalized neutropenic patients were treated for acute leukemia (38.3%), with non-Hodgkin and Hodgkin lymphomas accounting for 33% of cases. The cumulative incidence of probable and proven Invasive Pulmonary Aspergillosis (IPA) was 23%, as reported in a study conducted during hospital renovations. Galactomannan plasma levels are monitored biweekly. Computed tomography (CT) was conducted within two days after the onset of fever or respiratory symptoms. Of the patients who developed IPA, 64% were deceased. Disease progression was the primary cause of death, while fungal infections accounted for eight fatalities.

Zhang *et al.* (2024**)** Conducted a review of 19 studies involving a total of 2838 patients. Sensitivity was 77 percent for lateral flow assay (LFA) and 75 percent for GM-LFA. Specificity was 88 percent for LFA and 87 percent for GM-LFA. The positive probability ratio was 6.65 for LFA and 12.02 for GM-LFA, while the negative probability ratio was 0.26 for LFA and 0.27 for GM-LFA. The diagnostic odds ratio was 25.81 for LFA and 44.87 for GM-LFA, representing the pooled effect sizes for the various indicators. The area under the curve for LFA and GM-LFA, with a cut-off value of ≥ 0.5, was 0.91 and 0.94, respectively. The current meta-analysis indicates that LFA or GM-LFA with an optical density index (ODI) cutoff of ≥0.5 serves as an effective diagnostic tool for patients with IA. The data indicated no significant difference in the accuracy of LFA alone compared to GM-LFA in diagnosing IA.

Our findings align with those of Chun *et al.* (2024) of the 624 individuals with GM findings, 427 did not have invasive pulmonary aspergillosis (IPA), while 70 met the criteria for proven or probable IPA. Included were chronic forms of aspergillosis and potential invasive pulmonary aspergillosis (IPA). Serum, proximal airway, and Bronchoalveolar lavage fluid (BALF) GM demonstrated sensitivities of 78.9%, 70.6%, 93.1%, and 78.7%, as well as specificities of 78.6% and 91.0% for proven/probable IPA versus no IPA, respectively. This research demonstrates that non-invasive proximal airway samples can conduct the GM test with equal or superior efficacy compared to serum and distal airway samples (BALF).

Gefen *et al.* (2015) Investigated the efficacy of serial serum GM screening for identifying IA in pediatric patients with high-risk leukemia and those who underwent stem cell transplantation. The positive and negative predictive values of GM were 0.22 and 0.96, respectively, while its sensitivity and specificity were 0.8 and 0.66. Seventy-eight percent of the cases were identified as false positives.

The findings suggest that antifungal therapy should be initiated promptly upon the onset of fever in neutropenic patients who have positive Galactomannan enzyme immunoassay (GEI) results.

Conclusion

The incidence rates of invasive fungal infections (IFI) and invasive aspergillosis (IA) are increasing steadily. The diagnosis of IA is challenging due to the nonspecific nature of clinical symptoms, resulting in infrequent and difficult classical diagnosis. Diagnostic tools in mycology for invasive fungal infections (IFI) are currently limited. The GM test is available at various centers worldwide and should be employed to aid in diagnosis. Also, The GM test should be conducted on a regular basis to detect early fungal infections without exposing patients to the hazards of radiation from pulmonary CT, and to initiate empirical treatment that will either exacerbate or reduce mortality.

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