ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Utility of Galactomanann monitoring in predicting Response to Empiric Antifungal therapy in Neutropenic patients following intensive chemotherapy'

Hanan Hamed Abd Elhamid [1], Maryse Soliman Ayoub [2], Walaa Ali Elsalakawy [3], Ahmad A. Ali[5], Ghada Metwally El Gohary [4]

1, 2. Professor of Internal Medicine and Clinical Haematology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

3. Associate Professor of Internal Medicine and Clinical Haematology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

4. Associate Professor of Internal Medicine and Clinical Haematology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

5. Hematology and Bone Marrow Transplantation unit, Maady Armed Forces Medical compound, Cairo, Egypt

Manuscript Info

Manuscript History:

Received: 15 July 2015
Final Accepted: 26 August 2015
Published Online: September 2015

Key words:

Fungal infection,Neutropenia,Galactomannan

Corresponding Author

Ghada Metwally El Gohary [4]

Abstract

Azo dyes are widely used in the textile dyeing process due to the superior fastness for the fabric, high stability in light and washing, cost effectiveness of their synthesis and variety of colors are available in comparison to natural dyes and resistant to microbial attack. The discharge of these industrial effluent containing azo dyes results in creating undesirable conditions that are lethal to resident organisms. Various physical and chemical methods are employed for the remediation of the hazardous effluent but they may have disadvantages and limitations. Bioremediation is an effective technique, which is ecofriendly, cost effective, simple structural set up, less sludge producing properties. Realizing the importance of marine microbes in the degradation of azodyes, the present study focused on the azo dye degradation potential of marine bacterial strains *in vitro*. A total of 12 bacterial strains were isolated from marine samples collected from three sampling site, Sanghumukham, Veli and Vizhinjam coast along the Arabian Sea. The isolated strains were screened for their potential to tolerate Congo red and Methyl red. The strains which showed maximum tolerance were selected for the decolourisation assay. Effects of dye concentration and incubation period on decolourisation were studied. Corresponding growth of the bacterial strains were measured in terms of absorbance at 600 nm. The results revealed that most of the selected strains shown >50% decolourisation with slight variation and the growth and decolourisation are inter-related. The present study proved that marine bacterial strains are very effective in degrading azo dyes in an eco-friendly way.

INTRODUCTION

Neutropenia is defined as a neutrophil count of less than 500 cells per mm³, or a neutrophil count of less than 1000 cells per mm³, with a predicted decrease to less than 500 cells per mm³. **Hughes Wt., et al. 2002.**

Chemotherapy induced neutropenia (CIN) is the most serious hematologic toxicity of cancer chemotherapy – **Crawford J, et al., 2004.**

CIN predispose patients with cancer to life threatening infection particularly from gram negative bacilli, gram positive cocci and fungi by suppression of neutrophil production and by cytotoxic effects on alimentary tract.

The duration of CIN typically is 7 to 10 days. The severity and duration of a neutropenia episode with the presence of fever or febrile neutropenia increase the risk of further infection and of infection related mortality. - **Caggiano V, 2005.**

Despite advances in antimicrobial therapy and supportive care, invasive fungal infection remains a major clinical problem among patients with hematologic malignancies. Not only are fungal infections increasing in frequency in this patient population-, but they also are occurring earlier during the course of cytotoxic chemotherapy. The most common fungal infection is aspergillus 73%, candida 13%, and mucor 14% **Groll AH, Shah PM et al., 1996.**

Mortality from aspergillosis is variable but may be as high as 90 % despite therapy - **Richardson MD, et al., 1998.**

Although gold diagnostic standards for aspergillosis exist, they usually require invasive procedures to obtain specimens for histological examination and culture **Walsh TJ, et al., 1994.**

Unfortunately, such aggressive procedures are often precluded by cytopenia or by the critical condition of these patients. Hence, definite diagnosis is infrequently established before death or before fungal proliferation becomes overwhelming and therapy may no longer be successful - **Groll AH, et al., 1996.**

Newer diagnostic approaches have focused on the detection of surrogate markers such as circulating fungal antigens or metabolites. - **Roger V, et al., 1990.**

One such component is galactomannan (GM), a major aspergillar cell-wall constituent released during invasive disease.

Now a commercially available sandwich enzyme linked immunosorbent assay (ELISA) for the detection of GM was introduced **- Maertens J, et al., 1999.**

The assay employs the rat monoclonal antibody EB-A2 and recognizes the 135-b-D-galactofuranoside side chains of the GM molecule. By using the same antibody as both capture and detector antibody.

Also the cut off level is now world-wide lowered to 0.5 which will help to further standardize and compare this diagnostic tool **Stynen D, et al., 1995.**

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 - **Maertens J, et al., 2001.**

Furthermore, the concentration of circulating galactomannan corresponds with the fungal tissue burden Patterson TF, et al 1998 and may therefore be used to monitor the patient's response to antifungal treatment - **Verweij PE, et al., 1997.**

This study aims to Detecting the value of serum Galactomannan monitoring in predicting response to empiric antifungal therapy in neutropenic patients following intensive chemotherapy.

Materials and methods

Subjects

A total of 20 patients with hematological malignancies receiving intensive chemotherapy:

- 3 newly diagnosed AML patients receiving induction chemotherapy in form of (3+7) protocol
- 2 newly diagnosed ALL patients receiving induction chemotherapy in form of (VAPA) protocol.
- 5 refractory ALL patients receiving salvage chemotherapy with Fludara-Ara-C containing regimen.
- 3 refractory AML patients receiving salvage chemotherapy with Fludara, Ara-C containing regimen
- 4 ALL patients receiving intensification chemotherapy in form of Methotrexate, Ara-C
- 1 AML patient receiving intensification chemotherapy in form of HiDAC
- 2 refractory NHL patients receiving salvage chemotherapy one in form of DHAP with Etoposide and the other in form of FND.

Inclusion criteria

1. Patients with newly diagnosed acute leukemia receiving induction chemotherapy with expected neutropenia (absolute neutrophil count of <500/mL)
2. Patients with relapsed acute leukemia receiving salvage chemotherapy with expected neutropenia.
3. Patients with Hodgkin or Non Hodgkin Lymphoma receiving 2nd or 3rd line chemotherapy with expected neutropenia.
4. Patients on consolidation chemotherapy like HIDAC and Hyper CVAD with expected neutropenia.

Exclusion criteria

1. Patients who are human immunodeficiency virus (HIV) infected.
2. Patients with uncontrolled bacteremia.
3. Patients receiving concomitant piperacillin / tazobactam or co-amoxyclavulinic acid.
4. Patients with prior history of anaphylactic reaction to antifungal therapy eg; amphotericin B, voriconazole.
5. Patients with expected life expectancy < 72 hrs.

Methods

A. **New acute leukemia patients are diagnosed by** :

- CBC with differential
- Bone marrow aspirate and examination
- Immunophenotyping
- Cytogenetic study

B. **Routine labs**

- (Liver functions, kidney functions, Virology screen, PT-PTT, ESR, CRP, LDH, urine analysis and stool analysis) are done to all newly diagnosed patients.

C. **Routine Imaging**

- (Chest X-ray, Abdomen and Pelvis Ultrasound, ECG and Echocardiography) are done to all newly diagnosed patients; Neck and Axillary ultrasound and CT scan (chest, abdomen and pelvis) are done in patients suspected to be ALL or with those with clinically enlarged lymph nodes.

D. **Patients who are not already inpatient at time of febrile neutropenia are admitted.**

E. **Lymphoma patients are diagnosed by L.N biopsy and bone marrow examination**

- **All 20 patients are subjected to**
  - Full history taking : including
    - Date and nature of last cycle of chemotherapy
    - Prophylactic antibiotics used
    - Concomitant steroid use
    - Recent surgical procedure
    - Previous infections
    - Drug allergy
    - Previous G-CSF receiving
    - History of past positive microbiology
    - Presence of comorbidities
    - History of IV line infection
  - Initial assessment and investigations

    Symptoms or signs suggesting an infection focus with full questionnaire
    - Respiratory system; Upper respiratory tract infection (cough, Runny nose, sore throat, sneezing)
    - Lower respiratory tract infection (cough, a tight feeling chest, breathlessness, wheezing, sore throat, fever and chills, headaches, locked nose and sinuses).
    - Gastrointestinal tract (Nausea, vomiting, loss of appetite, dehydration, diarrhea, mucus or blood in stool and tenesmus).
    - Skin infection (Redness, hotness, pain, rash, pruritus)
    - Perianal region (Itching, burning sensation, constipation, mucus or blood in stool, mass in anal or rectal area)
    - Genitourinary infection (Flank pain, dysuria, frequency of micturition, urethral discharge, vomiting)
    - Oropharynx (Dysphagia, Odynophagia, oral thrush)
    - Central nervous system (Severe headache, stiff neck, blurring of vision, neck pain, drowsiness,

vomiting)
- Peripheral and central catheter sites infection

Investigations of febrile neutropenic patients

- Complete blood picture with differential
- Liver and renal functions
- Coagulation screen
- Electrolytes
- C reactive protein
- Blood cultures (minimum of two sets) including cultures from indwelling i.v. catheter
- Urinalysis and culture
- Stool microscopy and culture
- Sputum microscopy and culture
- Skin lesion (aspirate/biopsy/swab)
- Chest radiograph
- Further investigations may be done in case of suspecting invasive aspergillosis (High resolution CT chest and BAL)
- **Fungal serology examination:** Serial Serum Galactomanann testing is done using enzyme-linked immunosorbent assay with cut-off level of ≥0.5, testing is done on days 1, 3, 5, and 9 of febrile neutropenia for every patient. Correlation between results of Galactomanann test with need and/or response to empiric antifungal therapy regarding fever, clinical condition and aspergillous load, statistical analysis of the collected data has been performed and results have been attached.

➢ Treatment :

- Broad spectrum antibiotic (Carbapenems or Cefepime) had been started from day one of febrile neutropenia; additional antibiotics have been added according to each case.

Empiric systemic broad spectrum antifungal therapy including Aspergillus species (Amphotericin B) with dose of 0.5mg: 1 mg / kg / day over 4-6 hours started in patients with febrile neutropenia persistent despite 4-5 days of broad-spectrum antibacterial therapy or in those highly suspected to have invasive fungal infection with dosage modulation in case of renal or liver impairment.

Statistical analysis:

Statistical analyses were performed by using the statistical package for the social sciences (SPSS software version 17, Chicago, IL). A probability<0.05 was statistically significant and <0.001 was statistically highly significant. Differences between groups were assessed by one-way analysis of variance (ANOVA).

Results:

10 patients showed negative results all through the examined days of febrile neutropenia; 9 of those patients never needed antifungal therapy and clinically improved and remained afebrile after antibiotic therapy. While, 1 case showed persistent fever in spite of combination of antibiotics, CT imaging of this patient showed a halo sign of IA and improved after antifungal therapy. 10 patients showed positive results GM.; 2 of which showed positive test only in one day of the examined days, both of those 2 patients improved after combination of antibiotics.8 patients showed positive results in more than one day; 6 of which improved after use of antifungal therapy , improvement was regarding fever, clinical status and decrease in antigenic load in GM test .2 patients did not improve after antifungal therapy, however showed decrease in antigenic load in GM test. By D3 antifungal therapy was only used in 2 patients out of 10 patients who had ever positive results, by D5 antifungals had been started in 9 patients out of the 10 patients who had ever any

positive results, out of those 9 patients 7 patients improved by D9. Correlation between results of monitoring, use of systemic antifungal therapy and response-regarding fever subsidence and clinical improvement showed Sensitivity: 88.88 Specificity: 81.81 PPV: 80% NPV: 90%

Table (1): Correlation between empirical use of Fungizone D3 and GM results.

Fungizone D3		GM		
		Negative	Positive	Total
Not used	N	10	8	18
	%	55.56	44.44	100.00
used	N	0	2	2
	%	0.00	100.00	100.00
Total	N	10	10	20
	%	50.00	50.00	100.00
Chi-square	X2	2.995		
	P-value	0.084		

BY D3 Fungizone was empirically used only in 2 patients out of 10 patients with GM.

Table (2): Correlation between empirical use of Fungizone D5 and Response D9

Fungizone D5		Response at D9		
		Non response	Response	Total
Not used	N	0	11	11
	%	0.00	100.00	100.00
used	N	2	7	9
	%	22.22	77.78	100.00
Total	N	2	18	20
	%	10.00	90.00	100.00
Chi-square	X2	3.469		
	P-value	0.063		

By D5 Fungizone is empirically used in 9 cases (2 cases by D3), 7 of which responded at D9 regarding fever subside and clinical improvement, the non-responding 2 cases showed mixed infection and no recovery by D9.

Table (3): Statistical measures of Galactomannan test performance.

Sensitivity	88.88%
Specificity	81.81%
Positive predictive value	80%
Negative predictive value	90%

Discussion

In this study we evaluated the performance of the Aspergillus GM test for predicting response to empirical antifungal therapy in 80 serum samples for GM monitoring, samples were collected from 20 patients with hematological malignancies receiving intensive chemotherapy, the 20 patients were enrolled from Haematology and Bone Marrow Transplant inpatient department in Maadi Armed Forces Medical Compound in time period from May 2012 to July 2013. 5 female cases, 15 male cases with ranging age group from 4 years to 58 years old. Patients consisted of different diagnosis 11 cases of Acute lymhoblastic leukemia (5 are refractory receiving salvage chemotherapy, 2 are receiving phase 1 induction and 4 are receiving intensification therapy), 7 cases of Acute Myeloid Leukemia (3 are refractory receiving salvage chemotherapy, 3 are denovo AML receiving remission

induction chemotherapy and 1 case receiving intensification chemotherapy) and 2 cases of Refractory N.H.L receiving salvage chemotherapy.

AML patients received remission induction in form of 100- 200 mg/m² continuous infusion for 7 days and daunorubicin 60mg/ m² for 3 days (3+7) protocol.

Intensification of AML cases received intensification in form of HiDAC alone.

Refractory A.M.L cases received salvage chemotherapy in form of FLAG, one case and FLAG-ida in 2 cases.

A.L.L patients received remission induction in form of VAPA protocol, received intensification in form of Methotrexate, Ara-C and salvage therapy in form of FLAG-ida in 2 cases and FLAG-cyclo in 3 cases.

Refractory N.H.L patients received salvage chemotherapy in form of DHAP with etoposide.

IA is a leading cause of death among immunocompromised patients, especially among those patients with hematological malignancy or those who undergo bone marrow or solid-organ transplantation **Singh et al., (2009).**

Clinical and radiologic diagnosis of IA has limited sensitivity and specificity **Patterson (1999).** In patients with thrombocytopenia, a tissue diagnosis carries the risk of bleeding and is usually not advisable. The use of a biological marker as an adjunct for screening for IA in high-risk patients is attractive, because it is non-invasive and may detect evidence of IA prior to the appearance of clinical signs and symptoms. Galactomannan is a polysaccharide cell-wall component that is released by Aspergillus species. Among the many tests that have been developed for detection of galactomannan, the double-sandwich ELISA (Platelia; Bio-Rad), which incorporates the B 1-5 galactofuranose-specific EBA2 monoclonal antibody as both the acceptor and the detector for galactomannan, has the most promise. Although numerous studies have been performed to determine the sensitivity and specificity of the assay in various patients' populations, the results are variable.

The goal of this study is to correlate the results of galactomannan test with response to empiric use of systemic antifungal therapy, our main finding was that the test was useful in predicting response to systemic antifungal therapy in haemtological patients receiving intensive chemotherapy. Of 20 patients, 10 patients showed negative results all through the examined days 1,3,5,9 of febrile neutropenia; 9 of those patients never needed fungizone and improved-regarding fever subsidence and clinical improvement-after combination of antibiotic therapy, 6 of which according to C& S results and 3 after empirical antibiotics; 1 case showed persistent fever in spite of combination of antibiotics, this patient showed manifestations of chest infection, sputum culture showed no growth, CT imaging of this patient showed a halo sign of IA and the patient improved after fungizone.

10 patients showed positive results; 2 of which showed positive test only in one day of the examined days, both of those 2 patients improved after combination of antibiotics one received antibiotic according to C&S results and the other after empiric antibiotic usage. 8 patients showed positive results in more than one day of the examined days; 6 of which improved after use of fungizone, improvement was regarding fever, clinical status and decrease in antigenic load in galactomannan test in all 6 patients, 2 patients did not improve after fungizone therapy, however both of them show decrease in antigenic load in galactomannan test, both of them did not recover by D9; one of them showed infection with MRSA and the other has had both MRSA skin infection and ESBL in blood culture.

By D3 fungizone was only used of 2 patients out of 10 patients who has had ever positive results, by D5 fungizone had been to be started in 9 patients out of the 10 patients who has had ever any positive results, out of those 9 patients 7 patients improved by D9.

Correlation between results of galactomannan monitoring, use of systemic antifungal therapy and response-regarding fever subsidence and clinical improvement showed: Sensitivity: 88.88 Specificity: 81.81 PPV: 80% NPV: 90%. Table no (3).

In all 20 patients there was no significant difference in Hb level by D9 however both positive and negative cases showed significant difference in both platelets and neutrophil count arising a question of whether improvement was due to neutrophils recovery or use of proper anti-microbial therapy, to enhance this hypothesis the 2 galactomannan positive cases who received fungizone and the proper antibiotic therapy based on C, S results with no improvement regarding fever or clinical condition; both of them showed no hematological recovery by D9, however both of these cases showed antigenic load decrease in galactomannan results, whether neutrophils recovery is the key player in fighting fungal infection or more broad spectrum antifungal therapy would be more helpful in controlling fungal infection is still to be answered.

In comparison to meta-analysis Studies of the galactomannan assay that used the European Organization for Research and Treatment of Cancer or similar criteria as a reference standard and provided data to calculate sensitivity and specificity, Twenty-seven studies from 1966 to 28 February 2005 were included. Overall, the galactomannan assay had a sensitivity of 0.71 and specificity of 0.89 for proven cases of invasive aspergillosis, **Christopher, et al., (2006)** this study used patients with hematological malignancies, patients of bone marrow transplant and patients of solid organ transplant which may be a reason for decrease sensitivity than in our study, another variability from our study is that results of galactomannan test is tested against histopathological results from biopsy specimen not against the empirical use of fungizone and assessing response regarding fever and clinical improvement.

In comparison to another study which evaluated the performance of the Aspergillus GM test for the diagnosis of IA on 1812 serum samples obtained from 119 children undergoing aggressive chemotherapy or allogeneic HSCT at the Department of Paediatric Haematology and Oncology of the 'G. Gaslini' Children's Hospital, Genoa, Italy, and tested for the presence of serum GM antigen in the period 1999-2005, the performance of the test was found to be accurate with regard to specificity and NPV but the sensitivity and PPV were not accurate. The sensitivity was 0.32 and the specificity was 0.98; the positive predictive value was 0.70 and the negative predictive value was 0.92. The efficiency of the test was 0.91, the high NPV is consistent with our results, however sensitivity is quite different from ours, this could be attributed to many factors; 1st: use of Tazocin as a broad spectrum antibiotic with its known effect to produce false positive results which is excluded in our study **Steinbach, et al., (2007),** 2nd is categories of patients which included patients receiving allogeneic stem cell transplant, in patients exposed to mould-active antifungal agents as prophylaxis the Sensitivity is reduced to 20% compared with 80% in those not receiving these drugs **Castagnola, et al., (2009).**

Conclusion

GM test was useful in predicting response to systemic antifungal therapy in patients receiving intensive chemotherapy.

GM monitoring helps to exclude presence of circulating aspergillous antigen and hence guide management of persistent fever .

Sensitivity of GM test is yet to be explored due to high range of variations between different studies and multiple factors that may affect results.

Declaration of interest:

The authors declare no competing financial interests.

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