EVALUATION OF SERUM GALACTOMANNAN ASSAY FOR THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN CHILDREN WITH HEMATOLOGICAL MALIGNANCIES

DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR THE DEGREE

OF

D.M.
(PEDIATRIC HEMATOLOGY-ONCOLOGY)

OF THE

POST GRADUATE INSTITUTE OF MEDICAL EDUCATION AND RESEARCH,

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Contents

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As I complete this small contribution to health science, it's my immense pleasure to acknowledge the people who helped me in the completion of this study.

I was fortunate to have ***Dr.*** **Deepak Bansal,** *Additional Professor, Department of Paediatrics, PGIMER, Chandigarh as my guide. Mere words cannot express my heartfelt gratitude and reverence towards the immense help, invaluable suggestions and limitless patience.*

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I am grateful to my parents and parents in- law for their constant encouragements.

Above all, the unconditional love and support provided by my wife, Dr. Runa has been the force for me to go ahead.

December , 2011 ***Dr. Ajaya Kumar Jha***

Consent form

I ----------father/mother/guardian of ----------Cr no.----------hereby give consent for inclusion of my child in the research study entitled **"Evaluation of serum galactomannan assay for the diagnosis of invasive aspergillosis in children with hematological malignancies "** which is being conducted in the Division of Pediatric Hematology/Oncology, Department of Pediatrics, Post Graduate Institute of Medical Education and Research Chandigarh.

The purpose and procedures of the study have been explained to me in a language understandable to me. I understand that this study will require 3 ml of blood sample at the time of admission and their after weekly, till discharge. The sample will be drawn during regular sampling for complete blood count and blood culture and hence does not require a separate venepuncture. My decision to participate in this study is fully voluntary and I have full rights to withdraw any time during the course of this study. My child will continue to receive the same standard of medical care, irrespective of my decision. I have been assured that my/our identity and personal details will be kept confidential.

Signature
Name: …….
Relation with Child : ……

Witness signature
Name: …….
Date: …….

Signature of medical officer
Name:
Date:

(Annexture –II)

Table 1

Criteria for the Diagnosis of Aspergillosis

Proven aspergillosis

Microscopic demonstration of septate, acute-angle–branching, nonpigmented hyphae (without yeast forms) measuring 2 to 4 μm from a needle aspiration or biopsy in association with evidence of tissue damage, and a microbiological culture of a specimen obtained by an aseptic procedure from a normally sterile site yielding *Aspergillus* spp in association with clinical or radiologic evidence of focal infection at that site.

Probable aspergillosis

1. One host-related criterion,[a] and
2. One microbiological criterion,[b] and
3. One major, or two minor, clinical criteria[c] from an abnormal site consistent with infection

Possible aspergillosis

1. One host-related criterion,[a] and
2. One microbiological criterion,[b] or
3. One major, or two minor, clinical criteria[c] from an abnormal site consistent with infection

[a] Host criteria

1. Neutropenia defined by an absolute neutrophil count < 0.5 × 10^9/L for more than 10 days
2. Persistent fever for more than 96 hours refractory to broad-spectrum antibacterial agents
3. Body temperature > 38°C or < 36°C, and any one of the following:
   - Prolonged neutropenia of more than 10 days' duration within the last 60 days
   - Recent (within the preceding 30 days) or current use of immunosuppressive therapy
   - A previous episode of invasive aspergillosis
   - Coexistence of human immunodeficiency virus infection
4. Signs or symptoms of graft-vs-host disease
5. Prolonged (more than 3 weeks) use of corticosteroids

[b] Microbiological criteria

1. Positive culture for *Aspergillus* spp or microscopic appearance of hyphae consistent with *Aspergillus* spp from sputum or bronchoalveolar lavage specimens
2. Positive culture for *Aspergillus* spp or microscopic appearance of hyphae consistent with *Aspergillus* spp from a sinus aspirate
3. Positive antigen test for *Aspergillus* spp from a bronchoalveolar lavage specimen, a cerebrospinal fluid specimen, or in two or more blood specimens
4. Positive culture for *Aspergillus* spp or microscopic appearance of hyphae consistent with *Aspergillus* spp from tissue biopsy or otherwise sterile body fluid

[c] Clinical Criteria

Lower respiratory tract

Major: New pulmonary parenchymal infiltrates on CT scan of the chest including a halo sign, air-crescent sign, or cavitary disease within an area of consolidation

Minor: Symptoms of a lower respiratory tract infection including cough, pleuritic chest pain, hemoptysis, and dyspnea

Physical findings of a pleural rub or consolidation

Any new pulmonary infiltrate not fulfilling the major criterion

Sinonasal infection

Major: Radiologic evidence consistent with infection of the sinuses including opacification of the sinuses, air-fluid levels within the sinuses, erosion of the sinus walls, or extension of the process to neighboring structures

Minor: Upper respiratory tract symptoms including rhinorrhea or stuffiness

Presence of ulceration of nasal structures, nasal eschar, or epistaxis

Periorbital swelling

Maxillary tenderness

Perforation of the hard palate

Central nervous system

Major: Radiologic evidence consistent with central nervous system infection including intracranial space occupying lesions, meningeal enhancement associated with involved perinasal, auricular, or vertebral structures

Minor: Focal neurologic signs including seizures, hemiparesis, or cranial nerve palsies

Cognitive impairment

Signs of meningeal irritation

Abnormalities in the cerebrospinal fluid profile including elevated cell counts, elevated protein concentration, and a reduced cerebrospinal fluid–to–blood glucose ratio

Disseminated aspergillosis

Papular, nodular, or ulcerative skin lesions without other explanation

Small, peripheral, target-like ("bull's-eye") lesions observed on an imaging study (CT scan, MRI scan, ultrasonographic scan) of the liver and/or spleen (hepatosplenic fungal infections)

CT = computed tomography; MRI = magnetic resonance imaging. Adapted from Ascioglu et al.[19]

ANNEXURE – I : PROFORMA

Name: DOB/Age: Sex: M/F

CR No: POC: D.O.A. (Current):

Address:

Phone. No:

Diagnosis:

Phase of treatment:

Last chemotherapy received:

Last episode of FN (if any):

Chief complaints:

Localization of symptoms:

(i) Pulmonary (ii) CNS

(ii) Sinus (iv) GIT

(v) Other

ADMISSION DIAGNOSIS:

Investigation chart:

Date							
Hb							
TLC							
DLC							
ANC							
Plt							

Culture details:

Specimen	Date	Bacterial	Fungal	Others
Blood				
Sputum				
BAL				

Serum Galactomannan:

Date				
Value				

Microscopy of sputum/BAL/Sinus aspirate:

Antifungal prophylyxasis Y/N

If yes: name drug and duration

Radiological investigation:

Chest X-ray	
HRCT	
MRI/OTHERS	

Name of drug	From	To	Duration	Reason for stopping or changing

Major Complications:

Date of discharge:

Outcome of treatment:

(i) Improved

(ii) Improved with squeale

(iii) LAMA/DOR

(iv) Death

- Date
- Cause of death
- Autopsy: Y/N
- Postmortem sample

INTRODUCTION

Aspergillus is a ubiquitous soil-dwelling fungus. Invasive aspergillosis (IA) is the most common filamentous fungal infection in immuno–compromised patients. The incidence has increased in the past two decades due to wide spread use of chemotherapy and immunosuppressive agents. IA causes significant morbidity, mortality, and economic burden in patients with hematological malignancies.

Diagnosis of IA is challenging and is often a frustrating experiences for the treating physicians. Conventional diagnosis is dependent on culture and histopathologic examination of the involved tissue. Microscopy and culture of sputum and bronchoalveolar lavage samples are insufficiently sensitive for diagnosis. Biopsies are not always feasible in patients with a severe underlying condition. Radiology can provide clues towards diagnosis, however, lacks specificity. Blood, CSF and bone marrow specimen rarely yield Apergillus species. Thus, in majority, diagnosis depends on a combination of clinical signs, radiologic abnormalities, and clinical experience[1]. There are several limitations with the current diagnostic methods. There is a pressing need for a non-invasive, rapid, sensitive as well as specific diagnostic tool for IA. Galactomannan antigen detection test was introduced in the year 1995 and was approved by the Food and Drug Administration (FDA) in 2003. Its role in the diagnosis of IA is being actively researched.

Galactomannan is a heat-stable heteropolysaccharide released from the cell wall of aspergillus during the growth of hyphae in tissues the molecule has a non immunogenic mannan core with immunoreactive side chains containing galactofuranosyl units.[2] As it is a water-soluble carbohydrate, it can be detected in several body fluids, in addition to serum. [3,4,5] Detection of galactomannan is most popular in serum.

Initial diagnostic assays including latex agglutination had poor sensitivity. More recently, a double-sandwich ELISA that incorporates the B 1→5 glactofuranose–specific EBA2 monoclonal antibody as both the detector and acceptor for galactomannan has shown a high sensitivity.

The degree of antigenimia that indicates IA, is a subject of debate. Typical cut off values of Optical Density Index (ODI) of galactomannan range from 0.5-1.5. In a meta analysis the

sensitivity and specificity found was 33-100% and 60-100%, respectively, for proven and probable cases of IA.

The role of galactomannan assay in diagnosis of IA is evolving. There are several studies that have evaluated the assay in adult population, however, studies in children are limited. There are no published studies from India.

The current study is aimed at evaluating the role of galactomannan assay in the diagnosis of invasive aspergillosis in children on treatment for haematological malignancies.

LITERATURE REVIEW

The fungi classified as genus Aspergillus are anamorphic (asexual) filamentous organisms which reproduce by means of asexual spores termed conidia. There are eighteen groups of aspergillus classified by Raper and Thom. Most human infections are caused by A. fumigates, however, A. flavus, A. nidulans, A. niger and A. ferreus have also been implicated.

Inhalation of conidia leads to variety of diseases depending upon the degree of immunity of patients. In atopic person, asthma may occur. Other diseases caused by aspergillus include infection of paranasal sinuses, aspergilloma, chronic necrotizing aspergillosis, infection of eye, ear, heart, skin and central nervous system, and IA. [6]

Epidemiology of IA

IA is almost always seen in the setting of immunocompromised host and is often fatal. Those at risk include neutropenic patients with hematological malignancies, transplant recepients and children with chronic granulomatous diseases. Although lung is most common site of infection, aspergillosis can disseminate to any site and the true extent is often only apparent at autopsy.[7]

Variable incidence of IA is well discussed in different studies. The incidence rate of IA among immunocompromised children in the United States was 0.5% in 2000. A retrospective cohort study of 485 pediatric BMT patients at Duke University reported a frequency of aspergillosis of 4.79%, with the highest incidence reported in recipients of allogeneic BMTs. A Finnish study of 148 pediatric BMT recipients, similarly reported an IA incidence of 5%. Investigators from the Hospital for Sick Children in Toronto, reviewed 39 cases of pediatric IA from 1979 to 1988 and found that 74% of patients with IA had a hematologic malignancy.[8]

IA in children

Zaoutis et al, found children with malignancy to have a 13-fold higher risk for death when they developed IA. This rate varied between types of malignancies, with higher relative risks identified in patients with ALL, lymphoma, and CNS and bone malignancies. The risk was

lower in patients with AML, which likely reflects the higher mortality of the underlying disease even uncomplicated by IA and that death becomes a competing risk in the setting of high mortality rates.[9]

Diagnosis of aspergillosis

Establishing diagnosis of aspergillosis in immunocompromised patient is difficult because the clinical presentation is non specific and fungus is seldom isolated from blood or other body fluids.

Criteria for diagnosis of IA

The Mycoses Study Group of the European Organization for Research and Treatment of Cancer (EORTC-MSG) has laid down criteria for diagnosis of IA. According to this, IA is classified into four categories, namely, proven, probable, possible, and no IA. Briefly, proven IA includes all patients who have histologically proven disease or have a positive culture for Aspergillus obtained by percutaneous aspiration. Probable IA includes all patients who have a new opacity on their chest X-ray or High Resolution CT scan (HRCT), and repeated isolation of the same species of Aspergillus from sputum or Bronchoalveolar lavage. Possible IA includes all patients who have the development of a new opacity on the chest x-ray or HRCT, with no evident etiology.[10]

Methods for Detection of Aspergillosis

Microscopy

Aspergillus hyphea are seen as mycelia with regular septation and dichotomous branching at about 45^0, that advances in the same direction. Microscopic examination of sputum preparation is often helpful in diagnosing allergic aspergillosis but is seldom helpful in IA. Typical mycelium may also be detected in wet preparation of necrotic material from cutaneous lesions and sinus washing. However the most reliable is examination of stained tissue section. Aspergillus hyphae stain poorly with hematoxylin and eosin and are best highlighted by Gomori's methenamine silver stain.

Culture

The definitive diagnosis of aspergillosis depends upon isolation of etiological agent in culture. Because aspergillus species are commonly found in air, the isolation must be interpreted with caution. Aspergillus may be recovered from sputum or bronchial lavage (BAL) specimen, especially in patients with diffuse pulmonary infiltrate, however recovery with focal lesion is more difficult. Aspergillus species are seldom recovered from blood, urine or CSF, though culture has occasionally been rewarding in patients with endocarditis.

Detection of antibodies to aspergillus species

Detection of specific precipitating antibodies by various methods can be performed. However antibody production in immunocompromised host is often limited and its detection is a futile exercise.

Detection of Aspergillus antigen

Methods have been sought for dignosing IA which would rely upon the measurement of cell component of fungal origin and there by, be independent of host's ability to respond. Galactomannan is one such antigen. Galactomannan is a major heteropolysaccharide of the cell wall of Aspergillus species. During the course of infection, this carbohydrate antigen is expressed in tissue, the circulation, and the tracheobronchial tree.[11]

Circulating galactomannan may be detected at a median of five to eight days before the clinical manifestation of aspergillosis. Galactomannan detection also precedes the demonstration of abnormalities in high-resolution CT scan and the initiation of antifungal therapy by a median of 7.2 and 12.5 days, respectively[12].

Detection of aspergillus DNA by polymerase chain reaction (PCR)

The limitations of antibody detection and problems of sensitivity associated with antigen detection have prompted the evaluation of PCR. It can detect low levels of fungal genetic material and warns for the presence of possible IA.

Historical aspects of Galactomannan test

Antigen detection for diagnosis of IA was first reported in the late 1970s, and was made a reality by the production of monoclonal antibodies and creation of a standardized and reproducible assay in the early 1990s. Available in Europe for over 5 years, the Platelias Aspergillus antigen immunoassay produced by Bio Rad Laboratories (Hercules, CA, USA), was cleared by the FDA for diagnostic use in the USA in May 2003. The antigen can be detected using a commercially available sandwich ELISA (Platelia Aspergillus, BioRad, France) (PA-ELISA), which employs a monoclonal antibody (EB-A2) that binds to the galactofuran epitope of the GM antigen. The assay has been extensively studied and is now commonly used to monitor patients at high risk for invasive aspergillosis. [13]

Sensitivity and specificity of Galactomannan test

Galactomannan test shows a wide range of sensitivity and specificity. In a meta analysis by Pfeiffer et al, for proven or probable IA cases, the sensitivity was found to range from 33–100% and specificity was 60–100%. The pooled sensitivity of studies including pediatric population was 92% (range 82–100%) and the pooled specificity was 60% (range 36–81%).[14]

The performance of the PA-ELISA is most favorable in patients with hematological malignancy, especially those who have undergone a hematopoietic stem cell transplant and are neutropenic. However even within this group of patients significant variation in the sensitivity (33-100%) of the assay has been observed causes for the variability are not well understood, although some factors have been identified recently that have significant impact on the performance of the assay. For example, exposure of patients to mould-active antifungal agents reduced the sensitivity to 20% compared with 80% in those not receiving the drugs[15].

Most prospective clinical studies show high specificity of the PA-ELISA, with levels of false positive reactivity in adults of approximately 2.5%. Reported false positivity rates in pediatric patients is approximately 10% and the highest rates have been observed in neonates (83%), although the number of studies performed in this patient group is very limited.[16]

Several reasons that probably explain the reported differences in performance of the assay include the difference in the fungus (strain, growth phase and kinetics of Galactomannan

release), the host (preexisting condition, site and extent of fungal disease and antifungal treatment) and the definition (definition of case, cutoff Galactomannan index and results).[17] Cases have been documented in which, despite adequate sampling and proof of invasive aspergillosis, circulating Galactomannan was not detected. One explanation for lack of reactivity could be that in some patients the levels of circulating Galactomannan are below the detection limit of the PA-ELISA.[18]

The debate of cut off value in Galactomannan test

In order to improve results, some authors have suggested that Galactomannan index of 0.5 is a more definitive threshold. These modifications may increase the sensitivity of the test, with only a low decrease in specificity. In a meta analysis done by Leeflang et al, seven studies (901 patients) reported results for an Optical Density Index (ODI) of 0.5 as one cut-off value. The overall sensitivity in these studies was 78% (69-89%) and overall specificity was 81% (72-88%). Twelve studies (1744 patients) reported the results for cut-off value of 1.0 ODI. The overall sensitivity was 75% (59-86%) with a mean specificity of 91% (84-95%). Seventeen studies (2600 patients) reported the results for cut-off value of 1.5 ODI. Sensitivity was 64% (50-77%) and the mean specificity was 95% (91-97%). [19]

There is considerable variability in the literature regarding the cut-off values used for serum galactomannan testing. Although the manufacturer recommendes a cut-off of 1.5, most institutions used a lower cut-off, varying from 0.5-1.0. The approved cut-off in the USA is 0.5. Defining an appropriate cut-off is probably the most important variable influencing the sensitivity of the galactomannan test, considering that lower cut-off values result in higher sensitivity and lower specificity of the assay[20]. Debate still persists about the best cut-off for the galactomannan assays.

Galactomannan test in children

The highest false positive rates of Galactomannan test have been observed in neonates (83%), although the number of studies performed in pediatric patients is very small.[21,22] In study by Siemann et al, high false-positive results of 83% were observed. This may be related to cross-reactivity with *Bifidobacterium bifidum*, found in large inocula in the guts of breast- and formula-fed infants. The presence of damaged gut endothelium may increase the absorption of dietary galactomannan.

Galactomannan test in patients receiving antimicrobials

Serum cross reactivity has also been observed in patients that receive certain beta-lactam antibiotics, Piperacillin/tazobactam, amoxicillin/clavulanic acid, ampicillin and have been shown to contain PA-ELISA reactive material.[23].[24] Serum and BAL samples from patients who did not have the diagnostic criteria of invasive aspergillosis and received different antibiotics were prospectively analyzed for Galactomannan by Boonsarngsuk et al. Serum and BAL samples were also collected from patients who did not receive antibiotics. At the cut-off index of ≥0.5, false-positive serum results were found in patients who received amoxicillin-clavulanate and piperacillin-tazobactam (26.7%, and 58.3%, respectively).[25]

Galactomannan test in monitoring response to therapy

The concentration of circulating galactomannan corresponds with the fungal tissue burden and may therefore be used to monitor the response to treatment. [26] The course of the antigen titer generally corresponds with the clinical response to antifungal therapy. It is suggested that all high-risk patients with a respiratory tract infection or suspected extrapulmonary aspergillosis should be repeatedly tested with galactomannan ELISA, as the predictive positive value of the assay is highest in these groups.

Aim

To evaluate the role of serum galactomannan antigen assay in the diagnosis of invasive aspergillosis in children receiving treatment for hematological malignancies.

Objectives

1. To define invasive aspergillosis as proven, probable or possible on the basis of consensus definition given by the European Organization for Research and Treatment of Cancer /Mycology Study Group.

2. To study sensitivity, specificity, negative and positive predictive values of galactomannan antigen assay with various optical density index cut offs.

3. To evaluate the performance of galactomannan assay for monitoring the course of invasive aspergillosis.

Inclusion criteria

All of the following must be fulfilled to be considered for enrollement in the study.

1. Children on treatment for acute lymphoblastic leukemia, acute myeloid leukemia OR non Hodgkins lymphoma.

2. a. Febrile episodes requiring admission, irrespective of neutropenia OR
   b.Neutropenic children (absolute neutrophill count ≤750) admitted for sinus/pulmonary symptoms without fever.

3. Age ≤ 14 years.

Repeated inclusion

Patients can be included in the study on more than one occasion, although once diagnosed with IA, patients no longer be eligible for subsequent inclusion, until complete resolution of the infection.

Exclusion criteria

Any of the following will be exclusion criteria.

1. Patient who has received Piperacillin-Tazobactum and/or Amoxicillin –Clavulinic acid in the febrile episode under consideration.

2. Bone marrow transplant receipient

3. Denial of consent.

MATERIALS AND METHODS

- **Study design** – Prospective study
- **Study period** – July 2010 to June 2011
- **Seating** – 1. Pediatric Heamatology Oncology unit, Advanced Pediatric Center, PGIMER
  2. Department of Medical Microbiology, PGIMER.
- **Sample size** – At least 100 febrile episodes in whom the inclusion criteria are full filled, will be studied.

Methodology

Children aged ≤ 14 years on treatment for hematological malignancies, who are admitted with febrile episodes will be enrolled for the study. Children who are afebrile, however, neutropenic and admitted for pulmonary or sinus symptoms will be enrolled as well.

Management of febrile episodes will be as per the discretion of treating physician. Decision for antimicrobials (antibiotics and antifungals) will be taken by the treating team.

Blood samples for Galactomannan assay will be drawn on the day of admission along with complete blood count and Blood for bacterial and fungal culture. Serial estimation of galactomannan will be performed once weekly, till discharge or death of the patient. Investigations that aid in the diagnosis of IA, including, chest x-ray, CT scan lungs/sinuses, BAL and biopsy of the infected tissues will be performed, as clinically indicated.

Active attempts will be made to look for microbiological evidence for aspergillus in body fluids that are available, namely, sputum, sinus fluid, etc. Necropsy or autopsy will be persuaded in all fatal cases if a consent is obtained from the family. Galactomannan levels will be studied by sandwich ELISA.

Details of the episode of illness, examination findings, investigations performed during the hospital stay and treatment received will be documented in the appropriately designed proforma for this study. (Annexture – II)

Definition of Invasive Aspergillosis

Diagnosis of IA will be classified as definite, probable, or possible on the basis of criteria adapted from the European Organisation for Research and Treatment of Cancer/Mycology Study Group (EORTC/MSG) definitions. (Annexture – I)

Measurement of Galactomannan

Serum galactomannan levels will be measured using the *Platellia Aspergillus* enzyme immunoassay (EIA) test as per the manufacturer's instructions. Results will be recorded as the ratio of the optical density (OD) of the sample, to that of the threshold control samples provided in the kit.

Procedure

The Aspergillus Galactomannan EIA assay is an immunoenzymatic sandwich microplate assay for the detection of Aspergillus galactomannan antigen in adult and pediatric serum samples. The assay uses EBA-2 monoclonal antibodies which detect Aspergillus galactomannan.

Specimens

3 ml of blood in a plan vial (without anti-coagulants) will be collected. Specimen will be allowed to clot and centrifuged to obtained serum. A minimum volume of 1.0 ml of serum will be required. Specimen will be stored at 2 to 8°C until processed.

Testing procedure

The test serum will be first boiled for 3 min in the presence of 4% ethylene diamine tetra acetic acid (EDTA) to dissociate immune complexes and destroy interfering substances. The resultant coagulum will be centrifuged at 10, 000G for 10 min, and the supernatant removed. It may be stored at 2-8^{o}C until processed. Aspergillus grows well in contaminated

serum stored at 2-8 C, highlighting the importance of careful specimen processing and storage. Testing will be performed by adding a peroxidase-linked detector antibody followed by 50 microl of the test specimen into the pre-coated microplate wells, followed by incubation at 37^{o}C for 90 min. Next, a tetramethylbenzidine (TMB) chromogen substrate will be added, and the plate incubated in the dark for 30 min at 30^{o}C. If antigen is present, a blue color will appears. The enzyme reaction will be stopped by adding $H2SO_4$ stopping solution, which changes the color to yellow. The microplate wells will be aspirated and washed between steps and read in a microplate reader at both 450 and 620/630 nm wavelengths following the last step.

Cut-off value

Definition of positive galactomannan test – Galactomanan index of ≥1 is taken as positive result in our laboratory, however correlation of the result with the clinical diagnosis is suggested. In the context of equivocal result, further retesting is advised.

In different studies, variable cut off values ranging from 0.5 to 1.5 ODI have been reported. The course of the antigen titer is considered more important than a single cut-off. It is clear from the earlier studies that differences in cut-off level will cause substantial variation in performance of the assay. Appropriate cut off value in children population is a matter of debate. This study will help us to find an appropriate cut off value in pediatric patients on treatment for hematological malignancies in our millieu.

Ethical justification

The study will be undertaken after obtaining ethical clearance from the Institute's Ethics committee. Patients will be enrolled after obtaining informed consent from their parents/guardian. The participation in the study would in no way change or compromise the management. The study will require 3 ml of blood sample to be drawn at admission and weekly there after, until discharge or death of the patient. No separate venepuncturer will be done as the required sample will be collected at the time of venepuncture performed for regular investigations, namely, complete blood count, biochemistry, blood culture etc. Current cost of investigation is Rs 100 per test as per the hospital policy. The test will be done free of cost for patient who are admitted "poor free". The regular patient will pay for the investigation. The results of the study may aid in earlier detection and early intervention to prevent mortality and morbidity due to invasive fungal infection.

Statistical Analysis

Data analysis will be performed with the softwares SPSS version 10. In addition to the cut-off point of 1.5 that is originally recommended by the manufacturer of the Galactomannan Platelia kit, 1.0, 0.7 and 0.5 cut-off points will be used to calculate sensitivity, specificity, negative and positive predictive values as well.

Calculations will be made separately for single positive values and at least two consecutive positive results. The positive cases will be classified as proven plus probable cases or proven plus probable plus possible cases for the purpose of analysis.

Consent form

I ----------father/mother/guardian of ----------Cr no.----------hereby give consent for inclusion of my child in the research study entitled **"Evaluation of serum galactomannan assay for the diagnosis of invasive aspergillosis in children with hematological malignancies "** which is being conducted in the Division of Pediatric Hematology/Oncology, Department of Pediatrics, Post Graduate Institute of Medical Education and Research Chandigarh.

The purpose and procedures of the study have been explained to me in a language understandable to me. I understand that this study will require 3 ml of blood sample at the time of admission and their after weekly, till discharge. The sample will be drawn during regular sampling for complete blood count and blood culture and hence does not require a separate venepuncture. My decision to participate in this study is fully voluntary and I have full rights to withdraw any time during the course of this study. My child will continue to receive the same standard of medical care, irrespective of my decision. I have been assured that my/our identity and personal details will be kept confidential.

Signature	**Witness signature**
Name: …….	Name: …….
Relation with Child : ……	Date: …….

Signature of medical officer

Name: Date:

(Annexture –II)

Table 1

Criteria for the Diagnosis of Aspergillosis

Proven aspergillosis

Microscopic demonstration of septate, acute-angle–branching, nonpigmented hyphae (without yeast forms) measuring 2 to 4 µm from a needle aspiration or biopsy in association with evidence of tissue damage, and a microbiological culture of a specimen obtained by an aseptic procedure from a normally sterile site yielding *Aspergillus* spp in association with clinical or radiologic evidence of focal infection at that site.

Probable aspergillosis

1. One host-related criterion,[a] and
2. One microbiological criterion,[b] and
3. One major, or two minor, clinical criteria[c] from an abnormal site consistent with infection

Possible aspergillosis

1. One host-related criterion,[a] and
2. One microbiological criterion,[b] or
3. One major, or two minor, clinical criteria[c] from an abnormal site consistent with infection

[a] Host criteria

1. Neutropenia defined by an absolute neutrophil count < 0.5×10^9/L for more than 10 days
2. Persistent fever for more than 96 hours refractory to broad-spectrum antibacterial agents
3. Body temperature > 38°C or < 36°C, and any one of the following:
   - Prolonged neutropenia of more than 10 days' duration within the last 60 days
   - Recent (within the preceding 30 days) or current use of immunosuppressive therapy
   - A previous episode of invasive aspergillosis
   - Coexistence of human immunodeficiency virus infection
4. Signs or symptoms of graft-vs-host disease
5. Prolonged (more than 3 weeks) use of corticosteroids

[b] Microbiological criteria

1. Positive culture for *Aspergillus* spp or microscopic appearance of hyphae consistent with *Aspergillus* spp from sputum or bronchoalveolar lavage specimens
2. Positive culture for *Aspergillus* spp or microscopic appearance of hyphae consistent with *Aspergillus* spp from a sinus aspirate
3. Positive antigen test for *Aspergillus* spp from a bronchoalveolar lavage specimen, a cerebrospinal fluid specimen, or in two or more blood specimens
4. Positive culture for *Aspergillus* spp or microscopic appearance of hyphae consistent with *Aspergillus* spp from tissue biopsy or otherwise sterile body fluid

[c] Clinical Criteria

Lower respiratory tract

Major: New pulmonary parenchymal infiltrates on CT scan of the chest including a halo sign, air-crescent sign, or cavitary disease within an area of consolidation

Minor: Symptoms of a lower respiratory tract infection including cough, pleuritic chest pain, hemoptysis, and dyspnea

Physical findings of a pleural rub or consolidation

Any new pulmonary infiltrate not fulfilling the major criterion

Sinonasal infection

Major: Radiologic evidence consistent with infection of the sinuses including opacification of the sinuses, air-fluid levels within the sinuses, erosion of the sinus walls, or extension of the process to neighboring structures

Minor: Upper respiratory tract symptoms including rhinorrhea or stuffiness

Presence of ulceration of nasal structures, nasal eschar, or epistaxis

Periorbital swelling

Maxillary tenderness

Perforation of the hard palate

Central nervous system

Major: Radiologic evidence consistent with central nervous system infection including intracranial space occupying lesions, meningeal enhancement associated with involved perinasal, auricular, or vertebral structures

Minor: Focal neurologic signs including seizures, hemiparesis, or cranial nerve palsies

Cognitive impairment

Signs of meningeal irritation

Abnormalities in the cerebrospinal fluid profile including elevated cell counts, elevated protein concentration, and a reduced cerebrospinal fluid–to–blood glucose ratio

Disseminated aspergillosis

Papular, nodular, or ulcerative skin lesions without other explanation

Small, peripheral, target-like ("bull's-eye") lesions observed on an imaging study (CT scan, MRI scan, ultrasonographic scan) of the liver and/or spleen (hepatosplenic fungal infections)

CT = computed tomography; MRI = magnetic resonance imaging. Adapted from Ascioglu et al.[19]

(Annexture – I)

Proforma

Name: DOB/Age: Sex: M/F

CR No: POC: D.O.A. (Current):

Address:

Phone. No:

Diagnosis:

Phase of treatment:

Last chemotherapy received:

Last episode of FN (if any):

Chief complaints:

Localization of symptoms:

(i) Pulmonary (ii) CNS

(ii) Sinus (iv) GIT

(v) Other

ADMISSION DIAGNOSIS:

Investigation chart:

Date							
Hb							
TLC							
DLC							
ANC							
Plt							

Culture details:

Specimen	Date	Bacterial	Fungal	Others
Blood				
Sputum				
BAL				

Serum Galactomannan:

Date				
Value				

Microscopy of sputum/BAL/Sinus aspirate:

Antifungal prophylyxasis Y/N

If yes: name drug and duration

Radiological investigation:

Chest X-ray	
HRCT	
MRI/OTHERS	

Name of drug	From	To	Duration	Reason for stopping or changing

<u>Major Complications:</u>

Date of discharge:

<u>Outcome of treatment:</u>

(i) Improved

(ii) Improved with squeale

(iii) LAMA/DOR

(iv) Death

- Date
- Cause of death
- Autopsy: Y/N
- Postmortem sample

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ETHICAL JUSTIFICATION

A written ethical clearance was obtained from the Institute's Ethics committee prior to commencing the study. Patients were enrolled after obtaining informed consent from their parents/guardians. The participation in the study did not change or compromise the management of the patients. The study required 3 ml of blood sample to be drawn at admission and weekly there after until discharge or death of the patient. No separate venepuncturer were performed for the study. The required sample were collected at the time of venepuncture performed for regular investigations namely complete blood count, biochemistry, blood culture etc. It was expected that the results of the study would aid in earlier detection and early intervention to prevent mortality and morbidity due to invasive fungal infection.