Hematolojik kanserli hastalarda Invaziv fungal enfeksiyonun erken tanısındabeta glukan testi ne kadar etkilidir?

How effective is beta glucan test in early diagnosis of invasive fungal infection in patient with hematologic malignancy?

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ÖZET

Giriş ve Amaç: İnvaziv fungal enfeksiyonlar (IFE) sıklıkla immün süpresif hastalarda görülmektedir. Antifungal tedavi başarısı için erken tanı anahtar öneme sahiptir. Betaglucan (BG) testi mucormikoz ve kriptokoklar dışında birçok mantar patojeni tespit edebilir. Bu çalışmada betaglucan düzeyinin hematolojik kanserli ve IFE'li hastalarda tanısal bir araç olarak değerlendirmeyi amaçladık.

Yöntem ve Gereçler: Indüksiyon veya konsolidasyon tedavisi alan, klinik ve radyolojik IFE bulguları olmayan 14 günden uzun süreli nötropeni beklentisi olan 46 hasta çalışmaya dahil edildi. Hastanede yattıkları süre içerisinde haftada 2 defa kan galaktmannan (GM) ve BG düzeyleri ölçüldü. Sensitivite, spesifite ve öngörğlen değerlerin yorumlanması için Metod A (kanıtlanmış + muhtemel grup vs non-IFE) ve Metod B (kanıtlanmış + muhtemel +olası grup vs non-IFE) değişkenleri tanımlandı.

Bulgular: Metod A'da; BG testinin sensitivitesi, spesifitesi, pozitif öngörülen değer (PÖD), Negatif öngörülen değer (NÖD) sırasıyla %68.75, %84.1, %52.4, %91.4 olarak tespit edildi. Metod B'de; BG testinin sensitivitesi, spesifitesi, PÖD, NÖD sırasıyla %60, %88.9, %71.4, %82.8 olarak tespit edildi.

Hiçbir hastada ameliyat içi istenmeyen durum olmadı. Cerrahi gruptaki 3 hastaya embolizasyon uygulandı. Postoperatif takiplerde 1 hastada enfeksiyon, 2 hastada nüks saptandı.

Tartışma ve Sonuç:. Bizim verilerimiz ve mevcut literature dayanarak BG ve GM'nin IFE tanısına katkı sağlayan non-invaziv test olduğu sonucuna vardık.

Anahtar Kelimeler: İnvaziv fungal enfeksiyonlar, beta glukan, galaktomannan, sensitivite, spesifite

ABSTRACT

Introductıon: Invasive fungal infections (IFI) are commonly seen in immunosuppressive patients. Early diagnosis is key to optimizing antifungal treatment success. Betaglucan (BG) assay can detect most of the fungal pathogens except mucormycosis and Cryptococcus. In this study we aimed to evaluate the value of BG as a diagnostic tool in patients with hematological malignancy and IFI.

Methods: Forty-six hematological malignancy patients under induction and consolidation chemotherapy that expected to have neutropenia for more than 14 days with no clinical and radiological signs of IFI are included in this study. Blood Galactomannan (GM) and BG levels were measured 2 times in a week during the hospitalization period. Method A (proven + probable groups vs non IFI) and Method B (proven + probable + possible groups vs. non IFI) variables were determined to assess the sensitivity, specifity and predictive values.

Results: In method A; BG test's sensitivity, specifity, positive predictive value (PPV), negative predictive value (NPV) was determined as 68.75%, 84.1%, 52.4%, 91.4% respectively. In method B (proven + probable + possible groups vs. non IFI) BG test sensitivity, specifity, PPV, NPV was determined as 60%, 88.9%, 71.4%, and 82.8% respectively.

Discussion and Conclusıon: Depending on our data and present literature; we conclude that BG and GM is a non-invasive contributory test for the diagnosis of IFI The current treatment of the symptomatic extremity

Key words: Invasive Fungal Infections, Beta glucan, galactomannan, sensitivity, specifity

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Introduction

Invasive fungal infections (IFI) are commonly seen in immunosuppressive patients with hematological malignancy [1], solid organ and hematopoietic stem cell transplantation with serious morbidity and mortality [2, 3]. Routine azole prophylaxis decreases invasive Candida infections and mold infections become as a major concern. Aspergillus is responsible for approximately 90% of mold infections [4]. Early diagnosis is key to optimizing antifungal treatment success [5]. Diagnosis is challenging in patients with hematological malignancy due to hemorrhagic diathesis and poor performance status. Biomarker assays offer the advantage of ease of performance, safety, avoidance of invasive diagnostic procedures, and earlier diagnosis. Two tests - galactomannan (GM) and beta glucan (BG) - are globally commercialized. Survival rates from Invasive Aspergillosis (IA) in patients with AML and HCT have improved over the past decade due to earlier diagnosis and therapy [6 - 10].

BG is secreted from the pathogen fungi's wall during the infection and serum BG level is thought to be an indicator for fungal infection [11]. BG assay can detect most of the fungal pathogens except mucormycosis and Cryptococcus, encountered in patients with AML and undergoing HCT [5, 12]. A meta-analysis of more than 1700 patients with hematological malignancies showed an excellent specificity, but the sensitivity was less promising [13]. In this study we aimed to evaluate the value of BG as a diagnostic tool in patients with hematological malignancy and IFI.

Materials And Methods:

Eighty-one IFI episodes in 46 hematological malignancy patients over 18 years old were prospectively monitored between April 2008 and January 2009. Patients under induction or consolidation chemotherapy that expected to have neutropenia for more than 14 days with no clinical and radiological signs of IFI are included in this study.

Patients; under 18 years old, with neutropenia period lasting less than 14 days, poor performance condition with life expectancy less than one month, received antifungal medications one month prior to study were excluded from the study.

Patients were followed up closely for the signs and symptoms of sino-pulmonary infection during their hospitalization period. An episode was determined as a fever and/or infection period in post-chemotherapy neutropenia state.

Blood GM and BG levels were measured 2 times in a week during the hospitalization period. Samples collected for GM analysis were studied as manufacturer's (Platelia ® Aspergillus; Bio-Rad Labaratories, Marnes-la-Coquette, France) instructions with monostep sandwich Enzyme immunoassay (EIA) method. Samples with index value ≥ 0.5 were considered positive and underwent repeated testing to ensure positive results. Samples for BG were studied through manufacturer's (Fungitell assay; Cape-Cod Inc, USA) instructions. Results ≥80pg/ml was considered as positive. Serum samples for BG were collected, stored and studied, in endotoxin and glucan free equipments to avoid false positive results. Positive episode was determined based on 2 consecutive positive results for both GM and BG. Method A and Method B variables were determined to assess the sensitivity, specifity and predictive values. Proven and probable IFI patients were determined as true positive and non-IFI patients were determined as true negative for Method A. Proven, probable and possible IFI patients were determined as true positive and non-IFI patients were determined as true negative for Method B.

All the episodes were determined on the basis of European Organization for Research and Treatment of Cancer (EORTC), *National Institute of Allergy and Infectious Diseases (*NIAID) and Mycoses Study *Group* (MSG) criteria.

Statistical Analysis:We use SPSS 13 software for statistical analysis. Pearson chi-square test is used to investigate; whether sex, disease state is a risk factor for IFI. Forward logistic regression analysis was performed for variables considered to be a risk factor for IFI.

RESULTS:

Patients mean age was 40.3 ± 13 (18-66 years). Underlying diseases were AML (n=33), ALL (n=8), NHL (n=3), refractory MM (n=2).

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Twenty-seven (33.3%) patients were newly diagnosed malignancy, 31 (38.3%) patients had disease in remission, 11 (13,6%) patients had recurrence of primary disease and 12 (14.8%) patients had refractory disease. Totally, 18 of 46 patients died during follow up period. Mean episode duration was 31.3 ± 13.7 days (13-81 days). Seventy-six of the episodes were neutropenia and mean duration of neutropenia was 10 days. Patients' characteristics were depicted on Table 1.

We compared mean age, sex, neutropenia and hospitalization period, neutrophil count, underlying disease in IFI and non-IFI patients in order to determine the risk factors. Mean age was 43.4 ±12.5 (18-66 years) and male/female ratio was 11/7. No significant differences were observed about sex, age, underlying disease and disease status between the groups (P>0.05).

Days with neu #<500, days with neu #<100 and hospitalization period were all found to be risk factors for IFI (p=0.002, p=0.02 and p=0.01 respectively). Prolonged neutropenia and hospitalization period increased the risk for IFI.

The subgroup analysis of 81 IFI episodes were as follows: 2 proven sinonasal mucormycosis (2.5%), 2 proven sinonasal aspergillosis (2.5%), 14 probable invasive pulmonary aspergillosis (IPA) (17.3%), 7 possible IPA (8.6%), 2 possible invasive candidiasis (2.5%) and 54 non-IFI (66.7%).

Throughout the study 719 serum samples were analyzed to determine BG levels. Mean BG test number for each episode was 8.8. The number of samples studied for each episode was 10.7 in proven group, 11.5 probable group, 11.2 possible group, and 7.6 non-IFI groups. Mucormycosis cases (n=2) had negative test results as expected and they were excluded from the study.

In method A (proven + probable groups vs non IFI); BG test's sensitivity, specifity, positive predictive value (PPV), negative predictive value (NPV) was determined as 68.75%, 84.1%, 52.4%, 91.4% respectively. GM test sensitivity, specifity, PPV, NPV was determined as 68.75%, 98.4%, 91.7%, and 92.5% respectively (Table 1).

In method B (proven + probable + possible groups vs. non IFI) BG test sensitivity, specifity, PPV, NPV was determined as 60%, 88.9%, 71.4%, and 82.8% respectively. GM test sensitivity, specifity, PPV, NPV was determined as 47.8%, 98.2%, 91.7%, and 82.1% respectively (Table 2).

Table-1: Patients' Characteristics

	Proven IFI	**Probable IFI**	**Possible IFI**	**Non IFI**	**Total**
Episode #	4	14	9	54	81
Neutropenic episode #	4	14	8	50	76
Patients #	4	14	9	34	46
Mean Age **Range**	47.5±5.3 42-53	42.2±13.8 18-66	42.0±11.6 23-59	41.3±13.9 18-66	40.3±13.1 18-66
Expired patient #	0	6	3	9	18
Episode duration **Range**	35.2±16.4 14-54	39.8±18.5 21-81	40.6±19.5 18-21	27.3±8.7 13-46	31.3±13.7 13-81
Background diseases					
AML	4	9	9	40	62
ALL	0	4	0	7	11
NHL	0	0	0	4	4
MM	0	1	0	3	4
Host factors					
Episodes with steroid	0	3	0	10	13
Neu<500 **Range**	24±7.6 17-32	19.5±7.4 11-38	18.5±6.7 10-31	13.3±6.7 0-28	15.5±7.5 0-38
Neu<100 **Range**	15±3.8 10-18	12.2±7.5 3-28	14.8±9.3 0-31	7.7±4.7 0-21	9.7±6.4 0-31

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#: Number, IFI: Invasive Fungal Infection, AML: Acute Myeloid Leukemia, ALL: Acute Lymphoblastic Leukemia, NHL: Non-Hodgkin Lymphoma, MM: Multiple Myeloma, Neu: Neutrophil

Table-2: Comparison of BG and GM results for groups

	METHOD A (%)	METHOD B (%)
BG Senstivity	68.75	60
GM Senstivity	68.75	47.8
BG Specifity	84.1	88.9
GM Specifity	98.4	98.2
BG NPV	91.4	82.8
GM NPV	92.5	82.1
BG PPV	52.4	71.4
GM PPV	91.7	91.7

BG: Beta Glucan, GM: Galactomannan, NPV: Negative Predictive Value, PPV: Positive Predictive Value

Table-3: Studies about BG

Studies	Test	Sensitivity	Specifity	PPV	NPV
Ostrosk Z L (14)	*BG (60 pg/ml)*	69.9	87.1	83.8	75.1
Ostrosk Z L (14)	*BG (80 pg/ml)*	64.4	92.4	89	73
Kawazu M (16)	*GM*	100	93	55	100
Kawazu M (16)	*PCR DNA*	55	93	40	96
Kawazu M (16)	*BG (60 pg/ml)*	55	93	40	96
Obayashi T (18)	*BG (30 pg/ml)*	95.1	85.7	41.1	99.2
Obayashi T (18)	*BG (60 pg/ml)*	85.4	95.2	70.4	98
Obayashi T (18)	*BG (80 pg/ml)*	78	98.4	86.7	97.1
Senn L (19)	*BG*	63	96	79	91
Pazos C (20)	*BG*	87.5	89.6	70	96.3
Our present study	***BG (80 pg/ml Method A)***	**68.7**	**84.1**	**52.4**	**91.4**
Our present study	***BG (80 pg/ml Method B)***	**60**	**88.9**	**71.4**	**82.8**

14: Reference number 14, 16: Reference number 16, 18: Reference number 18, 19: Reference number 19, 20: Reference number 20, BG: Beta Glucan, GM: Galactomannan, NPV: Negative Predictive Value, PPV: Positive Predictive Value

DISCUSSION:

BG antigen is a fungal cell wall structure of all fungus except Zygomycetes and Cryptococcus neoformans [11]. This antigen can be detected in serum and other body fluids. It has been shown that BG can be positive before the clinical symptoms and radiological signs [14-20]. Various studies show that this test's sensitivity and specifity varies between 55-95% and 77-96%, respectively [14-20]. The differences among studies are due to population heterogeneity and 'positive result' description. Different cut-off values for positivity and the number of positive results all affect test results.

In this prospective study we evaluated the reliability of serial serum BG levels assessment for diagnosis of IFI in patients with hematological malignancies.

The cut-off values of non-invasive diagnostic tests affect from statistical parameters dramatically. BG levels increase

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during the progression of IFI, so low antigen levels are important in diagnosis of early IFI [19]. Actually, most of the patients with low antigen levels and in possible groups undergo empiric or prophylactic anti-fungal therapy, which is usually curative for most of the conditions among IFI population.

Some technical problems complicate BG test results such as requirement of glass tubes without endotoxin and glucan, false positivity with albumin and immunoglobulin and cross-reaction with some kind of medications. Because all these might affect the results it is crucial to interpret the results in view of clinical, radiological and other microbiological data. Some of the studies about BG are listed in Table-3

As a result, there are many conflicting results in different studies about sensitivity and specifity of BG as shown above. Test results were affected from many variables like IFI group description and reference values in statistical analysis. It is clear that there is still a need for non-invasive test with 100% sensitivity and specifity. Well-designed studies with large patient population are needed for BG. Depending on our data and present literature; we conclude that BG and GM is a non-invasive contributory test for the diagnosis of IFI.

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