GALACTOMANNAN ANTIGEN TEST AND REAL TIME PCR IN DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS IN NEUTROPENIC BONE MARROW TRANSPLANT PATIENTS

Original Article

GALAKTOMANNAN ANTİJEN VE REAL TİME PCR TESTLERİNİN NÖTROPENİK KEMİK İLİĞİ TRANSPLANT HASTALARINDAKİ İNVAZİF MANTAR ENFEKSİYONLARI TANISINDAKİ YERİ

Yesim GUROL

Yeditepe University Hospital Microbiology and Clinical Microbiology, Istanbul.

Yener KOC

Yeditepe University Hospital Stem Cell Transplant Unit,Istanbul.

Iskender KARALTI

Yeditepe University Hospital Microbiology and Clinical Microbiology, Istanbul.

Zehra KİPRİTCİ

Yeditepe University Hospital Microbiology and Clinical Microbiology, Istanbul.

Zuhal TEKKANAT TAZEGUN

Yeditepe University Hospital Microbiology and Clinical Microbiology, Istanbul.

Sesin KOCAGOZ

Yeditepe University Hospital Infectious Diseases,Istanbul.

Tanıl KOCAGOZ

Yeditepe University Hospital Microbiology and Clinical Microbiology, Istanbul.

Gulden CELİK

Yeditepe University Hospital Microbiology and Clinical Microbiology, Istanbul.

Corresponding Author
Yesim GUROL

Yeditepe University Hospital Microbiology and Clinical Microbiology. Devlet yolu Ankara cad. No 102-104.34755 Kozyatagi-Istanbul-TURKEY.
e-mail: yesimg@yeditepe.edu.tr

ABSTRACT

Background:In hematopoetic stem cell transplant (HSCT) recipients, moulds have emerged as a major cause of mortality and incidence of invasive aspergillosis has been shown to be increased. Rapid diagnostic procedures are needed in order to diagnose invasive fungal infections especially aspergillus infections.

METHODS

In this retrospective study, in their neutropenic fever episodes the results of galactomannan tests (GM) (Platelia Aspergillus test, Bio-Rad, France) applied 37 stem cell transplant patients in Yeditepe University Hospital, were handled. Galactomannan results were evaluated by blood or sputum cultures and/or fungal PCR. The total number of tests applied to these samples were 69.

RESULTS

Among four ex patients, everything was correlated in only one patient. From 66 PCR tests, 3 tests (4,5%); from 69 GM tests 6 tests (9%) were found as positive.

CONCLUSION

The definition of invasive fungal disease and treatment and diagnosis guidelines are revised and the place of the galactomannan test and PCR is still being discussed; such studies should take place for also standardization and validation of these tests.

Key words : *Aspergillus; fungal PCR;galactomannan;stemcell;transplantation.*

ÖZET

AMAÇ

Hematopoetik kemik iliği transplant alıcılarında, mantarlar mortalitenin en önemli nedenlerinden biri olarak ortaya çıkmış ve invazif aspergillozis insidansında artmış olmuştur. Hızlı tanı prosedürleri invazif fungal enfeksiyonlarını özellikle aspergillus enfeksiyonlarının tanısı için gereklidir.

GEREÇ-YÖNTEM

Retrospektif çalışmamızda nötropenik ateş ataklarında Yeditepe Üniversitesi Hastanesindeki 37 kemik iliği transplant hastasına uygulanan galaktomannan test (GM) (Platelia Aspergillus test, Bio-Rad, France) sonuçları ele alınmıştır.Galaktomannan sonuçları kan veya balgam kültürleri ve/veya fungal PCR sonuçlarıyla birlikte değerlendirilmiştir. Toplam değerlendirilen test sayısı 69'dur.

BULGULAR

Kaybedilen dört hastada yapılan testler sadece bir hastada ilişkili bulunmuştur. 66 PCR testinden 3 test (%4,5); 69 GM testinden 6 test (%9) pozitif olarak saptanmıştır.

SONUÇ

Invazif fungal hastalığın tanısı, tedavisi ve tanı rehberleri revize edilmekte ve galaktomannan testi ve PCR ın yeri tartışılmaktadır. Bu tür çalışmalar bu testlerin standardizasyonu ve validasyonu için de gereklidir.

Anahtar kelimeler: *Galaktomannan; aspergillus; kemik iliği; transplantasyon, fungal PCR.*

INTRODUCTION

Invasive fungal infections are known as a cause of morbidity and mortality in patients with allogeneic bone marrow transplantation (1). Especially in hematopoetic stem cell transplant (HSCT) recipients, moulds have emerged as a major cause of mortality and incidence of invasive aspergillosis (IA) has been shown to be increased(2).

Despite significant advances in the management of immunosuppressed patients, IA remains an important life-threatening complication. In the past two decades, the incidence of IA in this population increased (3). Factors that predispose patients to develop IA include prolonged granulocytopenia, the development of graft-versus-host disease, immunosuppressive therapy, the use of adrenal corticosteroids, and the prolonged impairment of host defenses associated with diseases such as chronic granulomatous disease.

Rapid diagnostic procedures are needed in order to diagnose invasive fungal infections especially aspergillus infections to improve survival , but they are difficult to diagnose and identify due to the difficulties of conventional diagnostic methods. At present, diagnosis generally depends upon the cultivation of Aspergillus from respiratory tract samples or the detection of hyphae within biopsy specimens. These approaches are restricted by the limited sensitivity of cultures and the invasiveness of

transbronchial biopsies.Presumptive diagnoses based on the evolution of lesions detected by thoracic computer tomography (CT) scanning in the absence of culture or biopsy results, but this strategy is also limited by low sensitivity and specifity (4).

New exciting developments in the early diagnosis of IA and the acceleration of antifungal drug discovery offer promise for the future(5).

The diagnosis of IA has been improved over the decades, and new methods like fungal antigen and DNA detecting tests in blood and other body fluids are used along with the use of high resolution CT (1). The use of GM test for high risk haematology patients twice a week and CT scan of the chest or bronchoscopy or both are used by many European countries (6). Early detection of IA by GM with early CT scanning reduces mortality. GM test needs to be also investigated in non haemotology patients.

The aim of this study is to evaluate retrospectively the diagnostic tools performed in IA patients in our hospital and to share the procedures to be applied as a policy in the neutropenic patients comparing with the other studies.

MATERIALS AND METHODS

GM tests were applied to 37 stem cell transplant patients in their neutropenic fever episodes in Yeditepe University Hospital. The GM results were evaluated by blood or sputum cultures and/or fungal PCR.

For this purpose primers previously described for panfungal PCR assay were employed (7,8). The reaction mix were loaded into light cycler capillaries and placed in the Light Cycler 2.0 instrument (Roche Diagnostics). After initial activation of fast start enzyme for 10 minutes at 95°C, 45 cycles constituting of 5 seconds at 95°C, 10 seconds at 50°C and 20 seconds at 72°C were performed. At the end a melting curve and a melting peak was obtained by increasing the temperature at a rate of 0.1°C/second from 72°C to 95°C. Candida albicans and Aspergillus niger DNA were used as positive control and deionized water as negative control in each assay.

GM antigen test is performed by direct double sandwich ELISA (Platelia Aspergillus test, Bio-Rad, France). The assay use the rat monoclonal antibodies EBA-2 , which are directed against Aspergillus galactomannan. The monoclonal antibodies are used to coat the wells of the microplate and bind the antigen and to detect the antigens bound the sensitized microplate. Samples were processed as the manufacturer's instructions. The index values of this test was <0.5 for negative, ≥0.5 for positive values.

All sera tested in routine for GM and fungal PCR were stored at –20oC until the time of testing.

RESULTS

GM tests were applied to 37 patients who were neutropenic and had fever episodes. Totally 66 samples were tested for fungal DNA by real time PCR with or without GM tests . The sample distribution was as follows; one sample was taken from 17 patients, two from 9 patients, three and four samples were taken from 5 patients and finally five samples from 1 patient. The number of tests applied to these samples were 69. The distribution of the results with applied therapy and exitus results is shown in **Table 1**.

Patient no	Age	Gender	Underlying disease	Number of samples tested	GM results	Culture results	PCR results	Exitus	Invazive fungal disease	Antifungal therapy
1	60	M	AML	1	-	-	-			
2	39	M	HL	1	-	-	-			
3	33	M	AML	1	-	-	-			
4	24	M	Burkitt	1	-	-	-			
5	28	F	AML	1	-	-	-			
6	40	F	NHL	1	-	-	-			
7	68	F	NHL	1	-	-	+			
8	40	M	MM	1	-	-	-			
9	46	F	AML	1	+	Aspergillus	-	+	+	+
10	21	M	HL	1	-	-	-			
11	49	M	AML	1	-	-	-			
12	23	M	AML	1	-	-	-			
13	34	M	CML	1	-	-	-			
14	27	M	CML	1	-	-	-			
15	63	F	CML	1	-	-	-			
16	28	F	AML	1	-	-	-			
17	23	F	HL	1	-	-	-			
18	32	M	Thalassemia	2	-	-	-			
19	56	M	NHL	2	+	-	-			
20	32	M	ALL	2	-	-	-			
21	26	F	HL	2	+	Aspergillus	+	+	+	+
22	25	F	NHL	2	-	-	-			
23	38	F	AML	2	-	-	-			
24	54	M	MM	2	-	-	-			
25	42	M	MM	2	-	-	-			
26	59	M	MDS	2	-	-	-			
27	45	F	Burkitt	3	+*	Candida	-		+	+
28	48	M	MDS	3	-	-	-			
29	48	F	MM	3	-	-	-			
30	62	M	AML	3	+	-	-	+		
31	33	M	AML	3	-	-	-			
32	22	F	ALL	4	-	-	-			
33	68	M	NHL	4	-	Candida	+*	+	+	+
34	35	F	AML	4	-	-	-			
35	52	M	CML	4	-	-	-			
36	34	M	AML	4	-	-	-			
37	19	F	AML	5	-	-	-			

*Test is positive in two samples.

Table 1: *The results overview.*

In patient number 7, PCR was positive although none of GM and culture was positive; it might be a false positivity. GM and aspergillus culture positivity were observed in only two patients number 9 and 21. In patient number 27, although GM was positive in two samples no positivity was observed in the cultures. Beyond PCR positivity in patient number 33 Candida spp. was positive in the culture and GM was negative. PCR could detect both candida and aspergillus, so this could not be a false positivity with culture positivity. Among four ex patients (number 9,21,30 and 33) everything was correlated in only number 21. From 66 PCR tests, 3 tests (4,5%); from 69 GM tests 6 tests (9%) were found as positive.

DISCUSSION

The GM test performs best among HSCT recipients and patients with hematologic malignancies, populations with the highest incidence of IA (9). Experience among patients undergoing solid organ

transplantation is much more limited. In studies of lung and liver transplant recipients, the sensitivities of the assay were 30% and 56%, respectively (10,11) with specificities of 93%-95% and 87%-94%, respectively (12).

Several studies have also indicated lower GM levels in patients undergoing antifungal treatment, which is an important issue in view of the increasing number of patients who receive antifungal prophylactic or preemptive treatment (13).

In some studies GM positivity was found higher than PCR results (14,15). In the follow up in the patients with hematological malignancies, GM was considered as a diagnostic tool (16,17,18). There are also studies about indicating higher PCR sensitivity against cultures and the other methods like ELISA (19,20).

The availability of the GM ELISA also may contribute substantially toward a non–culture-based diagnosis of IA. GM antigen has also been detected in CSF samples from patients with CNS aspergillosis and in bronchoalveolar lavage fluid specimens from patients with invasive pulmonary aspergillosis although the use of GM in such samples is investigational (21,22).

Antifungal therapy or prophylaxis, significantly reduce use of PCR-based diagnosis, which amplifies Aspergillus-specific fungal genes (23,24). However, these systems have not been standardized, are not commercially available, and remain investigational (25).

The definition of invasive fungal disease and treatment and diagnosis guidelines are revised and the place of the GM ELISA and PCR is still being discussed. Besides PCR, GM ELISA in brochoalveolar lavage and CSF needs the standardization and validation (26,27).

The limitation of our study is lack of information in follow up of some patients. The content of antifungal and the other therapies was not so clear. The number of patients is not enough to compare the sensitivity and specificity of GM and PCR tests. For all the samples were not applied to GM and PCR together, it was difficult to interpret the comparison of these tests.

As a microbiology laboratory we should convince the clinicians to demand GM and PCR tests together for the diagnosis and therapy. Follow up of GM or PCR tests , is recommended for the decision of the antifungal therapy.

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