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COMPARAÇÃO DE DESEMPENHO ENTRE A METODOLOGIA ASPERGILLUS POR ENSAIO DE FLUXO LATERAL E ELISA PARA DETECTAR O ANTÍGENO GALACTOMANNAN NO DIAGNÓSTICO DE ASPERGILOSE INVASIVA

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RESUMO

A Aspergilose Invasiva (AI) é uma doença infecciosa oportunista, causada pelo fungo do gênero *Aspergilllus* spp., o qual acomete principalmente pacientes imunocomprometidos. É responsável por elevada taxa de morbimortalidade nesses indivíduos, se não for diagnosticada o mais breve possível. Por quanto, a capacidade de tratar essas infecções depende diretamente do diagnóstico precoce, bem como da identificação das espécies presentes em cada hospedeiro. Entretanto, os métodos diagnósticos clássicos estão longe de ser adequados. Visto que, requer testes invasivos em pacientes gravemente enfermos, o que muitas vezes é inviável. O desenvolvimento de ensaios para detectar os biomarcadores do antígeno Galactomannan (GM) de pacientes com risco de AI melhorou significativamente o manejo dela. No entanto, o teste convencional imunoenzimático tem um alto custo e tempo de resposta. Devido a esses fatores tem sido desenvolvido metodologias menos laboriosas, como o teste de Fluxo Lateral (LFA – GM) para o diagnóstico AI. Diante do exposto o objetivo do presente trabalho, com base na literatura, foi avaliar o desempenho da metodologia de Fluxo Lateral (LFA - GM) e comparar com o teste ELISA – GM, usualmente utilizado para detecção do antígeno Galactomannan e contrapor com dados analisados do laboratório de microbiologia do Hospital de Clínicas de Porto Alegre. Por conseguinte, a metodologia GM-LFA mostrou excelente desempenho para o diagnóstico de IA em pacientes pré-dispostos a doença e pode ser uma opção confiável em ambientes onde o teste ELISA - GM não é viável.

Palavras-chave: Galactomannan, Fluxo lateral, Aspegilose invasiva, ELISA GM.

ABSTRACT

Invasive Aspergillosis (IA) is an opportunistic infectious disease caused by the fungus of the genus Aspergilllus spp. It is responsible for a high rate of morbidity and mortality in these individuals, if not diagnosed as soon as possible. Therefore, the ability to treat these infections depends directly on early diagnosis, as well as on the identification of the species present in each host. However, classical diagnostic methods are far from adequate. Since it requires invasive testing in severely ill patients, this is often not feasible. The development of assays to detect the galactomannan (GM) antigen biomarkers of patients at risk of IA has significantly improved the management of IA. However, the conventional enzyme immunoassay has a high cost and turnaround time. Due to these factors less laborious methodologies have been developed, such as the Lateral Flow Assay (LFA-GM) for IA diagnosis. The objective of this study, based on the literature, was to evaluate the performance of the LFA-GM methodology and compare it with the ELISA-GM test usually used to detect the galactomannan antigen and compare it with data analyzed in the microbiology laboratory of the Hospital de Clínicas de Porto Alegre. Therefore, the GM-LFA methodology showed excellent performance for AI diagnosis in patients pre-disposed to the disease and may be a reliable option in settings where the ELISA - GM test is not feasible.

Keywords: Galactomannan, lateral flow, Invasive aspegillosis, GM ELISA.

SUMÁRIO

1 INTRODUÇÃO 6

1.1 DIAGNÓSTICO DA ASPERGILOSE INVASIVA E A DETECÇÃO DO ANTÍGENO GALACTOMANNAN (GM)... 7

1.1.1 Detecção do antígeno Galactomannan por ELISA - GM ELISA 8

1.1.2 Ensaio de Fluxo Lateral para detecção de Galactomannan - (GM-LFA).................. 9

1.2 OBJETIVOS.. 11

1.2.1 Objetivo geral.. 11

1.2.2 Objetivos específicos.. 11

2 ARTIGO CIENTÍFICO.. 112

3 CONCLUSÃO E PERSPECTIVAS 22

REFERÊNCIAS 23

ANEXO A – NORMAS DE PUBLICAÇÃO DO BRAZILIAN JOURNAL OF INFECTIOUS DISEASES 27

1 INTRODUÇÃO

A Aspergilose Invasiva (AI) é uma doença fúngica invasiva (DFI) causada por fungos filamentosos do gênero *Aspergillus* spp. que acomete principalmente pacientes imunocomprometidos, sendo o principal fator de risco é a neutropenia prolongada.[1]

A doença é causada pelo microrganismo do gênero Aspergillus, os quais são caracterizados como fungos filamentosos, hialinos, septados. Estão presentes no ambiente, no solo, onde há matéria orgânica em decomposição. Reproduzem-se assexuadamente através da produção de conídios, estruturas esféricas de camada externa hidrofóbica e tamanho relativamente pequeno com facilidade de dispersão no ar.[2] Estas estruturas são produzidas em grandes quantidades e os humanos podem inalar centenas destas diariamente. A infecção em humanos se inicia pelo trato respiratório, pela inalação dos seus esporos presentes no ar. Além disso, há evidencias que fatores relacionados ao clima favorece a permanência do fungo no local e a sua relação hospedeiro parasita.[3,4]

Este gênero apresenta mais de 250 espécies, divididos em subgêneros ou complexos de espécies, no entanto apenas algumas são patogênicas ao homem, em destaque: *Aspergillus fumigatus,* o qual é responsável pela maioria das infecções, seguido por *A. flavus, A. niger e A. terreus*, há ainda evidências de outras espécies, que também tem destaque em pacientes imunodeprimidos.[3,5]

Eles têm a capacidade de causar uma variedade de doenças aos humanos, variando desde alergias até formas mais graves e invasivas. Esta última é definida como Aspergilose Invasiva, sendo a mais grave a Aspergilose Pulmonar Invasiva (API), a qual está relacionada a elevada taxa de morbidade e a mortalidade, que varia de 70 a 90% nesses pacientes.[6] No entanto a sua a gravidade dependerá da resposta imunológica do indivíduo ao patógeno, visto que, ela afeta principalmente pacientes imunocomprometidos.[7]

A Aspergilose Invasiva acomete principalmente pacientes Hematológicos, pós transplantados halogênicos de células tronco, transplantados de órgãos sólidos, indivíduos com alguma patologia pré-existente, com tumores sólidos, distúrbios autoimunes, imunodeficiência congênita ou doenças pulmonares crônicas, assim como, aqueles em tratamento com imunossupressores ou quimioterápicos. Entretanto, há também relatos na literatura do acometimento de pessoas fora do grupo de risco, mas que estão gravemente enfermos, internados em unidade de terapia intensiva.[3,4,7]

Além disso, recentemente AI tem acometido e agravado o prognóstico de pacientes internados em centro de terapia intensiva com influenza grave e infecção por síndrome respiratória aguda grave coronavírus 2 (SARS-CoV2). A mortalidade nesses indivíduos pode chegar a 50%.[8]

Por conseguinte, o prognóstico não favorável da API é consequência tanto da imunossupressão do paciente quanto da inexatidão do diagnóstico clínico, visto que, a doença não apresenta sinais e sintomas específicos. Logo, devido a esses fatores, ocorre um atraso no início do tratamento, o que resulta no agravamento do quadro ou até mesmo a morte do paciente. Portanto, o diagnóstico precoce e específico é um fator fundamental para a diminuição da mortalidade. No entanto, devido a limitação da identificação do fungo, existe uma busca constante de exames que possam indicar a presença de *Aspergillus* spp. no paciente imunodeprimido.[9]

Por quanto, a capacidade de tratar essas infecções depende diretamente do diagnóstico precoce, bem como da identificação das espécies presentes em cada hospedeiro.

1.1 DIAGNÓSTICO DA ASPERGILOSE INVASIVA E A DETECÇÃO DO ANTÍGENO GALACTOMANNAN (GM)

Em relação ao diagnóstico da Aspergilose Invasiva existe uma grande dificuldade para se chegar a um resultado específico e exato. Para o diagnóstico definitivo é necessário a visualização de elementos fúngicos nos tecidos obtidos por biópsia ou punção aspirativa com agulha do sítio de infecção, aliados com achados radiológicos e micológicos de evidência clínica.[10.11] No entanto, exames histológicos requerem procedimentos invasivos e frequentemente são excluídos, uma vez que os pacientes suspeitos são indivíduos imunodeprimidos, principalmente aqueles neutropênicos, os quais são submetidos a tratamentos quimioterápicos e aqueles pós transplantes de células tronco.[12,13]

Já a cultura a partir de amostras biológicas possui baixo valor preditivo positivo, visto que, nem sempre pode ser diferenciado se um resultado positivo é referente à doença invasiva, ou contaminação.[14] No entanto, novas abordagens diagnósticas têm se baseado na detecção de marcadores circulantes, antígenos ou constituintes da parede celular fúngica.

Estes biomarcadores foram incluídos como critério micológico em consenso sobre doenças fúngicas invasivas (DFI) elaborado pela European Organization for Research and Treatment of Cancer/ Invasive Fungal Infection Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG).[15] Este consenso foi projetado para pesquisa clínica e estudos epidemiológicos. Ademais, eles foram adotados por agências reguladoras para avaliação de antifúngicos e para avaliação de testes diagnósticos, todavia essas definições não são indicadas para direcionar ou guiar o atendimento ao paciente.[16]

O consenso elaborado pelo EORTC/MSG propõe definições padronizadas para o diagnóstico da DFI baseadas em fatores do hospedeiro, critérios clínicos e evidência micológica, dos quais faz parte a detecção do antígeno Galactomannan (GM) em plasma e no líquido cefalorraquidiano como critério comprovado para o diagnóstico da Aspergilose Pulmonar Invasiva. Os níveis de probabilidade atribuídos são: DFI “comprovada”, “provável” em pacientes com câncer e transplantados de células tronco hematopoiéticas ou transplantes de órgãos sólidos.[15]

O desenvolvimento de ensaios para detectar os biomarcadores de antígeno GM de pacientes com risco de AI melhorou significativamente o manejo dela. Ademais, estudos recentes apontaram que a negatividade persistente da reação em cadeia da polimerase (PCR de GM) e detecção do antígeno Galactomannan por ELISA (GM-ELISA) é suficiente para excluir a infecção e suspender ou interromper a terapia antifúngica sem afetar negativamente o prognóstico do paciente.[11] No entanto, para a realização da PCR-GM é necessário validação de ensaios e equipamentos de laboratório molecular, os quais estão disponíveis somente em grandes centros laboratoriais, assim como existe uma limitação da disponibilidade de testes GM-ELISA para pequenos centros diagnósticos.[17]

O diagnóstico definitivo é realizado através da combinação de achados clínicos, juntamente com métodos laboratoriais e estudos de imagem. Além disso, mesmo apresentando baixa sensibilidade e recuperação fúngica baixa, a cultura fúngica continua sendo o padrão-ouro diagnóstico e, se positiva, permite guiar a terapia através do teste de suscetibilidade, quando necessário.[18]

1.1.1 Detecção do antígeno Galactomannan por ELISA - GM ELISA

O Galactomannan (GM) é um heteropolissacarídeo termoestável que está presente na parede do *Aspergillus* spp. sendo liberada durante o crescimento da hifa e germinação de conídios no tecido que é infectado. A molécula imunogênica com sítios imunorreativos contém unidades de galactofuranose.[19,20] Cada espécie fúngica possui GM com propriedades químicas diferentes entre si. A GM é um biomarcador que contribui para diagnóstico da AI, uma vez que o antígeno é liberado na circulação sanguínea durante o crescimento das hifas nos tecidos do hospedeiro. Ainda, por ser hidrossolúvel, o antígeno pode ser encontrado em diferentes amostras clínicas, como no soro, urina, líquido cefalorraquidiano e no lavado bronco alveolar (BALF).[11,20-22]

A metodologia mais utilizada é realizada por ensaios imunoenzimáticos do tipo Elisa sanduíche, no qual é utilizado o anticorpo monoclonal de rato EBA-2 dirigido contra Aspergillus galactomannan. O antígeno é ligado aos poços da microplaca, a qual é revestida com o anticorpo EBA-2, o resultado, então, pode ser detectado por uma reação colorimétrica medida por densidade óptica.[23]

O desempenho do teste apresenta sensibilidade e especificidade variada, a depender do tipo de amostra, no soro e no líquido do lavado brônquico alveolar.[24] No entanto, em amostras de lavado bronco alveolar mostrou sensibilidade e especificidade próximas de 95 %, o que sugere boa precisão diagnóstica.[8.23] Ademais, as diretrizes europeias recomendam, atualmente, o uso de Galactomannan no lavado brônquio alveolar como um critério micológico para diagnóstico da AI.[25]

Entretanto, o teste pode apresentar reações cruzadas com outros fungos, mesmo sendo específico para *Aspergillus spp*, essas reações podem ocorrer com os microorganismos da família Trichocomaceae, *Fusarium spp. e Histoplasma capsulatum*.[23]

Além disso, o teste GM possui algumas limitações, pois apesenta resultados falsos positivos em pacientes com infeções bacterianas, em tratamento com antibióticos B lactmâmicos, em transfusões de sangue, e Hemodiálise, já os resultados falsos negativos estão relacionados a profilaxia antifúngica e a doença não invasiva.[6,11,23]

A cinética de liberação da GM ainda não é bem conhecida, o que se sabe é que fatores como o estado imunológico do hospedeiro, sítio da infecção, fase de crescimento do fungo, podem influenciar na concentração de GM liberada. Assim, quanto mais GM é detectada no tecido infectado, maior é o crescimento fúngico, mesmo que o paciente não tenha sinais clínicos da infecção.[6]

1.1.2 Ensaio de Fluxo Lateral para detecção de Galactomannan - (GM-LFA)

O Ensaio de Fluxo Lateral Galactomanana (GM-LFA) é um teste imunocromatográfico sanduíche para a detecção qualitativa e quantitativa de *Aspergillus* spp. a partir de amostras de soro e BAL. A metodologia é semelhante ao teste de gravidez amplamente utilizado, eliminando a necessidade de equipamentos avançados de laboratório, pela simplicidade de uso, é conhecido como teste Point Of Care, ou popularmente: como teste rápido.[26-28]

O princípio metodológico do Fluxo Lateral utiliza anticorpo monoclonal específicos de GM conjugados com ouro nano coloidal, os quais se ligam aos epítopos do antígeno GM, se estiver presente na amostra, à medida que acontece a corrida nas duas zonas testes da tira por fluxo capilar, o resultado positivo resulta na formação de uma linha de teste visível. O controle do teste se dá pelos anticorpos conjugados com ouro que fluem junto com a amostra, os quais são capturados pelos anticorpos presentes na linha de controle.[29]

Para realizar o teste são necessários em média 300 µl amostra, BALF ou soro, a qual é pré tratada para que ocorra a ligação eficaz dos anticorpos de detecção. A amostra, então, é desnaturada por aquecimento para minimizar a interferência das proteínas presentes, assim como para liberar o GM para a detecção, em seguida é centrifugada e acrescentada ao tampão de corrida. Após o tratamento as tiras testes são inseridas nessa mistura, que após 30 minutos os resultados já podem ser interpretados manualmente, como também através de leitor digital, o qual fornece um valor quantitativo, que corresponde aos títulos de GM presente na amostra.[22]

O teste apresenta reação cruzada com a cultura positiva para outros fungos que produzem galactofuranose, como por exemplo *Paracoccidiodes brasiliensis, Coccidioides* spp., *Histoplasma* spp., *Fusarium* spp. *e Candida* spp., no entanto não está claro a relevância clínica e se é verídica essa reatividade ou se esses pacientes estão coinfectados com os fungos relacionados acima.[30]

Estudos recentes encontraram uma sensibilidade e especificidade do GM-LFA para AI comprovada ou provável variando de 80% a 90% na leitura visual, o que pode ser uma alternativa confiante ao ELISA-GM para confirmar ou excluir um diagnóstico de AI.[27] Ainda, quando testado em amostras de BALF mostrou-se mais sensível e específico do que a amostra sorológica.[17]

Além do mais, quando utilizado a leitura automatizada o teste apresentou boa acurácia diagnóstica e uma boa concordância ao teste ELISA -GM, visto que remove a subjetividade do operador, como também fornece um valor quantitativo, o que permite a comparação direta com o GM-ELISA, logo, pode ser usado com confiança para monitorar a resposta à terapia ou determinar prognóstico do paciente.[17,26]

O teste GM-LFA apresenta bom desempenho diagnóstico, além de ser rápido e de fácil manuseio, pode superar a limitação do tempo de realização do teste convencional GM-ELISA, o que pode facilitar ainda mais o diagnóstico de AI no local de atendimento. Como também, é clinicamente relevante para direcionar a terapia precoce do paciente, o que pode determinar um bom prognóstico.[8,18,20,26,27]

1.2 OBJETIVOS

1.2.1 Objetivo geral

O trabalho visa comparar duas metodologias para detecção de antígenos do *Aspergillus* spp. no diagnóstico de Aspergilose Invasiva: Galactomannan pelo método de Elisa (GM Elisa) e por imunocromatografia GM-LFA (Ensaio de Fluxo Lateral Galactomannan).

1.2.2 Objetivos específicos

a) Revisar a literatura sobre a Aspergilose Invasiva;
b) Avaliar o perfil de pacientes para os quais são solicitados os exames de Galactomana no HCPA;
c) Revisar a metodologia diagnóstica não cultural para Aspergilose Invasiva;
d) Comparar o método ELISA- GM com o GM Lateral Flow para o diagnóstico;
e) Avaliar o desempenho da metodologia GM-LFA.

2 ARTIGO CIÊNTÍFICO

COMPARISON OF PERFORMANCE BETWEEN ASPERGILLUS METHODOLOGY BY LATERAL FLOW ASSAY AND ELISA TO DETECT THE ANTIGEN GALACTOMANNAN ANTIGEN IN THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS.

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ABSTRACT

Invasive Aspergillosis (IA) is an invasive fungal disease (IFD) caused by filamentous fungi of the genus Aspergillus spp. which is responsible for the majority of infections, affecting mainly immunocompromised patients, as well as being difficult to be diagnosed by traditional techniques. Therefore, the objective of this study is to compare the performance of the Lateral Flow (LFA) and ELISA methodologies for the detection of the galactomannan (GM) antigenin individuals predisposed to the disease. To this end, data from the literature will be compared with the findings in the laboratory routine of the Hospital de Clínicas de Porto Alegre. The data collected in the laboratory routine of the Hospital de Clínicas de Porto Alegre are in agreement with those found in the literature and confirm the diagnostic value of the Lateral Flow methodology, which can be a good alternative to the detection of galactomannan antigen in patients pre-disposed to Invasive Aspergillosis.

Keywords: Aspergillosis Invasive, Lateral Flow, Galactomannan, ELISA-GM

INTRODUCTION

Aspergillosis (IA) is an invasive fungal disease (IFD) caused by filamentous fungi of the genus *Aspergillus* spp., highlighting the species *Aspergillus fumigatus,* which is responsible for most infections, which mainly affect immunocompromised patients, in which the main risk factor is prolonged neutropenia. [1,2]

There are infections that are mild and others that are more serious, such as Invasive Pulmonary Aspergillosis (API), which is related to a high rate of morbidity and mortality, which varies from 70 to 90% in these patients, depending on the patient's immune status. [3,4]

Aspergillosis affects immunocompromised individuals, mainly Hematological patients, post-halogen stem cell transplants, solid organ transplants, or with some pre-existing pathology, as well as patients hospitalized under treatment in an intensive care unit. [4-6]

Therefore, the unfavorable prognosis of API is a consequence of both the patient's immunosuppression and the inaccuracy of the clinical diagnosis, since the disease does not present specific signs and symptoms. Therefore, due to these factors, there is a delay in the initiation of treatment, which results in the worsening of the condition or even the death of the patient. Therefore, early and specific diagnosis is a key factor in reducing mortality. However, due to the limited identification of the fungus, there is a constant search for tests that may indicate the presence of *Aspergillus* spp. in the immunosuppressed patient. [7]

Galactomannan antigen detection by ELISA - GM ELISA

Galactomannan (GM) is a thermostable heteropolysaccharide that is present in the wall of *Aspergillus* spp. being released during hyphal growth and conidia germination in the tissue that is infected. [8,9] Furthermore, GM is found in blood circulation, as well as in different clinical samples, such as urine, cerebrospinal fluid and bronchoalveolar lavage (BAL). [9-12]

However, the kinetics of GM release is still not well known, what is known is that factors such as the immune status of the host, site of infection, growth phase of the fungus, can influence the concentration of GM released. Thus, the more GM that is detected in infected tissue, the greater the fungal growth, even if the patient has no clinical signs of infection. [3]

The most used methodology is performed by immunoenzymatic assays of the Sandwich Elisa type, in which the mouse monoclonal antibody EBA-2 directed against Aspergillus galactomannann is used. [13]

Lateral Flow Assay for Galactomannan detection - (GM-LFA)

Galactomannan Lateral Flow Assay (GM-LFA) is an immunochromatographic test for the qualitative and quantitative detection of *Aspergillus* spp. from serum and BAL samples. The methodology is similar to the widely used pregnancy test, it is an easy-to-handle test, which eliminates the need for advanced laboratory equipment. [14-16]

The Lateral Flow methodological principle uses GM-specific monoclonal antibodies conjugated with nanocolloidal gold, which bind to the epitopes of the GM antigen, if present in the sample. Migration of the conjugate into the test zones of the strip is by capillary flow, and a positive result results in the formation of a visible test line. [17]

To perform the test, it is necessary to pre- treat the sample, which is performed by denaturing, followed by centrifugation, this treatment aims to minimize the interference of the proteins present, as well as to release the GM for detection. The results can be interpreted manually, as well as through a digital reader, which provides a quantitative value, which corresponds to the GM titles present in the sample. [12]

In view of the above, based on the available literature, the performance of these two methodologies mentioned above will be compared for the diagnosis of Invasive Aspergillosis and then, these data will be compared with the findings in the laboratory routine of the microbiology laboratory of the Hospital de Clínicas de Porto Alegre
(HCPA).

MATERIALS AND METHODS

The present work will be developed from data found in the literature on the methodologies mentioned above for the diagnosis of Invasive Aspergillosis, which detect the galactomannan antigen in patients predisposed to the disease, based on these findings and with the data collected at Hospital de Clínicas de Porto Alegre, a comparison will be made between the two methodologies to find out which one has better diagnostic performance.

17 samples from patients affected by hematological diseases were included, plus 3 internal control samples, to be analyzed using the two methodologies, GM - ELISA and GM - LFA.

RESULTS

Every 3 days, the HCPA performs screening for Invasive Aspergillosis through the investigation of galactomannan antigen, especially in patients with hematologic disease, including neutropenic patients, Acute Myelocytic Leukemia, Marrow Aplasia, Hematopoietic Stem Cell Transplants (HSCT), among others , this tracking is done using the GM - Elisa (BioRad ®) methodology, however another methodology was received to be tested comparatively with this one, the Lateral Flow (Immy ®) methodology.

These results can be seen in the table below, which compares the results of these two methods for detecting *Aspergillus* spp., performed in hematological patients at Hospital de Clínicas de Porto Alegre.

Table 1. Comparison of results between the GM - Elisa and GM - Lateral Flow methodology.

Patients	Characteristics	BioRAD GM - ELISA	Index	Immy – Sona GM - Lateral Flow	Lateral Flow-Index
1	M 16 - HSCT hello	Positive (False +)	2,329	Negative	0.03
2	QC	Positive	3.34	Positive	21.29
3	QC	Positive	3.79	Positive	19.57
4	QC	Positive	1.69	Positive	14.96
5	Neutral / Fever	Positive	0.55	Negative	9.49
6	Aplasia, Neutr /fever	Positive	1.06	Positive	17.14
7	M 3 - neutral	False + continuous	Repetition: 0.18	Negative	10.89
8	M 3 Neutr. aplasia	Positive	0.51	Positive	17.45
9	Neutral / Fever	Positive	0.54	Negative	11.2
10	Neutral / Fever	Positive	0.56	Negative	11
11	Neutral / Fever	Positive	4.2	Positive	14.2
12	Neutral / Fever	Positive	0.58	Negative	11.23
13	Neutral / Fever	Negative	0.48	Positive	13.82
14	Neutral / Fever	Negative	0.23	Negative	10.8
15	Neutral / Fever	Negative	0.26	Negative	10.8
16	Neutral / Fever	Negative	0.28	Negative	10.2
17	Neutropenia	Positive	0.68	Positive	14.4
18	Neutropenia	Positive	0.78	Positive	16
19	Neutropenia	Positive	0.72	Positive	14.5
20	Neutropenia	Positive	0.79	Positive	16.2

QC: quality control; Neutr : neutropenia; HSCT allo: hematopoietic stem cell transplantation; M3: leukemic cell line.

The Lateral Flow - Immy ® assay presented some advantages in relation to the currently used GM - ELISA method. We can highlight the greater speed in performing the other, in addition to practicality, ease of storage at room temperature, as well as excellent reproducibility, according to the data of the 20 samples that were compared.

Although a small sampling was observed according to the availability of tests, we can observe excellent reproducibility and precision, even in cases where we had low values (slightly greater than 0.5, that is, the cutoff point used routinely). In these cases, when they were submitted to the LFA test, they did not have a positivity. Furthermore, in these results observed in subsequent samples, 3 to 4 days later, there was no increase in the cutoff value by the standard test, that is, it was a false positive.

As a limitation, we can mention the impossibility of repeating the test in pediatric patients due to the larger amount of sample required.

DISCUSSION

In studies carried out to detect Aspergillus spp., using the GM - ELISA methodology, it showed varied sensitivity and specificity, depending on the type of sample, in serum and bronchial alveolar washing fluid. [18] However, in samples of bronchoalveolar lavage, it showed sensitivity and specificity close to 95%, which suggests good diagnostic accuracy. [13.19]

The ALF test, in a study carried out with 179 serum samples, showed sensitivity and specificity of 97% and 98%, respectively, confirming that the test can be used with confidence. [20] As well as in respiratory samples, which generated a sensitivity and specificity of 83 to 92% and 91 to 92%, respectively. [21.22]

In another study, where the clinical and analytical performance of the GM LFA for detecting the GM antigen in both serum and BAL was investigated, it was observed that the LFA detected the galactomannan antigen in more samples, especially when using the automatic reader, since LFA sensitivity was 100% for both serum and BAL, while specificity was 98% for serum and 93% for BAL. Unlike the GM ELISA, the GM LFA detected all proven AI cases. This study also quantitatively evaluated the index value for monitoring the galactomannan level, and found higher LFA values and moderate correlation compared to the GM-Elisa. [23]

Therefore, according to Autier et al. [14,] in a multicenter study carried out with serum and BAL samples using the LFA methodology, the sensitivity found in serum samples was low, ranging from 9-22%, however the specificity was 93%, whereas in BAL samples it ranged from 52% to 100%, depending on the cutoff point, which was from 0.5 to 1.0, it was soon observed that the lower the cutoff point, the greater the sensitivity. [14]

In other studies, reviewed by Jenks et al., [15] he observed that tests using the same methodology showed a sensitivity of 61% to 89% and specificity of 80% to 88% in visual reading, whereas with the use of a digital reader they were more accurate, showing good diagnostic agreement with the GM-ELISA test. [15]

Also, in a study with 200 BAL samples carried out by Scharmann et al., four criteria were evaluated to define Invasive Pulmonary Aspergillosis, namely: specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV). Such that a VPP and a NPV of 83.9% and 75% respectively were found, and a range of sensitivity from 85.3% to 93.3% and specificity from 46.1% to 72.9% according to the applied criteria. [17]

Despite being the test usually applied routinely, the GM - ELISA test has some limitations, as it presents false positive results in patients with bacterial infections, in treatment with B-lactam antibiotics, in blood transfusions, and hemodialysis, whereas false negative results are related to antifungal prophylaxis and non-invasive disease. [3,11,13] Whereas, the LFA is not interfered with by antifungal prophylaxis. [15.23]

Because the GM - ELISA and GM - LFA test cross-react with the positive culture for other fungi that produce galactofuranose, such as *Histoplasma* spp., It is *Fusarium* spp ., however, the clinical relevance is unclear and whether this reactivity is true or whether these patients are coinfected with the fungi listed above. [21]

Still, recent studies have found a sensitivity and specificity of GM-LFA for proven or probable AI ranging from 80% to 90% on visual reading, which can be a confident alternative to GM-ELISA for confirming or excluding an AI diagnosis. [15] In addition, when tested on BALF samples, it proved to be more sensitive and specific than the serological sample. [20]

Therefore, when automated reading was used, the test showed good diagnostic accuracy and good agreement with the ELISA-GM test, since it removes the subjectivity of the operator, as well as providing a quantitative value for monitoring the disease, which allows direct comparison with the GM-ELISA, therefore, can be used with confidence to monitor response to therapy or determine patient prognosis. [14.20]

Thus, the data collected in the HCPA laboratory routine refer to those found in the literature cited here, and confirm the diagnostic value of the GM - LFA methodology, which can be a good alternative for the detection of the galactomannan antigen in patients predisposed to Aspergillosis Invasive.

CONCLUSION

The LFA methodology shows promise for the diagnosis of Invasive Aspergillosis, in order to reduce the difficulty in establishing the disease, since, the standard GM - ELISA test is time-consuming and requires lots of samples for the diagnostic test, which makes it difficult to start treatment for the patient.

Therefore, the present work concludes that the GM-LFA test has good diagnostic performance, in addition to being fast, accurate and easy to handle, it not only overcomes the time limitation of performing the conventional GM-ELISA test, but can also facilitate even more diagnosis of UA at the point of care, as it does not require large laboratory equipment. In addition, the shorter trial time is clinically relevant to target early patient therapy, which may determine a good prognosis. [9, 14, 15, 19, 23]

However, the literature has few data related to non-hematological patients who underwent diagnosis using the aforementioned methodologies, which suggests the need for further studies with patients affected by pathologies other than hematological ones and at risk for Invasive Aspergillosis.

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3 CONCLUSÃO E PERSPECTIVAS

A metodologia de LFA mostra-se promissora para o diagnóstico da Aspergilose Invasiva, a fim de diminuir a dificuldade para o estabelecimento da doença, visto que~~,~~ o diagnóstico definitivo é feito através de procedimentos invasivos, o que muitas vezes é limitante, devido ao perfil de pacientes acometidos. Além do mais, o teste padrão GM – ELISA é demorado e necessita de lotes de amostras para o ensaio diagnóstico, também como de laboriosos equipamentos de laboratório, o que dificulta o diagnóstico, e consequentemente, o início do tratamento ao paciente.

Diante do exposto faz-se necessária a abordagem de metodologias simples e rápidas para o diagnóstico da Aspergilose Invasiva e que possam detectar marcadores da doença antes das suas manifestações clínicas desta. Além disso, o diagnóstico rápido e preciso visa adequar o tratamento desses pacientes aumentando sua sobrevida.

Portanto, o presente trabalho conclui que o teste GM-LFA apresenta bom desempenho diagnóstico, além de ser rápido e de fácil manuseio, não só supera a limitação do tempo de realização do teste convencional GM-ELISA, como também pode facilitar ainda mais o diagnóstico de AI no local de atendimento, pois não necessita de grandes equipamentos laboratoriais. Além disso, o menor tempo do ensaio é clinicamente relevante para direcionar a terapia precoce do paciente, o que pode determinar um bom prognóstico.[8,18,20,26,27]

No entanto há poucos estudos que abordam outros pacientes, que não sejam hematológicos que foram submetidos ao diagnóstico pelas metodologias acima mencionadas, o que sugere a realização de outros estudos, com pacientes de outros grupos de risco à Aspergilose Invasiva.

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ANEXO A – NORMAS DE PUBLICAÇÃO DO BRAZILIAN JOURNAL OF INFECTIOUS DISEASES

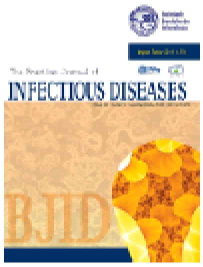
THE BRAZILIAN JOURNAL OF INFECTIOUS DISEASES

Official publication of the Brazilian Society of Infectious Diseases

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

- **Description** **p.1**
- **Audience** **p.1**
- **Impact Factor** **p.1**
- **Abstracting and Indexing** **p.1**
- **Editorial Board** **p.1**
- **Guide for Authors** **p.5**



ISSN: 1413-8670

DESCRIPTION

The Brazilian Journal of Infectious Diseases is the official publication of the Brazilian Society of Infectious Diseases (SBI). It aims to publish relevant articles in the broadest sense on all aspects of microbiology, infectious diseases and immune response to infectious agents.

The *BJID* is a bimonthly publication and one of the most influential journals in its field in Brazil and Latin America with a high impact factor, since its inception it has garnered a growing share of the publishing market.

AUDIENCE

Infectious Disease specialists

IMPACT FACTOR

2019: 1.971 © Clarivate Analytics Journal Citation Reports 2020

ABSTRACTING AND INDEXING

Science Citation Index Expanded
Scopus
Directory of Open Access Journals (DOAJ)
PubMed/Medline
PubMed/Medline
SciELO - Scientific Electronic Library Online

EDITORIAL BOARD

GUIDE FOR AUTHORS

Introduction

The Brazilian Journal of Infectious Diseases is the official publication of the Brazilian Society of Infectious Diseases (SBI). It aims to publish relevant articles in the broadest sense on all aspects of microbiology, infectious diseases and immune response to infectious agents. The BJID is a bimonthly publication and one of the most influential journals in its field in Brazil and Latin America with a high impact factor, since its inception it has garnered a growing share of the publishing market.

The article publishing charge (APC) that authors, their institutions or funding bodies pay, covers all expenses needed to support the publication process.

For articles submitted from 16th July 2018, the APC to publish a paper in the Brazilian Journal of Infectious Diseases is USD 1,500 for original and review articles, and USD 600 for case reports, short communications and letters.

Once the manuscript has been approved, the corresponding author will receive the instructions for the payment of the publication fee.

Types of article

Manuscripts may be submitted within designated categories of communication, including:
• Original basic or clinical investigation (original papers);
• Brief reports of new methods or observations (brief communications);
• State-of-the-art presentations or reviews (review or mini review papers);
• Case presentation and discussion (case reports);
• Clinical infectious diseases images;
• Letters to the editor concerning previous publications;
• Editor's corner, containing ideas, hypotheses and comments (Editorial).

Original articles
It is the most important section of the Journal. Original articles present new data about researches, issues and matters in the field of infectious diseases. These articles should conform strictly to the rules of publication, containing the following sections: abstract, objective or hypothesis, experimental design and methods used (statistical data), essential features of any interventions, main outcome measures, main results of the study, discussion and conclusion. An Original Paper should contain:
• An abstract of no more than 300 words;
• No more than 7 keywords;
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