Original Research

Use of Galactomannan Antigen and Aspergillus DNA Real-time Polymerase Chain Reaction as Routine Methods for Invasive Aspergillosis in Immunosuppressed Children in Greece

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ABSTRACT

Purpose: Invasive aspergillosis (IA) remains a critical issue in immunosuppressed patients. Detection of galactomannan antigen (GM) in serum samples is included as a criterion of IA by the European Organization for the Research and Treatment of Cancer/Mycoses Study Group. Nevertheless, *Aspergillus* DNA detection by polymerase chain reaction (PCR) has not yet been included because clinical data validation is lacking. The present study describes the simultaneous performance of GM and PCR tests as routine methods for IA diagnosis.

Methods: During the period January 2012 to December 2017, a total of 156 white children hospitalized in a tertiary children's hospital of Athens (97 boys and 59 girls; age range, 5 months–14 years) were examined as possible cases of IA. Patients were classified into 4 groups based on their underlying diseases: hematologic malignancies (107 of 156 [68.6%]), solid tumors (16 of 156 [10.2%]), primary immunodeficiency (12 of 156 [7.7%]), and hereditary immunodeficiency (21 of 156 [13.5%]). GM detection was made with the Platelia Aspergillus Ag kit (Bio-Rad Laboratories, Hercules, California). Sera with a cut-off index ≥0.5 on at least 2 separate blood collections were considered positive. Serum detection of *Aspergillus* DNA was conducted with real-time PCR MycAssay *Aspergillus* assay (Myconostica Ltd, Cambridge, United Kingdom). PCR positivity was determined by using a threshold of 38 cycles in at least 1 serum sample. Four or more successive samples per patient were tested.

Findings: Overall, 28 of 156 patients (53 of 744 serum samples) were found positive. Eleven patients were positive using both methods (24 samples). Four children were positive only by PCR (6 samples), whereas 13 (23 samples) were positive only with GM in consecutive samples. Agreement of both methods, GM(+)/PCR(+) or GM(–)/PCR(–), was found in 139 patients (90% of total patients) and 715 samples (96.1% of total samples). The agreement of both methods was found: (1) 85% in patients with hematologic malignancies; (2) 100% in patients with solid tumors; (3) 97.5% in patients with primary immunodeficiency; and (4) 98.8% in patients with hereditary immunodeficiency. Overall disagreement was observed in 17 patients, in which the positive result in any of the 2 methods was estimated as true positive in conjunction with radiologic and other clinical findings.

Implications: The combination of GM and PCR, provided high diagnostic accuracy in consecutive samples (twice a week). Clinical, radiologic, and other laboratory findings should be taken into consideration in the evaluation of GM and PCR. (*Clin Ther.* 2018;40:918–924)

Key words: *Aspergillus*, galactomannan, invasive aspergillosis, real-time PCR.

Accepted for publication May 2, 2018.
https://doi.org/10.1016/j.clinthera.2018.05.002
0149-2918/$ - see front matter

INTRODUCTION

Invasive aspergillosis (IA) is a serious threat among immunosuppressed patients.[1] The genus *Aspergillus* contains >250 species, although only a few are associated with disease in humans, particularly *Aspergillus fumigatus* and *Aspergillus flavus*. The infection is transmitted via inhalation of the 2.5- to 3.0-μm conidia (spores), and the most common sites of infection are the lungs and the brain.[2] The clinical spectrum includes primary cutaneous aspergillosis, (rhino)sinusitis, tracheobronchitis, pulmonary aspergillosis, and disseminated aspergillosis, with cerebral aspergillosis as its most serious manifestation.[3,4] During the last decades, many investigators have studied the role of the environment in the pathogenesis of IA such as the presence of *Aspergillus* in air and water.[4] Mortality due to IA, especially in hematologic patients and transplant recipients, is high, ranging from 50% to 100% in collected case series.[1]

The risk factors for IA involve granulocytopenia ($<0.5 \times 10^9$/L), especially in bone marrow transplant recipients; high-dose corticosteroid therapy; broad-spectrum antibiotic therapy; chronic granulomatous disease; AIDS with $CD4^+$ lymphocytes count <50/L; and treatment with cytotoxic drugs, such as cyclosporine.[2] The incidence of IA is estimated to range from 5% to >20% in high-risk groups.[5]

The problem of IA has been known from the late 1970s, and an increase of 158% was documented in the United States between 1970 and 1976.[6] Until the 1990s, the clinical, radiologic, and microbiologic diagnoses were unreliable, the antifungal treatment was toxic, and systemic prophylaxis was inadvisable.[7] Despite the evolution in diagnostic and therapeutic modalities, the mortality of IA still ranges from 50% to 100% in collected case series.[4–6]

Moreover, it seems that there has been a significant increase in the number of risk patients as candidates for developing IA.[2,8] There are many reasons for this increase, including the advent of AIDS; the development of new intensive chemotherapy regimens for solid tumors, difficult-to-treat lymphomas, myelomas, and resistant leukemias; a worldwide increase in the number of solid organ transplant recipients; and, finally, the increased use of immunosuppressive regimens for other autoimmune diseases such as lupus erythematosus.[2,3]

The diagnosis of IA among immunosuppressed patients is a controversial issue because clinical signs are nonspecific.[9,10] The cultures of sputum and bronchoalveolar lavage (BAL) have ~50% sensitivity in focal pulmonary lesions. According to the latest data, the cultures of respiratory specimens have relatively low positive value, which can decrease even more when testing nonhematologic patients.[2,11,12] Tissue biopsies may be required to confirm the diagnosis but are rarely performed due to complications.[1,3]

Most trials and guidelines have tried to circumvent the diagnostic difficulties by using the criteria developed by the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG).[11,13,14] The approach of using *Aspergillus* galactomannan antigen (GM) and/or polymerase chain reaction (PCR) reduced the empirical antifungal treatments and is an effective strategy for the management of IA in immunosuppressed patients.[15]

The present study describes the simultaneous performance of GM and *Aspergillus* DNA PCR tests as daily routine methods for IA diagnosis among immunosuppressed children with hematologic or other malignant diseases.

PATIENTS AND METHODS

The present study is an interpretation of routine laboratory data and conforms to the principles of the Declaration of Helsinki. The study does not contain any identifiable information of the patients (eg, names, initials, Social Security numbers, dates of birth).

In the framework of the routine examination of hospitalized immunosuppressed patients, results of GM and PCR routine tests were collected and analyzed. During the period January 2012 to December 2017, a total of 156 white children were hospitalized in a tertiary children's hospital of Athens (97 boys and 59 girls; age range, 5 months–14 years) with underlying immunodeficiency syndromes and were examined for probable/possible cases of IA (EORTC/MSG criteria). None of the children had undergone histopathologic examination and thus none could be categorized as a "proven" case.[13] The patients were classified into 4 groups according to their underlying diseases associated with immunodeficiency: (1) hematologic malignancies (107 of 156 [68.6%]); (2) solid tumors (16 of 156 [10.2%]); (3) primary immunodeficiency (12 of 156 [7.7%]); and (4)