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REVIEW

Challenges and Solution of Invasive Aspergillosis in Non-neutropenic Patients: A Review

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ABSTRACT

Invasive aspergillosis (IA) is a serious opportunistic infection, which has increasingly been recognized as an emerging disease of non-neutropenic patients. In this group of patients, the diagnosis of IA can be challenging owing to the lack of specificity of symptoms, the difficulty in discriminating colonization from infection, and the lower sensitivity of microbiological and radiological tests compared with immunocompromised patients. The aim of this article is to present to clinicians a critical review on the management of IA in non-neutropenic patients.

Keywords: *Aspergillus*; Biomarkers; Galactomannan; Invasive aspergillosis; Liver cirrhosis; Non-neutropenic patients

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INTRODUCTION

Invasive aspergillosis (IA) is a serious opportunistic infection that continues to increase its incidence in immunosuppressed or hospitalized patients with severe underlying diseases, with high rates of morbidity and mortality [1]. Over the past decades, the population of patients susceptible to develop IA has expanded significantly and IA has increasingly been recognized as an emerging disease of non-neutropenic patients with an incidence varying between 0.33–5.8% [2, 3]. In addition, the classical view of IA has been modified, since various clinical syndromes have been considered as a continuous spectrum of the disease, whose manifestations are defined by the complex interaction between pathogen and host factors [4].

Invasive aspergillosis in non-neutropenic patients is associated with bad prognosis, with mortality rates exceeding 80%, mainly due to delayed diagnosis [2, 3]. Difficulties in achieving a timely diagnosis of IA in non-neutropenic patients is related to the non-specificity of clinical presentation and to lower yields with diagnostic tests compared to neutropenic patients [2, 3].

The aim of this article is to present to clinicians a critical review on the recognition, risk factors, microbiological and radiological diagnosis, and management of IA in non-neutropenic patients.

Compliance with Ethical Guidelines

This article is based on previously conducted studies and does not involve any studies of human or animal subjects performed by any of the authors.

HOST RISK FACTORS AND SPECTRUM OF DISEASE

Invasive aspergillosis has been traditionally considered as an opportunistic infection mainly occurring in patients with well-established risk factors, such as neutropenia, hematologic malignancies, allogeneic bone marrow transplantation, solid organ transplantation, solid cancer or HIV [5].

However, an increasing number of reports have shown that *Aspergillus* spp. can cause invasive disease in other categories of non-neutropenic patients, including those with severe chronic obstructive pulmonary disease (COPD) requiring high-dose steroid therapy, with Child–Pugh C hepatic cirrhosis, and systemic diseases requiring immunosuppressive therapy, including new monoclonal agents in patients with autoimmune diseases [6].

An emerging broad group of patients who are admitted to the intensive care unit (ICU) may also be susceptible to IA [3, 7], with previously reported rates varying widely from 0.017% to as high as 6.9% [6, 8]. In addition to host underlying conditions (Table 1) [6], immunoregulatory abnormalities following critical illness can induce a state of immunoparalysis, hampering adequate host response to fungal disease in the ICU [9]. Other predisposing risk factors frequently met in ICUs include acute respiratory distress syndrome (ARDS), severe sepsis, acute renal failure, and H1N1 virus infection (especially if CS prior to ICU admission) [8]. Moreover, environmental factors including climatic variables, airborne mold concentrations, geographic area, remodeling or construction works and environmental quality of the air may influence IA development [1].

The nature of the immune suppression (the degree, duration, and type of immunodeficiency) influences the pathogenesis of disease.

Table 1 Risk of invasive aspergillosis among patients admitted to the intensive care unit by Meersseman et al. [6]

High-risk category
Neutropenia (neutrophil count, !500 neutrophils/ mm^3)
Hematological malignancy
Allogeneic bone marrow transplantation
Intermediate-risk category
Prolonged treatment with corticosteroids before admission to the ICU
Autologous bone marrow transplantation
Chronic obstructive pulmonary disease
Liver cirrhosis with a duration of stay in the ICU > 7 days
Solid-organ cancer HIV infection
Lung transplantation
Systemic diseases requiring immunosuppressive therapy
Low-risk category
Severe burns
Other solid-organ transplant recipients (e.g., heart, kidney, or liver transplant recipients)
Steroid treatment with a duration of < 7 days
Prolonged stay in the ICU (> 21 days)
Malnutrition
Post-cardiac surgery status

IA thus manifests as a spectrum of disease involving predominantly airway (tracheobronchitis), lung, or both. Another factor that makes IA in non-neutropenic patients difficult to diagnose is its non-specific symptomatology that makes clinical manifestations of IA (e.g., fever, cough, purulent sputum) indistinguishable from other bacterial bronchopneumonia [10]. Notably in previous studies, non-neutropenic patients have shown to be less

symptomatic than neutropenic patients regarding fever as well as cough and chest pain [3].

In this setting, clinical diagnosis of IA is a challenge, and initiation of additional diagnostic examinations is often delayed because clinical suspicion is low. Standard diagnostic definitions by the European Organization for Research and Treatment of Cancer (EORTC) have been developed but have been validated only for patients with cancer or after hematopoietic stem cell transplants and cannot be extrapolated to non-neutropenic patients [11]. Blot and colleagues validated a clinical diagnostic algorithm that aims to discriminate colonization from probable IA in ICU patients with *Aspergillus*-positive endotracheal aspirate cultures [12].

MICROBIOLOGICAL DIAGNOSIS

The suspicion of invasive aspergillosis in non-neutropenic patients may be delayed because paucisymptomatic disease is not uncommon [3, 13, 14]. Another factor that makes invasive aspergillosis in non-neutropenic patients difficult to diagnose is its non-specific symptomatology that makes clinical manifestations of IA (e.g., fever, cough, purulent sputum) indistinguishable from other bacterial bronchopneumonia [15]. A high index of suspicion is required to successfully achieve a positive diagnosis, and it is advisable to perform fungal cultures and non-culture-based methods in all patients with relevant risk factors who present with an infectious complication. This recommendation would apply even if other agents had already been isolated or even during necropsy or because a positive culture result might have been achieved accidentally (Fig. 1).

The cultures of lower respiratory tract are easy and cheap and enable *Aspergillus* species to be identified and their antifungal susceptibility testing performed. However, cultures are slow and their yield in respiratory sample is notoriously low, with a sensitivity ranging between 20% and 50% [16, 17]. In addition, the clinical significance of isolating *Aspergillus* from respiratory samples remains unclear, because differentiating true infection from simple colonization can be difficult [18]. Once the fungus is detected in respiratory samples, experts in the field should interpret the isolation in the clinical context of the patient and eventually start antifungal therapy, if indicated.

In recent years, surrogate markers have been developed for diagnosis of IA, based on the detection of fungal cell wall component or fungal DNA in clinical specimens. The Platelia (Sanofi Diagnostic Pasterur, Marnes la Coquette, France) sandwich-enzyme immunosorbent assay (ELISA) for the detection of galactomannan (GM) is currently one of the more used methods. It is based on the detection of circulating antigens in biologic fluids, such as serum, urine, or BAL fluid [19, 20]. A meta-analysis including 27 studies reported an overall sensitivity of serum galactomannan assay of 71% and specificity of 89%. However, when oncohematological patients were excluded from the analysis, the sensitivity and specificity of the test dropped to 22% and 84%, respectively [21].

In a recent prospective study of IA in non-neutropenic patients, Zhou et al. found a sensitivity of serum GM of 37.8% and a specificity of 87.1%, with a positive predictive value of only 60.8% [22]. Other specimens, such as bronchoalveolar lavage (BAL), have been proven to be more advantageous in the non-neutropenic population. At the index cutoff value of 0.5, the test yielded a sensitivity up to 100% and specificity ranging from 75% to 92% [22, 23]. Although in hematological patients the GM test may enable the early diagnosis of IA [24, 25] and monitoring the treatment response [26–28], further studies are mandatory in non-neutropenic patients since these aspects remain to be determined in this population [29].

Another test is the 1-3-β-D-glucan assay, a polysaccharide component of the cell wall of many pathogenic fungi other than *Mucoraceae* and *Cryptococcus*. Four previous meta-analyses, mainly including patients with hematological disease, reported a good sensitivity but a very low specificity and positive predictive value for the diagnosis of fungal infection [30–33]. In contrast, its negative predictive value was as high as 80–90%, thus making 1-3-β-D-glucan

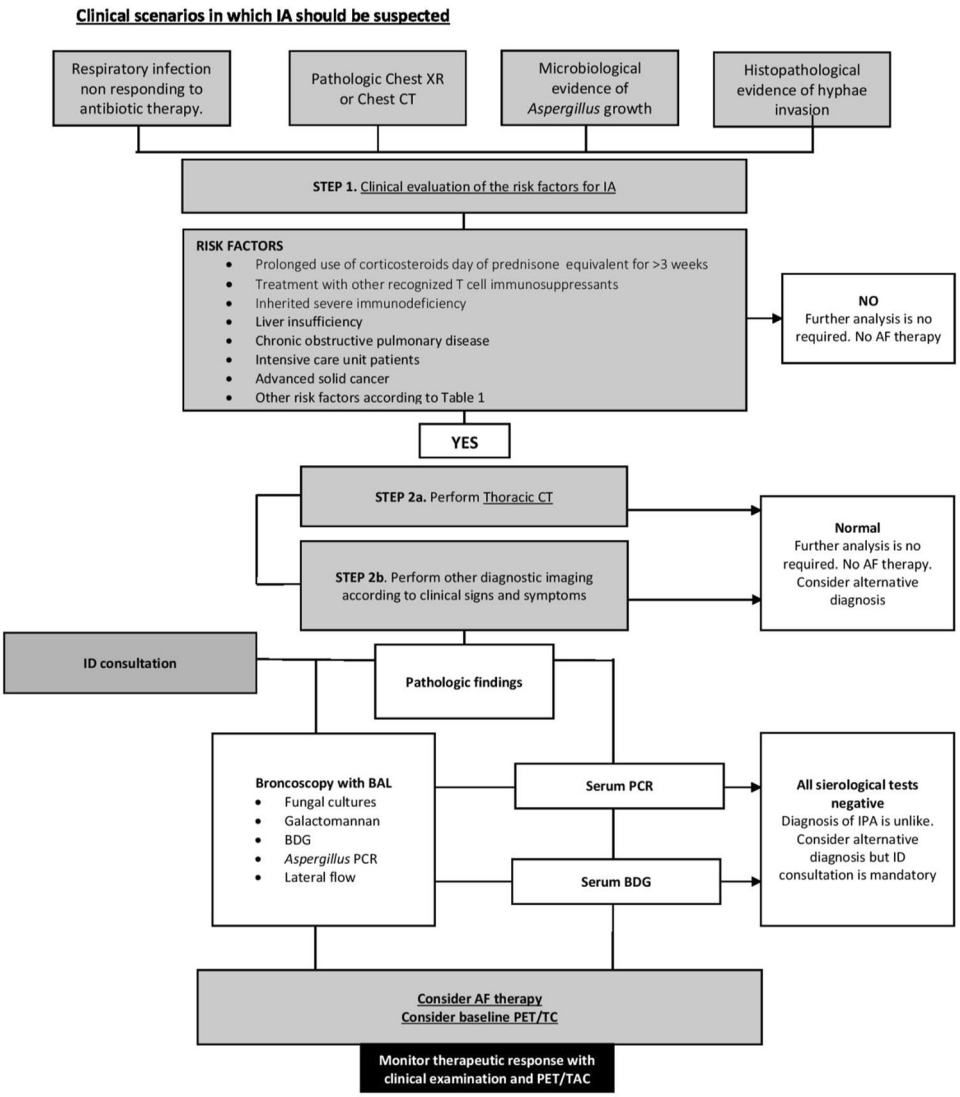

Fig. 1 Diagnostic and therapeutic approach in non-neutropenic patients with suspected IA

potentially useful to rule out the diagnosis of IA rather than to confirm it [30–33].

However, because the significant heterogeneity in testing strategies, inclusion criteria, and low number of patients, the role of this biomarker in the diagnosis of IA in non-neutropenic patients is still unknown and future studies appear to be appropriate. In addition, detection is also limited by the high frequency of false-positive results including semi-syntetic β-lactam antibiotics, human blood components, cellulose hemodialysis, and exposure to gauze [34]. A few studies have evaluated the role of BDG in BAL, indicating a very low specificity and positive predictive value (≈ 20%) for IA in immunocompromised patients [35].

One test that reduces the time required to diagnose invasive aspergillosis is the amplification of genetic material from *Aspergillus* spp., which detects genetic sequences (18S rDNA, 28SrDNA, 5.8 SrDNA, mithocondrial DNA) in cultures as well as in direct clinical samples within a few hours [34]. Unfortunately, PCR is not yet universally standardized [36, 37], and cannot yet be included as a mycological criterion in the EORTC/MSG guidelines [11]. In addition, its usefulness in non-neutropenic patients is not yet clear, although the

information that has been reported seems to be promising [38–41]. Theoretically, the test should not be affected either by the immune status of the patients or by the presence of other fungal or bacterial pathogens.

With two positive consecutive results, this kind of test has a specificity close to 95% with a high positive likelihood ratio (LR 12.8) and should be considered highly indicative of an active *Aspergillus* spp. infection [37]; on the other hand, a single negative PCR result is sufficient to exclude the diagnosis [42]. In addition, when combined with other fungal biomarkers in serum (either GM or BDG) or in BAL (GM), *Aspergillus* PCR has shown to increase the diagnostic sensitivity up to 100%, further supporting the implementation of this technique in the revised definition of invasive fungal infection by the EORTC/MSG. Experience with non-neutropenic patients is scarce, but the efficacy of molecular techniques seems to be similar to that for the population with hematological malignancies [43]. Another application of molecular biology techniques for the diagnosis and treatment of invasive aspergillosis is the ability to detect azole-resistant strains earlier than do conventional methods [44]. Finally, it is important to mention the contribution of molecular techniques in genotyping fungal strains directly from clinical samples.

Recently, a lateral flow device (LFD), detecting a glycoprotein antigen found in the serum and BAL of patients with IA [45], has been proposed as a new point-of care diagnostic approach for also detecting IPA in non-hematological populations, including SOT and ICU patients [46, 47]. A recent multicenter study evaluating the LFD device in BAL from 133 ICU patients showed a sensitivity, specificity, and positive and negative predictive values for probable IA of 80%, 81%, 96%, and 44%, respectively [48]. However, despite such promising results, further and larger studies are warranted before safe conclusions on the performance of *Aspergillus* LDF can be reached. A multicenter study evaluating the role of *Aspergillus* LFD as an alternative to GM in BAL fluid is currently underway (clinicaltrial.gov identifier NCT 02058316).

Finally, different technologies detecting volatile organic compounds exhaled in the breath of patients infected with IA have recently been tested [49, 50], with a sensitivity ranging from 94% to 100% and specificity form 83% to 93% [49, 50]. Also, other tests including gliotoxin, bis(methylthio)gliotoxin, have been analyzed for diagnosing IA with interesting results [51, 52]. Despite this, their role in non-neutropenic patients remains to be clarified and additional analysis with a larger cohort of patients are needed.

In conclusion, diagnosis of invasive aspergillosis remains challenging because none of the available diagnostic tests provides sufficient sensitivity and specificity alone, so the optimal approach relies on the simultaneous performance of several diagnostic strategies, including cultures, fungal biomarkers and molecular tools.

RADIOLOGICAL DIAGNOSIS

Pulmonary lesions caused by *Aspergillus* spp. can be responsible for a wide range of radiographic findings, as with disease manifestations potentially mutating depending upon the immune status of the host.

Chest radiographs are insensitive for detecting the earliest stages of pulmonary disease, but computed tomography (CT) scans typically demonstrate focal lesions. Several thoracic CT patterns are associated with pulmonary aspergillosis. Radiological patterns can be non-specific in non-neutropenic patients [9]. Radiographic signs of IA can vary from a single nodular lesions or larger masses to diffuse bilateral pulmonary infiltrates. The most typical imaging findings including the halo sign and the air crescent sign have shown high sensitivity (80%) and specificity (60–98%) in neutropenic patients. Nevertheless, both signs are uncommon, have a lower sensitivity (5–24%) and can be found even in non-infectious lesion processes in non-neutropenic patients [53].

A large study that included neutropenic and non-neutropenic patients showed that the more specific signs (nodules and cavitation) were infrequent, and that radiographic findings of

consolidation, ground-glass infiltrates, and pleural effusions were seen more commonly [3]. In addition, many ICU patients have radiologic abnormalities masked by underlying acute processes (pleural effusion, atelectasis or ARDS) [9]. Of interest, airway-invasive or angio-invasive radiological patterns have been described in non-neutropenic heart transplant recipients with IA and have been associated with different presentations, time to diagnosis and mortality rates [13].

Given the current limitations of CT, efforts to improve diagnostic performance in pulmonary aspergillosis have been pursued. High-resolution CT pulmonary angiography (CTPA) can detect angio-invasion and vessel occlusion signs (VOS). VOS has been shown to be superior to classic CT signs observed in non-contrast enhanced studies to diagnose invasive pulmonary aspergillosis in immunocompromised patients, including those with hematologic malignancies and other causes, with a sensitivity of 0.94, specificity of 0.71 and a diagnostic odds-ratio of 36.8 [54].

Combined anatomic and functional imaging with 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) is slowly gaining a foothold in the management of invasive fungal infections [55]. In current clinical practice, standalone FDG-PET/CT is not generally sufficient to differentiate IPA from malignancy or other active inflammatory lesions, such as active tuberculosis [55]. However, significant different FDG-PET/CT patterns in invasive and non-invasive forms of aspergillosis have been described [56]. Invasive aspergillosis usually presents with multiple hypermetabolic nodules and a higher peak (SUV 4.5; range 1.3–8.9), whereas non-invasive forms presents with isometabolic halo or nodule patterns with a relatively lower SUV peak value 1.6 (range 0.5–3.1) [56].

In real clinical practice, radiologic follow-up of aspergillosis is mostly carried out by serial CT scans and represents a challenge. FDG PET/CT has been found to be a valuable tool for early evaluation of treatment response in aspergillosis, particularly in patients with underlying pulmonary diseases (i.e., chronic obstructive pulmonary disease, previous TB, and cancer) and for establishing the appropriate treatment duration [57].

A recent study supported the use of a novel probe for detection of *A. fumigatus* lung infection based on antibody-guided positron emission tomography and magnetic resonance (immunoPET/MR). This promising imaging technique seems to allow accurate, non-invasive and rapid detection of fungal lung infection and discrimination of IPA from bacterial lung infections and general inflammatory responses [58].

In our opinion, the presence of a persistent pulmonary infection despite broad-spectrum antibiotics or abnormal thoracic imaging by CT scanning together with one of the host risk factors should trigger further diagnostic exploration through collection of respiratory secretions and/or laboratory markers. There is a potential role of FDG/PET-TC for radiological diagnosis and treatment monitoring in IA.

TREATMENT

Despite the many therapeutic options available today, the mortality rate of IA in non-neutropenic patients continues to be as high as 90% [6, 52], and is even higher than that reported in the hematological population, likely reflecting the lack of strategies of early diagnosis allowing early appropriate therapy in this population [1]. Indeed, in contrast to neutropenic patients, no consensus exists about the exact timeframe for starting empirical therapy in patients at high risk for *Aspergillus* infection and no microbiological evidence of IA [59].

In our opinion, non-neutropenic patients at high risk of IA (i.e., COPD, steroids and immunosuppressive therapy, hepatic failure, and ICU-related immunoparalysis) should receive adequate antifungal therapy upon suspicion of the fungal disease, even if a definitive proof of infection is still not obtained. Whenever possible, a CT scan, fungal cultures and a combination of serological biomarkers (GM, *Aspergillus* PCR and 1,3-b-D-glucan assay, as a screening strategy) should be performed and treatment should be revised and eventually withheld if the diagnosis of IA is not confirmed.

As for antifungal drugs, there has been considerable research in antifungal drugs targeted against IA over the past decade [16, 17, 60–62]. To date, the antifungal agents licensed for the first-line treatment of IA include voriconazole and isavuconazole or amphotericin B and its lipid formulations [5]. The severity of the infection, the clinical form, renal or hepatic insufficiency, drug–drug interactions, requirement for therapeutic drug monitoring and its cost are some of the factors that can help in selecting the best drug.

During the last 10 years, voriconazole use has progressively become widespread. The largest randomized trial for primary therapy of invasive pulmonary aspergillosis demonstrated that voriconazole was superior to amphotericin B deoxycholate, followed by other licensed antifungal therapy [63]. At week 12, successful outcomes among non-neutropenic population (31 patients) were observed in 50.0% of the patients in the voriconazole group and in 31.6% of those in the amphotericin B group. Moreover, patients treated with voriconazole had significantly fewer adverse events that were drug-related, except for transient visual disturbances. Therefore, the authors of this study concluded that voriconazole was more beneficial for the treatment of IA than amphotericin B.

Other series studying non-neutropenic patients, with proven or probable invasive pulmonary aspergillosis, confirmed a favorable response rate with voriconazole [64, 65]. Particularly remarkable is one study of pulmonary and disseminated IA, including 103 non-neutropenic patients, in which receiving voriconazole treatment was found to be the only factor associated with a reduced risk of death [1]. Because of better survival and improved response of initial therapy with voriconazole, this agent is now considered the drug of choice for primary therapy of IA in most patients, including non-neutropenic patients, by the recent Clinical Practice Guidelines of the Infectious Diseases Society of America (IDSA) (strong recommendation, high-quality evidence) [5].

Isavuconazole is a new triazole agent that can be given once daily and offers a wider spectrum of antifungal activity than voriconazole, including activity against most Mucorales infections. In addition, the intravenous formulation does not include cyclodextrin, a nephrotoxic and hepatotoxic compound, which is included in the intravenous formulations of the other triazoles in order to increase solubility. Compared to voriconazole, isavuconazole also has the advantages of linear and predictable pharmacokinetics which is likely to obviate the need for therapeutic drug monitoring and fewer CYP enzyme-mediated drug interactions [66]. A large randomized, double-blind trial has demonstrated non-inferiority or isavuconazole versus voriconazole in terms of all-cause mortality when used as primary treatment for invasive fungal disease caused by *Aspergillus* species or other filamentous fungi, with a superior safety profile [16].

Another alternative for primary therapy is represented by amphotericin B that was historically considered the mainstay of treatment for IA before the introduction of voriconazole. Development of lipid formulations improved the poor tolerability associated with the deoxycholate formulation, but the optimal dosage remains unconfirmed [67].

All echinocandins have been shown to exert in vitro and in vivo activity against *Aspergillus* spp., but only caspofungin is approved for the treatment of IA in patients who are intolerant to first-line therapy [5].

Other azoles such as itraconazole or posaconazole are considered as second line agents for the treatment of IA, particularly in severely ill patients [5]. Use of these drugs in non-life-threatening infections where the patient has been already stabilized with a more potent agent has been described [64]. However, their applicability in non-neutropenic patients is limited because of scarce clinical experience, poor oral bioavailability and restricted access to the intravenous formulation [64]. A study comparing intravenous posaconazole versus voriconazole for primary therapy of invasive aspergillosis is ongoing (clinicaltrial.gov identifier NTC 01782131).

Although still not approved, the other two echinocandins (anidulafungin and micafungin) are used in clinical practice, particularly when

non-neutropenic patients are involved. In breakthrough IA and in refractory disease, combination therapy (e.g., echinocandin plus voriconazole or liposomal amphotericin B) may be considered [5].

Adequate duration of antifungal therapy for IPA is an unresolved issue. Recent IDSA guidelines recommend treatment for IPA to be continued for a minimum of 6–12 weeks [5], depending on the clinical condition of the patients, as well as the extent of resolution of clinical disease. Different strategies including clinical evolution, serum biomarkers and CT scan should be considered for adequately monitoring therapeutic response for IA.

Some authors, including us, believe that emission tomography 18fluorodeoxygucose could be a new useful tool for monitoring response to IPA treatment [58, 68]. Although this technique is not specific, it may be helpful in monitoring clinical evolution of the patients, especially when biomarkers and CT scan are not enough.

CONCLUSION

There are several future challenges in the management of IA in non-neutropenic patients. Since new immunosuppressive regimens and ICU care are expected to continue increasing the incidence of populations at risk of IA, new criteria for diagnosis of IA in non-neutropenic patients are needed. We believe that there could be a potential role of FDG/PET-TC for treatment monitoring in IA. More pharmacokinetic/pharmacodynamics data on antifungal agents in non-neutropenic patients are needed to optimize drug exposure and to minimize adverse events, especially in patients with underlying severe disease and concomitant medications.

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Compliance with Ethical Guidelines. This article is based on previously conducted studies and does not involve any studies of human or animal subjects performed by any of the authors.

Data Availability. Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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