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LETTER TO THE EDITOR

(1-3)-β-D-Glucan serum increase and small-airway-invasive radiological findings as early signs of pulmonary aspergillosis in high-risk hematologic patients in the posaconazole era: preliminary observations

M. Picardi[1] · R. Della Pepa[2] · C. Giordano[2] · N. Pugliese[2] · C. Mortaruolo[2] · F. Trastulli[2] · F. Grimaldi[2] · I. Zacheo[2] · M. Raimondo[2] · C. Sirignano[3] · P. Salvatore[4] · F. Pane[2]

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Dear Editor,

Invasive pulmonary aspergillosis (IPA) is a severe complication in hematologic patients, including those with chemotherapy-induced profound neutropenia for acute myeloid leukemia (AML) remission induction [1]. The routine use of non-culture-based microbiological diagnostic tests and chest computed tomography (CT) scans has improved the diagnosis of IPA [2]. The integration of serum galactomannan index (s-GMI) levels and pre-specified pulmonary CT findings is crucial to categorizing suspected fungal infection episodes and starting targeted therapy [3–5]. However, these diagnostic features may vary according to several factors including mold-active antifungal prophylaxis with impairment of the performance of microbiological and radiological tests [6]. Among the different types of azoles that have been used over time, posaconazole, a next-generation of oral triazoles, has been recommended with the maximum level of evidence (A1) for IPA prophylaxis in several international guidelines [7]. With the intention of optimizing the yield of microbiological and radiological diagnostic work-up in hematological patients at very high risk of invasive fungal infection, we have determined if the 2008 European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [3] can be appropriately used to categorize IPA-suspected episodes in the era of prophylaxis with posaconazole.

This retrospective study was conducted in the hematology unit of the Federico II University of Naples (Italy). From January 2009 to December 2012, patients with AML who received intensive chemotherapy for hematological remission induction underwent itraconazole (Sporanox oral solution; 10 mg/ml) 200 mg two times daily as primary antifungal prophylaxis (Itra group), while, from January 2013 to December 2016, posaconazole (Noxafil suspension; 40 mg/ml) 200 mg three times daily was administered to the same setting of patients as primary antifungal prophylaxis (Posa group). In the event of febrile neutropenia, the same baseline evaluations based on blood cultures and conventional radiological examinations, as already reported, were performed on patients in both groups [8]. Among them, those patients with baseline negative results and persisting neutropenic fever after 96 h unresponsive to broad-spectrum antibiotics underwent intensive diagnostic work-up. This included s-GMI (Platelia Aspergillus; Bio-Rad, Marnes-la-Coquette, France) and (1-3)-β-D-glucan (BDG; Fungitel, Associates of Cape Cod, Inc.) assay three times a week, and thin-section chest CT scannings weekly (Lightspeed Ultra; GE Medical Systems, Milwaukee, WI) with collimation of 0.25–1 mm and dose optimization [2–6]. Finally, the results of the two groups were analyzed and compared by using the two-tailed Student *t* test and χ^2 test.

Overall, during the 8-year study period, 77 consecutive patients (36, Posa group; 41, Itra group) receiving the scheduled antifungal prophylaxis developed persisting neutropenic fever after 96 h unresponsive to broad-spectrum antibiotics with negative blood cultures, chest x-rays, and abdominal-pelvic ultrasonographies and were all assessed for the final analysis. The two groups were balanced regarding the baseline

✉ R. Della Pepa
roberta.dellapepa@unina.it

1 Department of Advanced Biomedical Sciences, Federico II University Medical School, Naples, Italy

2 Department of Clinical Medicine and Surgery, Federico II University Medical School, Via S. Pansini 5, 80131 Naples, Italy

3 Istituto di Biostrutture e Bioimmagini, Consiglio Nazionale delle Ricerche, Naples, Italy

4 Molecular Medicine and Medical Biotechnology, Federico II University Medical School, Naples, Italy

Table 1 Baseline clinical characteristics of analyzed patients

Patient characteristics	Total cases ($n = 77$)	Posa group (2013–2016) ($n = 36$)	Itra group (2009–2012) ($n = 41$)	P
Sex				0.73
N male, (%)	40 (51)	18 (50)	22 (54)	
Age				0.11
Median years, (range)	51 (18–72)	48 (18–72)	51 (18–70)	
WHO performance status, (%)				0.68
PS 0-1	34 (44)	14 (39)	20 (49)	
PS 2-4	43 (56)	22 (61)	21 (51)	
Acute myeloid leukemia subtypes*				0.99
N cases, (%)				
AML with maturation	53 (69)	25 (70)	28 (68)	
AML without maturation	16 (22)	7 (20)	9 (22)	
AML with minimal differentiation	4 (5)	2 (6)	2 (6)	
Acute myelomonocytic leukemia	2 (2)	1 (2)	1 (2)	
Acute monoblastic/monocytic leukemia	2 (2)	1 (2)	1 (2)	
Chemotherapy treatment for acute myeloid leukemia remission induction, (%)				1.89
Cytarabine + idarubicin†	50 (65)	20 (56)	30 (73)	
Cytarabine + daunorubicin‡	12 (16)	7 (19)	5 (12)	
Cytarabine + fludarabine + idarubicin§	15 (19)	9 (25)	6 (15)	
Antifungal prophylaxis compliance to scheduled dosage				0.70
N cases, (%)	77 (100)	36 (100)	41 (100)	
Severe neutropenia				0.70
N cases, (%)	77 (100)	36 (100)	41 (100)	
Time with ANC < 500/mm^3				
Median (range), days		12 (4–35)	11 (4–39)	
Time with ANC < 100/mm^3				
Median (range), days		8 (1–18)	8 (1–20)	
Type of broad-spectrum antibiotic regimen, (%)				0.47
Piperacillin-tazobactam + amikacin	27 (35)	10 (28)	17 (41)	
Piperacillin-tazobactam + gentamicin	14 (18)	7 (19)	7 (17)	
Meropenem + amikacin	26 (34)	15 (42)	11 (27)	
Ceftazidime + amikacin	10 (13)	4 (11)	6 (15)	

ANC absolute neutrophil count

Severe neutropenia is defined as an ANC of less than 500/μl

Antibiotic treatments were given according to standard schedules [9]

*Diagnosis was defined according to the 2016 World Health Organization classification of myeloid neoplasms and acute leukemia [10]

† Cytarabine + idarubicin: cytarabine 100 mg/m^2 /day as a continuous 7-day infusion plus idarubicin 13 mg/m^2 daily on the first 3 days of treatment [11]

‡ Cytarabine + daunorubicin: cytarabine 100 mg/m^2 /day as a continuous 7-day infusion plus daunorubicin 45 mg/m^2 daily on the first 3 days of treatment [11]

§ FLAI regimen: fludarabine 30 mg/m^2 plus cytarabine 2 g/m^2 on days 1 to 5 and idarubicin 10 mg/m^2 on days 1, 3, 5 [12]

clinical characteristics (Table 1). The 77 patients underwent intensive diagnostic work-up as planned. In particular, 280 determinations of s-GMI, 303 determinations of s-BDG, and 90 chest CT scans were performed. The median value of the serial determinations of s-BDG was 61.5 pg/ml (range, 13–180) in the Posa group and 35 pg/ml (range, 3–78) in the Itra group ($P < 0.001$); while, for the serial determinations of s-GMI, the median value was 0.39 (range, 0.1–0.79) in the Posa group and 0.59 (range, 0.1–1.13) in the Itra group ($P < 0.001$; Fig. 1). Regarding chest CT findings, the most frequent results for the Posa group were non-specific signs, i.e., different from those listed as the major criteria by the EORTC/MSG group, compatible with small-airway-invasive infiltrates including clusters of < 1 cm in diameter centrilobular nodular opacities, opacified segments of small branching bronchioles with a "tree-in-bud" appearance, peribronchial consolidations

consistent with bronchopneumonia, and ground-glass infiltrates, whereas in the Itra group, frequent chest CT findings were macronodules, halo signs, and air crescent signs (Fig. 1). According to the 2008 EORTC/MSG criteria, none of the patients in the Posa group and 14 out of 41 (34%) patients of the Itra group received a diagnosis of proven/probable IPA ($P < 0.001$). However, 10 cases (28%) in the Posa group, and no case in the Itra group ($P < 0.001$), met both non-conventional microbiological and radiological features, i.e., s-BDG test (≥ 80 pg/ml) and small-airway-invasive radiological infiltrates. Anti-*Aspergillus* targeted therapy was given to the 14 patients with proven/probable IPA in the Itra group (voriconazole, 10 cases, 6 mg/kg bid on day 1 then 4 mg/kg bid i.v.; liposomal amphotericin B, 4 cases, at 3 mg/kg i.v. daily) and, in view of the pre-emptive antifungal approach policy used in our Institution, to the 10 patients in the Posa group with clinically and microbiologically documented infection as reported above (voriconazole: 8 cases; liposomal amphotericin B: 2 cases; at the same dosage of the Itra group). The remaining 26 patients in the Posa group, and 27 patients in the Itra group, were considered not affected by IPA and continued antifungal prophylaxis as scheduled. Finally, IPA-attributable mortality at 30 days was less in the Posa group than in the Itra group [none (0/36) vs. 10% (4/41), respectively; $P = 0.05$]. Thus, at post hoc analysis, we classified the 10 patients with favorable outcome effect from anti-*Aspergillus* targeted therapy in the Posa group with a new IPA category defined as "probable invasive aspergillosis with positive s-BDG test (≥ 80 pg/ml) and small-airway-invasive radiological infiltrates."

Real-life studies show that about 30% of newly diagnosed AML patients receiving prophylaxis with posaconazole during intensive chemotherapy phase for hematological remission induction required systemic empirical antifungal treatment for persisting neutropenic fever unresponsive to broad-spectrum antibiotics [13, 14]. For these patients at very high risk of fungal disease, diagnosing cases harboring breakthrough IPA is a challenge for attending physicians [15]. The pathological changes of the angioinvasive phase in the evolution of IPA in neutropenic hosts are strictly associated with underlying antifungal prophylaxis [16, 17]. The fungal growth in the epithelial cells and macrophages of pulmonary alveoli in a few hours leads to mold

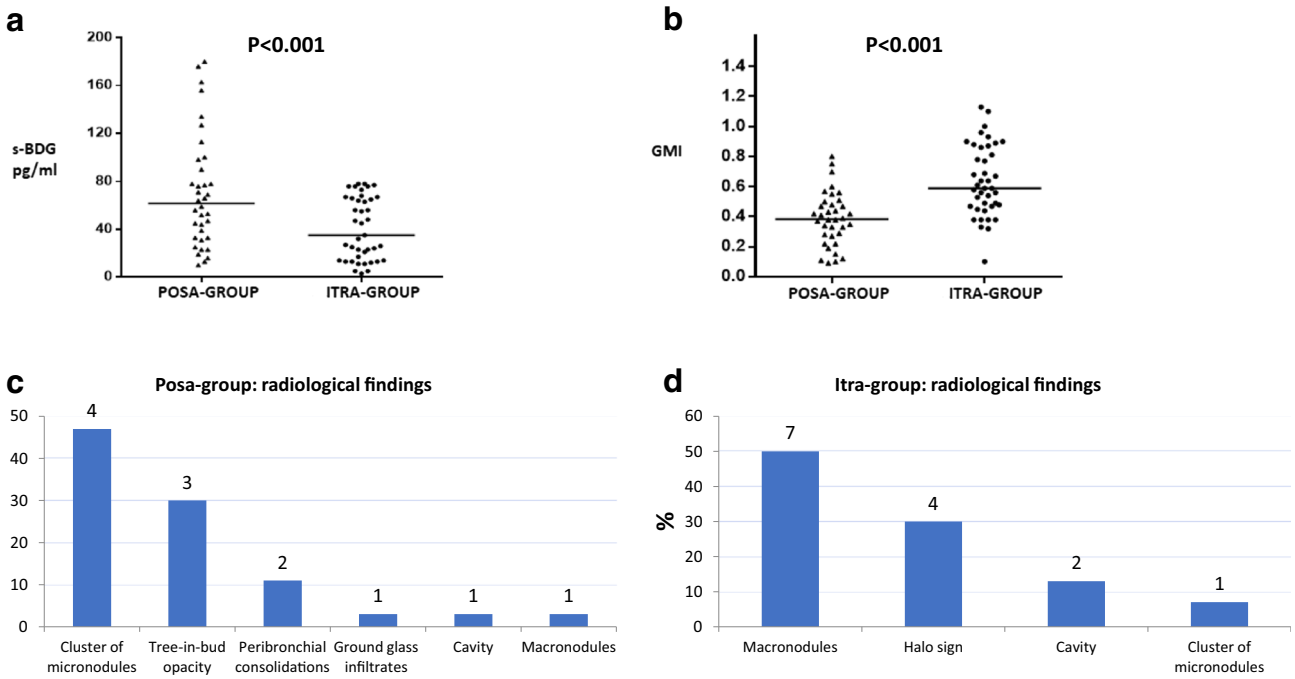

Fig. 1 Invasive pulmonary aspergillosis diagnostic work-up: comparison between the posaconazole group and the itraconazole group. **a** Comparison between median values of serum (1-3)-β-D-glucan (s-BDG) determinations. Each point represents the median of the s-BDG determinations for each patient in the study. **b** Comparison between median values of serum galactomannan index (GMI) determinations. Each point represents the median of the s-GMI determinations for each patient in the study. **c** Radiological diagnostic work-up results in the posaconazole prophylaxis group. The percentage is displayed on the *y*-axis, while the absolute numbers of observed cases with probable invasive aspergillosis according to a new IPA category (see the text) are shown on the top of the bar graph. In two patients, more than one of these radiological findings were simultaneously recorded. **d** Radiological diagnostic work-up results in the itraconazole-prophylaxis group. The percentage is displayed on the *y*-axis, while the absolute numbers of observed cases with proven/probable invasive aspergillosis according to the 2008 EORTC/MSG criteria (see the text) are shown on the top of the bar graph. Macronodule: ovoid and dense, well-circumscribed large lesion > 1 cm in diameter. Halo sign: macronodule surrounded for at least 75% by a perimeter of ground-glass opacity. Cavity (air crescent sign): crescentic pocket of gas occupying a separation interface between a lung sequestrum attributable to necrosis and a rim of viable lung. For cluster of micronodules, tree-in-bud sign, peribronchial consolidations, and ground-glass infiltrates, see text above

disease characterized by bloodstream invasion with typical radiological and non-culture-based microbiological findings [16, 17]. GMI increase is the serological representation of marked proliferation by *Aspergillus* hyphae [3–5]. The halo sign is the radiological representation of lung infarction: the nodule represents coagulation necrosis, and the halo is the edema and hemorrhage that surrounds the zone of infarction [3–5]. Posaconazole is highly lipophilic and has specific pharmacokinetic characteristics selectively partitioning into the extravascular compartment [18, 19] as proved in in vitro models of mammalian pulmonary epithelial cells membranes where the drug obtains concentrations 40–50-folds higher than in the serum [18]. This would explain posaconazole efficacy in reaching high levels in the target cells of *Aspergillus* spores and conidium, thus inhibiting hyphae proliferation [19]. Theoretically, posaconazole is able to immobilize fungal disease in the bronchoalveolar phase, i.e., in a stage with exclusively bronchial and alveolar involvement, preventing the occurrence of the angioinvasive phase [3–5]. In such instances, the crucial point is to recognize the images that precede the halo sign and to interpret them as early IPA in the context of positive serum surrogate biomarkers [20, 21]. Small-airway-invasive infiltrates are already considered the radiological expression of bronchoalveolar phase in the evolution of IPA in neutropenic patients [20, 21]. In our study, the kinetics of s-BDG correlated reversely with those of s-GMI. We hypothesized as possible pathogenetic pathway a multi-step mechanism: optimal fungistatic levels of posaconazole within epithelial cells and macrophages of pulmonary alveoli, accumulation of posaconazole within internal fungi membranes where the drug target is located (the enzyme CYP51a), fungal cell wall integrity alteration by the posaconazole activity with release of *Aspergillus* components [such as (1-3)-glucan] and consequently higher s-BDG levels, and inhibition of *Aspergillus* hyphae proliferation by the posaconazole activity resulting in lower s-GMI levels [16–19]. Based on such remarks, we propose a new category of probable IPA that includes an s-BDG positive test and small-airway-invasive radiological infiltrate without any macronodules revealing halo or air crescent sign. Applying our new infectious category in patients protected with posaconazole and with persisting neutropenic fever allows diagnosing IPA at an earlier stage, thus improving the outcome (likely resulting in the start of appropriate targeted antifungal therapy when the fungal burden is still low) [20–22]. By contrast, itraconazole having less lipophilic behavior was not able to prevent the rapid pathological changes of the angioinvasive phase in the evolution of fungal infection, thus quickly collapsing in a more advanced disease, associated with worse outcome of breakthrough IPA for disseminated fungal burden, as shown in our study and in others [14, 15].

In summary, in patients receiving prophylaxis with posaconazole at very high risk of fungal infection [13, 14], the application of serial s-BDG tests and an aggressive strategy of early chest CT scans may allow clinicians to diagnose breakthrough IPA with a low fungal burden before halo sign appearance and s-GMI increase, thus preventing lung infarction occurrence [3–5]. Our study presents limitations. It is a collection of retrospective data that relates to a small number of patients. In addition, BDG determination is described as having low sensibility in some settings of hematological patients with a certain rate of false-negative results [6]. The potential benefits and costs of such an approach should be evaluated by prospective studies.

Authors' contributions M.P. designed the research; M.P., R.D.P., and C.G. performed the research and wrote the paper; C.M., F.T., F.G., I.Z. and M.R. collected data; N.P. analyzed data; C.S. performed radiological exams; P.S. performed microbiological assays; and F.P. and M.P. performed the final revision of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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