# A Clinical Algorithm to Diagnose Invasive Pulmonary Aspergillosis in Critically Ill Patients

**Stijn I. Blot[1], Fabio Silvio Taccone[2], Anne-Marie Van den Abeele[3], Pierre Bulpa[4], Wouter Meersseman[5], Nele Brusselaers[1], George Dimopoulos[6], José A. Paiva[7], Benoit Misset[8], Jordi Rello[9], Koenraad Vandewoude[1], Dirk Vogelaers[1], and the *Asp*ICU Study Investigators***

[1]General Internal Medicine and Infectious Diseases, Ghent University, Ghent, Belgium; [2]Department of Intensive Care, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium; [3]Department of Microbiology, General Hospital St. Lucas, Ghent, Belgium; [4]Department of Intensive Care, Mont-Godinne University Hospital, Université Catholique de Louvain, Yvoir, Belgium; [5]Medical Intensive Care Unit, University Hospital Leuven, Leuven, Belgium; [6]Department of Critical Care Medicine, Attikon University Hospital, University of Athens Medical School, Athens, Greece; [7]Department of Emergency and Intensive Care, Centro Hospitalar S. Joao and University of Porto Medical School, Porto, Portugal; [8]Department of Intensive Care, Fondation Hôpital Saint-Joseph, Université Paris-Descartes, Paris, France; and [9]Hospital Universitari Vall d'Hebron, Vall D'Hebron Institute of Research, CIBERES, Universitat Autonoma de Barcelona, Barcelona, Spain

***Rationale*: The clinical relevance of *Aspergillus*-positive endotracheal aspirates in critically ill patients is difficult to assess.**

***Objectives*: We externally validate a clinical algorithm to discriminate *Aspergillus* colonization from putative invasive pulmonary aspergillosis in this patient group.**

***Methods*: We performed a multicenter (n = 30) observational study including critically ill patients with one or more *Aspergillus*-positive endotracheal aspirate cultures (n = 524). The diagnostic accuracy of this algorithm was evaluated using 115 patients with histopathologic data, considered the gold standard. Subsequently, the diagnostic workout of the algorithm was compared on the total cohort (n = 524), with the categorization based on the diagnostic criteria of the European Organization for the Research and Treatment of Cancer/Mycoses Study Group.**

***Measurements and Main Results*: Among 115 histopathology-controlled patients, 79 had proven aspergillosis. The algorithm judged 86 of 115 cases to have putative aspergillosis. This diagnosis was confirmed in 72 and rejected in 14 patients. The algorithm judged 29 patients to have *Aspergillus* colonization. This was confirmed in 22 and rejected in 7 patients. The algorithm had a specificity of 61% and a sensitivity of 92%. The positive and negative predictive values were 61 and 92%, respectively. In the total cohort (n = 524), 79 patients had proven invasive pulmonary aspergillosis (15.1%). According to the European Organization for the Research and Treatment of Cancer/Mycoses Study Group criteria, 32 patients had probable aspergillosis (6.1%) and 413 patients were not classifiable (78.8%). The algorithm judged 199 patients to have putative aspergillosis (38.0%) and 246 to have *Aspergillus* colonization (46.9%).**

***Conclusions*: The algorithm demonstrated favorable operating characteristics to discriminate *Aspergillus* respiratory tract colonization from invasive pulmonary aspergillosis in critically ill patients.**

### AT A GLANCE COMMENTARY

#### Scientific Knowledge on the Subject

The clinical relevance of *Aspergillus*-positive endotracheal aspirates in intensive care unit patients is difficult to assess, as clinical features are generally nonspecific.

#### What This Study Adds to the Field

A simple clinical algorithm demonstrated favorable operating characteristics to discriminate *Aspergillus* respiratory tract colonization from invasive pulmonary aspergillosis in intensive care unit patients. Particularly, its potential to rule out invasive pulmonary aspergillosis may support clinical decision-making.

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* A complete list of members may be found before the beginning of the REFERENCES.

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Correspondence and requests for reprints should be addressed to Stijn I. Blot, Ph.D., General Internal Medicine and Infectious Diseases, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium. E-mail: stijn.blot@ugent.be

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Invasive pulmonary aspergillosis (IPA) is a life-threatening disease generally occurring in hosts with impaired immune reaction (1, 2). The diagnosis of IPA is particularly problematic. According to the revised definitions for invasive fungal disease of the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG), IPA is categorized into proven, probable, and possible invasive fungal disease (Table 1) (3). A proven diagnosis requires histopathologic evidence of fungal invasion. A diagnosis of probable IPA is based on the presence of a combination of host factors, clinical features, and positive mycology. A diagnosis of possible IPA is made in the presence of host factors and clinical features but in the absence of or with negative mycological criteria. Cases that do not fall into one of these three categories are considered nonclassifiable.

These diagnostic criteria proved to be useful in research and practice in severely immunocompromised patients (4, 5). In intensive care unit (ICU) patients, however, diagnosing IPA according

**TABLE 1. DIAGNOSTIC CRITERIA FOR INVASIVE PULMONARY ASPERGILLOSIS ACCORDING TO THE EUROPEAN ORGANIZATION FOR THE RESEARCH AND TREATMENT OF CANCER/MYCOSIS STUDY GROUP (3) AND THE CLINICAL ALGORITHM (7)**

| EORTC/MSG criteria |
|---|
| Proven invasive pulmonary aspergillosis |
| Microscopic analysis on sterile material: histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or sterile biopsy in which hyphae are seen accompanied by evidence of associated tissue damage. Culture on sterile material: recovery of *Aspergillus* by culture of a specimen obtained by lung biopsy |
| Probable invasive pulmonary aspergillosis (all three criteria must be met) |
| 1. Host factors (one of the following) |
| • Recent history of neutropenia (<500 neutrophils/mm$^3$) for 110 d |
| • Receipt of an allogeneic stem cell transplant |
| • Prolonged use of corticosteroids at a mean minimum dose of 0.3 mg/kg/d of prednisone equivalent for 13 wk |
| • Treatment with other recognized T-cell immunosuppressants |
| • Inherited severe immunodeficiency |
| 2. Clinical features (one of the following three signs on CT) |
| • Dense, well-circumscribed lesion(s) with or without a halo sign |
| • Air-crescent sign |
| • Cavity |
| 3. Mycological criteria (one of the following) |
| • Direct test (cytology, direct microscopy, or culture) on sputum, BAL fluid, bronchial brush indicating presence of fungal elements or culture recovery *Aspergillus* spp. |
| • Indirect tests (detection of antigen or cell-wall constituents): galactomannan antigen detected in plasma, serum, or BAL fluid |
| Possible invasive pulmonary aspergillosis |
| Presence of host factors and clinical features (cf. probable invasive aspergillosis) but in the absence of or negative mycological findings. |

| Alternative clinical algorithm |
|---|
| Proven invasive pulmonary aspergillosis |
| Idem EORTC/MSG criteria |
| Putative invasive pulmonary aspergillosis (all four criteria must be met) |
| 1. *Aspergillus*-positive lower respiratory tract specimen culture (= entry criterion) |
| 2. Compatible signs and symptoms (one of the following) |
| • Fever refractory to at least 3 d of appropriate antibiotic therapy |
| • Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause |
| • Pleuritic chest pain |
| • Pleuritic rub |
| • Dyspnea |
| • Hemoptysis |
| • Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support |
| 3. Abnormal medical imaging by portable chest X-ray or CT scan of the lungs |
| 4. Either 4a or 4b |
| 4a. Host risk factors (one of the following conditions) |
| • Neutropenia (absolute neutrophil count <500/mm$^3$) preceding or at the time of ICU admission |
| • Underlying hematological or oncological malignancy treated with cytotoxic agents |
| • Glucocorticoid treatment (prednisone equivalent, >20 mg/d) |
| • Congenital or acquired immunodeficiency |
| 4b. Semiquantitative *Aspergillus*-positive culture of BAL fluid (+ or ++), without bacterial growth together with a positive cytological smear showing branching hyphae |
| *Aspergillus* respiratory tract colonization |
| When ⩾1 criterion necessary for a diagnosis of putative IPA is not met, the case is classified as *Aspergillus* colonization. |

*Definition of abbreviations*: BAL = bronchoalveolar lavage; CT = computed tomography; EORTC/MSG = European Organization for the Research and Treatment of Cancer/Mycosis Study Group; ICU = intensive care unit.

to this strict classification is problematic for a number of reasons. First, open lung biopsy might be contraindicated because of coagulation disorders and/or severe respiratory failure; as such, a diagnosis of proven IPA is rare. Second, current definitions of probable or possible IPA have been validated only in immunocompromised patients. This is a serious drawback, as IPA may develop in ICU patients without classic host factors (6, 7). Third, radiological findings in mechanically ventilated patients are nonspecific in the majority of cases (7), in contrast to the very strict definitions of radiological lesions in the EORTC/MSG criteria (3). Finally, galactomannan antigen detection on serum is of little value in nonneutropenic patients (8). The lack of specific criteria for diagnosing IPA in critically ill patients hampers timely initiation of appropriate antifungal therapy and may, as such, compromise the odds of survival (5, 9, 10). Hence, EORTC/MSG criteria have recently been questioned as an approach to encompass the true burden of disease (11–13).

The detection of *Aspergillus* in endotracheal aspirate cultures is observed in up to 2% of mechanically ventilated ICU patients and presents a dilemma, as it may represent only colonization rather than infection (7, 14, 15). The relevance of *Aspergillus*-positive endotracheal aspirates was assessed by Vandewoude and colleagues, who proposed a clinical diagnostic algorithm to discriminate *Aspergillus* colonization from IPA (7). The algorithm was derived from the EORTC/MSG definitions (16) and considers an endotracheal aspirate culture to represent probable IPA in the presence of compatible signs, abnormal thoracic medical imaging, and either host factors or bronchoalveolar lavage (BAL) fluid positive for *Aspergillus* on direct microscopy and culture (Table 1). In a cohort of 172 ICU patients with *Aspergillus*-positive endotracheal aspirate cultures, 83 were judged to have probable IPA (48.3%). Histopathology data were available in 26 patients, 19 in the probable IPA group and 9 in the colonization group. In all 26 cases the diagnosis as based on the clinical algorithm was confirmed. These data

**TABLE 2. CHARACTERISTICS OF CRITICALLY ILL PATIENTS WITH *ASPERGILLUS*-POSITIVE ENDOTRACHEAL ASPIRATE CULTURES**

| | All Patients ($n = 524$) | Nonhistopathology-controlled Patients ($n = 409$) | Histopathology-controlled Patients ($n = 115$) |
|---|---|---|---|
| Demographics | | | |
| Sex, male | 313 (59.7) | 249 (60.9) | 64 (55.6) |
| Age, yr | 65 (53–74) | 67 (54–75) | 61 (51–71)* |
| Body mass index, weight (kg)/height $(m)^2$ | 24 (21–27) | 24 (21–27) | 24 (20–26) |
| ICU stay before first positive culture, d | 4 (1–9) | 4 (1–9) | 4 (1–9) |
| Admission data | | | |
| Type of ICU admission | | | |
| Medical | 372 (71.0) | 276 (67.5) | 96 (83.5)† |
| Elective surgery | 79 (15.1) | 63 (15.4) | 8 (7.0)* |
| Emergency surgery | 68 (13.0) | 54 (13.2) | 14 (12.2) |
| Trauma | 39 (7.4) | 36 (8.8) | 3 (2.6)* |
| Diagnostic category | | | |
| Respiratory | 214 (40.8) | 161 (39.4) | 53 (46.1) |
| Cardiac | 137 (26.1) | 105 (25.7) | 32 (27.8) |
| Neurological | 29 (5.5) | 24 (5.9) | 5 (4.3) |
| Gastrointestinal | 34 (6.5) | 27 (6.6) | 7 (6.1) |
| Endocrine | 17 (3.2) | 9 (2.2) | 8 (7.0) |
| Postoperative and trauma | 81 (15.5) | 75 (18.3) | 6 (7.8) |
| Other | 4 (0.8) | 3 (0.7) | 1 (0.9) |
| APACHE II score | 23 (17–28) | 23 (16–28) | 24 (18–28) |
| Underlying conditions | | | |
| Insulin-dependent diabetes | 68 (13.0) | 53 (13.0) | 15 (13.0) |
| Chronic heart failure | 54 (10.3) | 45 (11.0) | 9 (7.8) |
| Chronic renal failure | 21 (4.0) | 18 (4.4) | 3 (2.6) |
| Acute liver failure | 11 (2.1) | 6 (1.5) | 5 (4.3) |
| Chronic liver failure | 26 (5.0) | 17 (4.2) | 9 (7.8) |
| Myelodysplastic syndrome | 11 (2.1) | 9 (2.2) | 2 (1.7) |
| Acute leukemia | 25 (4.8) | 19 (4.6) | 6 (5.2) |
| Chronic leukemia | 7 (1.3) | 2 (0.5) | 5 (4.3)† |
| Bone marrow transplantation | 5 (1.0) | 4 (1.0) | 1 (0.9) |
| Solid tumor | 54 (10.3) | 38 (9.3) | 16 (13.9) |
| Neutropenia | | | |
| $<500$ neutrophils/$mm^3$ | 24 (4.6) | 19 (4.6) | 5 (4.3) |
| $<500$ neutrophils/$mm^3$ for $>10$ d | 2 (0.4) | 2 (0.5) | 0 |
| Solid organ transplantation | 51 (9.7) | 34 (8.3) | 17 (14.8)* |
| Chronic obstructive pulmonary disease | 168 (32.1) | 138 (33.7) | 30 (26.1) |
| Treatment with cytotoxic agents | 55 (10.5) | 30 (7.3) | 25 (21.7) |
| Corticosteroid use | | | |
| Chronic corticosteroid use | 161 (30.7) | 115 (28.1) | 46 (40.0)* |
| Stress doses of hydrocortisone for sepsis | 31 (5.9) | 21 (5.1) | 10 (8.7) |
| Chronic inhalational corticosteroids | 62 (11.8) | 52 (12.7) | 10 (8.7) |
| EORTC host factor present | 237 (45.2) | 176 (40.8) | 70 (60.9)‡ |
| Severity of acute illness at time of diagnosis | | | |
| SOFA score | 7 (4–12) | 7 (4–12) | 10 (6–14)‡ |
| Supportive therapy on time of diagnosis | | | |
| Vasopressive or inotropic agents | 321 (61.3) | 246 (60.0) | 75 (65.2) |
| Mechanical ventilation | 452 (86.3) | 337 (82.4) | 115 (100)‡ |
| Renal replacement therapy | 145 (27.7) | 94 (23.0) | 51 (44.3)‡ |

*Definition of abbreviations*: APACHE = acute physiology and chronic health evaluation; EORTC = European Organization for the Research and Treatment of Cancer; ICU = intensive care unit; SOFA = sepsis-related organ failure assessment.

Data are summarized as n (%) or median (interquartile range); one patient can have more than one underlying condition.

* $P < 0.05$ Compared with nonhistopathology-controlled patients.

† $P < 0.01$.

‡ $P < 0.001$.

appeared promising, but the low number of histopathology-controlled cases and single-center design called for external validation in a larger cohort.

The objective of this study was to externally validate a clinical diagnostic algorithm, which aims to discriminate colonization from probable IPA in ICU patients with *Aspergillus*-positive endotracheal aspirate cultures. First, the predictive value of the algorithm was evaluated in a subset of histopathology-controlled cases. Thereafter, in a cohort of 524 ICU patients with *Aspergillus*-positive endotracheal aspirate cultures, a comparison was made between the diagnostic categorization as based on the clinical algorithm and the EORTC/MSG criteria. Pilot results of this study have been previously reported in the form of an abstract (17).

## METHODS

The *Asp*ICU project is an international, multicenter (n = 30) observational cohort study conducted between November 2006 and January 2011. Adult ICU patients were eligible for inclusion when they had evidence of *Aspergillus* involvement by at least one positive direct test on any body site, sampled during the ICU course. Because we anticipated a relative paucity of patients in which histopathology data were available, we accepted patients from historical cohorts (as from January 2000) on the condition that none of the requested data were missing. The results are reported according to the Standards for the Reporting of Diagnostic Accuracy Studies (18).

Data recorded included demographics, diagnostic category, and acute and underlying conditions. Clinical data included signs compatible

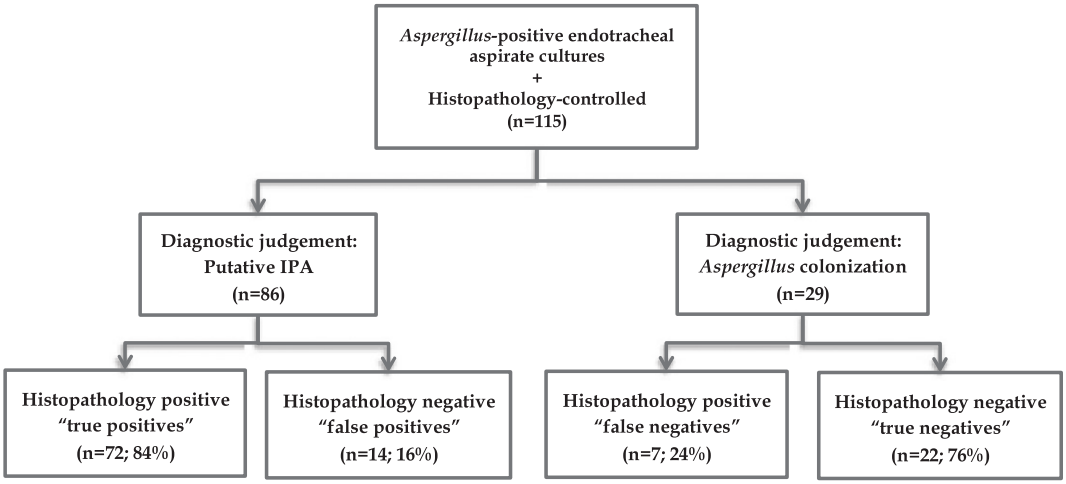


***Figure 1.*** Predictive value of the clinical algorithm to discriminate invasive pulmonary aspergillosis (IPA) from *Aspergillus* colonization in 115 critically ill patients with *Aspergillus*-positive endotracheal aspirates and histopathologic examination.

with invasive fungal disease. Mycological data included sampling techniques, mycological tests, and test results. Mycological tests were considered positive according to international consensus criteria (16). Data on radiographic assessment included findings from chest CT scan or X-ray and CT scan of the sinuses, abdomen, and central nervous system.

Time of diagnosis was considered as the date of diagnosis of invasive aspergillosis or of the first positive culture in case of *Aspergillus* colonization.

The clinical relevance of the *Aspergillus*-positive endotracheal aspirates was assessed by means of the EORTC/MSG criteria and a clinical algorithm as described earlier (7). Table 1 summarizes the diagnostic criteria of both approaches. First, we selected patients with *Aspergillus*-positive endotracheal aspirate cultures in which histopathology data were available. A comparison was made between the judgment as based on the algorithm and results of the pathology examination. When histopathology data were positive, the diagnosis was considered proven; when histopathologic examination was negative for invasive disease, the *Aspergillus*-positive endotracheal aspirates were considered to represent *Aspergillus* colonization. As a second objective, we compared the classification of all ICU patients with *Aspergillus*-positive endotracheal aspirates into diagnostic categories according to the EORTC/MSG criteria and the clinical algorithm. The category of proven IPA is identical in both (Table 1). For semantic clarity, the classification of probable IPA in the clinical algorithm was renamed to putative IPA, to distinguish from probable IPA in the EORTC/MSG criteria for invasive fungal disease.

The validity of the diagnostic algorithm was assessed with results of the biopsy or necropsy data as reference standard. Predictive value of the algorithm to diagnose IPA was assessed by specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV), and their 95% confidence intervals (CIs). Because predictive values greatly depend on the particular case mix within an ICU, adjustment was crucial to extrapolate these results to the clinical setting: estimates of PPV and NPV were adjusted according to an assumed range of 20 to 50% prevalence of IPA within a population of ICU patients with *Aspergillus*-positive endotracheal aspirates as reported previously in consecutive series (7, 14, 15).

Additionally, the diagnostic algorithm and the EORTC/MSG criteria were evaluated using receiver operating characteristic curve analysis. This analysis graphically represents the sensitivity and specificity of the algorithm for predicting IPA. The area under the curve is reported with 95% CIs.

## RESULTS

Thirty centers from eight countries (Belgium, Brazil, China, France, Greece, India, Portugal, and Spain) participated in the *Asp*ICU study. The final cohort included 563 patients. In 529 patients the lung was the affected site, as evidenced by an *Aspergillus*-positive culture from endotracheal aspirate, BAL fluid, lung biopsy, or autopsy. Five additional patients were excluded because the diagnosis of proven IPA was made *post mortem*, without previous *Aspergillus*-positive endotracheal aspirate cultures present. Characteristics of the study cohort (n = 524) are shown in Table 2. Medical diseases, especially involving the respiratory and cardiac systems, were the principle reasons for ICU admission. Patients presented in the ICU with a high severity of disease index, as evidence by the Acute Physiology and Chronic Health Evaluation II scores (19). About one-fifth of the patients had malignancy as underlying condition, and nearly one-third suffered from chronic obstructive pulmonary disease (COPD). There were 52 solid organ transplant patients

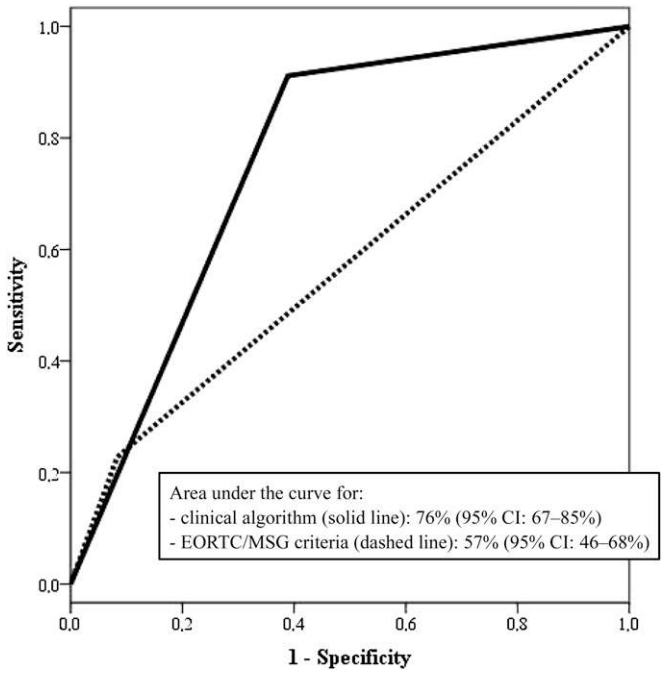


***Figure 2.*** Receiver operating curve analyses for diagnosing invasive pulmonary aspergillosis by means of the clinical algorithm and the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria in 115 histopathology-controlled cases.

(10%). EORTC host factors were present in 240 patients (45%), mostly because of treatment with immunosuppressive agents for COPD, malignancy, or solid organ transplantation.

### Predictive Value of the Clinical Algorithm

The cohort contained 115 critically ill patients with at least one *Aspergillus*-positive endotracheal aspirate culture and in which pathology data were available, either through lung biopsy or autopsy. This subgroup was used to evaluate the predictive value of the algorithm to discriminate *Aspergillus* colonization from putative IPA. Characteristics of this subgroup are shown in Table 2. Compared with patients without histopathologic examination, histopathology-controlled cases appeared to have a higher risk profile for invasive fungal disease as they had more EORTC/MSG host factors, and higher severity of disease as evidenced by higher Sepsis-Related Organ Failure Assessment scores and more need for supportive therapy.

Among histopathology-controlled patients, lung biopsy was performed in 61 patients (53.0%) and autopsy in 54 patients (47.0%). Figure 1 shows a breakdown of the diagnostic judgement according to the algorithm and results of the histopathologic examination. A diagnosis of proven IPA was found in 79 patients (68.7%). According to the clinical algorithm, 86 of 115 patients had IPA, and 29 had *Aspergillus* colonization. IPA diagnosis was confirmed in 72 (true positives) and rejected in 14 patients (false positives). Seven patients with proven IPA were incorrectly classified by the algorithm as colonized (false negative). The remaining 22 patients were correctly classified as *Aspergillus* colonized. Consequently, the algorithm for diagnosing IPA had a sensitivity of 92% (95% CI, 83–96%) and a specificity of 61% (95% CI, 45–75%). Figure 2 illustrates receiver operating characteristic curve analysis for the diagnosis of IPA according to the clinical algorithm and the EORTC/MSG criteria. For the clinical algorithm, the area under the curve was 76% (95% CI, 67–85%). In contrast, for the EORTC/MSG criteria, the area under the curve was only 57% (95% CI, 46–68%). In the (presumed) absence of histopathologic data, a diagnosis of probable IPA would have been achieved in only 20 of 79 patients (25.3%), as clinical features and/or host factors were missing in 55 (69.6%) and 22 patients (27.8%), respectively.

Table 3 shows the diagnostic criteria for IPA observed in distinct diagnostic groups. Compatible signs and symptoms were present in all but nine patients (92.2%). Abnormal thoracic medical imaging was present in nearly all patients (98.3%). However, in patients with proven IPA, radiological features, as defined by the EORTC/MSG (3), were present in only one-third of the patients (24 of 79; 30.4%). Differences in clinical judgment were mainly due to the presence or absence of risk factors and direct mycological tests on BAL fluid.

Estimates of PPV and NPV for different prevalence assumptions are listed in Table 4. Adjusted for an assumed prevalence of 20 to 50%, the PPV ranged from 50 to 70%, whereas the NPV ranged 94 to 87%. In immunocompromised patients (n = 70), the PPV ranged 38 to 59%, whereas the NPV was always 100%, as there were no false negatives in this

**TABLE 3. DIAGNOSTIC CRITERIA FOR INVASIVE PULMONARY ASPERGILLOSIS IN INTENSIVE CARE UNIT PATIENTS, CLASSIFIED ACCORDING TO THE RESULTS OF HISTOPATHOLOGY EXAMINATION AND CLINICAL ALGORITHM**

| | Pathology Positive (Proven IPA) | | Pathology Negative (Proven Colonization) | | Operating Characteristics for Distinct Criteria within the Algorithm | | | |
|---|---|---|---|---|---|---|---|---|
| | TP (*n* = 72) | FN (*n* = 7) | FP (*n* = 14) | TN (*n* = 22) | Sens (%) | Spec (%) | PPV (%) | NPV (%) |
| Criteria of the clinical algorithm for diagnosing probable IPA | | | | | | | | |
| 1. *Aspergillus*-positive endotracheal aspirate | 72 (100) | 7 (100) | 14 (100) | 22 (100) | | | | |
| 2. Compatible signs | 72 (100) | 7 (100) | 14 (100) | 13 (59.1) | 100 | 25 | 47 | 100 |
| Fever refractory to at least 3 d of appropriate antibiotic therapy | 40 (55.6) | 6 (85.7) | 4 (28.6) | 3 (13.6) | | | | |
| Recrudescent fever after ⩾48 h of defervescence while still on antibiotics and without other apparent cause | 2 (2.8) | 0 | 1 (7.1) | 2 (9.1) | | | | |
| Pleuritic chest pain | 5 (6.9) | 0 | 0 | 0 | | | | |
| Pleuritic rub | 3 (4.2) | 0 | 0 | 0 | | | | |
| Dyspnea | 37 (51.4) | 3 (42.9) | 6 (42.9) | 7 (31.8) | | | | |
| Hemoptysis | 13 (18.1) | 0 | 3 (21.4) | 0 | | | | |
| Worsening respiratory insufficiency despite appropriate antibiotic therapy and ventilatory support | 51 (70.8) | 4 (57.1) | 8 (57.1) | 4 (18.2) | | | | |
| 3. Abnormal thoracic medical imaging on CT scan or X-ray | 72 (100) | 7 (100) | 14 (100) | 20 (90.9) | 100 | 6 | 41 | 100 |
| Diffuse reticular or alveolar opacities | 17 (23.6) | 3 (42.9) | 8 (57.1) | 5 (22.7) | | | | |
| Nonspecific infiltrates and consolidation | 49 (68.1) | 4 (57.1) | 5 (35.7) | 15 (68.2) | | | | |
| Pleural fluid | 28 (38.9) | 3 (42.9) | 6 (42.9) | 5 (22.7) | | | | |
| Wedge-shaped infiltrate | 8 (11.1) | 3 (42.9) | 0 | 0 | | | | |
| Well-shaped nodule(s) | 19 (26.4) | 2 (28.6) | 3 (21.4) | 4 (18.2) | | | | |
| Air-crescent sign | 1 (1.4) | 0 | 1 (7.1) | 0 | | | | |
| Halo sign | 5 (6.9) | 0 | 0 | 1 (4.5) | | | | |
| Cavitation | 7 (9.7) | 0 | 0 | 1 (4.5) | | | | |
| 4a. Host risk factors | 67 (93.1) | 0 | 14 (100) | 5 (22.7) | 84 | 47 | 51 | 81 |
| Neutropenia (<500 neutrophils/mm$^3$) | 6 (8.3) | 0 | 2 (14.3) | 0 | | | | |
| Malignancy treated with cytotoxic agents | 16 (22.2) | 0 | 4 (28.6) | 0 | | | | |
| Glucocorticoid treatment | 52 (72.2) | 0 | 12 (85.7) | 5 (22.7) | | | | |
| Inherited severe immunodeficiency | 3 (4.2) | 0 | 0 | 0 | | | | |
| 4b. Semiquantitative *Aspergillus*-positive culture of BAL fluid + positive direct microscopy | 31 of 51 (60.8) | 0 of 3 (0) | 1 of 11 (9.1) | 0 of 5 (0) | 94 | 57 | 87 | 77 |
| Criteria for proven IPA present | | | | | | | | |
| Biopsy positive | 34 of 34 (100) | 3 of 3 (100) | 0 of 9 (0) | 0 of 15 (0) | | | | |
| Autopsy positive | 38 of 38 (100) | 4 of 4 (100) | 0 of 5 (0) | 0 of 7 (0) | | | | |

*Definition of abbreviations*: BAL = bronchoalveolar lavage; FN = false negatives; FP = false positives; IPA = invasive pulmonary aspergillosis; NPV = negative predictive value; PPN = positive predictive value; Sens = sensitivity; Spec = specificity; TP = true positives.

**TABLE 4. POSITIVE PREDICTIVE VALUE AND NEGATIVE PREDICTIVE VALUE OF THE CLINICAL ALGORITHM TO DIAGNOSE PROBABLE INVASIVE PULMONARY ASPERGILLOSIS ACCORDING TO ITS ASSUMED PREVALENCE**

| | | Assumed Prevalence of IPA in ICU Patients with *Aspergillus*-Positive Endotracheal Aspirate Cultures (%) | | | |
|---|---|---|---|---|---|
| | | 20 | 30 | 40 | 50 |
| All histopathology-controlled patients (n = 115) | PPV | 37 | 50 | 61 | 70 |
| | NPV | 97 | 95 | 92 | 89 |
| Immunocompromised patients* (n = 70) | PPV | 27 | 38 | 49 | 59 |
| | NPV | 100 | 100 | 100 | 100 |
| Patients with COPD receiving prolonged corticosteroid therapy (n = 30) | PPV | 45 | 59 | 69 | 77 |
| | NPV | 100 | 100 | 100 | 100 |
| Nonimmunocompromised patients (n = 45) | PPV | 44 | 57 | 68 | 76 |
| | NPV | 91 | 85 | 79 | 71 |

*Definition of abbreviations*: COPD = chronic obstructive pulmonary disease; EORTC/MSG = European Organization for the Research and Treatment of Cancer/Mycoses Study Group; ICU = intensive care unit; IPA = invasive pulmonary aspergillosis; NPV = negative predictive value; PPV = positive predictive value.

*According to definitions of the EORTC/MSG; this includes all patients with COPD receiving prolonged corticosteroid therapy and hence qualifying within the EORTC/MSG criteria.

subgroup. When only nonimmunocompromised patients were considered (n = 45), the PPV ranged 57 to 76%, whereas the NPV ranged from 91 to 71%.

### Diagnostic Evaluation in 524 ICU Patients with *Aspergillus*-Positive Endotracheal Aspirate Cultures

Figure 3 shows a breakdown of the diagnostic workout according to the EORTC/MSG criteria and the clinical algorithm. For each diagnostic category, the proportion of immunocompromised patients (presence of host factors as defined by the EORTC/MSG), patients under antifungal therapy, and associated 12-week mortality are reported.

Of the 524 patients with *Aspergillus*-positive endotracheal aspirate cultures, 79 patients had proven IPA (16%). According to the EORTC/MSG criteria, 32 patients had probable IPA (6%). None of the patients had possible IPA, because positive mycology was present in all patients (study entry criterion). Of the 524 patients, 413 could not be classified according to the EORTC/MSG criteria (78%).

According to the clinical algorithm, 199 patients had a diagnosis of putative IPA. The remaining 246 patients were considered to have *Aspergillus* respiratory tract colonization (47%).

Of the 32 patients diagnosed as probable IPA, 31 were judged as putative IPA according to the algorithm. One case with probable IPA was—according to the algorithm—considered to be only colonized with *Aspergillus*, as no compatible sings were present. Forty percent of patients who were not classified following the EORTC/MSG definitions (n = 168) were classified as putative IPA (algorithm-based diagnosis). The remaining 245 nonclassifiable patients were categorized as *Aspergillus* colonization following the algorithm.

### Proportion of Immunocompromised Patients in Distinct Diagnostic Categories

Among proven cases, 71% were immunocompromised. As per definition, all of the cases with probable IPA had host factors. Host factors were present in 36% of patients who could not be classified according to the EORTC/MSG criteria. Seventy percent of patients with putative IPA had host factors. Among patients judged to have *Aspergillus* colonization, 16% were immunocompromised.

### Mortality in Distinct Diagnostic Categories

Figure 4 illustrates survival distributions for distinct diagnostic categories as defined by EORTC/MSG criteria (Figure 4a) and the clinical algorithm (Figure 4b). In cases with IPA, either proven, probable, or putative, a majority of patients deceased within 2 weeks after the first *Aspergillus*-positive culture. Mortality observed among cases with probable (71.9%), putative (67.5%), and proven IPA (76.9%) was not significantly different ($P = 0.302$) as was the proportion of patients under antifungal therapy (88.6, 90.6, and 72.4%, respectively; $P = 0.063$). Mortality in nonclassified cases (EORTC/MSG approach) was significantly higher compared with cases judged as being colonized with *Aspergillus* ($P = 0.011$) despite a significantly higher proportion of patients under antifungal therapy (39.5 vs. 23.6%; $P < 0.001$). Table E5 in the online supplement reports mortality rates in distinct diagnostic categories and whether or not patients received antifungal therapy.

## DISCUSSION

In this multicenter study we externally validated a clinical algorithm for diagnosing IPA by discriminating *Aspergillus* colonization from invasive disease in ICU patients with *Aspergillus*-positive endotracheal aspirate cultures. With histopathology as the gold standard, the algorithm demonstrated 61% specificity and 92% sensitivity. Because the prevalence of IPA in ICU patients remains uncertain, and may vary according to the risk profile of the index population (20), predictive values were calculated for different possible prevalences. For an assumed IPA prevalence of 40%, the PPV and NPV were 61 and 92%, respectively.

The need for a modified diagnostic approach for IPA in ICU patients has been repeatedly underlined (6, 21, 22). Autopsy series indicated that strict interpretation of the host factors for invasive fungal disease contributes to the risk of missed diagnosis (6, 23, 24). Underlying conditions, such as COPD, nonhematological malignancy, diabetes, liver cirrhosis, chronic alcohol abuse, HIV, malnutrition, and severe burn injury, have been described in association with invasive aspergillosis (15, 20, 25–29). In the present study, 22 of 79 proven IPA cases (27.8%) lacked host factors. This implies that, according to EORTC/MSG criteria, diagnosis of IPA was impossible without performing a lung biopsy. Fifteen of these 22 cases were identified by the algorithm (data not shown), thereby stressing the clinical relevance of an extended interpretation of risk profile. Host risk factors as defined in the algorithm showed an NPV of 81% (Table 3). Broadening the radiological criteria for diagnosing IPA in ICU patients also appears valuable, as in 69.6% of proven IPA cases (55 of 79) typical radiological lesions suggestive for invasive fungal disease were absent. This is in agreement with the low sensitivity of 24% of these features in patients without hematologic malignancy compared with 82% in neutropenic patients (30). As radiological lesions are generally nonspecific, the evaluated algorithm accepts any radiological abnormality. However, abnormal thoracic imaging in mechanically ventilated critically ill patients is nearly ubiquitous. In our study, for example, only two patients (evaluated as true negatives) had normal medical imaging. Consequently, the algorithm's radiological criteria as such have poor discriminative value, albeit with a high NPV (Table 3).

The final prerequisite for the diagnosis of probable IPA is either the presence of host factors or a stringent mycological criterion. All patients evaluated as false negatives (n = 7) failed to comply with both conditions. Host factors, mostly treatment with immunosuppressive agents, were found in 84.8% of proven cases (67 of 79) but were also present in 52.8% of patients

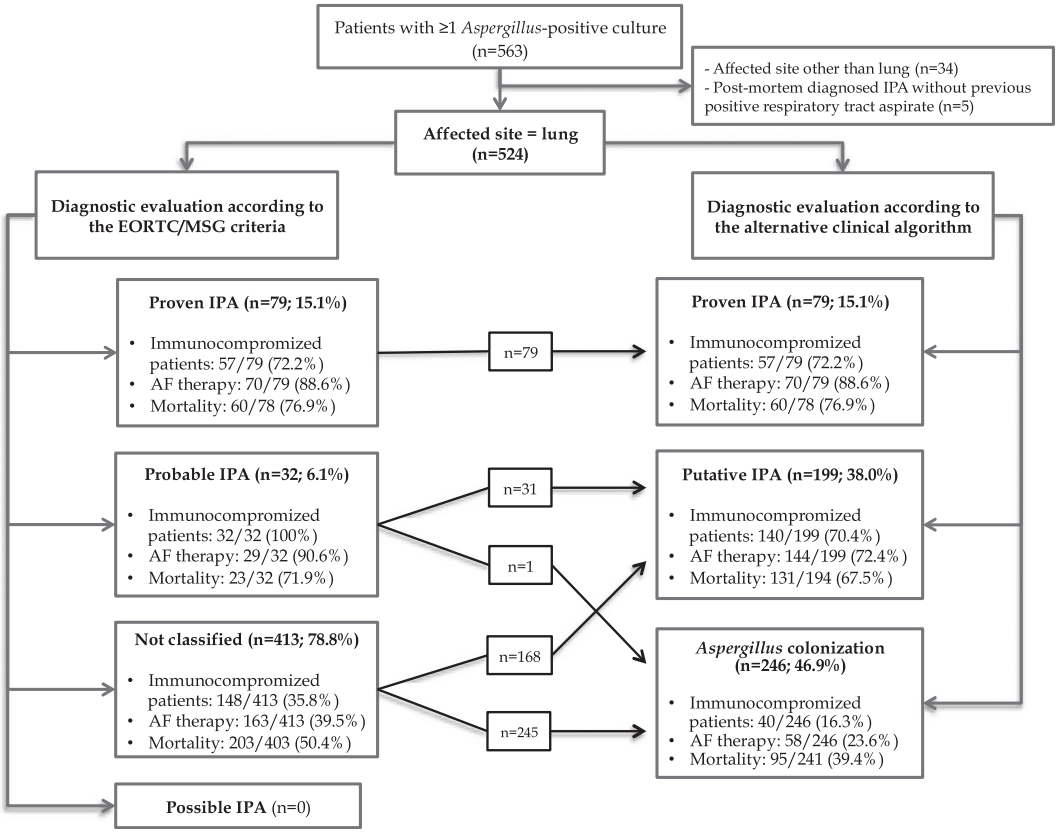


***Figure 3.*** Diagnostic breakdown of critically ill patients with *Aspergillus*-positive endotracheal aspirate cultures, according to the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria (3) and the clinical algorithm (7). Immunocompromised status indicates the presence of host factors as defined by the EORTC/MSG (c.f. Table 1). AF therapy = antifungal therapy with mold activity initiated; IPA = invasive pulmonary aspergillosis.

without IPA (19 of 36). As such, host factors, as a single criterion, demonstrated to have high sensitivity but lacked specificity in this cohort. As IPA may develop in patients without apparent risk factors, the algorithm incorporated an alternative mycological criterion with stronger discriminative value than the endotracheal aspirate culture. Culture- and microscopy-positive BAL fluid proved to have high specificity, as it was only positive in one of 16 cases without IPA, but poor sensitivity (57.4%). Unfortunately, BAL was only performed in 70 patients (60.9%).

Crucial in the diagnostic algorithm for IPA is the risk of false negatives. In our cohort of histopathology-controlled patients, seven cases of proven IPA were judged as colonized (7 of 79; 8.9%). All these patients were nonimmunocompromised (Table 4). For a prevalence of 40%, the NPV was high (91%). Nevertheless, false-negative diagnoses remain a matter of concern, especially in nonimmunocompromised patients. In patients without risk factors or culture-positive BAL fluid, additional galactomannan antigen detection on BAL fluid can be recommended to further strengthen the diagnostic certainty. Meersseman and colleagues evaluated the value of galactomannan detection in BAL fluid in 72 pathology-controlled nonneutropenic ICU patients with an overt risk profile for aspergillosis, as evidenced by thoracic CT scan and underlying and acute conditions (8). Using a cutoff index of 0.5, the sensitivity and specificity of galactomannan detection in BAL fluid was 88 and 87%, respectively.

We evaluated the diagnostic classification of all 524 patients with *Aspergillus*-positive endotracheal aspirates according to the EORTC/MSG criteria and the alternative clinical algorithm. With the exception of proven IPA for which equal definitions are used, differences in diagnostic approaches result in vast differentiations in classification. Out of 435 nonproven cases, only 32 were considered probable IPA, whereas 413 were nonclassifiable. Following the clinical algorithm, 199 patients were classified as putative IPA. Importantly, this number contained 168 cases that were considered not classifiable with the EORTC/MSG diagnostic workout. The prognostic profile associated with the diagnostic categories provides indirect support that the algorithm can better discriminate between significant disease and colonization. Despite including a great number of nonclassifiable cases (EORTC/MSG-approach), putative IPA had a similar mortality (70%) to probable (72%) and proven IPA (78%) ($P = 0.193$). Another indirect indication for a better classification by means of the algorithm is found in the number of early deaths. In Figure 4a, the survival curve representing nonclassifiable patients follows the curves of probable and proven IPA for nearly 2 weeks. On the contrary, the survival curve of patients with *Aspergillus* colonization (Figure 4b) diverges from those of putative and proven IPA immediately after the first positive culture. We assume that this difference in survival pattern is due to the proportion of ill-classified cases according to the EORTC/MSG diagnostic workout. A (nonsignificantly) smaller proportion of patients with putative IPA received antifungal therapy (72.4 vs. 88.6 and 90.6% for probable and proven IPA, respectively). Seventeen patients with putative IPA survived while not receiving adequate antifungal therapy (data not shown). As IPA is considered lethal in the absence of antifungal therapy, these cases must be considered as false positives. Yet, in the light of a fatal outcome if left untreated, not missing the diagnosis is a greater concern. As such, a discrete loss in specificity seems acceptable to increase sensitivity.

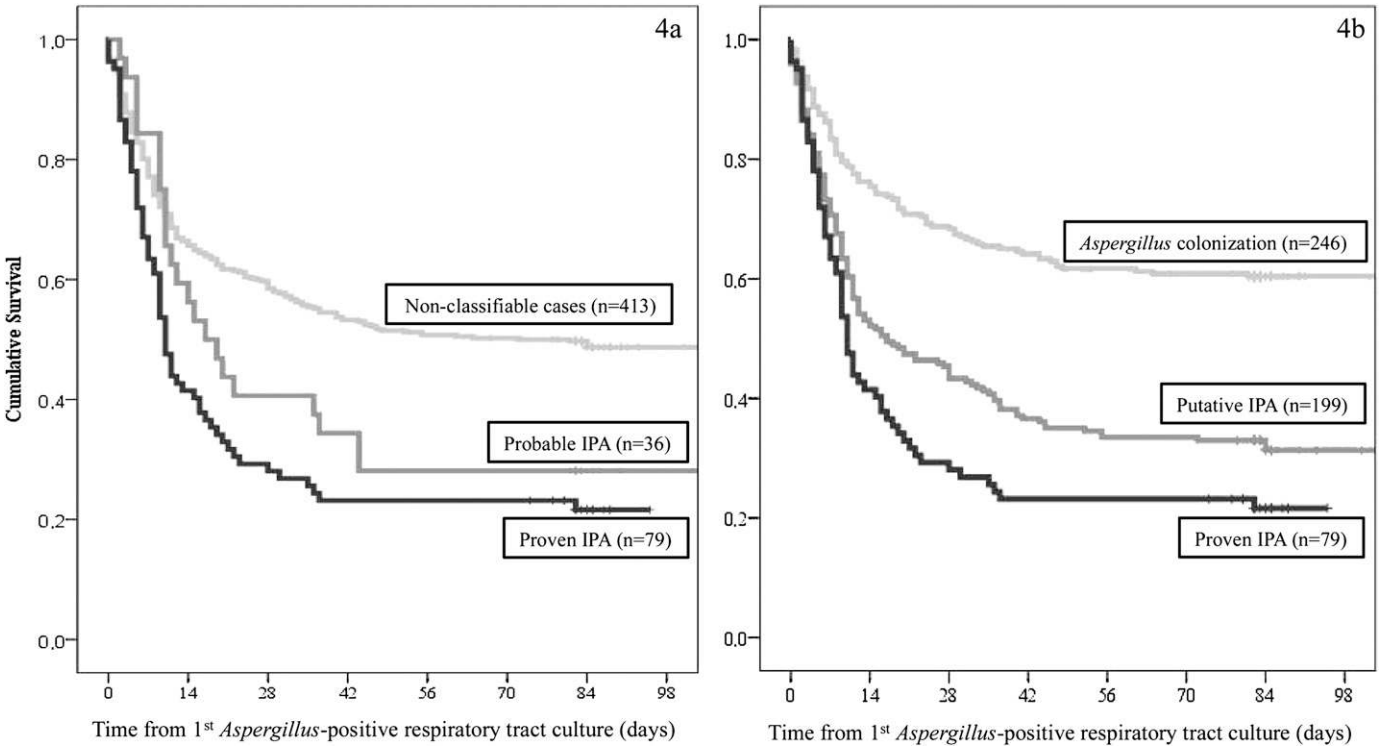


***Figure 4.*** Survival curves for distinct categories for the diagnosis of invasive pulmonary aspergillosis (IPA) according to criteria of the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (*a*) and the clinical algorithm (*b*). Log rank for survival distributions in *a* and *b*, $P < 0.001$.

A drawback of the algorithm is the requirement of an *Aspergillus*-positive culture as entry criterion because IPA may develop in the absence of positive cultures. In the study by Meersseman and colleagues, cultures on BAL fluid appeared negative in 42% of cases with proven aspergillosis (11 of 26) (8). Consequently, by adopting the algorithm in clinical practice, only the fraction that is preceded by a positive culture can be assessed.

Strengths of this study include the extensive series of histopathology-controlled cases and the multicenter approach. Thirty centers from eight different countries contributed to the cohort, thereby backing up the external validity of the study results.

Limitations of the study include the nonconsecutive series and the bias resulting from the selection of only histopathology-controlled cases. It is expected that biopsies are more likely to be performed in patients with a high index of suspicion. For example, classic host factors were present in 61% of histopathology-controlled patients, whereas in the total cohort 41% were immunocompromised according to the EORTC/MSG definitions (Table 2). Therefore the proportion of patients with proven IPA (79 of 115; 68.7%) in this study cohort is considered to be higher than in clinical practice. Therefore, PPV and NPV estimates were adjusted for an assumed prevalence ranging 20 to 50%. This prevalence will depend on the case mix of a particular ICU population. A greater proportion of patients at high risk for developing invasive fungal disease will probably reflect in a higher prevalence of such infections.

Furthermore, autopsy as well as biopsy data were considered as gold standard, but false-negative lung biopsies cannot be ruled out. Also, due to the observational nature of the study BAL was not performed in all patients. In 17 patients without host factors no BAL was performed; 7 of these had proven IPA. In these cases a diagnosis of putative IPA (in the absence of histopathology data) was per definition impossible. Excluding patients without BAL data did not substantially alter the operating characteristics of the algorithm (51% PPV, 94% NPV).

In conclusion, a simple clinical algorithm demonstrated reasonable operating characteristics, in particular regarding its sensitivity for a lethal disease if left untreated. This suggests its usefulness as a tool to discriminate *Aspergillus* respiratory tract colonization from putative IPA in ICU patients. In comparison to the EORTC/MSG criteria, this algorithm probably encompasses a larger proportion of the true burden of IPA in the ICU.

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***AspICU Study Investigators*:** Miguel Blasco-Navalpotro, Hospital Universitario Severo Ochoa (Madrid, Spain); Stijn Blot, Ghent University (Ghent, Belgium); Nele Brusselaers, Ghent University Hospital, (Ghent, Belgium); Pierre Bulpa, Mont-Godinne University Hospital (Yvoir, Belgium); Teresa Cardoso, Hospital de Santo Antonio (Porto, Portugal); Pierre-Emmanuel Charles, Dijon University Hospital (Dijon, France); Didier Clause, Cliniques de l'Europe (Brussels, Belgium); Patricia Courouble, Cliniques Universitaires Saint Luc (Brussels, Belgium); Emmanuel De Laere, Heilig Hartziekenhuis Roeselaere-Menen (Roeselaere, Belgium); George Dimopoulos, University Hospital Attikon (Athens, Greece); Frédéric Forêt, Centre Hôspitalier Régional Mons-Warquignies (Mons, Belgium); Dan Li, Shangai Public Health Clinical Center (Shangai, China); Claude Martin, Assistance publique hôpitaux de Marseille, (Marseille, France); Shahram Mashayekhi, Centre Hospitalier Grand Hornu (Hornu, Belgium); Wouter Meersseman, Universitair Ziekenhuis Gasthuisberg (Leuven, Belgium); Benoit Misset, Hôpital Saint-Joseph (Paris, France); José Artur Paiva, Hospital de Sao Joao, (Porto, Portugal); Paulo Mergulhao, Hospital de Sao Joao (Porto, Portugal);Alessandro Pasqualotto, Santa Casa-Complexo Hospitalar (Porto Allegre, Brazil); Marcos Pérez, Vall d'Hebron University Hospital (Barcelona, Spain); Ratna Rao, Apollo Hospital (Hyderabad, India); Jordi Rello, Joan XXIII University Hospital (Tarragona, Spain) and Vall d'Hebron University Hospital (Barcelona, Spain); Jessica Souto, Vall d'Hebron University Hospital (Barcelona, Spain); Herbert Spapen, Brussels University Hospital (Brussels, Belgium); Fabio Silvio Taccone, Hôpital Erasme (Brussels, Belgium); Anne-Marie Van den Abeele, AZ Sint Lucas, (Ghent, Belgium); Koenraad Vandewoude, Ghent University Hospital, (Ghent, Belgium); and Dirk Vogelaers, Ghent University Hospital (Ghent, Belgium).

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