Additional File 1: Diagnostic algorithms for IPA

A) Proven IPA [1-4]

Microscopic analysis on sterile material: histopathological, cytopathological or direct microscopic examination of a sample obtained by needle aspiration or sterile biopsy in which hyphae are seen together with evidence of associated tissue damage.

B) AspICU for the diagnosis of IPA in critically ill patients [1]

1. aspergillus (+) culture
2. clinical signs and symptoms (one of the following) • Fever after at least 3 days of appropriate antibiotic therapy • Pleuritic chest pain • Pleural rub • Dyspnea • Hemoptysis • Increase in respiratory insufficiency despite appropriate antibiotic therapy and ventilatory support.
3. pathological X-ray or computed tomography of the thorax
4. either
4a. Risk factors (one of the following conditions)
• Neutropenia (absolute neutrophil count of less than 500/mm^3) before or at the time of admission to the ICU. • Hematological or oncological malignancy treated with chemotherapy • Treatment with glucocorticoids (prednisone or equivalent, >20 mg/day) • Congenital or acquired immunodeficiency
OR
4b. Semiquantitative Aspergillus positive
Culture of BAL fluid (+ or ++) without bacterial growth together with a positive cytological smear showing branched hyphae.
• probable invasive pulmonary aspergillosis if 1 + 2 + 3 + either 4a or 4b • if criterion ≥1 is not met, the patient is classified as having Aspergillus colonization

C) Modified clinical algorithm for the diagnosis of probable IPA in critically ill patients according to Schauwvlieghe [2]

1. clinical signs and symptoms (<u>one</u> of the following)
• Fever after at least 3 days of appropriate antibiotic therapy. • Pleuritic chest pain • Pleural rubbings • Dyspnea • Hemoptysis • Increase in respiratory insufficiency despite appropriate antibiotic therapy and ventilatory support.
2. any infiltrate on X-ray or computed tomography of the thorax
3. mycological examinations of <u>one</u> of them
• Aspergillus (+) culture and in BAL • positive cytological smear of BAL or sputum showing branched hyphae • Aspergillus antigen (galactomannan-EIA) in Bronchoalveolar Lavage Fluid: ≥ 1.0 Optical Density Index • Aspergillus antigen (galactomannan-EIA) in serum: ≥ 0.5 Optical Density Index

D) Modified clinical algorithm for the diagnosis of probable IPA in critically ill patients according to Loughlin [3]

1. clinical signs and symptoms (<u>two</u> of the following)
• Fever < 35°C or >38°C • Leukocytes < 4×10^9 or > 11×10^9 • Purulent tracheal secretion
2. new or increasing infiltrates in the X-ray of the lung or a computed tomography of the thorax
3. mycological examinations of <u>one</u> of them
• Aspergillus (+) culture and in BAL • positive cytological smear of BAL or sputum showing branched hyphae • Aspergillus antigen (galactomannan-EIA) in BAL: ≥ 1.0 OD, • Aspergillus antigen (galactomannan-EIA) in serum: ≥ 0.5 OD

E) EORTC/MSGERC ICU Working Group new definition of IPA [5]

Proven IPA:
1. Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy in which hyphae compatible with Aspergillus spp. Are seen accompanied by evidence of associated tissue damage (with necessary confirmation by means of culture or PCR)
2. Recovery of Aspergillus spp. by culture of a specimen obtained by a sterile procedure from a normally sterile site and clinically or radiologically abnormal site consistent with an infectious-disease process

1. Mycological evidence of Aspergillus spp. at least 1 of the following:.
• cytology, direct microscopy, and/or culture indicating presence of Aspergillus spp. in a lower respiratory tract specimen • galactomannan antigen index >0.5 in plasma/serum and/or galactomannan antigen >0.8 in BALF
2. 1 Clinical/radiological abnormality
• Dense, well-circumscribed lesions with or without a halo sign • Air crescent sign • Cavity • Wedge-shaped and segmental or lobar consolidation • Tracheobronchial ulceration, pseudomembrane, nodule
3. Host factors (one of the following conditions)
• Glucocorticoid treatment with prednisone equivalent of 20 mg or more per day • Qualitative or quantitative neutrophil abnormality (inherited neutrophil deficiency, absolute neutrophil count of ≤500 cells/mm3) • Chronic respiratory airway abnormality (chronic obstructive lung disease, bronchiectasis) • Decompensated cirrhosis • Treatment with recognized immunosuppressants (eg, calcineurin or mammalian target of rapamycin inhibitors, blockers of tumor necrosis factor and similar antifungal immunity pathways, alemtuzumab, ibrutinib, nucleoside analogues) during the past 90 days • Hematological malignancies/HSCT • Solid organ transplantation • Human immunodeficiency virus infection • Severe influenza (or other severe viral pneumonia, such as COVID-19)

F) Diagnosis criteria of the EORTC (European Organisation for Research and Treatment of Cancer) [4]

Mycological investigations of one of them

- Aspergillus (+) Culture and in BAL or sputum
- positive cytological smear of BAL or sputum showing branched hyphae
- Aspergillus antigen (galactomannan-EIA) in BAL or serum individually ≥ 1.0 OD, serum or plasma together: ≥ 0.7 OD and BAL fluid ≥ 0.8 OD.

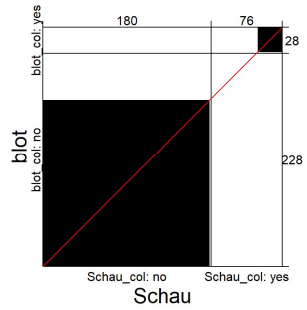
Host factors of one of them

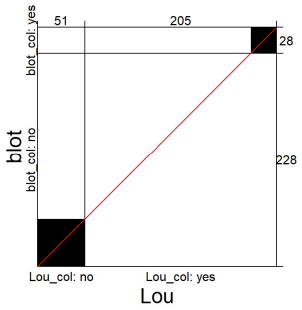
- Neutropenia (<0.5 × 10 ^{9}neutrophils for >10 days)
- Hematological malignancy
- allogeneic stem cell transplantation
- Solid organ transplantation
- Prolonged use of corticosteroids at a therapeutic dose of ≥ 0.3 mg/kg corticosteroids for ≥ 3 weeks in the past 60 days
- T-cell immunosuppressant, such as calcineurin inhibitors, TNF-a blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues during the last 90 days.
- B-cell immunosuppressant
- Congenital immunodeficiency
- Therapy refractory GVHD

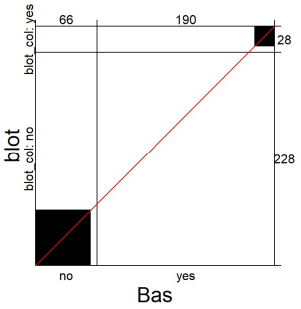
Computed tomography of the thorax with dense, well-circumscribed lesion(s) with or without one of them

- Halo sign
- Cavity
- Air crescent sign
- Wedge-shaped segmental or lobar consolidation

Additional File 2: Agreement Plots subgroup analysis of severely immunocompromised patients (n=256)





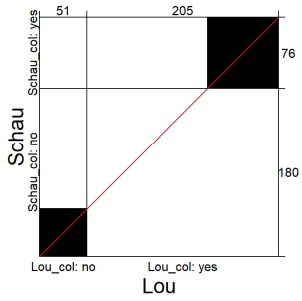


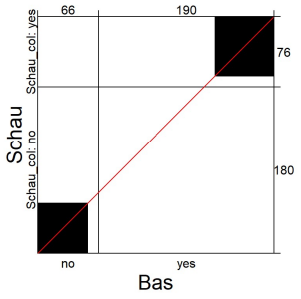
Cohens kappa (95%CI):

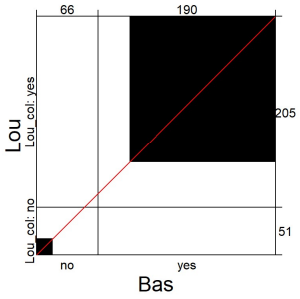
0.40 (0.26, 0.55)

0.05 (-0.03, 0.13)

0.00 (-0.08, 0.08)







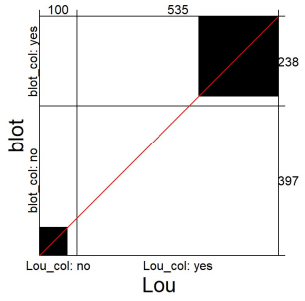
Cohens kappa (95%CI):

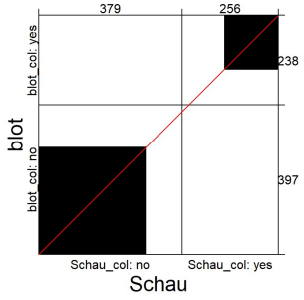
0.19 (0.09, 0.29)

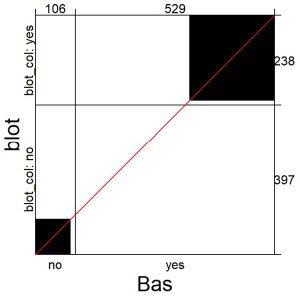
0.10 (-0.00, 0.20)

0.08 (-0.08, 0.25)

Additional File 3: Agreement Plots subgroup analysis of patients without viral pneumonia (influenza and COVID-19; n=635)

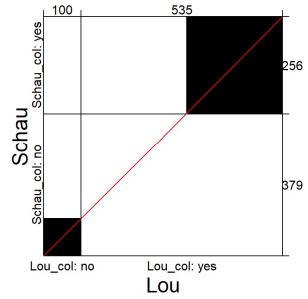


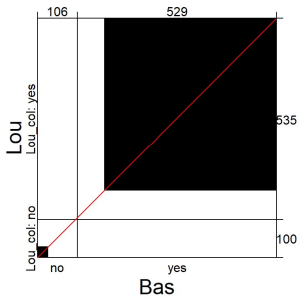


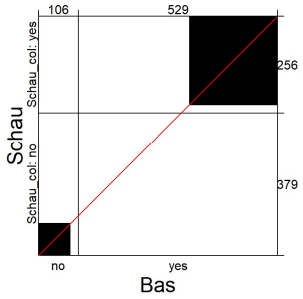


Cohens kappa (95%CI):

0.06 (-0.00, 0.13) 0.31 (0.23, 0.39) 0.14 (0.08, 0.21)







Cohens kappa (95%CI):

0.22 (0.15, 0.29) 0.13 (0.01, 0.25) 0.12 (0.05, 0.18)

References:

1. Blot SI, Taccone FS, Van den Abeele AM, Bulpa P, Meersseman W, Brusselaers N, Dimopoulos G, Paiva JA, Misset B, Rello J *et al*: A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med* 2012, 186(1):56-64.
2. Schauwvlieghe A, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, Lagrou K, Verweij PE, Van de Veerdonk FL, Gommers D *et al*: Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med* 2018, 6(10):782-792.
3. Loughlin L, Hellyer TP, White PL, McAuley DF, Conway Morris A, Posso RB, Richardson MD, Denning DW, Simpson AJ, McMullan R: Pulmonary Aspergillosis in Patients with Suspected Ventilator-associated Pneumonia in UK ICUs. *Am J Respir Crit Care Med* 2020, 202(8):1125-1132.
4. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, Clancy CJ, Wingard JR, Lockhart SR, Groll AH *et al*: Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis* 2020, 71(6):1367-1376.
5. Bassetti M, Azoulay E, Kullberg BJ, Ruhnke M, Shoham S, Vazquez J, Giacobbe DR, Calandra T: EORTC/MSGERC Definitions of Invasive Fungal Diseases: Summary of Activities of the Intensive Care Unit Working Group. *Clin Infect Dis* 2021, 72(Suppl 2):S121-S127.