Supplementary methods

As for the original systematic review [1], this update follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [2]. The full Fungal Infections Definitions in Intensive Care Unit (FUNDICU) protocol has been published in 2018 [3].

Data sources and data management

We searched PubMed and EMBASE (OvidSP), CINAHL (EBSCOHost), and the Cochrane Database (Wiley) using a pre-defined search string [3]. The search period of the original study was from January 2003 to December 2018, whereas the search period of the present update was from January 2019 to Marche 2022. All abstracts and full texts were imported and managed into an EndNote Web database, and shared between Daniele Roberto Giacobbe (DRG), Erika Asperges (EA), and Valentina Zuccaro (VZ). Abstract and full text review was performed independently by EA and VZ. References of retrieved full texts were also screened to identify further studies suitable for inclusions. Finally, independent decisions on inclusion were compared between EA and VZ, with disagreement being resolved by a third reviewer (DRG).

Inclusion and exclusion criteria

We included cross-sectional studies, longitudinal (cohort) prospective or retrospective studies, randomized controlled trials, single-arm studies, quasi-experimental studies that assessed the diagnostic performance for invasive aspergillosis (IA) of a definition/s and/or laboratory/radiology test/s vs. a reference standard (histology) or a reference definition.

Studies were excluded if: (*i*) they were conducted exclusively in the paediatric population (<18 years) (*ii*) the diagnostic performance of tests/definitions for IA cannot be separated from the diagnostic performance for other invasive fungal diseases (IFD) considered in the given study; (*iii*) patients who were classified in specific reference categories (e.g., possible IA) were excluded from the analysis of the diagnostic performance of the evaluated test/s or definition/s. We also excluded

those studies in which the population was composed for ≥50% by hematological and/or solid organ transplant (SOT) patients. However, if mixed populations were present but no information about the proportion of hematological and/or SOT patients was provided, studies were retained if it was clearly stated in methods that they involved also wards other than hematology/SOT (e.g., hematology plus ICU).

Data extraction

Data were extracted on a standard form. The form was drafted by VZ and DRG, with supervision by Luigia Scudeller (LS). For each study, the following data was extracted by VZ and EA: first author; publication year; type of study (randomized controlled trial [RCT], observational); study timeline (retrospective, prospective, cross-sectional); site of IA (e.g., pulmonary, cerebral, any); study population; setting (e.g., ICU, other wards); number of enrolled patients; reference definition/test applied in the study; reference diagnostic categories (e.g., IA/non-IA, non-IA/possible IA/probable IA/proven IA) and number of patients classified in the different reference categories; tests/definitions evaluated in the study; used cut-offs (where applicable); diagnostic performance of the evaluated tests/definitions with respect to the reference (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV]; positive likelihood ratio [LR+], negative likelihood ratio [LR-], diagnostic odds ratio [DOR]).

Risk of bias

The risk of bias in included studies was assessed using a scoring tool specifically designed for this project. One point was assigned for each of the following potential sources of bias, with higher total scores thus corresponding to higher risk of bias:

- retrospective study
- missing IFD classification of >10% of included patients (for loss of follow-up or other reasons)

- study population including also hematological and/or SOT patients
- exclusion of patients difficult-to-diagnose from the study
- combination of adults and children
- *ad hoc* selection of the cut-off value (where applicable)
- unreliability of the reference standard (defined as any reference standard different from histology)
- classification as IA after knowledge of the result of the reference standard (where applicable, i.e., when classification of IA was based upon arbitrary clinical criteria)

Data synthesis

No formal data synthesis was applicable to the present systematic review [3].

References

[1] Bassetti M, Giacobbe DR, Grecchi C, Rebuffi C, Zuccaro V, Scudeller L*, et al.* Performance of existing definitions and tests for the diagnosis of invasive aspergillosis in critically ill, adult patients: A systematic review with qualitative evidence synthesis. J Infect. 2020; 81: 131-146.

[2] Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009; 6: e1000097.

[3] Bassetti M, Scudeller L, Giacobbe DR, Lamoth F, Righi E, Zuccaro V*, et al.* Developing definitions for invasive fungal diseases in critically ill adult patients in intensive care units. Protocol of the FUNgal infections Definitions in ICU patients (FUNDICU) project. Mycoses. 2019; 62: 310-319.