The Clinical Impact of Rapid Molecular Microbiological Diagnostics for Pathogen and Resistance Gene Identification in Patients With Sepsis: A Systematic Review

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Fast microbiological diagnostics (MDx) are needed to ensure early targeted antimicrobial treatment in sepsis. This systematic review focuses on the impact on antimicrobial management and patient outcomes of MDx for pathogen and resistance gene identification compared with blood cultures. PubMed was searched for clinical studies using either whole blood directly or after short-term incubation. Twenty-five articles were retrieved describing the outcomes of 8 different MDx. Three interventional studies showed a significant increase in appropriateness of antimicrobial therapy and a nonsignificant change in time to appropriate therapy. Impact on mortality was conflicting. Length of stay was significantly lower in 2 studies. A significant decrease in antimicrobial cost was demonstrated in 6 studies. The limitations of this systematic review include the low number and observed heterogeneity of clinical studies. In conclusion, potential benefits of MDx regarding antimicrobial management and some patient outcomes were reported. More rigorous intervention studies are needed focusing on the direct benefits for patients.

Keywords. antimicrobial therapy; bacteremia; molecular microbiological diagnostics; patient outcomes.

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection and can lead to septic shock, which increases mortality considerably [1]. The mortality rate of patients with sepsis is 10%–20%, which increases to 40%–80% in patients developing septic shock [2]. Additionally, hospital length of stay (LOS) increases in patients with bloodstream infection (BSI), especially due to antimicrobial-resistant pathogens [3, 4].

Antimicrobial treatment should start as soon as possible and certainly within the first hour of recognizing sepsis. This should be preceded by taking blood cultures, if this does not cause any significant delay (>45 minutes) [5]. Kumar et al. showed a 7.6% decrease in survival rate for each hour of delay in administering antimicrobial therapy after the start of hypotension in patients with septic shock [6].

Blood culture (BC) is the gold standard for identifying bacteria that cause sepsis [7]. A serious limitation is that culture-based diagnostics are time-consuming, and the time to positivity is pathogen dependent [8, 9].

Due to this long turnaround time (TAT) and the need for immediate antimicrobial treatment, physicians start treatment empirically with broad-spectrum antibiotics, which contributes to antibiotic resistance [10, 11]. Thus, faster microbiological diagnostics are necessary to ensure an early targeted treatment. Molecular microbiological diagnostic techniques (MDx) could provide relevant results within a few hours [11, 12].

Ideally, results should be available within a single workday, and the time-consuming culture step should be avoided. Starting directly from the clinical specimen or after a short-term (a few hours) incubation of blood is beneficial [13–15]. In 2016, Timbrook et al. performed a meta-analysis of 5920 patients to assess the impact of rapid MDx starting from positive blood cultures, such as Matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF), on clinical outcomes. They found that these MDx were associated with significant decreases in time to effective antibiotic therapy, in mortality risk in the presence of an antimicrobial stewardship plan, and in LOS [16]. The goal of this systematic review was to identify the clinical impact of rapid MDx for the identification of pathogens directly in whole-blood samples or after short-term incubation in terms of antimicrobial therapy management and patient outcomes.
METHODS

Literature Search
This systematic review was done according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [17]. PubMed was searched through October 2019 for all types of studies evaluating MDx to identify pathogens and resistance genes either directly in whole-blood samples or after a short-term incubation step in patients with sepsis (cutoff date October 22, 2019). The search terms are shown in Supplementary Figure 1. No restrictions on the year of publication or language were set. Two researchers independently screened the corresponding studies by title and abstract. Studies were included in consensus and excluded according to criteria shown in Figure 1.

Study Selection Criteria
The objective was to identify articles that evaluated the clinical impact of rapid MDx on patients with sepsis or (suspected) BSI. Studies assessing the use of MDx to identify causative pathogens (bacteria and/or fungi) and/or genetic susceptibility directly from whole-blood specimens or after short-term incubation (shorter than BC incubation to positivity) in comparison with BCs were included. Both pathogen-specific and multiplex tests were eligible. The study population was any patients with sepsis, BSI, or suspected BSI of bacterial or fungal origin, from whom BCs were drawn. Studies concerned all settings, such as the emergency department, the intensive care unit (ICU), and the hospital. All study types (interventional, prospective, and retrospective), except case reports and reviews, were eligible.

Reasons for exclusion (Figure 1) were the use of other types of specimen than whole blood or the use of positive blood culture bottles. Nonmolecular techniques such as antigen detection or biomarkers were excluded as well. Studies on viral, protozoan, and parasitic infections were excluded. Studies using spiked samples or analyzing only performance characteristics were excluded.

Data Extraction
Data collected from included studies were study design, diagnosis at inclusion, population, number of patients included, type and (branded) name of MDx used, study location, funding source, number of antimicrobial therapy adjustments, number of patients on appropriate antimicrobial therapy, time to any antimicrobial therapy adjustment, time to appropriate antimicrobial therapy, LOS in-hospital and in-ICU, mortality, and costs or expenses. Additionally, test characteristics and test performances of MDx were extracted from studies that were found in the search but were excluded because they did not contain clinical data on antimicrobial therapy or patient outcomes.

Outcomes
The aims were to assess the impact of MDx on the antimicrobial management of patients with bacterial and fungal BSI or sepsis and the impact on all patient-related outcomes. Appropriateness of antimicrobial therapy regardless of definition, any treatment change (escalation, de-escalation, discontinuation), and the time to appropriateness or change of antimicrobial therapy was assessed. Eligible patient-related outcomes were mortality,
LOS, ICU-LOS, sepsis severity, destination at discharge, and readmission. Lastly, costs associated with the use of MDx in the clinical setting were analyzed.

**Risk of Bias**

The risk of bias of randomized controlled trials (RCTs) was assessed by 1 reviewer using the Cochrane risk of bias tool for randomized trials (RoB2) [18]. This tool assesses 5 elements (sequence generation, allocation concealment, blinding, incomplete outcome data, and selective outcome reporting) of potential bias to estimate the effectiveness of an intervention. The risk of bias for prospective (nonrandomized) studies was also evaluated by 1 reviewer using the Cochrane risk of bias tool for nonrandomized studies of interventions (ROBINS-I) [19].

**RESULTS**

**Study Design and Population**

The search resulted in 692 records (Figure 1). The study characteristics of the included articles are presented in Table 1. In total, 25 eligible articles were retrieved. Three interventional studies (including 2 RCTs), 12 prospective studies, and 7 retrospective studies performed MDx directly on blood samples. Three prospective studies on different MDx used short-term BC incubation. MDx were compared with BC in all studies.

All patients in all studies had (suspected) bacterial or fungal BSI or (suspected) sepsis. However, inclusion criteria were not defined homogeneously. Suspected infection was the criterion for inclusion in 12 studies. However, criteria for suspicion were variable or not mentioned, and patients could be included based on BC draw or if the patient presented with fever or hypothermia. Ten studies concerned patients with proven sepsis, a subset of sepsis (severe sepsis, septic shock), or a systemic infection. Again, these terms were not always uniformly defined: Sepsis was diagnosed based on either the prevalence of systemic inflammatory response syndrome (SIRS) along with a suspicion of BSI or other definitions or national guidelines. All patients were sampled in-hospital in different types of wards. The ICU was represented in most studies (13/25), the emergency department in 3/25. Furthermore, 2/25 studies concerned neutropenic patients or patients with malignancies, and 2/25 concerned pediatric patients.

**Antimicrobial Management**

Antimicrobial management was the most frequently analyzed outcome parameter in 21/25 articles. However, the definitions differed among the studies. A currently accepted general definition of appropriate antimicrobial therapy is the use of antimicrobials with in vitro activity against the causative microorganism. Data on antimicrobial management from included studies are summarized in Table 2. Various terms and definitions were used for appropriateness or effective treatment and for change and/or adjustments (escalation, de-escalation, discontinuation). These terms and synonyms were used interchangeably. Presence and tasks of antimicrobial stewardship programs during the study periods were not always described.

In 2 randomized intervention studies by Tafelski et al. and Bhat et al., there were significantly fewer adjustments made when using SeptiFast (13.5% vs 9.8%) [20, 21] and another intervention study by Cambau et al. found a significant increase in the use of appropriate antimicrobials (23.6% vs 33.6%) [22]. In prospective studies by Mancini et al. and Tschiegel et al., the use of SeptiFast resulted in higher rates of antibiotic changes [23, 24]. Lodes et al. found appropriate changes in 16.9% of cases [25], and in the study conducted by Bravo et al. this was true in 77% of ICU cases [26]. Ten percent of patients would have had an earlier or improved initial treatment, observed by Maubon et al. [27]. Contrarily, Tran et al. found an inappropriate use of antibiotics in 29.2% of polymerase chain reaction (PCR)–positive patients, while this was 18.7% in BC-positive patients [28]. In addition, Lehmann et al. calculated that there was a 57-day reduction of inadequate antibiotic therapy when SeptiFast was used and a 22.5-day reduction per 100 tests done [29]. Retrospectively, SeptiFast would have resulted in adjustment of antibiotic therapy in 29.6%–38.9% of cases [29–31]. One prospective study by Bloos et al. concluded that a de-escalation was suggested in 24.2% of cases based on the VYOO results [32]. A retrospective analysis by Bloos et al. found a shorter time to appropriate therapy when using VYOO (67.5 hours vs 31 hours) [33]. Based on the PCR-ESI-MS results, Vincent et al. concluded that there would have been a recommendation for a treatment change in 41% of the cases [34]. Bhat et al. used SES in a randomized controlled trial with neonates, which resulted in a significantly higher rate of adjustments of antibiotics (15 vs 165 changes) but an overall lesser use of antibiotics per patient (5.8 vs 2.6 antibiotics/patient) [20, 21]. Retrospectively assessed by Patch et al., the use of T2MR was significantly associated with initiation of appropriate antifungal therapy 6 hours after blood draw, while this was 32 hours when using BCs [35]. Last, using ETGA for confirmation of negative samples after ≥ 12 hours of incubation (overnight) instead of 7 days, Dryden et al. found 73.6% appropriate stewardship outcomes [36].

**Patient Outcomes**

There were limited published data on the impact of MDx on clinical outcomes of patients. We were able to find data on mortality and/or LOS in-hospital or in-ICU in 11/25 articles (Table 3). Eleven studies described mortality differences when performing MDx directly on whole-blood samples. Mortality was assessed differently between studies and could be in-hospital or in-ICU, at 7 or 28 days. In the randomized intervention study by Cambau et al., there was no significant difference in mortality in the postintervention group [22], which was confirmed by the retrospective analysis by Alvarez et al. [37]. In the RCT by Bhat et al. using SES, in-hospital
| Author | Study Design | Diagnosis of Inclusion | Population | No. of Patients | Diagnostic Test | Study Location | Funding Source |
|--------|--------------|------------------------|------------|----------------|----------------|----------------|----------------|
| Tafelski [20] | RCT | Suspected sepsis of abdominal or pulmonary origin | ICU patients | 41 intervention, 37 control | SeptiFast, Roche | Germany | Roche Deutschland GmbH |
| Bhat [21] | RCT | Sepsis | Neonates | 183 intervention, 185 control | Syndrome evaluation system | India | Council for Scientific and Industrial Research, New Millennium India Technological Leadership Initiative |
| Cambau [22] | Cluster-randomized interventional | Severe sepsis, Septic shock, Infective endocarditis | In-hospital patients | 731 intervention, 684 control | SeptiFast, Roche | France | French Ministry of Health |
| Lodes [25] | Prospective intervention | SIRS | ICU patients | 104 intervention, No control | SeptiFast, Roche | Germany | Not known |
| Mancini [23] | Prospective, pre/postintervention | Suspected sepsis | Hematological patients | 137 intervention, 138 control | SeptiFast, Roche | Italy | Roche Diagnostics |
| Bloos [42] | Prospective controlled observational | Severe sepsis, Septic shock | ICU patients | 142 prospective, 63 control | SeptiFast, Roche | Germany, France | Roche Diagnostics |
| Wallet [43] | Prospective observational | Suspected BSI | ICU patients | 72 | SeptiFast, Roche | France | Roche Diagnostics provided materials |
| Bravo [26] | Prospective observational | Suspected BSI | Neutropenic and ICU patients | 86 neutropenic, 53 ICU | SeptiFast, Roche | Spain | Not known |
| Maubon [27] | Prospective observational | Suspected sepsis | Patients with solid or hematological malignancies | 110 | SeptiFast, Roche | France | Roche Diagnostics, academic grants, Brahms Diagnostics, Gilead sciences, Merck, Pfizer |
| Bloos [32] | Prospective observational | Suspected sepsis | ICU patients | 245 | VYOO, SIRS-Lab | Germany | Thuringian Ministry of Education; the Thuringian Foundation for Technology, Innovation, and Research; STIFT, the German Sepsis Society; SIRS-Lab GmbH supplied the VYOO Kits and personnel |
| Tran [28] | Prospective observational | Suspected sepsis | Trauma, emergency, and burn surgery patients | 76 | SeptiFast, Roche | US | Roche Diagnostics, National Institute of Biomedical Imaging and Bioengineering |
| Vincent [34] | Prospective observational | Suspected/proven sepsis | ICU patients | 529 | PCR/ESI-MS | Belgium, France, Germany, UK, Switzerland, Poland | Ibis Biosciences, Abbott |
| O’Dwyer [40] | Prospective observational | Suspected/proven sepsis | ICU patients | 439 | PCR/ESI-MS | Belgium, France, Germany, UK, Switzerland, Poland | Ibis Biosciences, Abbott |
| Muñoz [38] | Prospective observational | Suspected invasive candidiasis | In-hospital patients | 54 | T2MR | Spain | European Regional Development Fund, T2 Biosystems |
| Muñoz [39] | Prospective observational | Candidemia | In-hospital | 44 | T2MR | Spain | European Regional Development Fund, T2 Biosystems |
| Dierkes [30] | Retrospective, pre/postintervention | Septicemia | In-hospital patients | 77 post | SeptiFast, Roche | Germany | Not known |
| Alvarez [37] | Retrospective, pre/postintervention | Severe sepsis, Septic shock | ICU patients | 48 post, 54 pre | SeptiFast, Roche | Spain | Not known |
| Lehmann [29] | Retrospective | Suspected sepsis | ICU and emergency patients | 436 | SeptiFast, Roche | Germany, Spain, Italy | Supported in part by Roche Diagnostics |
| Tschiebel [24] | Retrospective | Systemic infection | Pediatric ICU patients | 75 | SeptiFast, Roche | Germany | Not known |
| Herne [31] | Retrospective | Proven sepsis | Septic shock, Severe infection | 144 | SeptiFast, Roche | Estonia | Not known |
| Bloos [33] | Retrospective | Candidemia | ICU patients | 874 | VYOO, SIRS Lab | Germany | Pfizer Pharma GmbH |
mortality was significantly lower when using SES [21]. Other differences in mortality were not significant. Interestingly, Lehmann et al. and Bloos et al. observed a higher mortality for PCR-positive patients compared with BC-positive patients [29, 32]. Additionally, the absence of *Candida* spp. DNA was found to be an independent predictor of survival [28], and a positive T2MR at baseline was an independent predictor of 7-day mortality [38].

Bhat et al. reported a significantly lower in-hospital LOS in their RCT using SES [21]. Alvarez et al. retrospectively found a significantly decreased in-hospital LOS and ICU LOS when using SeptiFast compared with BC, respectively [37]. Other tests did not result in significant differences in any LOS. In the study by Muñoz et al., the role of T2MR in predicting complicated candidemia, which was defined as an episode involving metastatic spread to other organs or with attributable mortality, was assessed. Patients with complicated candidemia, thus having a more severe disease, were more likely to have positive T2MR results. Furthermore, a positive T2MR result early in the disease episode was an independent predictor of complicated candidemia [39]. Our search did not reveal MDx studies reporting data on the clinical outcomes transfer to ICU, destination at discharge, or readmissions.

**Costs**

Four of 25 studies reporting cost calculations are presented in Table 3. Mostly, cost identification studies that compared total costs and spending during hospitalizations [23, 37] were performed, rather than cost-effectiveness analyses. Only Cambau et al. calculated cost-effectiveness, together with cost identification, when using SeptiFast. The costs for SeptiFast, BCs, other diagnostic procedures, and anti-infective treatments were identified per patient and were adjusted for their LOS. They did not find a difference in costs in the complete study population between the control group and intervention group, nor for severe sepsis patients. Furthermore, the cost-effectiveness analysis did not show a significant increase in effectiveness, due to an ICU LOS that was not affected by an earlier microbiological identification [22].

In 2 other studies, cost calculations included diagnostic procedures and anti-infective treatment per patient during hospitalization [23, 37]. In both studies, it was concluded that, despite the high costs of tests, MDx were cost saving because of lower costs of antibiotic treatment. Contrary to Cambau et al., Mancini et al. found significant savings for patients with sepsis when using MDx [23]. The retrospective study by Alvarez et al. calculated that there was a 96.3% probability of cost savings when using SeptiFast (321 EUR/analysis) [37].

**Molecular Diagnostics**

Details on the MDx that were used in the 25 eligible articles assessing clinical impact, including performance characteristics, are shown in Supplementary Table 1. Eight different MDx, of which 5 commercial MDx used whole blood directly as sample material and 3 techniques used short-term incubation, were reported. None of the articles reported on in-house assays. One MDx, SeptiFast (Roche, Mannheim, Germany), was evaluated in 14/25 (63.6%) of the included studies. The VYOO system (SIRS-Lab, Jena, Germany) and PCR followed by electrospray ionization–mass spectrometry (PCR/ESI-MS; Plex-ID, Ibis/Abbott Inc., Abbott Park, IL, USA) were each evaluated in 2/25 (9.1%) studies, respectively. The T2 *Candida* magnetic resonance assay (T2MR, T2 Biosystems, Lexington, MA, USA) was studied in 3/25 articles, and the Syndrome Evaluation System (SES, Xcyton Diagnostics, Bangalore, India) was assessed in 1/25 (4.5%) study. Three are CE-IVD marked tests. Only T2MR has Food and Drug Administration approval. Of note, the most recent publications on SeptiFast dated from 2017, and the test was discontinued after the end of 2019. Additionally, VYOO was bought by Analytik Jena in 2013 and is not marketed anymore. Last, Ibis Biosciences, Abbott, has discontinued the PCR/ESI-MS system.

Three techniques used short-term incubation; PCR + pyrosequencing after 8 hours of incubation, Molyss + PCR with 2.6-hour and 6.3-hour incubation, and the Enzyme Template Generation and Amplification (ETGA) test (Cognitor Minus,
Table 2. Data on Defined Antimicrobial Management Outcomes of Included Studies

| Author         | Antimicrobial Treatment Adjustments | Appropriate Treatment | Time to Treatment Adjustment, h | Time to Appropriate Treatment, h |
|----------------|-----------------------------------|-----------------------|-------------------------------|---------------------------------|
| Tafelski [20]  | Adjustments: 13.5% vs 9.8%<sup>a</sup> | -                     | 38.8 vs 18.8 (ns)<sup>b</sup> | -                               |
| Bhat [21]      | Total adjustments: 15 vs 165 ($P < .001$)<sup>b</sup>; 5.8 vs 2.6 ($P < .001$)<sup>b</sup> antibiotics/patient | -                     | -                             | -                               |
| Cambau [22]    | -                                 | 23.6% vs 33.6% ($P < .001$)<sup>b</sup> of patients | -                             | -                               |
| Lodes [25]     | Adjustment: 25 (16.9%)            | -                     | -                             | -                               |
| Mancini [23]   | 61.67% vs 62.5% ($P = .1$)        | -                     | -                             | -                               |
| Bloos [42]     | -                                 | -                     | -                             | -                               |
| Wallet [43]    | Adjusted: 3 patients based on SF  | -                     | -                             | -                               |
| Bravo [26]     | Inappropriate addition: 2 neutropenic cases | Appropriate adjustment: 5 neutropenic cases | -                             | -                               |
| Mauzon [27]    | -                                 | Adequate treatment: 4/62 | Earlier treatment: 4/32 BC+    | -                               |
| Mauzon [38]    | -                                 | Adequate duration: 1/32 | Immediate prescription: 3.2% (2.8%) | -                               |
| Mauzon [39]    | -                                 | Improved initial treatment: 11 (10%) | -                             | -                               |
| Tschiedel [24] | Adjustment: 8/27 would be triggered | -                     | 3 of 8 adjustments would have been made earlier | -                               |
| Lehmann [29]   | Adjustment suggested by PCR result: 46 episodes | -                     | 22.8 days on early adequate treatment per 100 tests | -                               |
| Tschiedel [24] | Adjustment: 18%, 9 positive and 5 negative cases | -                     | -                             | -                               |
| Herne [31]     | Adjustment: 38.9% of positive cases (74% de-escalation, 25.9% escalation) | -                     | -                             | -                               |
| Bloos [32]     | -                                 | Antifungal treatment without positive blood cultures or positive T2MR: 42.8% and 41.6% | -                             | 67.5 vs 31.0 ($P < .01$)<sup>a</sup> |
| Patch [35]     | -                                 | Antifungal treatment without positive blood cultures or positive T2MR: 42.8% and 41.6% | -                             | 34 h vs 6 h ($P = .0013$)<sup>b</sup> |
| McCann [44]    | -                                 | -                     | 5.7 h time difference between PCR result and positive signaling | -                               |
| Gebert [45]    | -                                 | -                     | -                             | -                               |
| Dryden [36]    | -                                 | Appropriate stewardship outcome: 73.6% | -                             | -                               |

Abbreviations: BC, blood culture; ICU, intensive care unit; ns, not significant; PCR, polymerase chain reaction.

<sup>a</sup>Control vs intervention.

<sup>b</sup>These studies did not evaluate the impact on antimicrobial therapy. They were included because they assessed the impact on clinical severity; results are shown in Table 3.
Table 3. Data on Defined Patient Outcomes (Hospital and ICU LOS, Mortality, and Costs) of Included Studies

| Author       | Hospital LOS, d | ICU LOS, d | Mortality                        | Costs                                           |
|--------------|-----------------|------------|----------------------------------|------------------------------------------------|
| Tafelski [20]| -               | -          | -                                | -                                              |
| Bhat [21]    | 19 vs 15 (P < .001)* | 11 vs 7 (P < .001)* | 17.8% vs 3.2% (P < .001)* in-hospital mortality | -                                              |
| Cambau [22]  | -               | -          | 15.8 vs 18.7% (P = .38) 7-d mortality | Total cost per patient: €20,995 vs €19,329 (P = .09)* |
| Lodes [25]   | -               | -          | 45/104 (43.2%)                  | -                                              |
| Mancini [23] | -               | -          | -                                | Total cost per patient: €2010.53 vs €1932.90 (P = .05)* |
| Bloos [42]   | -               | -          | In-hospital mortality:           | Price of antimicrobial therapy: €1384.87 vs €927.01 (P = .02)* |
| Wallet [43]  | -               | -          | -                                | -                                              |
| Bravo [26]   | -               | -          | -                                | -                                              |
| Mauton [27]  | -               | -          | -                                | -                                              |
| Bloos [32]   | -               | -          | -                                | -                                              |
| Tran [28]    | -               | -          | DNA absence is an independent predictor of survival (OR, 0.194; 95% CI, 0.046–0.840; P = .028) | -                                              |
| Vincent [34] | -               | -          | -                                | -                                              |
| O'Dwyer [40] | BC+ vs BC-: 27.8% | PCR+: 36% | PCR+ vs PCR+: 34.5%            | -                                              |
| Muñoz [38]   | -               | -          | T2MR positivity is an independent predictor of poor outcome (7-d mortality; OR, 26.4; 95% CI, 2.1–327.3; P = .01) | -                                              |
| Muñozb [39]  | -               | -          | -                                | -                                              |
| Dierkes [30] | -               | -          | -                                | -                                              |
| Álvarez [37] | 21.3 vs 18.3 (P < .05)* | 31.0 vs 22.9 (P < .05)* | 24% vs 29% 28-d mortality | Total cost per patient: €42,198 vs €32,228 (P < .05)* |
| Lehmann [29] | -               | -          | 30-d mortality: PCR+/BC-: 14.1% | Costs of antibiotics: €3576 vs €2812 (P < .05)* |
| Tschiedel [24]| -               | -          | -                                | -                                              |
| Herno [31]   | -               | -          | -                                | -                                              |
| Bloos [33]   | 27.5 vs 19 (P = .386)* | 17 vs 3 (P = .138)* ICU mortality | -                                              | -                                              |
| Patch [35]   | 20 vs 12 (P = .041)* | 4 vs 6 (P = .039)* | 9 vs 8 (P = .75) in-hospital mortality | -                                              |
| McCann [44]  | -               | -          | -                                | -                                              |
| Gebert [45]  | -               | -          | -                                | -                                              |
| Dryden [36]  | -               | -          | -                                | -                                              |

Abbreviations: BC, blood culture; ICU, intensive care unit; LOS, length of stay; PCR, polymerase chain reaction.
*Control vs intervention.
†This study did not evaluate any of these outcomes but evaluated the impact on the severity of the disease course.
Momentum Bioscience, Long Hanborough, UK) for the confirmation of negative samples after 12 hours of incubation.

**Supplementary Table 1** also shows 2 CE-IVD marked tests, SepsiTest-UMD (Molzym, Bremen, Germany) and the MagicPlex Sepsis Test (Seegene, Seoul, South-Korea), which were identified by our search but excluded because of lack of reported clinical outcomes.

**Risk of Bias**

Using the Cochrane risk of bias tool, the 3 intervention studies were assessed for bias at the study level. The risk of bias assessment is summarized in **Supplementary Table 2**. The study by Cambau et al. scored best (low level of bias), while the 2 other studies showed some concerns in missing outcome data. The overall level of bias was considered to be moderate with some concerns. The overall risk of bias in the included nonrandomized studies was moderate to serious. The assessment is shown in **Supplementary Table 3**. There was serious risk of bias for the studies assessing therapeutic impact, as most studies used a nonblinded method of chart review, which is a subjective method. In addition, no information on confounders was given in most studies, and no information was provided on missing data, which are both factors that are likely to occur in prospective observational studies. Lastly, bias in the selection of the reported results is mostly moderate, as no protocol reviews have been done, which is needed to reach the level of certitude needed for a low risk of bias assessment in this domain.

**DISCUSSION**

The most striking finding of this systematic review was the scarcity of reports describing the impact on patient outcomes and antimicrobial management of rapid MDx either directly on whole blood or after short-term incubation. Our search yielded only 25 eligible articles on 8 commercially available tests, and no in-house test with data on clinical impact was identified. The heterogeneity of these 25 articles was too large and the number was too small to perform a meta-analysis.

The use of MDx could potentially result in better antimicrobial management before BC results become available, and thus in higher rates of appropriate antimicrobial therapy. However, in the few studies describing antimicrobial management, no uniform terminology was used, making comparisons very difficult. An earlier change in antimicrobial therapy has additional benefits for patients. However, time to change of antimicrobial therapy was only evaluated in 3 studies, of which only 1 found a significant reduction of inadequate antibiotic therapy [20, 29, 33]. Additionally, a faster detection of the causative agent of sepsis, but more importantly its susceptibility, could lead to an earlier de-escalation from broad-spectrum to narrow-spectrum, thereby reducing the selective pressure for antibiotic resistance.

The only patient outcomes studied for the use of MDx were LOS, ICU LOS, and mortality. Furthermore, few studies found improved patient outcomes, while others did not find a significant improvement or did not study these outcomes at all. Two studies found a significantly decreased hospital LOS and ICU LOS with the use of MDx. Again, a comparison between the different methods and studies is difficult, as different definitions for LOS were used. Additionally, conflicting results were reported on mortality. Interestingly, fungal DNA absence, determined via PCR, was found to be an independent predictor for survival, and a positive PCR result was found to be an independent predictor for mortality, which could be valuable in the fast identification of the most critical patients [28, 40]. Only 1 study evaluated disease severity [38]. Other important patient outcomes such as ICU transfer or 30-day readmission were not evaluated. Other outcomes relevant to the patient and society, such as destination upon discharge and return to former trajectory, need to be explored further. Studies show that inadequate antibiotic treatment is associated with higher mortality [41]. However, the lack ofRCTs evaluating antibiotic therapy changes makes the added value of these MDx unknown at the patient level.

Lastly, MDx seemed to have an impact on cost. Even with expensive tests, 4 studies showed a significant decrease in costs. Of note: This decrease is not related to the test itself but mostly to reduced antimicrobial therapy. Although no cost-effectiveness studies were included here, 3 model calculations were identified in the search but were excluded as they were beyond the scope of our review.

All of the above findings, and especially the large gap of knowledge, could have led to a reluctance of physicians and clinical microbiologists to implement MDx starting directly from whole blood in clinical practice. These tests are reported to be expensive, and the research and production as well, which may have led to the discontinuation of 3 out of the 5 systems.

This review on rapid MDx is, to our knowledge, the first to report on the added clinical value of MDx either directly from blood or after short-term incubation. The strengths of this analysis were the evaluation of outcomes that affect the individual patient directly (mortality and LOS) and indirectly (antimicrobial management). These outcomes also have an impact on society. However, this review has several limitations. We only searched 1 database and could only include 25 studies in this review. Only 3 of those were intervention studies. Most studies retrospectively evaluated hypothetical changes in antibiotic therapy and were not designed to find statistically significant differences. Even among this limited number of studies, the observed heterogeneity of studies was high. Studies used different terms, outcome measures, and analyses and reported their findings differently. This hampers comparisons and general conclusions. Furthermore, the target population, (suspected) BSI or sepsis, is also heterogeneous, making comparison between studies even more challenging.
In conclusion, data on the clinical evaluation of rapid MDx in sepsis are limited. Only a handful of studies showed clear benefits in antimicrobial therapy management and patient outcomes. Commercially available MDx on whole blood have important shortcomings, such as low sensitivity, limited antibiotic resistance detection, and high cost. These are all probable reasons for discontinuation by companies. MDx combined with cultivation, for example, short-term incubation, seem more performant. In the future, more robust intervention studies should be performed on newly developed MDx, focusing on the added value of MDx in clinical practice and the possible benefits for critically ill patients.

**Supplementary Data**

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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