Systematic review of host genomic biomarkers of invasive bacterial disease: Distinguishing bacterial from non-bacterial causes of acute febrile illness

Eimear Kelly, a* Seán Olann Whelan, b Eli Harriss, c Sarah Murphy, d Andrew J. Pollard, a and Daniel O’Connor a

a Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, UK; NIHR Oxford Biomedical Research Centre, Oxford, UK
b Department of Clinical Microbiology, Galway University Hospital, Galway, Ireland
c Bodleian Health Care Libraries, University of Oxford
d Department of Paediatrics, Cork University Maternity Hospital, Wilton, Cork, Ireland

Summary

Background Infectious diseases play a significant role in the global burden of disease. The gold standard for the diagnosis of bacterial infection, bacterial culture, can lead to diagnostic delays and inappropriate antibiotic use. The advent of high-throughput technologies has led to the discovery of host-based genomic biomarkers of infection, capable of differentiating bacterial from other causes of infection, but few have achieved validation for use in a clinical setting.

Methods A systematic review was performed. PubMed/Ovid Medline, Ovid Embase and Scopus databases were searched for relevant studies from inception up to 30/03/2022 with forward and backward citation searching of key references. Studies assessing the diagnostic performance of human host genomic biomarkers of bacterial infection were included. Study selection and assessment of quality were conducted by two independent reviewers. A meta-analysis was undertaken using a diagnostic random-effects model. The review was registered with PROSPERO (ID: CRD42021208462).

Findings Seventy-two studies evaluating the performance of 116 biomarkers in 16,216 patients were included. Forty-six studies examined TB-specific biomarker performance and twenty-four studies assessed biomarker performance in a paediatric population. The results of pooled sensitivity, specificity, negative and positive likelihood ratio, and diagnostic odds ratio of genomic biomarkers of bacterial infection were 0.80 (95% CI 0.78 to 0.82), 0.86 (95% CI 0.84 to 0.88), 0.18 (95% CI 0.16 to 0.21), 5.5 (95% CI 4.9 to 6.3), 30.1 (95% CI 24 to 37), respectively. Significant between-study heterogeneity (I² 77%) was present.

Interpretation Host derived genomic biomarkers show significant potential for clinical use as diagnostic tests of bacterial infection however, further validation and attention to test platform is warranted before clinical implementation can be achieved.

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Introduction

Infectious diseases account for a large proportion of the global burden of disease.1 Infants, children and the elderly experience the highest burden of disease and are especially susceptible to serious bacterial infection (SBI).2 Worldwide, infectious diseases remain the leading cause of death in children under five.3 Improvements in the prevention and treatment of infectious disease in children is a priority for global health. The development of more efficient and accurate diagnostics may play a vital role in this global health initiative.1,4

The gold standard for diagnosis of bacterial infection remains bacterial culture from a normally sterile site but may require several days to achieve a result. In ill-appearing infants and children with suspected SBI, current practice is to initiate antimicrobial therapy while
Research in context

Evidence before this study

Infectious diseases contribute significantly to the global burden of diseases and worldwide, remain the leading cause of death in children under five. Although infants and children are at increased risk of invasive bacterial infection, most infections in children are attributable to self-limited viral infections. The gold standard of diagnosis of bacterial infection remains culture of bacteria from a normally sterile site, which can lead to diagnostic delays, unnecessary antibiotic use, and prolonged hospitalisation. In resource-limited settings especially, the paucity of inexpensive, reliable, rapid of point-of-care (POC) diagnostic tests of bacterial infection frequently leads to empiric antimicrobial use, contributing to the global crisis of antimicrobial resistance. Host genomic biomarkers, reflective of a specific host immune response to infection, offer the potential to differentiate bacterial from non-bacterial causes of infection and febrile illness. To date, most of these biomarkers remain restricted to laboratory-based research and have yet to achieve validation for use in a clinical setting.

Added value of this study

In this systematic review and meta-analysis of host genomic biomarkers of bacterial infection, we show that these novel biomarkers demonstrate comparable and often superior diagnostic performance to routinely used biomarkers in clinical practice with pooled sensitivity of 0.80 (95% CI 0.78-0.82) and specificity of 0.86 (95% CI 0.84-0.88). However, a high degree of study heterogeneity was present (I² 77%) and several significant sources of potential bias identified on the assessment of study quality.

Implications of all the available evidence

Genomic biomarkers show considerable promise for clinical application as diagnostic tests of bacterial infection and for development into POC diagnostic tests. However, most are still in an early stage of development and require further validation before clinical use can be considered. Most genomic biomarkers also use testing platforms confined to laboratory use and will require translation into inexpensive, accessible POC tests suitable for use by non-specialists before they can be implemented in routine clinical practice.

This can lead to diagnostic delays, unnecessary antibiotic use (viral infection being most often causative in febrile children), and prolonged hospitalization, contributing a financial burden to health services.1-5 Many causes of infection and febrile illness are clinically indistinguishable from each other, contributing to diagnostic uncertainty.10 Moreover, concurrent viral and bacterial infection is a well-recognized phenomenon.11 Rapid molecular diagnostic tests capable of differentiating bacterial from viral infection, frequently identify those viruses shown to reside in the nasopharynx of healthy children and thus, are unable to eliminate the possibility of bacterial infection nor provide guidance regarding the need for antimicrobial therapy in the febrile unwell child.12 Moreover, these expensive tests are not widely available and may not be feasible for use in resource limited settings.13 Direct detection of pathogen deoxyribonucleic acid (DNA) in blood using real-time polymerase chain reaction (PCR) has provided an alternative to culture and though capable of delivering results more rapidly, its inadequate sensitivity in some settings limits its potential for the diagnosis of bloodstream infections.14 Pathogen detection does not always infer causation and bacterial colonization in healthy children is a well-recognized phenomenon. In resource poor settings especially, there may be limited capacity for even conventional laboratory-based diagnostic testing and this, in addition to the present paucity of inexpensive, and accurate, rapid point-of-Care (POC) diagnostic tools, is contributing to a rising crisis in global antimicrobial resistance.15,16

There is a clear need for rapid POC tests capable of detecting and differentiating bacterial from other causes of infection. Host-pathogen interaction has already been shown to elicit a reproducible immune response at a genomic level.17,18 The induction of this ‘host gene pattern’ (“RNA biosignatures”) in response to infection has shown significant promise as a novel diagnostic tool.19,20 More recently, research has focussed on translating these RNA biosignatures into a platform capable of performance as an affordable and easily accessible POC test.21 Indeed, many of these biomarkers have been shown to outperform those routinely used in clinical practice.22

To date however, most host genomic biomarkers have been restricted to laboratory-based research and few have achieved validation for use in a clinical setting. The aim of this review is to evaluate the current state and diagnostic performance of genomic biomarkers in differentiating bacterial from other causes of infection and to explore their potential future clinical application.

Methods

Search strategy and selection criteria

A systematic review and meta-analysis of genomic biomarkers capable of differentiating bacterial from non-

 awaits the results of culture of blood, urine, cerebrospinal fluid (CSF).7 However, many flaws exist to this practice; antibiotic administration prior to sampling, risk of specimen contamination with skin flora if an incorrect sampling technique is used, and the nidus of infection may reside at an inaccessible site. Culture results are often, therefore, unreliable or difficult to interpret.7 Moreover, blood volume attainment is often small leading to the risk of false negative results and associated inadequate sensitivity has been reported.7

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Methods

Search strategy and selection criteria

A systematic review and meta-analysis of genomic biomarkers capable of differentiating bacterial from non-
bacterial sources of infection was conducted according to the ‘preferred reporting items for systematic reviews and meta-analyses (PRISMA)’ statement.\(^{23}\) PubMed/Ovid Medline, Ovid Embase and Scopus databases were searched from inception up to 30/03/2022. The search strategies applied both the SIGN diagnostics search filter\(^{24}\) and the search filter for identifying paediatric papers by Leclercq et al.\(^{53}\) to text words and relevant index terms to retrieve studies relating to host genomic biomarkers capable of differentiating bacterial from non-bacterial causes of infection (see Supplement for full search strategy). There were no limits applied to the search results. A forward and backward citation search was conducted for all key references. The review was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration ID: CRD42021208462.

Studies which compared the diagnostic performance of human host genomic biomarkers of bacterial infection to those with non-bacterial sources of infection were included. There were no age restrictions, nor any restrictions applied to study design type eligible for inclusion. Studies were restricted to those that examined the performance of human host genomic biomarkers. Non-bacterial sources of infection included fungal, viral, parasitic and protozoan infections. Systemic inflammatory conditions were also included as a comparator group as their presentation may be similar to that of bacterial infection. Excluded studies included those involving animals or if insufficient information provided for analysis. Database search outputs were screened independently by two reviewers (EK and SW). Publications were initially screened by title and abstract and thereafter by full text. Decisions regarding study inclusion and exclusion were made independently and any discrepancies resolved through discussion. If consensus could not be reached, adjudication was provided by a third reviewer (DOC).

### Data extraction and management

Data extracted included information relating to study population, study groups, sample size, study design, test specimen, biomarker, and biomarker discovery. Biomarker performance metrics were collated: sensitivity (Sen), specificity (Spec), true positives (TP), true negatives (TN), false positives (FP), false negatives (FN), area under the curve (AUC) values. Missing data were requested from authors by email. Author and year of publication were documented. Quantitative data relating to biomarker performance was entered into an Excel spreadsheet. Descriptive data was documented using a Microsoft word template.

### Assessment of methodological quality of study

The quality of the included studies and risk of bias were independently assessed by two reviewers (EK and SM) using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) assessment tool.\(^{26}\) Adjudication was provided by a third reviewer (SW) if agreement could not be reached. As recommended by QUADAS-2 guidelines, each of the four key domains (patient selection, index test, reference standard, flow and timing) were classified as “high”, “low” or “unclear” depending on the information available in each paper and the signalling questions provided to assist judgements regarding risk of bias.\(^{26}\) If insufficient information was available for any domain, the risk was classified as “unclear”. If all responses to signalling questions in a domain were answered in the affirmative, the risk of bias was deemed “low”. However, if more than one question elicited a “no” response then that domain was flagged as “high” risk.

### Data synthesis and statistical analysis

Data were extracted to form 2 \(\times\) 2 contingency tables of reference versus index test results. The performance of the diagnostic index test was assessed using Sen, Spec, positive or negative likelihood ratios (PLR or NLR), diagnostic odds ratio (DOR), summary receiver operating characteristic curve (ROC) and AUC measured with 95% confidence interval (95% CI). A meta-analysis was performed using a diagnostic random-effects model to estimate summary diagnostic performance: forest plots of pooled Sen and Spec, pooled DOR and NLR. A hierarchical summary receiver operating curve (HSROC) was generated to account for the variance in threshold effect in each study. The shape of the ROC curve was used to determine the discriminatory ability of the diagnostic test. The closer the curve to the upper-left corner and the larger the AUC, the better the ability of the test to discriminate between bacterial and other causes of infection. Heterogeneity was explored using meta-regression models and a subgroup analysis was performed to investigate the effect of paediatric-related studies and those which related to TB-specific biomarkers. The meta-analysis (including forest plots of Sen, Spec, DOR, NLR, SROC and heterogeneity assessments) were created using OpenMeta-Analyst.\(^{27}\) All other analyses were carried out using RevMan version 5.4.\(^{28}\)

### Results

A total of 8788 studies were identified following a search of three electronic databases. Following the removal of 1264 duplicate results, 7524 studies were screened using title and abstract. One hundred and eighty-four articles were identified for full text review, of which 38 met the criteria for inclusion in the review. Sufficient data for quantitative analysis were available for 31 of these studies. Studies were excluded if the control or comparator group contained patients with...
bacterial infection or if the biomarker was discovered and validated using only public gene expression repositories or retained databases as an additional potential source of bias. Forward and backward citation search of key references revealed a further 68 studies eligible for inclusion, of which 41 were included in the final review. A total of 72 studies was finally included in the review (Figure 1).

Of the 72 studies included in the review, most were published between 2013 and 2022 (69/72; 96%). Most studies were conducted using adult patients (49/72; 68%) and performed outside of Europe (61/72; 88%). Except for five studies, a case control design was used, and most did not attempt to validate the biomarker(s) discovered (41/72; 57%). TB-specific biomarkers were the focus of many of the studies (46/68; 68%).

Tables 1a and 1b. The performance of 116 biomarkers in 16,216 patients was evaluated. Biomarker sensitivity ranged from 0.21 (95% CI 0.1 to 0.39) to 0.98 (95% CI 0.74 to 0.99). The pooled sensitivity was 0.80 (95% CI 0.78-0.82), p < 0.001; the I² value was 75%. Biomarker specificity varied between 0.38 (95% CI 0.26-0.52) to 0.99 (95% CI 0.83-1.0). The pooled specificity was 0.86 (95% CI 0.84-0.88) with significant between-study heterogeneity, I² value 77%, p < 0.001. Pooled NLR, PLR, and DOR results were 0.18 (95% CI 0.16-0.21), I² 84%; 5.5 (95% CI 4.9-6.3), I² 79%, and 30.1 (95% CI 24-37), I² 77%, respectively (all p-values < 0.001) (Figures 2 and 3). To account for the threshold variability of the included studies, a HSROC was used to summarise biomarker diagnostic performance. (Figure 5). A diagnostic random-effects model was used for all analyses.

Heterogeneity was significant and was investigated using meta-regression models and subgroup analyses. In the meta-regression analysis, the effect of study population on sensitivity, specificity and DOR was significant; p value < 0.001, respectively. The effect of TB-related biomarker studies on overall diagnostic performance values was statistically significant; p-values for sensitivity, specificity and DOR: < 0.001, < 0.001, and < 0.001, respectively. Heterogeneity was further explored using subgroup analysis. There was no clear difference in biomarker performance between paediatric and adult study populations, with similar sensitivity, specificity, NLR, PLR, and DOR values observed in both populations on subgroup analysis (Table 3). Performance metrics were also similar between TB and non-TB biomarkers: sensitivity 0.78 (95%CI 0.76-0.81) vs 0.85 (95%CI 0.81-0.89), specificity 0.85 (95%CI 0.83-0.87) vs 0.88 (0.85-0.91), respectively. The DOR for the non-TB biomarkers was 53.39 (95%CI 31.94-89.23) compared to 25.02 (95%CI 19.74-31.73) for studies of TB-related biomarkers. Heterogeneity remained significant on subgroup analysis (Table 3).

Regarding test platform, PCR-based techniques (43/72; 60%), followed by microarray (26/72; 36%), and RNA-sequencing (11/72; 15%) were most often used. Dual colour multiplex ligation-dependent probe amplification (dcRT-MLPA) was used in three studies and NanoString Technologies (gene expression panel) in two. Further validation with qRT-PCR following Microarray or RNA-sequencing occurred in 13 studies. Except for two studies, blood was used for testing purposes. Two studies evaluated biomarker performance using CSF (Table 2).

An appraisal of the methodological quality and assessment of the risk of bias was performed using the QUADAS-2 tool (Figure 4a, b)). Regarding patient selection, 32% of studies were deemed high risk, most often as a result of the use of inappropriate exclusion criteria or a case control study design. Most of the control groups consisted of healthy volunteers, and those with immunodeficiency were frequently ineligible to participate. Insufficient information pertaining to the patient selection process occurred in 33% of cases. The highest level of risk occurred in the index test domain with 86% of studies deemed to be ‘high risk’. The index test results were frequently interpreted with prior knowledge of the results of the reference standard and a prespecified threshold was rarely established. Most studies (88%) employed an appropriate reference standard, the results of which were often interpreted without knowledge of the index test results. No concern regarding applicability occurred for most domains and there were minimal concerns regarding flow and timing (76%).

Discussion

The global burden of infectious diseases and rising antimicrobial resistance necessitate the development of improved diagnostic tools to ameliorate treatment strategies in healthcare and rationalise the use of antibiotics. In this review, we found genomic biomarkers demonstrated comparable (and in some cases superior) performance to biomarkers routinely used in clinical practice in their ability to differentiate bacterial from other causes of infection and febrile illness. Disappointingly, many of these newly discovered biomarkers are still in an early stage of development and have not yet been validated in independent cohorts. Furthermore, many of the included studies were found to be at significant risk of bias, and most biomarkers were still reliant on expensive testing platforms confined to laboratory use.

In terms of individual biomarker performance, wide-ranging sensitivity and specificity values were present (Figures 2 and 3). When combined however, the results of pooled sensitivity and specificity compared favourably with those of more established biomarkers of bacterial infection (C-reactive protein, procalcitonin). Indeed, many genomic biomarkers demonstrated impressive diagnostic performances when compared to conventional diagnostic biomarkers such as the 2-transcript...
signature (FAM89A and IFI44L genes) of Gomez-Carballa et al.37 capable of differentiating bacterial from viral infection in febrile children with 90.9% (95% CI 72.7% -100%) sensitivity and 85.7% (95% CI 64.3% - 100%) specificity. The diagnostic capabilities of the 2-transcript signature were further demonstrated by Herberg et al.18 [sensitivity 100%, (95% CI, 100%-100%), specificity 96.4%, (95% CI, 89.3%-100%)]. Similarly impressive results were also illustrated for other biomarkers included in this review.45-49 This was
| Article (reference) | Study Design | Study setting | Age range | Biomarker | Measure of effect | Test platform | Control group |
|---------------------|--------------|---------------|------------|-----------|------------------|--------------|---------------|
| Herberg 2016<sup>16</sup> | Cross-sectional | Hospitals in United Kingdom, Spain, Netherlands, United States | Febrile children $<17$ yrs | 2-transcript RNA signature (FAM89A, IFI44L) (DRS) | Sens 1.0 (95% CI 0.85-1.0) Spec 0.96 (95% CI 0.82-1.0) | Microarray | N/A. Population also included healthy children |
| El-Hefnawy (2021)<sup>34</sup> | Case control | Paediatric Department, Faculty of Medicine, Menoufi University, Egypt | Neonates; 1-3 days | miRNA-16a | Sens 0.88 (95% CI 0.69-0.97) Spec 0.98 (0.80-1.0) | RT-PCR | Healthy newborns |
| Fouda (2021)<sup>30</sup> | Case control | NICU of Menoufi University Hospital, Egypt | Neonate; 1-4 days | miRNA 15b miRNA 378a | Sens 0.76 (95% CI 0.55-0.91) Spec 0.88 (95% CI 0.69-0.97) Sens 0.60 (95% CI 0.39-0.79) Spec 0.88 (95% CI 0.69-0.97) | RT-PCR | Healthy newborns |
| Tian (2021)<sup>31</sup> | Case control | Shenzhen Children’s Hospital and Beijing Children’s Hospital | Children $<14$ years | FAM89A and IFI44L (DRS) | Sens 0.78 (95% CI 0.61-0.89) Spec 0.77 (95% CI 0.63-0.86) | RT-PCR | Children with viral infection |
| Barral-Arca 2018<sup>13</sup> | Cross-sectional | Mexican children (GEO database) | Children $<10$ years | 2-transcript RNA signature (FAM89A, IFI44L) (DRS) | Sens 0.68 (95% CI 0.59-0.76) Spec 0.85 (95% CI 0.72-0.93) | RNA-seq | N/A. Population also included children with viral infection |
| Berner 2000<sup>13</sup> | Case control | University Children’s Hospital and the University Hospital of Obstetrics and Gynaecology in Freiburg, Germany | Neonates | IL-8 mRNA | Sens 0.89 (95% CI 0.52-1.0) Spec 0.95 (95% CI 0.77-1.0) | RT-PCR | Healthy neonates |
| Cernada 2014<sup>14</sup> | Case control | University and Polytechnic Hospital La Fe | VLBW infants (birth weight $<1500$g) | GWEP | Sens 1.0 (95% CI 0.80-1.0) Spec 0.68 (95% CI 0.43-0.87) | RT-PCR | Healthy neonates |
| Ge 2013<sup>13</sup> | Case control | China | Infants $<12$ months old | 5 miRNA profile (miR-202, miR-342-5p, miR-206, miR-487b, miR-576-5p) | Sens 0.97 (95% CI 0.89-1.0) Spec 0.94 (95% CI 0.86-0.98) | Microarray, qRT-PCR (further evaluation)<sup>13</sup> | Healthy children |
| Article (reference) | Study Design | Study setting | Age range | Biomarker | Measure of effect | Test platform | Control group |
|---------------------|--------------|---------------|-----------|-----------|------------------|---------------|---------------|
| Gjoen 2017<sup>16</sup> | Cross-sectional | Tertiary hospital, Delhi, Palamaner Taluk, India | Children 6 months to 15 years | 10-transcript signature: IFNG, NLRP1, NLRP3, TGFBR2, TAGAP, NOX2, GBP5, IFITM1/3, KIF1B and TNIP | Sens 0.92 (95%CI 0.73-0.99) Spec 0.88 (95%CI 0.70-0.98) | dcRT-MLPA | N/A |
| Gomez-Carballa 2019<sup>11</sup> | Case control | Hospital Clinico Universitario from Santiago de Compostela (Spain) | Children 1 to 10 years | FAM89A and IFI44L genes | Sens 0.93 (95%CI 0.66-1.0) Spec 0.82 (95%CI 0.48-0.98) | RT-qPCR | Children with viral infection and healthy children |
| Kaforou 2017<sup>10</sup> | Cross-sectional | US emergency departments | Infants <60 days old | 2-transcript RNA signature (FAM89A, IFI44L) (DRS) | Sens 0.89 (95%CI 0.80-0.94) Spec 0.94 (95%CI 0.87-0.97) | Microarray | N/A |
| Liu, G 2020<sup>9</sup> | Case control | Yidu Central Hospital of Weifang China | Neonates | Microrna (miR)-181a | Sens 0.83 (95%CI 0.75-0.90) Spec 0.84 (95%CI 0.71-0.93) | RT-qPCR | Healthy neonates |
| Mahajan 2016<sup>11</sup> | Cross-sectional | Emergency Departments in PECARN | Infants <60 days old | 66 classifier genes | Sens 0.87 (95%CI 0.73-0.95) Spec 0.89 (95%CI 0.81-0.95) | Microarray | N/A |
| Pan 2017<sup>10</sup> | Case control | Third Affiliated Hospital of Zhengzhou University, the First Affiliated Hospital of Zhengzhou University and Children’s Hospital of Zhengzhou | Children 1 to 8 years old | MiR-29a | Sens 0.67 (95%CI 0.58-0.75) Spec 0.89 (95%CI 0.83-0.94) | RT-qPCR | Healthy children |
| Ng 2019<sup>9</sup> | Case control | University affiliated tertiary neonatal centre | Preterm infants 28-32 weeks (GA) | miR-1290 | Sens 0.83 (95%CI 0.67-0.94) Spec 0.92 (95%CI 0.88-0.95) | Microarray, RT-qPCR (further analysis)<sup>1</sup> | Healthy preterm neonates 28-32 weeks (GA) |
| Smith 2014<sup>42</sup> | Case control | Neonatal Unit, Royal Infirmary of Edinburgh, and the Division of Pathway Medicine, University of Edinburgh | Preterm and term neonates (23-41 weeks GA) | 52-gene-classifier | Sens 1.0 (95%CI 0.79-1.0) Spec 1.0 (95%CI 0.69-1.0) | Microarray | Healthy preterm and term neonates (24-44 weeks GA) |

Table 1a (Continued)
| Article (reference) | Study Design | Study setting | Age range | Biomarker | Measure of effect | Test platform | Control group |
|---------------------|--------------|---------------|-----------|-----------|------------------|---------------|--------------|
| Tornheim 2020 | Case control | Byramjee Jeejeebhoy Government Medical College, tertiary hospital in Pune, India | Children <15 years old | TB Risk Signature | Sens 0.63 (95%CI 0.35-0.85) Spec 0.78 (95%CI 0.60-0.91) | RNA-seq | Healthy children |
| Verhagen 2013 | Case control | Venezuela (Warao Amerindian population; GEO) | Children 1 to 15 years old | 5-gene signature (S100P, HBD, PIGC, CHRM2 and ACOT7) | Sens 0.78 (95%CI 0.40-0.97) Spec 0.96 (95%CI 0.88-0.99) | Microarray | Healthy children |
| Wang 2015 | Case control | Department of Infectious Diseases in People’s Hospital of Laiwu City, Shandong Province | Range not provided; 21 < 3 and 44 > 3 years old | microRNA-31 | Sens 0.98 (95%CI 0.92-1.0) Spec 0.87 (95%CI 0.75-0.94) | RT-PCR | Healthy children |
| Zhou 2016 | Case control | Children's Hospital of Chongqing Medical University, China | Children 4 to 10 years old | 8 mRNA (miR-1, miR-10a, miR-125b, miR-146a, miR-150, miR-155 and miR-31, miR-29) | Sens 0.96 (95%CI 0.80-1.0) Spec 1.0 (95%CI 0.84-1.0) | Microarray, RT-qPCR (validation)* | Healthy children |
| Salim 2020 | Case control | NICU, Paediatric Dept. | Neonates (term) <2 weeks old | miR-187, miR-101 | Sens 0.84 (95%CI 0.71-0.93) Spec 0.83 (95%CI 0.65-0.94) | RT-qPCR | Healthy neonates |
| Kathirvel 2020 | Case control | Tertiary hospital JIPMER, Puducherry | Children <14 years old | miR-31 | Sens 0.90 (95%CI 0.73-0.98) Spec 0.90 (0.73-0.98) | RT-qPCR | Healthy children |
| Pennisi 2021 | Case control | Hospitals in United Kingdom, Spain, Netherlands, United States | Children <17 years old | 2-transcript signature (IFI44L and EMR1-ADGRE1) | Sens 1.0 (95%CI 0.74-1.0) Spec 1.0 (95%CI 0.74-1.0) | Electronic RT-LAMP (RT-eLAMP) | Viral infection |

Table 1A: Summary characteristics of included studies which explored genomic biomarker performance using a paediatric population. An outline of the study setting and design, biomarker, biomarker performance and platform used in each of the included studies has been provided. Further information available in the Supplement.

Sens: sensitivity; Spec: specificity; 95%CI: 95% Confidence Interval; NICU: Neonatal Intensive Care Unit; DRS: Disease Risk Score; RT-qPCR: Quantitative reverse transcription PCR; dcRT-MLPA: Dual colour multiplex ligation-dependent probe amplification; VLBW: Very Low Birth Weight; PECARN: Paediatric Emergency Care Applied Research Network; GA: gestational age.

* Analytical validation/further evaluation: assessment of biomarker performance using routinely available clinical laboratory tools.
| Article (reference) | Study Design | Study setting | Age range | Biomarker | Measure of effect | Test platform | Control group |
|----------------------|-------------|---------------|-----------|-----------|------------------|---------------|---------------|
| Barry 2018<sup>10</sup> | Case control | Ningxia Hui Autonomous region in north-western China | 18 to 91 years old | 5-miRNA signature: miRs – 29a, –99b, –21, –146a, –652 | Sens 0.94 (95%CI 0.87-0.98) Spec 0.88 (95%CI 0.80-0.94) | qRT-PCR | Healthy adults |
| Mahle (2021)<sup>11</sup> | Case control | Emergency Departments of Duke University Medical Center, Durham VA Health Care System, UNC Health Care, and Henry Ford Hospital | 14 to 94 years old | 81-gene signature | Sens 0.80 (95%CI 0.72-0.88) Spec 0.80 (95%CI 0.74-0.86) | RT-PCR | Viral infection and non-infectious illness |
| Mendelsohn (2021)<sup>12</sup> | Cross sectional | Five communities in South Africa with a high TB burden | 28 to 42 years | RISK11 signature | Sens 0.88 (95%CI 0.58-1.0) Spec 0.66 (95%CI 0.63-0.69) | RT-PCR | N/A |
| Xu (2021)<sup>13</sup> | Case control | Four hospitals in Shandong province, China | 17 to 85 years | 2-transcript biomarker (IFI44L and PI3 transcripts) | Sens 0.86 (95%CI 0.71-0.94) Spec 0.95 (95%CI 0.85-0.99) | RT-PCR | Viral infection; SLE |
| Francisco 2017<sup>14</sup> | Case control | China | 18 to 84 years | GBP5,DUSP3,KLF2 | Sens 0.76 (95%CI 0.71-0.81) Spec 0.86 (95%CI 0.81-0.90) | RT-PCR | Healthy adults |
| Pan 2019<sup>15</sup> | Case control | Beijing Chest Hospital, Beijing Chao-yang Hospital, Beijing Tiantan Hospital, Beijing Ditan Hospital, Xuanwu Hospital and People’s Liberation Army 263 hospital | 18 to 80 years | 4 miRNA panel (miR-126-3p, miR-130a-3p, miR-151a-3p, and miR-199a-5p) | Sens 0.82 (95%CI 0.48-0.98) Spec 0.90 (0.55-1.0) | Microarray | Viral meningitis |
| Penn-Nicholson 2020<sup>16</sup> | Cross sectional | Worcester region of the Western Cape, South Africa | > 18 years old | RISK6 transcriptomic signature (GBP2, FCGR1B, SERPING1, TUBGCP6,TRMT2A, and SDR39U1) | Sens 0.92 (95%CI 0.80-0.98) Spec 0.74 (95%CI 0.60-0.86) | RT-qPCR | N/A |
| Cui 2017<sup>17</sup> | Case control | Harbin Chest Hospital (Harbin, China) | 25 to 56 years (Mean age 43 years) | Risk Score Analysis (3-miRNA signature) | Sens 0.79 (95%CI 0.68-0.88) Spec 0.86 (95%CI 0.71-0.95) | RNA seq, RT-qPCR (validation)<sup>28</sup> | Healthy adults |

Table 1b (Continued)
| Article (reference) | Study Design | Study setting | Age range | Biomarker | Measure of effect | Test platform | Control group |
|---------------------|--------------|---------------|-----------|-----------|------------------|---------------|---------------|
| Warsinske 2018<sup>57</sup> | Case control | Estabelecimento Penal Jair Ferreira de Carvalho, Dourados. State Prison in Campo Grande, Brazil | Age > 30 | TB Risk Score (DUSP3, GBP5, KLF2) | Sens 0.91 (95%CI 0.76-0.98) Spec 0.69 (95%CI 0.54-0.81) | Microarray, RNA seq, RT-qPCR | Healthy adults |
| Berry 2010<sup>58</sup> | Case control | St. Mary’s Hospital and Hammersmith Hospital, London, Hillingdon Hospital, Uxbridge, UK. Ubuntu TB/HIV clinic Khayelitsha, Cape Town, South Africa. | Age > 18 years | 86-gene signature | Sens 0.90 (95%CI 0.68-0.99) Spec 0.83 (95%CI 0.77-0.88) | Microarray | Healthy adults |
| Kelly 2018<sup>59</sup> | Case control | Brigham and Women’s Hospital emergency department | 40-65 years | 3-predictor gene expression model (RAD18, MAPKAPK3, JAG1) | Sens 1.0 (95%CI 0.59-1.0) Spec 0.86 (95%CI 0.65-0.97) | RNA seq | Healthy adults |
| Gliddon 2021<sup>60</sup> | Case control | Study sites in Cape Town, South Africa and Karonga District, Malawi | 25-68 years | FS-PLS signature for TB/OD (4-transcript signature) (GBP6, TMCC1, PRDM1, and ARG1) | Sens 0.95 (95%CI 0.75-1.0) Spec 0.85 (95%CI 0.62-0.97) | Microarray, RT-dPCR | Non-infectious illness |
| Abd-El-Fattah 2013<sup>61</sup> | Case control | Chest department, Al-Kasr Al-Eni Hospital, Faculty of Medicine, Cairo University, Egypt | 30-65 years | miR-155, miR-197 | Sens 1.0 (95%CI 0.86-1.0) Spec 1.0 (0.91-1.0) | Microarray, RT-qPCR | Healthy adults |
| Bloom 2013<sup>62</sup> | Case control | Royal Free Hospital NHS Foundation Trust, London. | > 18 years | 144-transcript signature | Sens 0.88 (95%CI 0.47-1.0) Spec 0.91 (95%CI 0.76-0.98) | Microarray | Healthy adults and sarcoidosis |
| Darboe 2019<sup>63</sup> | Case control | eThekwini clinic in Durban, KwaZulu-Natal, South Africa | 25-53 years | 11-gene ACS COR signature | Sens 0.60 (95%CI 0.44-0.75) Spec 0.75 (95%CI 0.64-0.83) | Microarray | TB-free controls |
| de Araujo 2016<sup>64</sup> | Case control | Rio de Janeiro state, Brazil | 25-55 years | NPC2, mRNA, EP1A4 mRNA, DOCK9 mRNA | Sens 0.86 (95%CI 0.68-0.96) Spec 0.92 (95%CI 0.62-1.0) | RNA seq | Healthy adults |

Table 1b (Continued)
| Article (reference) | Study Design | Study setting | Age range | Biomarker | Measure of effect | Test platform | Control group |
|---------------------|-------------|---------------|-----------|-----------|------------------|---------------|---------------|
| Ho 2020[1]          | Case control | Ca Mau Province, Vietnam | 41-66 years | 7-gene signature (IFI6, TGF1, GZMA, DHRS9, APOL6, FCGR1C, IFI35) | Sens 0.80 (95%CI 0.70-0.89) | RNA seq | Healthy adults |
| Jorge 2017[2]       | Case control | University hospitals, the Federal University of Minas Gerais and the Federal University of Sergipe (Brazil). | 18 to 60 years | 3-gene signature (RAP1A, C11orf2, SEPT4) Four miRNAs (miR-101, miR-196b, and miR-27b, and miR-29c) Four miRNAs (miR-101, miR-196b, miR-27b, and miR-29c) | Sens 0.65 (95%CI 0.54-0.75) | Microarray, RT-qPCR | Healthy adults |
| Latorre 2015[3]     | Case control | Barcelona, Spain | Age range not provided | miRNA-signature for rapid pulmonary TB diagnosis (hsa-miR-150, hsa-miR-21, hsa-miR-29c and hsa-miR-194) | Sens 0.88 (95%CI 0.64-0.99) | Microarray, RT-qPCR | Healthy adults |
| Lee 2016[4]         | Case control | Taoyuan General Hospital, Taoyuan, Taiwan | 20-40 years | PTPRC, ASUN, DHX29 | Sens 0.97 (95%CI 0.84-1.0) | Microarray, RT-qPCR | Healthy adults |
| Lei 2021[5]         | Case control | 2 tertiary hospitals | 20-50 years | 2-gene model (S100A12 + CD177) | Sens 0.94 (95%CI 0.87-0.97) | RT-qPCR | Healthy adults |
| Li 2020[6]          | Case control | Beijing Chest Hospital, Capital Medical University, Beijing, China | 18–73 years | miRNA-29a | Sens 0.90 (95%CI 0.85-0.94) | RT-qPCR | Healthy adults |
| Lydon 2019[7]       | Case control | Emergency departments at Duke University, Durham VA Health Care System, Henry Ford Hospital, and University of North Carolina | Ave. 42-58 years | 87-transcript signature | Sens 0.75 (95%CI 0.60-0.86) | RT-qPCR | Viral and non-infectious illness |
| Maertzdorf 2016[8]  | Case control | St. John’s hospital, Bangalore, India | > 18 years | 4-gene signature (GBP1, ID3, P2RY14, IFITM3) | Sens 0.85 (95%CI 0.69-0.95) | RT-qPCR | Healthy adults |
| Mihret 2014[9]      | Case control | Addis Ababa, T/Haimanot, Kirkos and W-23 health centres in Addis Ababa | Av. 32 years | 8-gene signature (BLR1, BLR2, IL10, FCGR1A, MARCO, CCL19, and LTF, TGFB1, and Foxp3) | Sens 0.92 (95%CI 0.73-0.99) | dcRT-MLPA | Healthy adults |

*Table 1b (Continued)*
| Article (reference) | Study Design | Study setting | Age range | Biomarker | Measure of effect | Test platform | Control group |
|---------------------|--------------|---------------|-----------|-----------|-------------------|---------------|---------------|
| Miotto 2013         | Case control | San Raffaele Hospital (Milano, Italy), Ifakara Health Institute, Tanzania, St. Francis Nsambya Hospital (Kampala, Uganda) | 19-90 years | 10 miRNA signature (European) | Sens 0.78 (95%CI 0.52-0.94) Spec 0.89 (95%CI 0.65-0.99) | Microarray | Healthy adults |
| Ndzi 2019           | Case control | Jamot hospital Yaounde, Cameroon | 16-76 years | miR-29a-3p | Sens 0.80 (95%CI 0.70-0.88) Spec 0.72 (95%CI 0.56-0.85) | RT-qPCR | Healthy adults |
| Satproedprai 2015   | Case control | Chiangrai Prachanukroh Hospital, Thailand | 21 to 79 years | TB Sick Score (FCGR1A, FCGR1B variant 1, FCGR1B variant 2, APOL1, STAT1, MAFB and KAZN) | Sens 0.82 (95%CI 0.67-0.93) Spec 1.0 (95%CI 0.91-1.0) | Microarray | Healthy adults |

Table 1b (Continued)
| Article (reference) | Study Design | Study setting | Age range | Biomarker | Measure of effect | Test platform | Control group |
|---------------------|--------------|---------------|-----------|-----------|------------------|---------------|---------------|
| Sampson 2020⁹¹      | Case control | University College London Hospitals Emergency Department | 19 to 99 years | SeptiCyte™ TRIAGE (DIAPH2/IL7R, GBP2; GMAP4, TLR5/FRG2) | Sens 0.87 (95%CI 0.76-0.94) Spec 0.79 (95%CI 0.49-0.95) | NanoString platform | Viral infection |
| Serrano 2016⁹²      | Case control | Mexico | 22 to 65 years | PSTPIP1 | Sens 0.70 (95%CI 0.35-0.93) Spec 1.0 (95%CI 0.83-1.0) | RT-qPCR | Healthy adults |
| Sivakumaran 2021⁹³ | Case control | Palamaner and Kuppam Taluk, Chittoor district, Andhra Pradesh, India | 19 to 70 years | 11-gene signature (CASP8, CD3E, CD8A, CD14, GBP5, GNLY, NLRP2, NOD2, TAGAP, TLR5, and TNF) | Sens 0.77 (95%CI 0.64-0.88) Spec 0.92 (95%CI 0.81-0.98) | dcRT-MLPA | Healthy adults |
| Sodersten 2021⁹⁴    | Case control | South African district hospital and a Peruvian referral hospital | > 18 years | Xpert-MTB-HR-Prototype (GBP5, DUSP3, and KLF2) | Sens 0.78 (95%CI 0.66-0.87) Spec 0.92 (95%CI 0.86-0.96) | Xpert assay (RT-PCR) | Healthy adults |
| Suarez 2015⁹⁵       | Case control | Rochester General Hospital, New York | > 21 years | 10 classifier genes | Sens 0.95 (95%CI 0.77-1.0) Spec 0.92 (95%CI 0.78-0.98) | Microarray | Healthy adults |
| Wang 2018⁹⁶        | Case control | Sixth Hospital of Shaoxing and the First Hospital of Jiaxing, China | 20 to 60 years | miR-21-5p, miR-92a-3p, miR-125a-5p, miR-148b-3p | Sens 0.65 (95%CI 0.56-0.73) Spec 0.75 (95%CI 0.68-0.82) | Solexa seq | Healthy adults |
| Wu 2007⁹⁷          | Case control | TB Clinic at San Francisco Department of Public Health/San Francisco General Hospital, Stanford University Medical Center | 19 to 66 years | IFN-γ mRNA | Sens 0.65 (95%CI 0.51-0.77) Spec 1.0 (95%CI 0.69-1.0) | qPCR | Healthy controls |
| Wu 2012⁹⁷          | Case control | Huashan Hospital, School of Medicine, Fudan University | 16 to 85 years | miR-155, miR-155* | Sens 0.43 (95%CI 0.22-0.66) Spec 0.95 (95%CI 0.74-1.0) | Microarray (RT-qPCR (validation)) | Healthy adults |
| Zhang 2019⁹⁸       | Case control | Shanghai Public Health Clinical Center Shanghai, China | 16 to 85 years | MIR-892b | Sens 0.50 (95%CI 0.27-0.73) Spec 0.80 (95%CI 0.56-0.94) | RT-qPCR | Healthy adults |

Table 1b (Continued)
| Article (reference) | Study Design | Study setting | Age range | Biomarker | Measure of effect | Test platform | Control group |
|------------------|-------------|---------------|-----------|-----------|-----------------|--------------|---------------|
| Burel 2018       | Case control | University of California, San Diego Anti-Viral Research Center clinic and the Universidad Peruana Cayetano Heredia | > 18 years | 74-gene signature | Sens 0.93 (95%CI 0.78-0.99) Spec 0.83 (95%CI 0.64-0.94) | RNA seq | Healthy adults |
| Sun 2021         | Case control | Shanxi Provincial Institute for Tuberculosis Control and Prevention | Majority > 18 years | miR-125b | Sens 0.90 (95%CI 0.76-0.97) Spec 0.93 (95%CI 0.80-0.98) | RT-qPCR | Healthy “volunteers” |
| Nabil 2019       | Case control | Tropical Medicine Department, Mansoura University Hospitals, Egypt | > 18 years | microRNA-155 | Sens 0.95 (95%CI 0.87-0.99) Spec 0.96 (95%CI 0.87-1.0) | RT-PCR | Decompensated cirrhotic non-infectious ascites |
| Dawany 2014      | Case control | Themba Lethu Clinic, Johannesburg, South Africa | 31-39 years | 251-gene TB signature | Sens 0.92 (95%CI 0.64-1.0) Spec 0.97 (95%CI 0.82-1.0) | Microarray | TB free controls |
| Mamishi 2021     | Case control | Masih Daneshvari Hospital, Tehran, Iran | 20-60 years | PTPRC | Sens 0.64 (95%CI 0.44-0.81) Spec 0.71 (95%CI 0.52-0.86) | RT-PCR | Healthy adults |
| Chen 2017        | Case control | Six Hospital of Shaoxing (China) | 20-50 years | Four lncRNAs (NR_03822, NR_003142, ENST00000570366, ENST000004422183) | Sens 0.79 (95%CI 0.65-0.89) Spec 0.75 (95%CI 0.61-0.86) | RT-qPCR | Healthy adults |
| de Araujo 2019   | Case control | TB Control Program of Clementino Fraga Filho University Hospital, Rio de Janeiro | 35-50 years | 4 sncRNA (let-7a-5p, miR-589-5p, miR-196b-5p, and SNORD104) | Sens 1.0 (95%CI 0.63-1.0) Spec 0.98 (95%CI 0.87-1.0) | RNA seq | Non-TB cases |
| Huang 2018       | Case control | First Affiliated Hospital of Nanchang University and Jiangxi Chest Hospital, China | 30 -60 years | hsa_circ_0001953 | Sens 0.69 (95%CI 0.60-0.77) Spec 0.89 (0.81-0.94) | Microarray | Healthy adults |

Table 1b: Summary characteristics of included studies which explored genomic biomarker performance using an adult population. An outline of the study setting and design, biomarker, biomarker performance and platform used in each of the included studies has been provided. Further information available in the Supplement.

Sens: sensitivity; Spec: specificity; 95%CI: 95% Confidence Interval; SLE: Systemic Lupus Erythematosus; RT-qPCR Quantitative reverse transcription PCR; dcRT-MLPA Dual colour multiplex ligation-dependent probe amplification; lncRNA Long noncoding RNA; sncRNA Small noncoding RNA; RT-eLAMP/electronic RT-lamp Reverse Transcription Loop-mediated Isothermal Amplification.

* Analytical validation/further evaluation: assessment of biomarker performance using routinely available clinical laboratory tools.
reflected in the HSROC despite moderate performance metrics attained with respect to PLR, NLR and DOR. However, significant heterogeneity was detected on all analyses (Figures and 3, Table 3). Although both study population and TB-related studies were significant covariates, similar performance values occurred on subgroup analysis, and heterogeneity persisted. It is also possible that the wide-ranging confidence interval of the DOR of the non-TB subgroup undermines the value attained and is the consequence of the small study numbers present in this subgroup. The accuracy of the slightly superior performance metrics observed in the paediatric subgroup is also questionable given the smaller number and study population of the included paediatric studies. The significant between-study heterogeneity may also be the result of the variability in molecular functionality of the included biomarkers, however the possibility of publication bias cannot be excluded.

The choice of test threshold in diagnostic test accuracy studies often affects the sensitivity or specificity of
the test depending on the optimum threshold chosen to determine the presence or absence of a disease or condition. The high sensitivity (and specificity) values on pooled (and individual) analyses of biomarker performance observed here, reflect the significant potential role of genomic biomarkers in clinical practice to help exclude the presence of SBI in those disease-free, which would be of vital importance in clinical decision-making and emphasises the importance of their development into much needed POC tests. However, there exist many obstacles to their implementation in clinical settings.

Most biomarkers were assessed in an adult population, using a case-control study design, an inherent source of bias in the appraisal of test performance. The use of a healthy population as a control group can contribute to an overestimation of biomarker specificity. Participants with immunodeficiencies were frequently ineligible to participate. Genomic biomarkers of infection, which rely on an appropriate host immune response to infection, may not perform to the same extent in this patient population and their exclusion may have led to falsely elevated levels of sensitivity.
The most significant risk of bias related to the index test domain as most studies were discovery/early-phase explorative studies comparing gene expression between affected and matched healthy control groups and which selected the transcript or gene that illustrated the most discriminatory potential as a novel biomarker for investigation. Fewer than half of the studies in this review validated the discovered biomarker in an independent group. Furthermore, the index test threshold was frequently determined by choosing the optimal cut-off value which provided the best trade-off between sensitivity and specificity, thereby providing an impressively accurate performance result in the selected cohort, but without validating its reproducibility and performance in an independent group. Indeed, the optimal threshold may vary between studies and when selected for each study, will have the highest accuracy for that study. Therefore, biomarker performance accuracy in this review may have been artefactually increased as a consequence of study design.

### Table 2: Frequency of test specimen and assay used in each of the included studies (n = 72).

| Platform test                  | Frequency of test utilisation |
|--------------------------------|------------------------------|
| RT-PCR                         | 43                           |
| Microarray                     | 26                           |
| RNA sequencing                 | 11                           |
| dcRT-MLPA                      | 3                            |
| NanoString technologies        | 2                            |
| (gene expression panel)        |                              |

| Specimen                      | Frequency of specimen utilisation |
|--------------------------------|----------------------------------|
| Blood                         | 70                               |
| CSF                            | 2                                |
| Ascitic fluid                  | 1                                |
| Skin biopsy                    | 1                                |

In 13 studies, qRT-PCR was used following Microarray or RNA to further validate the initial results attained. CSF was used to assess biomarker performance in two studies.

### Table 3: Diagnostic performance of genomic biomarkers according to subgroup analysis and assessment of heterogeneity ($I^2$). Biomarker performance was assessed according to study population (paediatric or adult) and TB disease (TB or non-TB related biomarker).

| Subgroup                        | No. studies | Sens (95% CI) | Spec (95% CI) | PLR (95% CI) | NLR (95% CI) | DOR (95% CI) |
|---------------------------------|-------------|---------------|---------------|--------------|--------------|--------------|
| **Study population**            |             |               |               |              |              |              |
| Adults                          | 49          | 0.79 (0.76-0.81) | 0.86 (0.84-0.88) | 5.36 (4.7-6.2) | 0.20 (0.17-0.23) | 27.15 (21.2-34.8) |
| Heterogeneity ($I^2$)           |             | 75.24         | 79.0          | 77.67 | 81.76 | 76.56 |
| Children                        | 23          | 0.85 (0.80-0.89) | 0.86 (0.83-0.90) | 6.13 (4.60-8.23) | 0.15 (0.10-0.23) | 44.34 (24.23-81.17) |
| Heterogeneity ($I^2$)           |             | 72.41         | 66.71         | 71.07 | 85.50 | 79.40 |
| **Biomarkers specific to TB disease** | | | | | | |
| TB                              | 47          | 0.78 (0.76-0.81) | 0.85 (0.83-0.87) | 5.05 (4.40-5.79) | 0.20 (0.17-0.23) | 25.02 (19.74-31.73) |
| Heterogeneity ($I^2$)           |             | 75.47         | 75.47         | 78.73 | 85.27 | 76.01 |
| Non-TB                          | 25          | 0.85 (0.81-0.89) | 0.88 (0.85-0.91) | 7.23 (5.49-9.54) | 0.15 (0.11-0.19) | 53.39 (31.94-89.23) |
| Heterogeneity ($I^2$)           |             | 65.80         | 65.80         | 71.57 | 73.72 | 76.37 |

Notes.
- Sens = sensitivity.
- Spec = specificity.

### Figure 4.
(a) Summary of the results of the risk of bias and applicability concerns assessment outlined in graphical format for the 72 included studies in the review. The numbers of studies which fall into each category of risk (differentiated by colour) are indicated on the plot. Assessment of applicability is not relevant to flow and timing and therefore this component of the assessment has not been applied to this domain. (b) Risk of bias and applicability concerns summary: review authors’ judgements for each included study according to risk category.
RT-qPCR was most often used to assess biomarker performance. The challenge now is to move from the discovery and validation of promising diagnostic transcript signatures to clinical application, and to translate laboratory-based analysis to platforms which are affordable, easy-to-use POC tests, particularly for resource-limited settings. Unfortunately, the techniques currently required for the analysis of nucleic acids are expensive, require skilled technicians, and are time-consuming to perform. Of the studies included in this review, few utilised the recently developed microfluidics and lab-on-a-chip technologies which would facilitate the conversion of transcriptomic analysis to a POC test.

Although RNA-sequencing has shown superior performance in gene expression profiling, RT-PCR and microarray are often preferred. RT-PCR offers the benefits of rapidity, sensitivity, accuracy and a more targeted approach to gene expression analysis. It is often used to validate the results of high-throughput studies and indeed, was used for this purpose in many studies in this review. Given very few of the included studies used an RNA-sequencing platform, it is not possible to accurately determine the extent to which the platform-type may have influenced biomarker performance. The variety of platforms featured may also have contributed to the notable heterogeneity seen in the analysis and further research is warranted to observe the effect, if any, that these differences may have had on biomarker performance. The adaptability of these techniques must also be considered, as ease of clinical application, as well as accuracy, are required for true clinical value to be ascertained.

Blood was used for almost all test platforms. Blood is relatively straightforward to obtain, compared with CSF, ascitic fluid or skin biopsy used in other featured studies, facilitating its use as a POC test. In two studies, biomarker performance was also assessed in CSF in children with tuberculous meningitis. The biomarkers in each study performed comparably well in both blood and CSF (Pan et al. 2017, AUC 0.852, 0.890 respectively; Pan et al 2019, AUC 0.716, 0.784, respectively). Bartholomeus et al. (though not included in this review due to insufficient data for analysis) also investigated the possibility of a blood transcriptomic signature as an alternative to CSF for the diagnosis of enterovirus meningitis in children. Considering viral and bacterial causes of meningitis are clinically indistinguishable and children are particularly susceptible, the potential development of a blood-based biomarker to replace or guide decision-making regarding the need for lumbar puncture, would revolutionise current paediatric practice and minimise the need for broad-spectrum antibiotics if translated into a rapid POCT.

The cause of febrile illness, particularly in children, may not be the result of a single pathogen, and
determination of the aetiology of infection is challenging in most clinical settings. Serious infection in children and infants, can lead to significant morbidity and mortality, with the emphasis in this vulnerable group on early recognition and treatment to minimise the risk of damaging sequelae. Empiric antibiotic therapy, and hospitalisation, with resultant costs to healthcare and antibiotic resistance, are frequently incurred. A rapid POC diagnostic tool capable of accurately differentiating bacterial from potential co-existent pathogens is needed. Although many of the biomarkers reviewed here show great potential to correctly identify bacterial infection, most studies assessed biomarker performance in cohorts infected with a single pathogen or used a healthy comparator group. Further studies are needed to determine the performance of such biomarkers in the setting of co-infection, which would in turn, enhance the use of healthcare resources, and facilitate targeted antibiotic usage.

The source of infection is also dependent on geographic location, associated endemcity and pathogen prevalence. In areas of South and Southeast Asia, dengue fever and leptospirosis account for most cases of acute fever. Here, and in other malaria-endemic areas, bacterial co-infection is a frequent and significant risk. It is possible that the high burden of such diseases may affect the performance of genomic biomarkers in these settings where such diagnostic tools are urgently needed. However, there is promising evidence to suggest that their diagnostic accuracy is preserved under such conditions.

To assess the diagnostic capability of genomic biomarkers, a meta-analysis was undertaken. Although useful, the results reported here require cautious interpretation. Each genomic biomarker is reflective of a unique host-pathogen interaction, has varying underlying molecular functionality, and may be disease-specific. Therefore, the determination of overall effect

Figure 5. Hierarchical summary receiver operating curve (HSROC) illustrating the diagnostic accuracy of the included genomic biomarkers of bacterial infection. The receiver operating curve depicted uses hierarchical modelling to account for the variability in study threshold of each of the included studies and between-study heterogeneity.
achieved through pooled meta-analysis, may not accurately reflect the ability of this type of biomarker to differentiate bacterial from other causes of infection and may account for the significant heterogeneity observed.

Genomic biomarkers show considerable promise as diagnostic tests of bacterial infection and for development into POC tests. Most genomic biomarkers, however, are still in an early stage of development and require further investigation and validation before clinical use can be considered. Further work is needed to assess their performance in different clinical settings using improved study designs (randomised, with adequate blinding to index and standard test results, and pre-defined test thresholds) in order to minimise the risk of bias and achieve reliable and reproducible results of genomic biomarker performance.

Contributors
EK and DO’C contributed to the conception and design of the study. Database search outputs were screened by EK and SW and data collected by EK and SW. An assessment of risk of bias and critical appraisal quality of the included studies was performed by EK and SM. EK completed the literature review, extracted and collated the data and performed the data analysis, interpreted the results, and wrote the first draft of the manuscript. DO’C accessed and verified the underlying data analysis and assisted in the interpretation of the results. DO’C and AJP critically reviewed the manuscript and provided guidance in the writing of the manuscript. EH provided guidance and expertise in devising the database search strategy.

Data sharing statement
The data collected for this study can be provided upon reasonable request.

Declaration of interests
AJP is chief investigator of clinical trials of the SARS-CoV-2 vaccine (ChAdOx-1 nCoV-19). These clinical trials are funded by UK Research and Innovation, Coalition for Epidemic Preparedness Innovations, the National Institute for Health Research, and the National Institute for Health Research Oxford Biomedical Research Centre. AJP is Chair of UK Department of Health and Social Care’s (DHSC) Joint Committee on Vaccination and Immunisation (JCVI). He is a member of the Academy of Medical Sciences and an expert in an advisory capacity for WHO’s SAGE. The views expressed in this article do not necessarily represent the views of DHSC, JCVI, NIHR, or WHO.

Supplementary materials
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104110.

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