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

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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 (I^2 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 diseases.[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.[3,4]

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

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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 (I^2 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.

awaiting the results of culture of blood, urine, cerebrospinal fluid (CSF).[5] 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.[6] Moreover, blood volume attainment is often small leading to the risk of false negative results and associated inadequate sensitivity has been reported.[7]

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.[8,9]

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-recognised 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-recognised 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-

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.[25] 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 2×2 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 (63/72; 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 I^2 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, I^2 value 77%, p < 0.001. Pooled NLR, PLR, and DOR results were 0.18 (95% CI 0.16-0.21), I^2 84%; 5.5 (95% CI 4.9-6.3), I^2 79%, and 30.1 (95% CI 24-37), I^2 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 qRTPCR 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 4(a), (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).[97] Indeed, many genomic biomarkers demonstrated impressive diagnostic performances when compared to conventional diagnostic biomarkers such as the 2-transcript
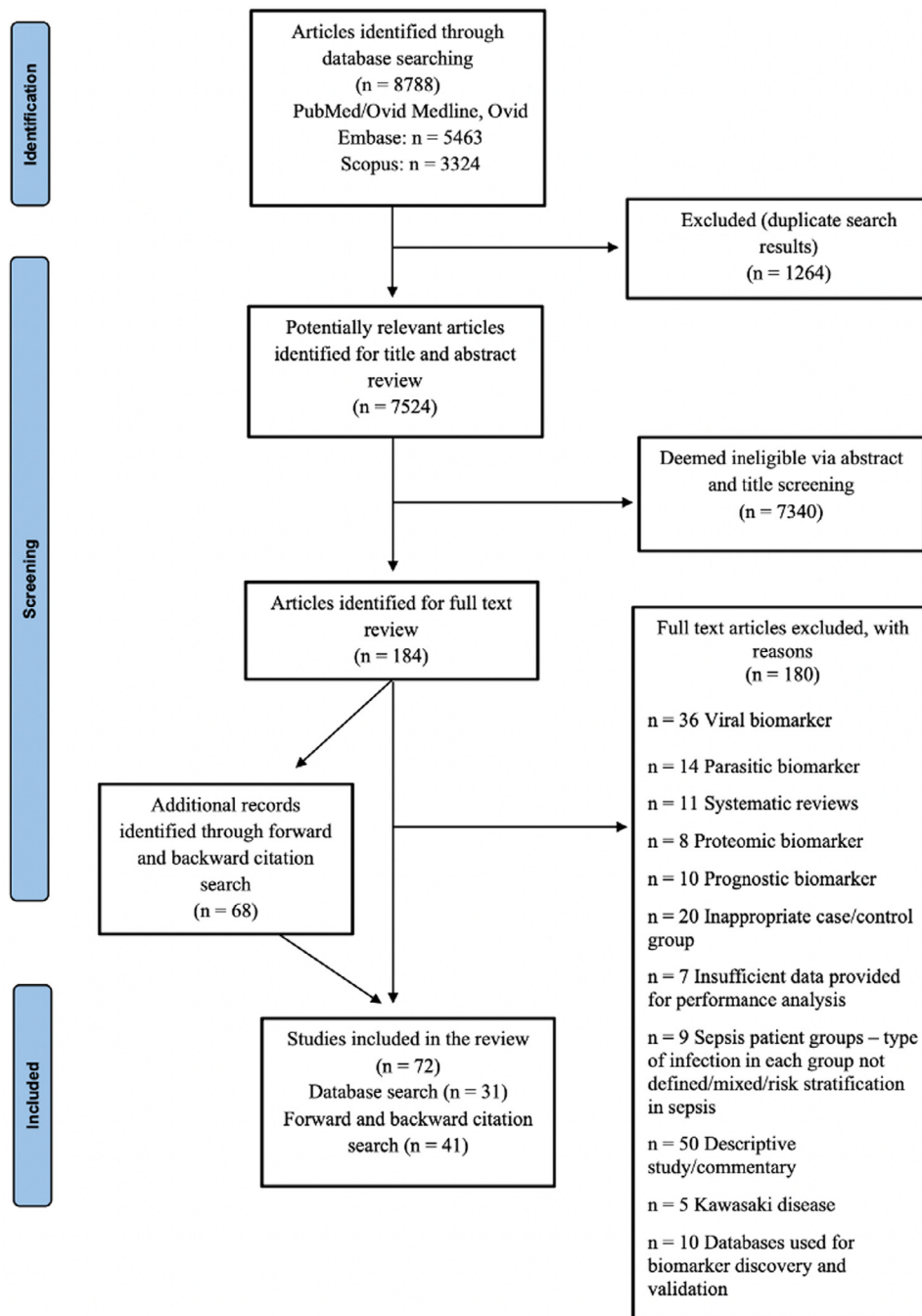
Figure 1. Flow diagram of the study selection process.
    Overview of database search results, abstracts identified for full text review and reasons for study exclusion provided. The study selection process was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.

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[18]	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)[29]	Case control	Paediatric Department, Faculty of Medicine, Menoufia 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
				miRNA-451	Sens 0.64 (95%CI 0.43-0.82) Spec 0.60 (95%CI 0.39-0.79)		
Fouda (2021)[30]	Case control	NICU of Menoufia University Hospital, Egypt	Neonate; 1-4 days	miRNA 15b	Sens 0.76 (95%CI 0.55-0.91) Spec 0.88 (95%CI 0.69-0.97)	RT-PCR	Healthy newborns
				miRNA 378a	Sens 0.60 (95%CI 0.39-0.79) Spec 0.88 (95%CI 0.69-0.97)		
Tian (2021)[31]	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[32]	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[33]	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[34]	Case control	University and Polytechnic Hospital La Fe	VLBW infants (birth weight <1500g)	GWEP	Sens 1.0 (95%CI 0.80-1.0) Spec 0.68 (95%CI 0.43-0.87)	RT-PCR Microarray (validation)*	Healthy neonates
Ge 2013[35]	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)	Micoarray, qRT-PCR (further evaluation)*	Healthy children

Table 1a (Continued)

Article (reference)	Study Design	Study setting	Age range	Biomarker	Measure of effect	Test platform	Control group
Gjoen 2017[36]	Cross-sectional	Tertiary hospital, Delhi, Pala-maner Taluk, India	Children 6 months to 15 years	10-transcript signature: *IFNG, NLRP1, NLRP3, TGFBR2, TAGAP, NOD2, 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
				7-transcript signature: *MMP9, CD3E, NOD2, GBP5, IFITM1/3, KIF1B* and *TNIP1*	Sens 0.92 (95%CI 0.73-0.99) Spec 0.81 (95%CI 0.61-0.93)		
Gomez-Car-balla 2019[37]	Case control	Hospital Clínico Universi-tario 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[38]	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[39]	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[17]	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
				10 classifier genes	Sens 0.95 (95%CI 0.75-1.0) Spec 0.95 (95%CI 0.88-0.98)		
Pan 2017[40]	Case control	Third Affiliated Hospital of Zhengzhou University, the First Affiliated Hospi-tal of Zhengzhou Univer-sity 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[41]	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)*	Healthy preterm neo-nates 28-32 weeks (GA)
Smith 2014[42]	Case control	Neonatal Unit, Royal Infir-mary 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[43]	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[44]	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[45]	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[46]	Case control	Children's Hospital of Chongqing Medical University, China	Children 4 to 10 years old	8 miRNA (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[47]	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[48]	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[21]	Case control	Hospitals in United Kingdom, Spain, Netherlands, United States	Children <17 years	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[49]	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)[50]	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)[51]	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)[52]	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[53]	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[54]	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[55]	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[56]	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)*	Healthy adults

Table 1b (Continued)

Article (reference)	Study Design	Study setting	Age range	Biomarker	Measure of effect	Test platform	Control group
Warsinske 2018[57]	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[58]	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 393-transcript signature	Sens 0.90 (95%CI 0.68-0.99) Spec 0.83 (95%CI 0.77-0.88) Sens 0.62 (95%CI 0.38-0.82) Spec 0.94 (95%CI 0.80-0.99)	Microarray	Healthy adults
Kelly 2018[59]	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[60]	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[61]	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) Sens 1.0 (95%CI 0.88-1.0) Spec 0.95 (0.82-0.99)	Microarray, RT-qPCR	Healthy adults
Bloom 2013[62]	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[63]	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[64]	Case control	Rio de Janeiro state, Brazil	25-55 years	*NPC2,* mRNA *EPHA4* mRNA *DOCK9* mRNA	Sens 0.86 (95%CI 0.68-0.96) Spec 0.92 (95%CI 0.62-1.0) Sens 0.55 (95%CI 0.36-0.74) Spec 0.92 (95%CI 0.62-1.0) Sens 0.21 (95%CI 0.08-0.40) 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[65]	Case control	Ca Mau Province, Vietnam	41-66 years	7-gene signature (IFI6, TGIF1, GZMA, DHRS9, APOL6, FCGR1C, IFI35)	Sens 0.80 (95%CI 0.70-0.89) Spec 0.84 (95%CI 0.77-0.89)	RNA seq	Healthy adults
				3-gene signature (RAP1A, C11orf2, SEPT4)	Sens 0.65 (95%CI 0.54-0.75) Spec 0.53 (95%CI 0.42-0.64)		
Jorge 2017[66]	Case control	University hospitals, the Federal University of Minas Gerais and the Federal University of Sergipe (Brazil).	18 to 60 years	Four miRNAs (miR-101, miR-196b, miR-27b, and miR-29c) Four miRNAs (miR-101, miR-196b, miR-27b, and miR-29c)	Sens 0.79 (95%CI 0.58-0.93) Spec 0.92 (95%CI 0.62-1.0)	Microarray, RT-qPCR	Healthy adults
Latorre 2015[67]	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) Spec 0.88 (95%CI 0.72-0.97)	Microarray, RT-qPCR	Healthy adults
Lee 2016[68]	Case control	Taoyuan General Hospital, Taoyuan, Taiwan	20-40 years	PTPRC, ASUN, DHX29	Sens 0.97 (95%CI 0.84-1.0) Spec 0.93 (95%CI 0.68-1.0)	Microarray, RT-qPCR	Healthy adults
Lei 2021[69]	Case control	2 tertiary hospitals	20-50 years	2-gene model (S100A12 + CD177)	Sens 0.94 (95%CI 0.87-0.97) Spec 0.97 (95%CI 0.92-0.99)	RT-qPCR	Healthy adults
Li 2020[70]	Case control	Beijing Chest Hospital, Capital Medical University, Beijing, China	18−73 years	miRNA-29a	Sens 0.90 (95%CI 0.85-0.94) Spec 0.71 (95%CI 0.64-0.77)	RT-qPCR	Healthy adults
Lydon 2019[71]	Case control	Emergency departments at Duke University, Durham VA Health Care System, Henry Ford Hospital, and University of North Carolina	Av. 42-58 years	87-transcript signature	Sens 0.75 (95%CI 0.60-0.86) Spec 0.92 (95%CI 0.85-0.97)	RT-qPCR	Viral and non-infectious illness
Maertzdorf 2016[72]	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) Spec 0.76 (95%CI 0.60-0.88)	RT-qPCR	Healthy adults
Mihret 2014[73]	Case control	Arada, T/Haimanot, Kirkos and W-23 health centres in Addis Ababa	Av. 32 years	*BLR1, Bcl2, IL4d2, FCGR1A, MARCO, CCL19*, and *LTF, TGFB1*, and *Foxp3, FPR1* and *TGFB1*.	Sens 0.92 (95%CI 0.73-0.99) Spec 0.96 (95%CI 0.78-1.0)	dcRT-MLPA	Healthy adults

Table 1b (Continued)

Articles

Article (reference)	Study Design	Study setting	Age range	Biomarker	Measure of effect	Test platform	Control group
Miotto 2013[74]	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
				12 miRNA signature (African-specific sig.)	Sens 1.0 (95%CI 0.69-1.0) Spec 0.90 (95%CI 0.55-1.0)		
Ndzi 2019[75]	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
				MiR-155-5p	Sens 0.80 (95%CI 0.70-0.88) Spec 0.50 (95%CI 0.35-0.65)		
				MiR-361-5p	Sens 0.88 (95%CI 0.79-0.94) Spec 0.58 (95%CI 0.42-0.73)		
Perumal 2021[76]	Case control	All India Institute of Medical Sciences, New Delhi and The Jawaharlal Institute of Postgraduate Medical Education & Research Puducherry Guy's and St Thomas', Royal Free London	>18 years	*GBP1, IFIT3, IFITM3, SAMD9L*	Sens 0.80 (95%CI 0.73-0.86) Spec 0.94 (0.89-0.98)	qPCR	Healthy adults
Petrilli 2020[77]	Case control	Instituto Brasileiro para Investigação de Tuberculose and 2°Centro de Saúde Rodrigo Argolo, Bahia, Brazil.	25 to 60 years	*CEACAM1*	Sens 1.0 (95%CI 0.80-1.0) Spec 1.0 (95%CI 0.48-1.0)	NanoString platform	Healthy adults
				CR1	Sens 1.0 (95%CI 0.80-1.0) Spec 1.0 (95%CI 0.48-1.0)		
				FCGR1A/B	Sens 1.0 (95%CI 0.80-1.0) Spec 1.0 (95%CI 0.48-1.0)		
Poore 2018[78]	Case control	Emergency Dept at Duke Hospital, UNC-Chapel Hill, and Henry Ford Hospital	19 to 76 years	Bacterial vs viral miRNA signature (40 DE miRNA)	Sens 1.0 (95%CI 0.69-1.0) Spec 0.85 (95%CI 0.55-0.98)	Microarray	Viral infection
				Bacterial vs healthy miRNA signature (67 DE miRNA)	Sens 1.0 (95%CI 0.69-1.0) Spec 1.0 (95%CI 0.84-1.0)		Healthy adults
Roe 2020[79]	Case control	South Africa, The Gambia	>18 years	3-gene transcript signature (*BATF2, GBP5, SCARF1*)	Sens 0.83 (95%CI 0.52-0.98) Spec 0.96 (95%CI 0.86-0.99)	RNA seq	Healthy adults
Satproedprai 2015[80]	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 2020[81]	Case control	University College London Hospitals Emergency Department	19 to 99 years	SepticyteTM TRIAGE (*DIAPH2/IL7R, GBP2/ GIMAP4, TLR5/FGL2*)	Sens 0.87 (95%CI 0.76-0.94) Spec 0.79 (95%CI 0.49-0.95)	NanoString platform	Viral infection
				Combined *SepticyteTM*	Sens 0.94 (95%CI 0.86-0.98) Spec 0.93 (95%CI 0.66-1.0)		
Serrano 2016[82]	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
				NCF1 and *ORM*	Sens 0.95 (95%CI 0.75-1.0) Spec 0.80 (0.64-0.91)		
Sivakumaran 2021[83]	Case control	Palamaner and Kuppam Taluks, 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 2021[84]	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 2015[22]	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 2018[85]	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 RT-qPCR (validation)*	Healthy adults
Wu 2007[86]	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 2012[87]	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 2019[88]	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[89]	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[90]	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"
Nabiel 2019[91]	Case control	Tropical Medicine Department, Mansoura University Hospitals, Egypt	>18 years	microRNA-155	Sens 0.95 (95%CI 0.87-0.99) Spec 0.97 (95%CI 0.87-1.0)	RT-PCR	Decompensated cirrhotic non-infectious ascites
Dawany 2014[92]	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[93]	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
				ASUN	Sens 0.75 (95%CI 0.53-0.90) Spec 0.77 (95%CI 0.59-0.90)		
Chen 2017[94]	Case control	Six Hospital of Shaoxing (China)	20-50 years	Four lncRNAs (NR_03822, NR_003142, ENST00000570366, ENST00000422183)	Sens 0.79 (95%CI 0.65-0.89) Spec 0.75 (9%%CI 0.61-0.86)	RT-qPCR	Healthy adults
de Araujo 2019[95]	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[96]	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
				hsa_circ_0009024	Sens 0.60 (95%CI 0.51-0.69) Spec 0.86 (95%CI 0.78-0.92)		

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* Quantiative 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.

Figure 2. Forest plot of pooled sensitivity and specificity of diagnostic genomic biomarkers of bacterial infection.

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

Figure 3. Forest plot of pooled negative likelihood ratio and positive likelihood ratio of diagnostic genomic biomarkers of bacterial infection.

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.[98] The use of a healthy population as a control group can contribute to an overestimation of biomarker specificity.[98] 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[99] and their exclusion may have led to falsely elevated levels of sensitivity.

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

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

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

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.

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

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).**
Notes.
  * Sens = sensitivity.
  ** Spec = specificity.
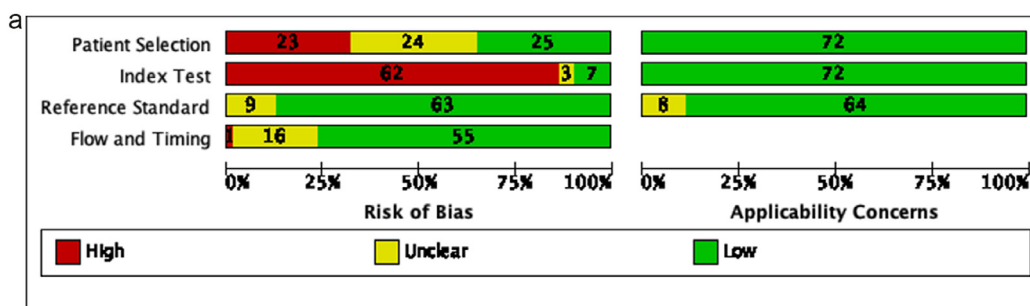
Figure 4. (a) Summary of the results of the risk of bias and applicability concerns assessment outlined in graphical format for the 72 included studies in the review. The numbers of studies which fall into each category of risk (differentiated by colour) are indicated on the plot. Assessment of applicability 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.
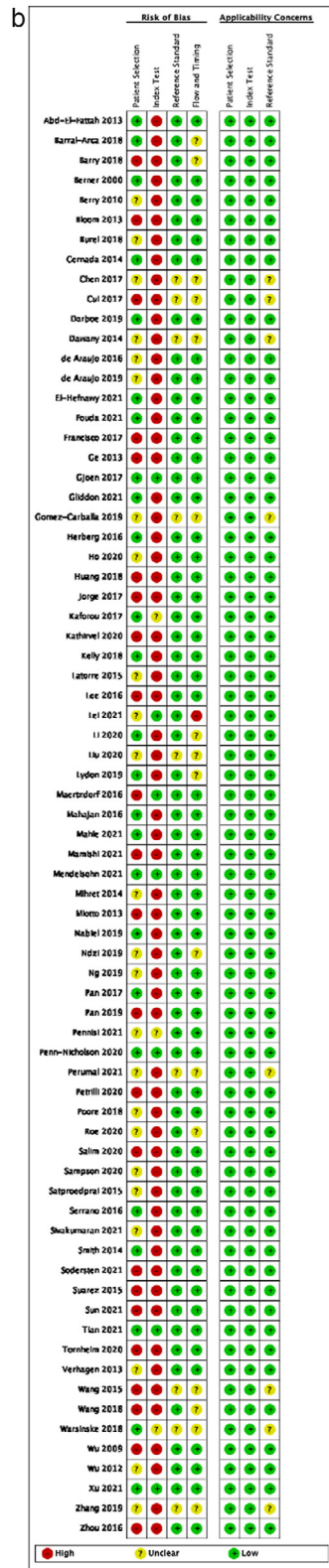
Figure 4 Continued.

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.[100]

Although RNA-sequencing has shown superior performance in gene expression profiling, RT-PCR and microarray are often preferred.[101] RT-PCR offers the benefits of rapidity, sensitivity, accuracy and a more targeted approach to gene expression analysis.[102] 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.[40,54] 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 entero-virus meningitis in children.[103] 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[104] 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
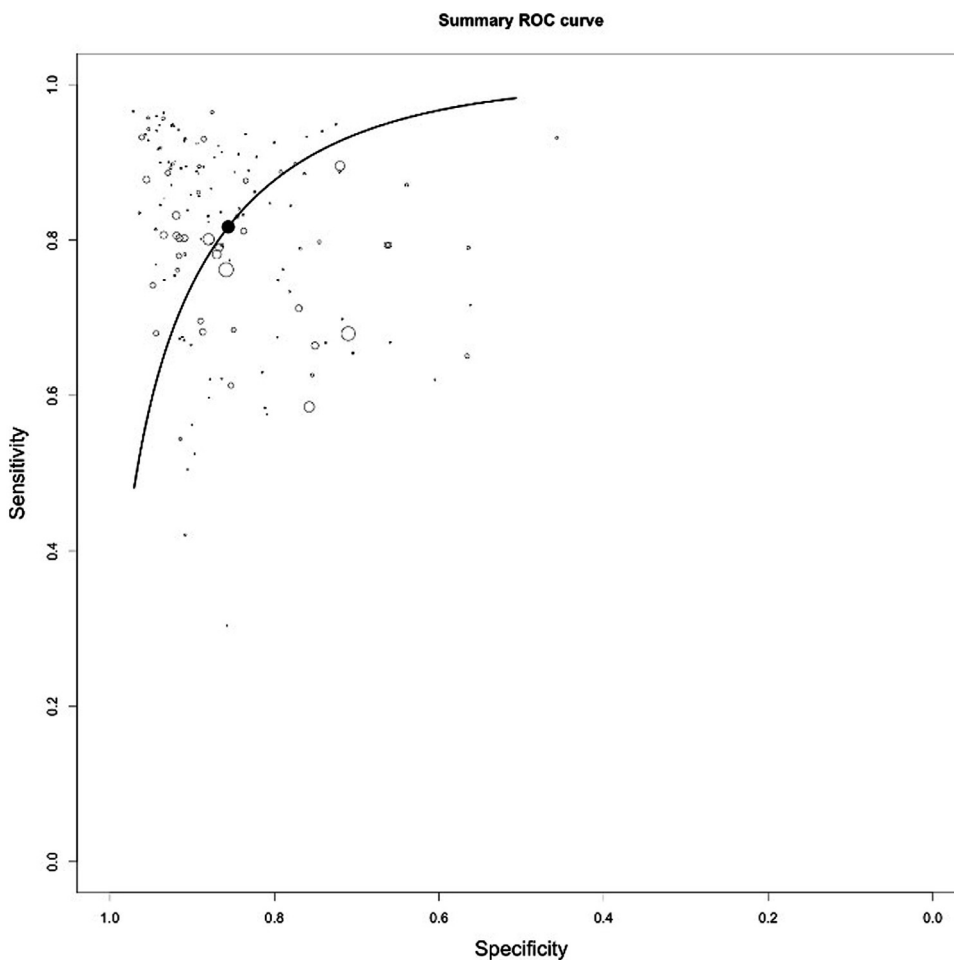
Figure 5. Hierarchical summary receiver operating curve (HSROC) illustrating the diagnostic accuracy of the included genomic biomarkers of bacterial infection. The receiver operating curve depicted uses hierarchical modelling to account for the variability in study threshold of each of the included studies and between-study heterogeneity.

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.[20] Empiric antibiotic therapy, and hospitalisation, with resultant costs to healthcare and antibiotic resistance, are frequently incurred.[21] 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 endemicity 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.[105] 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.[11]

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

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

Supplementary materials

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

References

1 The Global Health Observatory WHO. Global Health Estimates: Life expectancy and leading causes of death and disability 2021.

2 Byington CL, Rittichier KK, Bassett KE, et al. Serious bacterial infections in febrile infants younger than 90 days of age: the importance of ampicillin-resistant pathogens. *Pediatrics*. 2003;111(5 Pt 1):964–968.

3 World Health Organization. Children: improving survival and well-being 2020. Available from: https://www.who.int/news-room/fact-sheets/detail/children-reducing-mortality.

4 Centers for Disease Control and Prevention. Top 10 Global Health Priorities 2017.

5 Arora R, Mahajan P. Evaluation of child with fever without source: review of literature and update. *Pediatr Clin North Am*. 2013;60(5):1049–1062.

6 Iroh Tam PY, Bernstein E, Ma X, Ferrieri P. Blood culture in evaluation of pediatric community-acquired pneumonia: a systematic review and meta-analysis. *Hosp Pediatr*. 2015;5(6):324–336.

7 Connell TG, Rele M, Cowley D, Buttery JP, Curtis N. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. *Pediatrics*. 2007;119(5):891–896.

8 DeAngelis C, Joffe A, Wilson M, Willis E. Iatrogenic risks and financial costs of hospitalizing febrile infants. *Am J Dis Child*. 1983;137(12):1146–1149.

9 Zwang O, Albert RK. Analysis of strategies to improve cost effectiveness of blood cultures. *J Hosp Med*. 2006;1(5):272–276.

10 Sadarangani M, Willis L, Kadambari S, et al. Childhood meningitis in the conjugate vaccine era: a prospective cohort study. *Arch Dis Child*. 2015;100(3):292–294.

11 Silterra J, Gillette MA, Lanaspa M, et al. Transcriptional categorization of the etiology of pneumonia syndrome in pediatric patients in malaria-endemic areas. *J Infect Dis*. 2017;215(2):312–320.

12 Hjuler IM, Hansen MB, Olsen B, Renneberg J. Bacterial colonization of the larynx and trachea in healthy children. *Acta Paediatr*. 1995;84(5):566–568.

13 Mizusawa M. Updates on rapid diagnostic tests in infectious diseases. *Mo Med*. 2020;117(4):328–337.

14 Warhurst G, Dunn G, Chadwick P, et al. Rapid detection of health-care-associated bloodstream infection in critical care using multi-pathogen real-time polymerase chain reaction technology: a diagnostic accuracy study and systematic review. *Health Technol Assess*. 2015;19(35):1–142.

15 Dupuy AM, Philippart F, Pean Y, et al. Role of biomarkers in the management of antibiotic therapy: an expert panel review: I - currently available biomarkers for clinical use in acute infections. *Ann Intensive Care*. 2013;3(1):22.

16 Lee CR, Cho IH, Jeong BC, Lee SH. Strategies to minimize antibiotic resistance. *Int J Environ Res Public Health*. 2013;10(9):4274–4305.

17 Mahajan P, Kuppermann N, Mejias A, et al. Association of RNA biosignatures with bacterial infections in febrile infants aged 60 days or younger. *JAMA*. 2016;316(8):846–857.

18 Herberg JA, Kaforou M, Wright VJ, et al. Diagnostic test accuracy of a 2-transcript host rna signature for discriminating bacterial vs viral infection in febrile children. *JAMA*. 2016;316(8):835–845.

19 Hu X, Yu J, Crosby SD, Storch GA. Gene expression profiles in febrile children with defined viral and bacterial infection. *Proc Natl Acad Sci U S A*. 2013;110(31):12792–12797.

20 Ramilo O, Allman W, Chung W, et al. Gene expression patterns in blood leukocytes discriminate patients with acute infections. *Blood*. 2007;109(5):2066–2077.

21 Pennisi I, Rodriguez-Manzano J, Moniri A, et al. Translation of a host blood rna signature distinguishing bacterial from viral infection into a platform suitable for development as a point-of-care test. *JAMA Pediatr*. 2021;175(4):417–419.

22 Suarez NM, Bunsow E, Falsey AR, Walsh EE, Mejias A, Ramilo O. Superiority of transcriptional profiling over procalcitonin for

distinguishing bacterial from viral lower respiratory tract infections in hospitalized adults. *J Infect Dis.* 2015;212(2):213–222.

23 Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 2009;6(7):e1000097.

24 Scottish Intercollegiate Guidelines Network (SIGN). SIGN Database Search Filters Diagnostic studies, 2020. Available from: https://www.sign.ac.uk/assets/search-filters-diagnostic-studies.docx.

25 Leclercq E, Leeflang MM, van Dalen EC, Kremer LC. Validation of search filters for identifying pediatric studies in PubMed. *J Pediatr.* 2013;162(3):629–634.e2.

26 Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med.* 2011;155(8):529–536.

27 Casey-Wallace B TT, Lau J, Schmid C. *OpenMeta-Analyst: Open-Source, Cross-Platform Software for Advanced Meta-Analysis.* 2010. Keystone.

28 Review Manager (RevMan) Version 5.4. The Cochrane Collaboration, 2020.

29 El-Hefnawy SM, Mostafa RG, El Zayat RS, Elfeshawy EM, Abd El-Bari HM, El-Monem Ellaithy MA. Biochemical and molecular study on serum miRNA-16a and miRNA- 451 as neonatal sepsis biomarkers. *Biochem Biophys Rep.* 2021;25:100915.

30 Fouda E, Elrazek Midan DA, Ellaban R, El-Kousy S, Arafat E. The diagnostic and prognostic role of MiRNA 15b and MiRNA 378a in neonatal sepsis. *Biochem Biophys Rep.* 2021;26:100988.

31 Tian S, Deng J, Huang W, et al. FAM89A and IFI44L for distinguishing between viral and bacterial infections in children with febrile illness. *Pediatr Investig.* 2021;5(3):195–202.

32 Barral-Arca R, Pardo-Seco J, Martinón-Torres F, Salas A. A 2-transcript host cell signature distinguishes viral from bacterial diarrhea and it is influenced by the severity of symptoms. *Scientific Reports.* 2018;8(1):8043.

33 Berner R, Tuxen B, Clad A, Forster J, Brandis M. Elevated gene expression of interleukin-8 in cord blood is a sensitive marker for neonatal infection. *Eur J Pediatr.* 2000;159(3):205–210.

34 Cernada M, Serna E, Bauerl C, Collado MC, Perez-Martinez G, Vento M. Genome-wide expression profiles in very low birth weight infants with neonatal sepsis. *Pediatrics.* 2014;133(5):e1203–e1211.

35 Ge Y, Zhao K, Qi Y, et al. Serum microRNA expression profile as a biomarker for the diagnosis of pertussis. *Mol Biol Rep.* 2013;40(2):1325–1332.

36 Gjøen JE, Jenum S, Sivakumaran D, et al. Novel transcriptional signatures for sputum-independent diagnostics of tuberculosis in children. *Scientific Reports.* 2017;7(1):5839.

37 Gómez-Carballa A, Cebey-López M, Pardo-Seco J, et al. A qPCR expression assay of IFI44L gene differentiates viral from bacterial infections in febrile children. *Scientific Reports.* 2019;9(1):11780.

38 Kaforou M, Herberg JA, Wright VJ, Coin LJM, Levin M. Diagnosis of bacterial infection using a 2-transcript host RNA signature in febrile infants 60 days or younger. *JAMA.* 2017;317(15):1577–1578.

39 Liu G, Liu W, Guo J. Clinical significance of miR-181a in patients with neonatal sepsis and its regulatory role in the lipopolysaccharide-induced inflammatory response. *Exp Ther Med.* 2020;19(3):1977–1983.

40 Pan D, Pan M, Xu YM. Mir-29a expressions in peripheral blood mononuclear cell and cerebrospinal fluid: diagnostic value in patients with pediatric tuberculous meningitis. *Brain Res Bull.* 2017;130:231–235.

41 Ng PC, Chan KYY, Yuen TP, et al. Plasma miR-1290 is a novel and specific biomarker for early diagnosis of necrotizing enterocolitis-biomarker discovery with prospective cohort evaluation. *J Pediatr.* 2019;205:83–90.e10.

42 Smith CL, Dickinson P, Forster T, et al. Identification of a human neonatal immune-metabolic network associated with bacterial infection. *Nature Communications.* 2014;5(1):4649.

43 Tornheim JA, Madugundu AK, Paradkar M, et al. Transcriptomic profiles of confirmed pediatric tuberculosis patients and household contacts identifies active tuberculosis, infection, and treatment response among Indian children. *J Infect Dis.* 2020;221(10):1647–1658.

44 Verhagen LM, Zomer A, Maes M, et al. A predictive signature gene set for discriminating active from latent tuberculosis in Warao Amerindian children. *BMC Genomics.* 2013;14:74.

45 Wang JX, Xu J, Han YF, Zhu YB, Zhang WJ. Diagnostic values of microRNA-31 in peripheral blood mononuclear cells for pediatric pulmonary tuberculosis in Chinese patients. *Genet Mol Res.* 2015;14(4):17235–17243.

46 Zhou M, Yu G, Yang X, Zhu C, Zhang Z, Zhan X. Circulating microRNAs as biomarkers for the early diagnosis of childhood tuberculosis infection. *Mol Med Rep.* 2016;13(6):4620–4626.

47 Salim RF, Sobeih AA, Abd El Kareem HM. Evaluation of the clinical value of circulating miR-101, miR-187 and miR-21 in neonatal sepsis diagnosis and prognosis. *Egyptian J Med Human Genetics.* 2020;21(1):12.

48 M K, S S, S M. Expression levels of candidate circulating microRNAs in pediatric tuberculosis. *Pathog Glob Health.* 2020;114(5):262–270.

49 Barry SE, Ellis M, Yang Y, et al. Identification of a plasma microRNA profile in untreated pulmonary tuberculosis patients that is modulated by anti-mycobacterial therapy. *J Infect.* 2018;77(4):341–348.

50 Mahle RE, Suchindran S, Henao R, et al. Validation of a host gene expression test for bacterial/viral discrimination in immunocompromised hosts. *Clin Infect Dis.* 2021;73(4):605–613.

51 Mendelsohn SC, Fiore-Gartland A, Penn-Nicholson A, et al. Validation of a host blood transcriptomic biomarker for pulmonary tuberculosis in people living with HIV: a prospective diagnostic and prognostic accuracy study. *Lancet Glob Health.* 2021;9(6):e841–e853.

52 Xu N, Hao F, Dong X, et al. A two-transcript biomarker of host classifier genes for discrimination of bacterial from viral infection in acute febrile illness: a multicentre discovery and validation study. *The Lancet Digital Health.* 2021;3(8):e507–e516.

53 Francisco NM, Fang YM, Ding L, et al. Diagnostic accuracy of a selected signature gene set that discriminates active pulmonary tuberculosis and other pulmonary diseases. *J Infect.* 2017;75(6):499–510.

54 Pan L, Liu F, Zhang J, et al. Genome-wide miRNA analysis identifies potential biomarkers in distinguishing tuberculous and viral meningitis. *Front Cell Infect Microbiol.* 2019;9:323.

55 Penn-Nicholson A, Mbandi SK, Thompson E, et al. RISK6, a 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment response. *Scientific Reports.* 2020;10(1):8629.

56 Cui JY, Liang HW, Pan XL, et al. Characterization of a novel panel of plasma microRNAs that discriminates between Mycobacterium tuberculosis infection and healthy individuals. *PLoS One.* 2017;12(9):e0184113.

57 Warsinske HC, Rao AM, Moreira FMF, et al. Assessment of validity of a blood-based 3-gene signature score for progression and diagnosis of tuberculosis, disease severity, and treatment response. *JAMA Netw Open.* 2018;1(6):e183779.

58 Berry MPR, Graham CM, McNab FW, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature.* 2010;466(7309):973–977.

59 Kelly RS, Lasky-Su J, Yeung SJ, et al. Integrative omics to detect bacteremia in patients with febrile neutropenia. *PLoS One.* 2018;13(5):e0197049.

60 Gliddon HD, Kaforou M, Alikian M, et al. Identification of reduced host transcriptomic signatures for tuberculosis disease and digital PCR-based validation and quantification. *Front Immunol.* 2021;12:637164.

61 Abd-El-Fattah AA, Sadik NAH, Shaker OG, Aboulftouh ML. Differential MicroRNAs expression in serum of patients with lung cancer, pulmonary tuberculosis, and pneumonia. *Cell Biochem Biophys.* 2013;67(3):875–884.

62 Bloom CI, Graham CM, Berry MP, et al. Transcriptional blood signatures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. *PLoS One.* 2013;8(8):e70630.

63 Darboe F, Mbandi SK, Naidoo K, et al. Detection of tuberculosis recurrence, diagnosis and treatment response by a blood transcriptomic risk signature in HIV-infected persons on antiretroviral therapy. *Front Microbiol.* 2019;10:1441.

64 de Araujo LS, Vaas LA, Ribeiro-Alves M, et al. Transcriptomic biomarkers for tuberculosis: evaluation of DOCK9. EPHA4, and NPC2 mRNA expression in peripheral blood. *Front Microbiol.* 2016;7:1586.

65 Ho J, Bokil NJ, Nguyen PTB, et al. A transcriptional blood signature distinguishes early tuberculosis disease from latent tuberculosis infection and uninfected individuals in a Vietnamese cohort. *J Infect.* 2020;81(1):72–80.

66 Jorge K, Souza RP, Assis MTA, et al. Characterization of Micro-RNA expression profiles and identification of potential biomarkers in leprosy. *J Clin Microbiol.* 2017;55(5):1516–1525.

67 Latorre I, Leidinger P, Backes C, et al. A novel whole-blood miRNA signature for a rapid diagnosis of pulmonary tuberculosis. *Eur Respir J*. 2015;45(4):1173–1176.

68 Lee SW, Wu LS, Huang GM, Huang KY, Lee TY, Weng JT. Gene expression profiling identifies candidate biomarkers for active and latent tuberculosis. *BMC Bioinformatics*. 2016;17(suppl 1):3.

69 Lei H, Xu X, Wang C, Xue D, Wang C, Chen J. A host-based two-gene model for the identification of bacterial infection in general clinical settings. *Int J Infect Dis*. 2021;105:662–667.

70 Li Z, Li C, Bao R, Liu Z. Expressions of miR-29a, TNF-οificance. *Iran J Public Health*. 2020;49(9):1683–1691.

71 Lydon EC, Henao R, Burke TW, et al. Validation of a host response test to distinguish bacterial and viral respiratory infection. *EBioMedicine*. 2019;48:453–461.

72 Maertzdorf J, McEwen G, Weiner 3rd J, et al. Concise gene signature for point-of-care classification of tuberculosis. *EMBO Mol Med*. 2016;8(2):86–95.

73 Mihret A, Loxton AG, Bekele Y, et al. Combination of gene expression patterns in whole blood discriminate between tuberculosis infection states. *BMC Infectious Diseases*. 2014;14(1):257.

74 Miotto P, Mwangoka G, Valente IC, et al. miRNA signatures in sera of patients with active pulmonary tuberculosis. *PLoS One*. 2013;8(11):e80149.

75 Ndzi EN, Nkenfou CN, Mekue LM, et al. MicroRNA hsa-miR-29a-3p is a plasma biomarker for the differential diagnosis and monitoring of tuberculosis. *Tuberculosis (Edinb)*. 2019;114:69–76.

76 Perumal P, Abdullatif MB, Garlant HN, et al. Validation of differentially expressed immune biomarkers in latent and active tuberculosis by real-time PCR. *Front Immunol*. 2020;11:612564.

77 Petrilli JD, Araújo LE, da Silva LS, et al. Whole blood mRNA expression-based targets to discriminate active tuberculosis from latent infection and other pulmonary diseases. *Scientific Reports*. 2020;10(1):22072.

78 Poore GD, Ko ER, Valente A, et al. A miRNA host response signature accurately discriminates acute respiratory infection etiologies. *Front Microbiol*. 2018;9:2957.

79 Roe J, Venturini C, Gupta RK, et al. Blood transcriptomic stratification of short-term risk in contacts of tuberculosis. *Clin Infect Dis*. 2020;70(5):731–737.

80 Satproedprai N, Wichukchinda N, Suphankong S, et al. Diagnostic value of blood gene expression signatures in active tuberculosis in Thais: a pilot study. *Genes Immunity*. 2015;16(4):253–260.

81 Sampson D, Yager TD, Fox B, et al. Blood transcriptomic discrimination of bacterial and viral infections in the emergency department: a multi-cohort observational validation study. *BMC Med*. 2020;18(1):185.

82 Serrano CJ, Cuevas-Cordoba B, Macias-Segura N, et al. Transcriptional profiles discriminate patients with pulmonary tuberculosis from non-tuberculous individuals depending on the presence of non-insulin diabetes mellitus. *Clin Immunol*. 2016;162:107–117.

83 Sivakumaran D, Ritz C, Gjoen JE, et al. Host blood RNA transcript and protein signatures for sputum-independent diagnostics of tuberculosis in adults. *Front Immunol*. 2020;11:626049.

84 Sodersten E, Ongarello S, Mantsoki A, et al. Diagnostic accuracy study of a novel blood-based assay for identification of tuberculosis in people living with HIV. *J Clin Microbiol*. 2021;59(3):e01643–20.

85 Wang C, Yang S, Liu CM, et al. Screening and identification of four serum miRNAs as novel potential biomarkers for cured pulmonary tuberculosis. *Tuberculosis (Edinb)*. 2018;108:26–34.

86 Wu B, Huang C, Kato-Maeda M, et al. Messenger RNA expression of IL-8, FOXP3, and IL-12beta differentiates latent tuberculosis infection from disease. *J Immunol*. 2007;178(6):3688–3694.

87 Wu J, Lu C, Diao N, et al. Analysis of microRNA expression profiling identifies miR-155 and miR-155* as potential diagnostic markers for active tuberculosis: a preliminary study. *Hum Immunol*. 2012;73(1):31–37.

88 Zhang Y, Zhang X, Zhao Z, Zheng Y, Xiao Z, Li F. Integrated bioinformatics analysis and validation revealed potential immune-regulatory miR-892b, miR-199b-5p and miR-582-5p as diagnostic biomarkers in active tuberculosis. *Microb Pathog*. 2019;134:103563.

89 Burel JG, Lindestam Arlehamn CS, Khan N, et al. Transcriptomic analysis of CD4(+) T cells reveals novel immune signatures of latent tuberculosis. *J Immunol*. 2018;200(9):3283–3290.

90 Sun X, Liu K, Wang X, Zhang T, Li X, Zhao Y. Diagnostic value of microRNA125b in peripheral blood mononuclear cells for pulmonary tuberculosis. *Mol Med Rep*. 2021;23(4).

91 Nabiel Y, Barakat G, Abed S. Serum CD64 and ascitic fluid calprotectin and microRNA-155 as potential biomarkers of spontaneous bacterial peritonitis. *Eur J Gastroenterol Hepatol*. 2019;31(8):1064–1069.

92 Dawany N, Showe LC, Kossenkov AV, et al. Identification of a 251 gene expression signature that can accurately detect M. tuberculosis in patients with and without HIV co-infection. *PLoS One*. 2014;9(2):e89925.

93 Mamishi S, Pourakbari B, Sadeghi RH, Marjani M, Mahmoudi S. Differential gene expression of ASUN, NEMF, PTPRC and DHX29: candidate biomarkers for the diagnosis of active and latent tuberculosis. *Infect Disord Drug Targets*. 2021;21(2):268–273.

94 Chen Z-l, Wei L-L, Shi L-Y, et al. Screening and identification of lncRNAs as potential biomarkers for pulmonary tuberculosis. *Scientific Reports*. 2017;7(1):16751.

95 de Araujo LS, Ribeiro-Alves M, Leal-Calvo T, et al. Reprogramming of small noncoding RNA populations in peripheral blood reveals host biomarkers for latent and active mycobacterium tuberculosis infection. *mBio*. 2019;10(6):e01037-19.

96 Huang Z, Su R, Qing C, Peng Y, Luo Q, Li J. Plasma circular RNAs hsa_circ_0001953 and hsa_circ_0009024 as diagnostic biomarkers for active tuberculosis. *Front Microbiol*. 2018;9:2010.

97 Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clin Infect Dis*. 2004;39(2):206–217.

98 Rutjes AW, Reitsma JB, Vandenbroucke JP, Glas AS. Bossuyt PM. Case-control and two-gate designs in diagnostic accuracy studies. *Clin Chem*. 2005;51(8):1335–1341.

99 Steinbrink JM, Zaas AK, Betancourt M, Modliszewski JL, Corcoran DL, McClain MT. A transcriptional signature accurately identifies Aspergillus Infection across healthy and immunosuppressed states. *Transl Res*. 2020;219:1–12.

100 Dincer C, Bruch R, Kling A, Dittrich PS, Urban GA. Multiplexed point-of-care testing - xPOCT. *Trends Biotechnol*. 2017;35(8):728–742.

101 Zhao S, Fung-Leung WP, Bittner A, Ngo K, Liu X. Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. *PLoS One*. 2014;9(1):e78644.

102 Kolodziejczyk AA, Lonnberg T. Global and targeted approaches to single-cell transcriptome characterization. *Brief Funct Genomics*. 2018;17(4):209–219.

103 Bartholomeus E, De Neuter N, Lemay A, et al. Diagnosing enterovirus meningitis via blood transcriptomics: an alternative for lumbar puncture? *J Transl Med*. 2019;17(1):282.

104 Obaro S. Updating the diagnosis of bacterial meningitis. *Lancet Infect Dis*. 2019;19(11):1160–1161.

105 Nielsen MV, Amemasor S, Agyekum A, et al. Clinical indicators for bacterial co-infection in Ghanaian children with P. falciparum infection. *PLoS One*. 2015;10(4):e0122139.