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Review

Distinguishing bacterial versus non-bacterial causes of febrile illness – A systematic review of host biomarkers

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SUMMARY

Background: Acute febrile illnesses (AFIs) represent a major disease burden globally; however, the paucity of reliable, rapid point-of-care testing makes their diagnosis difficult. A simple tool for distinguishing bacterial versus non-bacterial infections would radically improve patient management and reduce indiscriminate antibiotic use. Diagnostic tests based on host biomarkers can play an important role here, and a target product profile (TPP) was developed to guide development.

Objectives: To qualitatively evaluate host biomarkers that can distinguish bacterial from non-bacterial causes of AFI.

Data sources: The PubMed database was systematically searched for relevant studies published between 2015 and 2019.

Study eligibility criteria: Studies comparing diagnostic performances of host biomarkers in patients with bacterial versus non-bacterial infections were included.

Participants: Studies involving human participants and/or human samples were included.

Methods: We collected information following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. A risk of bias assessment was performed, based on a modified QUADAS-2 (Quality Assessment of Diagnostic Accuracy Score 2).

Results: We identified 1107 publications. Following screening, 55 publications were included, with 265 biomarker entries. Entries mostly comprised protein biomarkers (58.9%), followed by haematological, RNA, and metabolite biomarkers (15.5%, 8.7%, 12.5%). Sensitivity/specificity was reported for 45.7% of biomarker entries. We assessed a high overall risk of bias for most entries (75.8%). In studies with low/medium risk of bias, four biomarker entries tested in blood samples had sensitivity/specificity of more than 0.90/0.80. Only 12 additional biomarker entries were identified with sensitivity/specificity of more than 0.65/0.65.

Conclusions: Most recently assessed biomarkers represent well-known biomarkers, e.g. C-reactive protein and procalcitonin. Some protein biomarkers with the highest reported performances include a combined biomarker signature (CRP, IP-10, and TRAIL) and human neutrophil lipocalin (HNL). Few new biomarkers are in the pipeline; however, some RNA signatures show promise. Further high-quality studies are needed to confirm these findings.

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Introduction

Severe and non-severe fevers are a major cause of morbidity and mortality around the world and are one of the primary reasons patients seek healthcare services, both in high-income countries (HICs) and low- and middle-income countries (LMICs).¹ Fever, also referred to as acute febrile illness (AFI), may result from a variety

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of infectious or non-infectious causes. However, infections are the leading cause of AFI; particularly in LMICs, where AFI-caused infections represent a major disease burden for children.^{2,3} There is considerable heterogeneity, by geography, season, and comorbidities such as HIV infection, in the incidence and aetiology of AFIs of infectious origin.^{4,5} While the widespread use of rapid diagnostic tests for malaria has transformed the management of fevers in tropical settings, it has been accompanied by an increase in antibiotic prescriptions, as in the absence of further diagnostics malaria-negative patients with fever are often treated for bacterial infection.^{6,7} Unnecessary use of antibiotics is a major driver of antimicrobial resistance (AMR), a serious threat to global public health resulting in worse patient outcomes and increased health expenditure.^{7,8}

To reduce the indiscriminate use of antibiotics, a fever triage test capable of distinguishing between bacterial and non-bacterial infections is desirable. It is thought that one of the most promising solutions for a point-of-care (POC) diagnostic test to support triage of bacterial/non-bacterial cases of AFI is a test that measures the levels of host biomarkers. Ideally, such a test could be deployed in conjunction with integrated disease management guidelines.^{7,9} Certain host biomarkers are widely used in clinical practice in HICs, e.g. C-reactive protein (CRP) and procalcitonin (PCT).^{10–13} However, several studies have revealed that these biomarkers perform poorly in LMICs, mostly due to widespread malnutrition and high levels of co-infections in these settings, which themselves lead to increased CRP and PCT levels.^{3,9} Therefore, biomarkers that demonstrate good performance in LMICs are still required. Such tools could ultimately support patient care at the primary care level, indicate to healthcare providers if antibiotic treatment is needed, and identify a need for referral in cases of bacterial co-infections or patients with signs of severe infection. Furthermore, targeted antibiotic treatment for patients with AFI will reduce the long-term negative effects of antibiotic overuse, including avoidable health costs, adverse events, and AMR.

In 2016, a target product profile (TPP) was developed to assist in working towards a next-generation host biomarker test.⁹ A 2016 systematic review of the literature published between 2010 and 2015 summarised the performance data of host biomarkers for distinguishing bacterial from non-bacterial causes of AFI.³ The present systematic review of literature published between 2015 and 2019 expands on this work – incorporating the TPP criteria – and provides a novel view of the findings.

Methods

We conducted a systematic review of the PubMed database for literature published in English, between 1 January 2015 and 31 December 2019, and pertaining to host biomarkers differentiating bacterial from non-bacterial infections. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines,¹⁴ using similar methods and criteria to those employed previously,³ briefly outlined below.

Search strategy

A PubMed database search was conducted using a search strategy developed and validated based on key publications chosen by experts in the field. Any key publications identified by field experts not found in the PubMed search results were also included in this review (Supplementary Table S1 shows the full strategy).

Eligibility criteria

Study population

We included studies comparing the diagnostic performance of host biomarkers in patients with bacterial versus non-bacterial infections. We also captured studies that included other comparator groups, such as healthy participants, in addition to the bacterial/non-bacterial infection groups. Case reports were excluded, as were studies involving host biomarker comparisons with non-infectious diseases or studies investigating biomarkers of non-infectious conditions.

Biomarker, sample, and study types

Studies of host biomarkers included host proteins, RNA transcripts, biochemical reactions, and cellular processes; clinical signs and symptoms were excluded unless they were used in combination with host biomarkers or were part of an objective, computerised fever management algorithm. Studies of pathogen markers alone or in combination with host biomarkers were excluded. Studies involving human participants and/or human samples were included; animal studies and human tissue culture studies were excluded. Studies using biomarkers to answer research questions unrelated to using host biomarkers to differentiate AFIs of bacterial/non-bacterial origin were excluded. Only studies reporting the diagnostic performance of a quantitative biomarker (sensitivity/specificity or area under the receiver operating characteristic curve [AUROC]) or providing statistical significance were included.

Study screening, selection, and data extraction

All publications identified were stored in Endnote. Following de-duplication, one reviewer (EM) screened all publications by title and abstract prior to full-text screening. A second reviewer (BLFC) screened any studies with an unclear reason for inclusion.

Quality control and validation

Information was collected in a standardised manner (see Supplementary Table S2), similar to that used in a review of active tuberculosis biomarkers; data quality control and validation also followed this previously reported approach.¹⁵ Sensitivity/specificity values were recalculated and compared with reported values. We assessed study quality and risk of bias using a modified Quality Assessment of Diagnostic Accuracy Score 2 (QUADAS-2) tool. For simplicity and in line with related work,¹⁵ five quality items within three QUADAS-2 domains were used: study recruitment timing, study design, sampling, blinding of index test, and reference standard¹⁶ (Fig. 1). As per QUADAS-2 guideline, the selected questions were those deemed most relevant for identifying biases for those studies included in the review.

Biomarker entries

A biomarker entry was defined as either an individual biomarker or a signature (the latter comprising several biomarkers), together with performance data.¹⁵ A publication that reported performance data for several biomarkers resulted in an entry for each biomarker in that publication. Multiple performance data points for one biomarker in a publication led to multiple entries in that publication. No meta-analyses were performed due to high between-study heterogeneity and/or insufficient studies per biomarker.

Results

Search results

The database search and subsequent de-duplication of records identified 1107 publications. Most were excluded following abstract

Quality and bias of a publication									
The risk of bias assessment consisted of five selected quality items, representing three domains of QUADAS-2.									
Individual quality items									
Study recruitment timing		Study design		Sampling		Blinding of index test		Reference standard	
Prospective	Retrospective, unclear	Cohort, cross-sectional	Case-control, unclear	Random, consecutive	Convenience, unclear	Yes	No, unclear	Not clinical assessment only	Clinical assessment only, unclear
Low	High	Low	High	Low	High	Low	High	Low	High
Overall risk of bias									
≥ 4 quality items = Low			3 quality items = Low			≤ 2 quality items = Low			
Low			Medium			High			

Fig. 1. Criteria used to assess the quality and risk of bias of a publication. QUADAS: quality assessment tool for diagnostic accuracy studies. Individual quality items were scored as 'yes', 'no', or 'unclear'; then each study was assigned an overall risk of bias of 'low', 'medium', or 'high'.

screening (957/1107), leaving 150 publications for full-text screening; this elicited 55 studies for inclusion (Supplementary Fig. S1 shows PRISMA flow-chart of publication selection).

Entries per biomarker category

This review included 265 biomarker entries from 55 studies (full dataset available as Supplementary material). Entries were classified into: protein, haematological, RNA, metabolites, and signatures with combined biomarker types. Each category was sub-classified according to the type of quantitative diagnostic performance measures provided (sensitivity/specificity, AUROC, p-values only; Fig. 2).

Most entries were proteins (156/265, 58.9%), followed by haematological, RNA, and metabolites (41/265, 15.5%; 23/265, 8.7%; and 33/265, 12.5%, respectively). Sensitivity/specificity were reported for 121/265 (45.7%) entries, AUROC values without sensitivity/specificity were included for 54/265 (20.4%), statistical significance data only were available for 90/265 (34.0%).

Table 1 shows all biomarkers and signatures with three or more associated entries.

Study size

Just 20 biomarker entries (20/265, 7.5%) were evaluated in large studies (>500 samples or patients); 29/265 (10.9%) entries were assayed in sample sizes of 251 to 500; and 79/265 (29.8%) entries were tested in sample sizes of 100 to 250. Most studies were small (137/265, 51.7%), with entries evaluated on <100 samples.

Biomarkers with reported diagnostic performance

All biomarker entries with reported sensitivity and specificity (121/265, 45.7%) were plotted on a sensitivity–specificity scatter plot (Fig. 3),^{17–21 24 26–28,30,32,35–42,44,47,48,52–57,59,61–66,69}

Protein markers were the most frequent biomarker category for which sensitivity/specificity was reported. Study size varied in this biomarker category, as did sensitivity/specificity values. Some studies examining protein signatures (pink) reported higher sensitivity/specificity values than CRP, PCT, and other protein markers alone. RNA biomarkers were the next most frequently reported biomarker category with sensitivity/specificity data. Although RNA biomarker studies tended to be smaller than the other studies, they generally reported high sensitivity/specificity values.

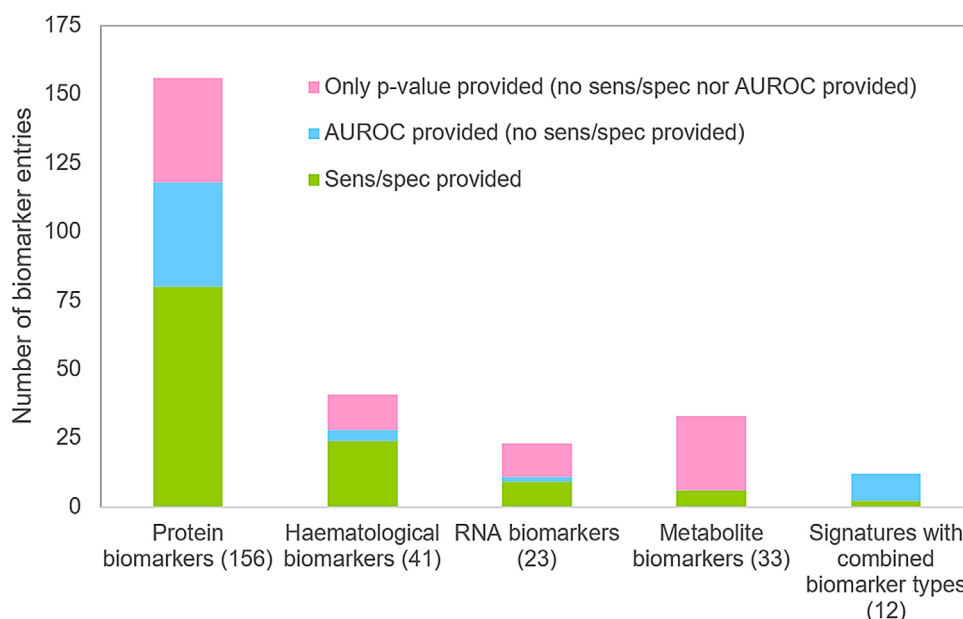


Fig. 2. Number of biomarker entries per biomarker category and subgroups based on performance measures. Biomarker entries represented in green reported sensitivity and specificity, in blue provided AUROC (and no sensitivity and specificity), and in pink included values of statistical significance only.^{17–71}

Table 1

Number of biomarker entries per biomarker or signature from 55 studies, subdivided based on performance measures.^{17–71} For example, CRP is the protein biomarker with the highest number (32) of entries. Sensitivity/specificity and/or AUROC values were reported in 22 CRP entries, and AUROC without sensitivity/specificity was provided for four. The other CRP entries reported only statistical significance.

Biomarker name	Number of markers	Number of entries	Reported S/C	No S/C, reported AUROC	No S/C, no AUROC, $p \leq 0.05$	No S/C, no AUROC, $p > 0.05$
<i>Protein biomarkers</i>						
CRP	1	32	22	4	5	1
PCT	1	21	16	3	2	0
IL-6	1	9	3	2	3	1
MxA	1	6	4	1	1	0
HNL	1	5	3	2	0	0
IL-10	1	3	1	1	1	0
IL-4	1	3	1	1	0	1
IL-2	1	3	1	1	1	0
Other protein biomarkers	1	56	20	15	12	9
CRP; IP-10; TRAIL	3	5	4	1	0	0
CRP; PCT	2	3	2	1	0	0
Signatures of protein biomarkers	2–3	10	3	6	1	0
<i>Haematological biomarkers</i>						
WBC	1	13	8	1	4	0
ANC	1	5	5	0	0	0
Neutrophils	1	5	3	1	0	1
PLT	1	3	1	0	1	1
Other haematological biomarkers	1	8	4	1	2	1
Signatures of haematological biomarkers	2	7	3	1	0	3
<i>RNA biomarkers</i>						
Single RNA transcript biomarkers	1	13	0	1	6	6
71-RNA transcript signature	71	5	5	0	0	0
Signatures of RNA biomarkers	2–66	5	4	1	0	0
<i>Metabolite biomarkers</i>						
Lactate	1	3	1	0	2	0
Other metabolite biomarkers	1	29	5	0	24	0
CSF glucose: blood glucose ratio	2	1	0	0	1	0
<i>Signatures with combined biomarker types (e.g. protein, clinical symptoms, haematology, and metabolite markers)</i>						
Signatures with combined individual biomarker types	2–4	12	2	10	0	0

Biomarkers with less than three biomarker entries were combined. The 'Number of markers' column denotes whether a biomarker is an individual biomarker or a signature. Within each biomarker category, individual biomarkers (top) and signatures (bottom) are organised by the number of biomarker entries (highest to lowest), thus reflecting the most frequently reported biomarkers. **Abbreviations:** ANC, absolute neutrophil count; AUROC, area under the receiver operating characteristic curve; CRP, C-reactive protein; CSF, cerebrospinal fluid; HNL, human neutrophil lipocalin; IL-6, interleukin 6; IL-10, interleukin 10; IL-4, interleukin 4; IL-2, interleukin 2; IP-10, interferon-gamma-inducible protein 10; MxA, myxovirus resistance protein 1; PCT, procalcitonin; PLT, platelets; S/C, sensitivity/specificity; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; WBC, white blood cells.

Fig. 4 shows the same 121 biomarker entries plotted by disease group.

The number of biomarker entries varied by disease group (Fig. 4). Febrile illness and pneumonia included a high number of entries (37 and 36, respectively), while few were seen for sepsis (6).

Biomarker categories varied by disease groups. Protein and haematological biomarkers comprised most biomarker entries for febrile illness (35/37, 95%), sepsis (6/6, 100%), and pneumonia (34/36, 94%), with CRP and PCT the most frequently evaluated protein biomarkers. RNA biomarkers comprised most entries for respiratory tract infections (7/13, 54%).

Sample types

Most biomarkers were measured in blood samples (190/265, 71.7%). However, 58 (21.9%) entries were measured exclusively in cerebrospinal fluid (CSF) and 11 (4.2%) in blood and CSF; all of these were focused on meningitis, neurological infections, and encephalopathy. One entry, measured in stool samples, focused on gastrointestinal diseases, and five entries in urine focused on leptospirosis.

Study quality of included biomarker entries

Overall risk of bias was high for most entries (201/265, 75.8%), primarily because of retrospective and case-control designs, a lack

of random/consecutive sampling, and a lack of blinding (Fig. 5). Few entries showed a low (33/265, 12.5%) or medium (31/265, 11.7%) overall risk of bias.

Most promising biomarkers

The objective here was to assist in the development of assays to differentiate bacterial from non-bacterial infections. Thus, we included biomarkers with the most potential for this application, i.e. entries from studies with low/medium risk of bias that met the TPP minimal performance criteria ($\geq 0.90/0.80$ sensitivity/specificity) in accessible, commonly collected specimen types (including blood, saliva, and urine, and excluding CSF and stool samples). Fig. 6 shows the performance of entries from all studies with low/medium overall risk of bias. Table 2 gives details of entries (plotted in Fig. 6) with high (meet current TPP minimum diagnostic performance criteria, i.e. ≥ 0.90 and 0.80 sensitivity/specificity) and moderate (≥ 0.65 and 0.65 sensitivity/specificity but not ≥ 0.90 and 0.80 sensitivity/specificity) performance. Sensitivity/specificity was not reported for any entries evaluated in urine and no entries were evaluated in saliva. Thus, all entries plotted in Fig. 6 and summarised in Table 2 were evaluated in blood.

Only four entries met the minimal TPP performance: CRP, the CRP, IP-10, and TRAIL signature, and two RNA signatures^{20,21,36,38} (Table 2). Even when considering entries with moderate performance, only 12 additional entries were identified (Table 2).

Table 2

Details of 16 biomarker entries (plotted in Fig. 6) evaluated in blood samples from studies with low or medium overall risk of bias and with either (1) high performance (meet the current TPP minimum diagnostic performance criteria, i.e. ≥ 0.90 and 0.80 sensitivity/specificity) or (2) moderate performance (≥ 0.65 and 0.65 and < 0.90 and 0.80 sensitivity/specificity).^{19–21,36–39,59} To avoid overlooking other potentially useful biomarkers, biomarker entries with medium or high performance evaluated in studies with a high risk of bias were also identified (see Supplementary Table S3). All biomarkers/signatures and their corresponding performances listed in the table are biomarker entries from unique publications. Performances are not summaries from all studies related with a given biomarker/signature.

Biomarker name	Number of markers	Biomarker category	Study	Sample size	Sensitivity	Specificity	Risk of bias	Disease group
<i>Biomarker entries with high performance</i>								
CRP	1	Protein	Dashti, 2017	39	0.91	1.00	Low	Meningitis
CRP; IP-10; TRAIL	3	Protein	Ashkenazi-Hoffnung, 2018	314	0.94	0.94	Low	Febrile illness; Respiratory tract infection
CRP; IP-10; TRAIL	3	Protein	Stein, 2018	108	0.93	0.91	Medium	Respiratory tract infection
RNA signature (FAM89A; IFI44L)	2	RNA biomarker	Herberg, 2016	51	1.00	0.96	Medium	Febrile illness
<i>Biomarker entries with moderate performance</i>								
CRP	1	Protein	Ashkenazi-Hoffnung, 2018	314	0.91	0.78	Low	Febrile illness; Respiratory tract infection
CRP	1	Protein	Zarkesh, 2015	195	0.82	0.90	Medium	Febrile illness/Severe bacteraemia
CRP	1	Protein	Van Houten, 2017	443	0.82	0.83	Medium	Febrile illness
CRP	1	Protein	Stein, 2018	124	0.79	0.97	Medium	Respiratory tract infection
HNL	1	Protein	Ashkenazi-Hoffnung, 2018	78	0.71	0.78	Low	Febrile illness; Respiratory tract infection
IL-17a	1	Protein	Liu, 2017	124	0.70	0.66	Medium	Pneumonia
IL-6	1	Protein	Zarkesh, 2015	195	0.79	0.92	Medium	Febrile illness/Severe bacteraemia
PCT	1	Protein	Van Houten, 2017	419	0.80	0.85	Medium	Febrile illness
PCT	1	Protein	Esposito, 2016	265	0.67	0.65	Low	Pneumonia
PDGF-BB	1	Protein	Liu, 2017	124	0.82	0.71	Medium	Pneumonia
CRP; IP-10; TRAIL	3	Protein	Van Houten, 2017	443	0.87	0.91	Medium	Febrile illness
ESR	1	Haematological marker	Dashti, 2017	46	0.86	0.68	Low	Meningitis

Abbreviations CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HNL, human neutrophil lipocalin; IFI44L, interferon-induced protein 44-like; IL-6, interleukin 6; IL-17a, interleukin 17a; IP-10, interferon-gamma-inducible protein 10; PCT, procalcitonin; PDGF-BB, platelet-derived growth factor homodimer BB; TPP, target product profile; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand.

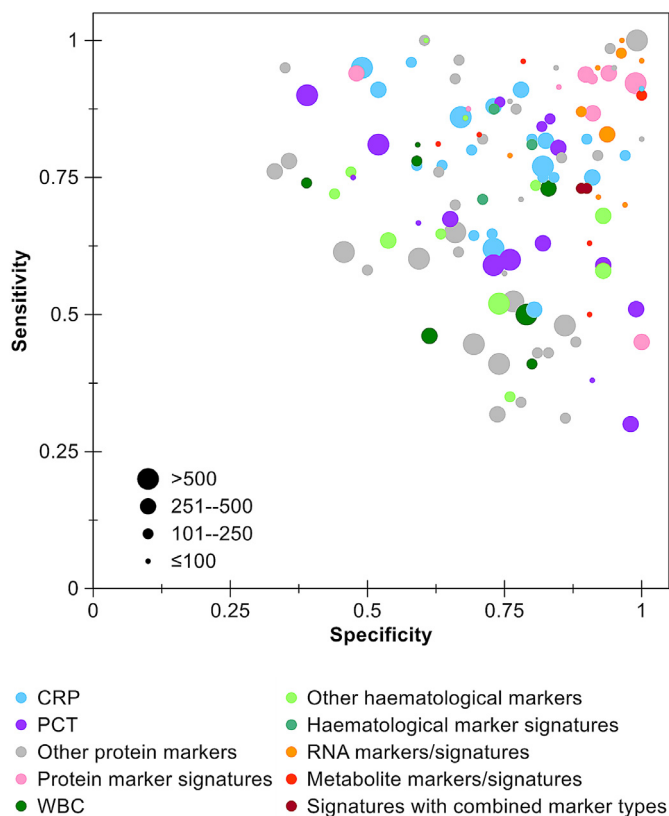


Fig. 3. Sensitivity–specificity scatter plot of 121 biomarker entries with reported sensitivity and specificity. Symbol colours represent different biomarker categories and the size of the symbol represents the study sample size. **Abbreviations** CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood cells.

Biomarker entries with $AUROC \geq 0.8$ without sensitivity/specificity are shown in Table S4. In low/medium risk of bias studies, 11 biomarker entries have $AUROC \geq 0.8$, all from the same study in the context of meningitis and evaluated in blood and CSF samples (Anahita Sanaei Dashti 2017). In high risk of bias studies, there are 28 biomarker entries mainly focused on meningitis (9/28), and febrile illnesses and respiratory tract infections (18/28).

Protein biomarkers

Three protein entries met the minimal TPP performance criteria and were evaluated in studies at low/medium risk of bias: one CRP entry in the context of meningitis, and two entries for the CRP, IP-10, and TRAIL signature in the context of febrile illnesses and respiratory tract infections.^{20,21,38}

CRP was the most extensively evaluated biomarker. One entry showed high performance in a study at low risk of bias while four and eight entries performed moderately in studies with low/medium and high risk of bias, respectively (Table 2 and Supplementary Table S3).^{19–21,24,27,32,37,41,52,66} CRP cut-off values used in these studies varied widely: ~ 20 (2 entries), ~ 40 (4), ~ 60 (1), and ~ 80 mg/L (5).^{19–21,24,27,32,38,41,52,66} PCT was another widely evaluated biomarker, with reported cut-off values of 0.19 (pneumonia), 0.50 (febrile illness), 0.88 (sepsis), and 0.99 ng/mL (sepsis).^{19,54,55,59}

Human neutrophil lipocalin (HNL)^{20,40,47} and some interleukins (IL-6, IL-17a, and the IL-6, IL-10 signature)^{37,39,65} demonstrated high and/or moderate performances in studies with different risk of bias levels (Table 2 and Supplementary Table S3). The myxovirus A (MxA) and CRP signature showed high performance (0.92/0.85 sensitivity/specificity, 0.94 AUROC) in a study at high risk of bias.³⁵ Another study involving this signature, with a high risk of bias, only reported AUROC (0.77).²⁵ Other protein entries with moderate

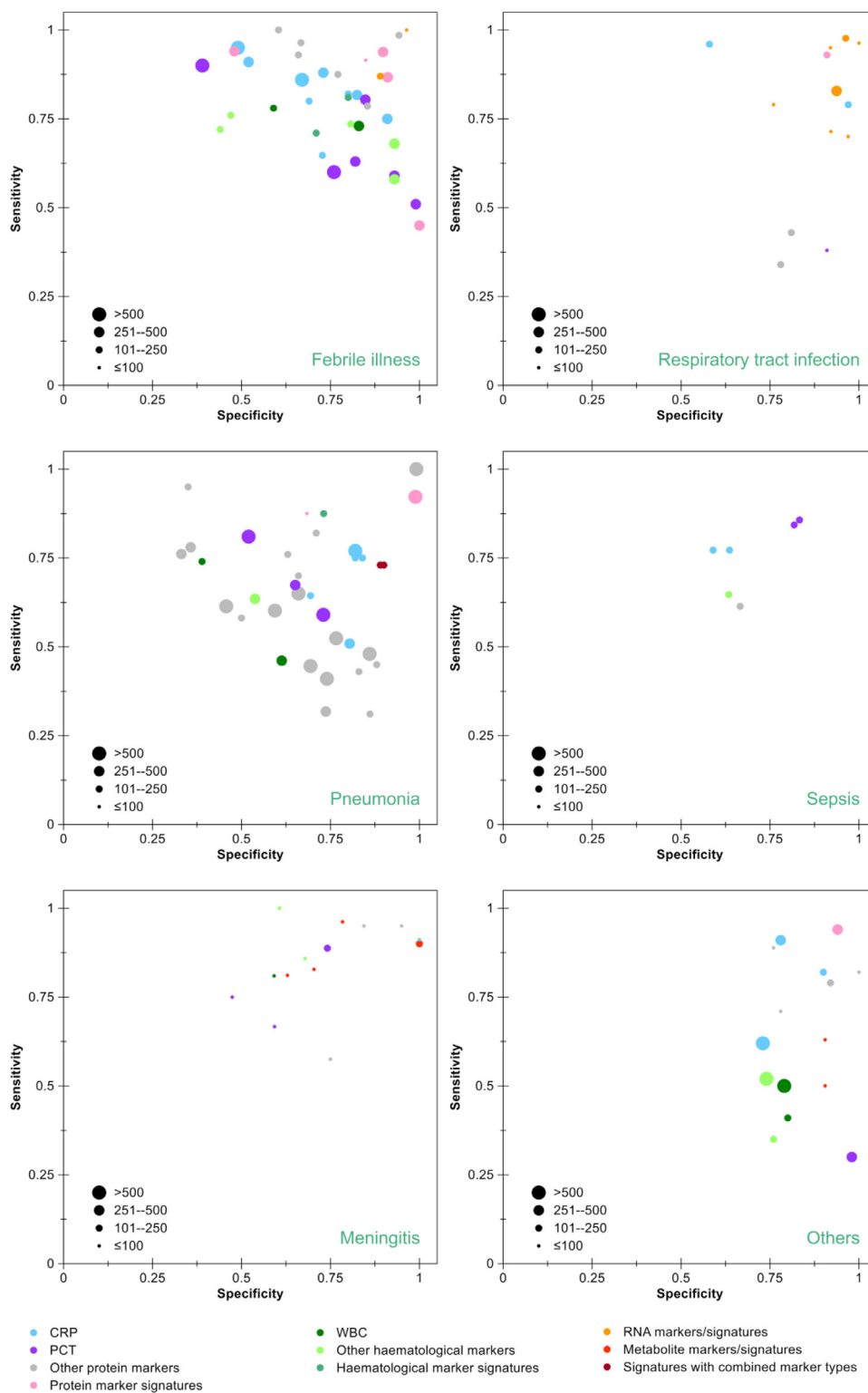


Fig. 4. Sensitivity–specificity scatter plots of 121 biomarker entries with reported sensitivity and specificity, clustered by disease group. Number of biomarker entries by disease group: febrile illness, 37; sepsis, 6; respiratory tract infection, 13; pneumonia, 36; meningitis/neurological infection, 14; and ‘others’, 15. (‘Others’ included dengue fever, leptospirosis, gastroenteritis, and combinations such as febrile illness and respiratory tract infections). Symbol colours represent different biomarker categories, and the size of the symbol represents the study sample size. **Abbreviations** CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood cells.

performances were platelet-derived growth factor homodimer BB (PDGF-BB)³⁹ and a three-protein signature (haptoglobin, IL-10, and tissue inhibitor of metalloproteinases 1 [TIMP1]),⁵⁷ which were evaluated for pneumonia.

Haematological biomarkers

No haematological biomarkers showed a high performance in differentiating bacterial from non-bacterial infections; how-

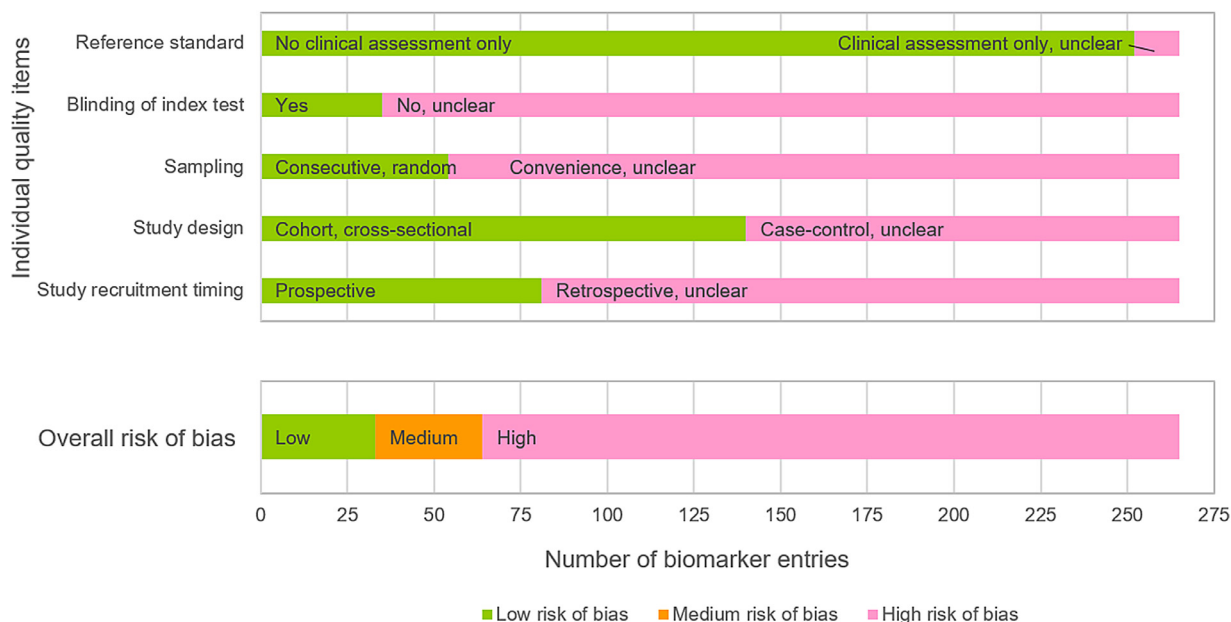


Fig. 5. Summary of the modified QUADAS-2 assessment for study quality and bias risk. Responses in pink and green represent high or low risk of bias, respectively. Bar lengths represent the proportion of answers to each question. Some studies are represented more than once if they reported multiple biomarker entries.

ever, several biomarkers/signatures displayed moderate performance (Table 2 and Supplementary Table S3).^{24,38,40,61,66}

RNA biomarkers

The only RNA biomarker with high performance evaluated in a study with low risk of bias was the FAM89A and IFI44L RNA signature.³⁶ Three gene-expression studies with high risks of bias reported RNA signatures comprising 10, 11, and 71 transcripts that demonstrated high/moderate performances with respiratory tract infections^{44, 62, 64}; a 7-RNA transcript signature with AUROC 0.91 (no sensitivity/specificity data provided) was also reported in a study with a high overall risk of bias (Supplementary Table S4).⁵⁸

Biomarkers and signatures with low performance

Table 1 includes biomarkers with non-significant p-values ($p > 0.05$). For several of the biomarkers, some studies reported a significant result and others did not. This was the case for CRP (5/1, number of studies in which $p \leq 0.05/p > 0.05$); IL-6 (3/1); polymorphonuclear neutrophils (PMN) (3/1); and platelets (PLT) (1/1).

Fig. 3 shows all biomarker entries with a reported sensitivity/specificity. Biomarkers from each category appear in the low-performance area (< 0.65 or 0.65 sensitivity/specificity). Entries evaluated in studies with relatively large sample sizes (> 250) and low performances included CRP, PCT, neutrophils, and white blood cells (WBCs) (Supplementary Table S5).

Discussion

This review identified 265 biomarker entries, from 55 studies published from January 2015 through December 2019, that were reported for use in discriminating bacterial from non-bacterial infections. The most frequently evaluated host biomarkers identified in this work were CRP, PCT, IL-6, and WBCs, similar to findings from an earlier review.³ Protein and RNA signatures were the best performing entries. The CRP, IP-10, and TRAIL signature was the only entry with high performance evaluated in studies with low/medium risk of bias and > 250 patients or samples. This three-protein signature has been evaluated multiple times in HIC

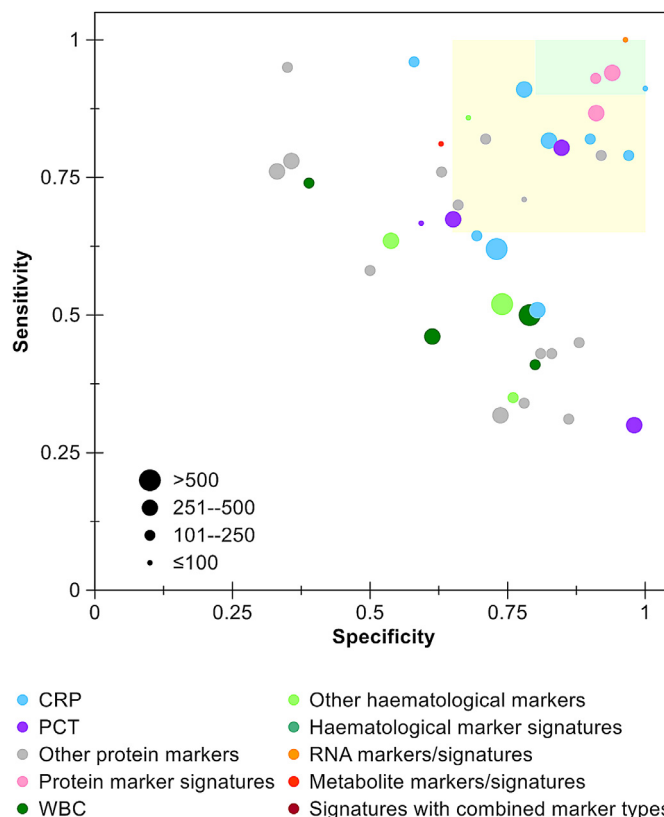


Fig. 6. Sensitivity-specificity scatter plot for a selection of 16 biomarker entries (from Fig. 3) evaluated in blood samples, from studies with low or medium overall risk of bias and with moderate or high performance. The symbol colours represent different biomarker categories, and the size of the symbol represents the study sample size. The yellow and green shaded areas represent moderate and high performance, respectively. **Abbreviations** CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood cells.

in ELISA format and showed high levels of performance.^{19–22,66} MeMed Diagnostics utilizes this signature in a POC device, MeMed BV™.⁷² HNL, the MxA and CRP signature, and the IL-6 and IL-10 signature were evaluated in studies with high risk of bias.^{35,40,65} These biomarkers and signatures should be investigated in studies with low risk of bias, particularly because AgPlus Diagnostics is developing a POC test based on HNL,⁷³ which could be suitable for LMICs. The MxA and CRP signature is utilised by the FebriDx® test (Lumos Diagnostics) to provide qualitative results on CRP and MxA levels in capillary blood.⁷⁴ The CRP, IP-10, and TRAIL signature, the MxA and CRP signature, and HNL were evaluated in the context of febrile illness and in studies specifically designed to differentiate bacterial from non-bacterial infections, in line with the TPP. The IL-6 and IL-10 signature was evaluated in the context of pneumonia and it would be interesting for it to be evaluated within additional illnesses. Most biomarker entries were evaluated in laboratories using research techniques (ELISA, cytometry, microarrays, etc.) and in few studies. Ideally, for all promising protein-based biomarkers/signatures highlighted here, independent studies should be performed using POC tests suitable for deployment in settings with poorly accessible healthcare.

Other high performing entries were three RNA signatures comprising 2, 10, and 71 RNA-transcript signatures. These signatures were evaluated in the context of febrile illness (2-RNA transcript signature) and respiratory tract infections (10 and 71-RNA transcript signatures). It would be desirable to evaluate the latter signatures for other febrile illnesses, in addition to respiratory tract infections. Although RNA signatures are promising, they are currently less likely to be developed into rapid, low-cost POC tests accessible to LMICs; additionally, the more RNA markers needed to comprise a signature, the more costly and complex it is to translate it into a POC test. It is therefore important to determine whether the diagnostic performances of these signatures remain acceptable using fewer RNA transcripts. Furthermore, it would be interesting to determine whether any of these RNA signatures contain protein-encoding RNAs; these proteins could potentially then be tested as diagnostic targets, e.g. protein based lateral flow assays, making them more suitable as low-cost POC tests.

Other notable biomarkers included widely evaluated biomarkers such as CRP and PCT, as well as the moderately performing biomarkers platelet-derived growth factor BB homodimer (PDGF-BB) and a three-marker signature (haptoglobin; IL-10; and tissue inhibitor of metalloproteinases 1 [TIMP1]), which should be assessed in studies with lower risks of bias and larger sample sizes.

Biomarker entries were subcategorised by disease, given that some biomarkers might perform better with certain diseases. It is challenging to identify host biomarkers for differentiating bacterial from non-bacterial infections in the general febrile illness population as this population encompasses a broad group of conditions; therefore, identifying biomarkers at the required standard of performance for the TPP may be over-ambitious. However, in this review, some biomarkers tested in specific disease groups (e.g. pneumonia) did not show an improved performance compared with the results of the same biomarker in the general febrile illness population.

A previous review³ highlighted seven strong performing biomarkers: the CRP, IP-10, and TRAIL signature; HBP, PCT, and a 10-gene classifier signature; PMN counts; a 48-gene classifier signature; a CD35, CD32, CD88, and MHC1 signature; MxA; and IL-4. Of which only the CRP, IP-10, and TRAIL signature continues to be reported as a high-performing biomarker, while MxA showed moderate performance in a study with a high risk of bias, and IL-4 showed low performance. The other four biomarkers/signatures were not evaluated in the current review. Hence, the recommendation to further assess any strongly performing biomarkers identified in the previous review remains valid.

Most entries were evaluated in hospital settings in HICs, with only around one-third evaluated in LMICs. The scarcity of rapid diagnostic tests in LMICs means AFIs tend to be indiscriminately treated with antibiotics, leading to increased AMR.³ Of the promising biomarkers, the CRP, IP-10, and TRAIL signature, the MxA and CRP signature, and the 2, 10, and 71-RNA transcript signatures were evaluated in HICs, while HNL and IL-6 were tested in an LMIC (China). Applying biomarker that have been studied in HIC to a LMIC can be misleading due to the different patients' demographics (immune function, co-infections, nutrition status, age groups, etc.). Further multi-site clinical evaluations in LMICs, using POC tests when available, is desirable for all promising biomarkers.

Most studies identified in this review were of low quality based on QUADAS criteria, with a high overall risk of bias for 75.8% of entries. There were various reasons for this. First, because of retrospective and case-control designs, a lack of random/consecutive sampling, and a lack of blinding. More blinded, prospective studies should be conducted, with consecutive or random sampling, and cohort or cross-sectional study designs. Second, incomplete results were reported for many entries, and only p-values (and no sensitivity/specificity or AUROC data) were reported for 34.0% of entries. AUROC values and sensitivity/specificity values are essential when describing diagnostic performance. Third, the reference methods used to determine whether patients had bacterial or non-bacterial infections varied and were poorly defined, adding further complexity. Additionally, most entries were evaluated in studies with small sample sizes (51.7% of entries were evaluated in <100 samples/patients), increasing the probability of recruiting unrepresentative study populations.

When matching the TPP⁹ with the findings of this systematic review, we identified 12 entries that met the minimum performance criteria. Only one of the high performing biomarker entries was evaluated in a study at low/medium risk of bias and with >250 samples or patients. Based on evidence collected thus far in this and prior systematic reviews, the performance requirements of this ambitious TPP appear to be unachievable, at least in the short-term. Therefore, the TPP might need revisiting and reassessed. Host biomarker tests may need to form part of a suite of tools, including good clinical guidance and training, to ensure any potential lack of accuracy of such tests is acknowledged and considered when making any diagnosis or decision relating to patient management.

Limitations

While this comprehensive review has many strengths, there are some limitations. First, the time frame was limited to January 2015 to December 2019. Literature from 2010 to 2015 was reviewed previously.³ The current review is complementary to the 2016 publication, providing data over a longer timeframe. COVID-19 pandemic started after the time frame of this study and thus articles related with COVID-19 are not included in this review. Second, the study quality assessment method we used was based on five QUADAS-2 questions deemed most relevant; therefore, other biases could have been overlooked, although we consider the included questions covered the most critical risks. If information to assess specific quality criteria was not reported or was insufficiently or poorly described, then we assumed the study did not meet those criteria. Therefore, it is possible that the number of low-quality studies was over-estimated due to poor quality reporting rather than poor quality methodology.

Conclusions and outlook

This review sought to identify promising host biomarkers for distinguishing between bacterial and non-bacterial infections; it is

hoped this will help guide future research and development of novel biomarker tests. Interestingly, the best known and most frequently assessed biomarkers (e.g. CRP, PCT, WBCs, and neutrophils) are not the best performing ones but they do represent those most frequently used in routine care and hence are the most accessible. The most promising recently reported protein biomarkers are the CRP, IP-10, and TRAIL signature, the MxA and CRP signature, the IL-6 and IL-10 signature, and HNL. Several RNA signatures demonstrated high performance, although rapid POC tests based on these signatures may be prohibitively expensive for many LMICs. There is a clear need for more quality studies to be conducted, in both LMICs and HICs, to fully explore the potential of host biomarkers or combinations in distinguishing bacterial from non-bacterial causes of acute febrile illness.

Contributors

SD conceived the study. BLF-C, CE, EM, and AK collected the data. BLF-C prepared the first draft of the manuscript. All authors contributed to the final version of the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Access to data

Collected information in a standardized manner from the 55 studies included in this work provided as Supplementary material.

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Supplementary materials

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