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Original Article

A host signature based on TRAIL, IP-10, and CRP for reducing antibiotic overuse in children by differentiating bacterial from viral infections: a prospective, multicentre cohort study

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ABSTRACT

Objectives: Identifying infection aetiology is essential for appropriate antibiotic use. Previous studies have shown that a host-protein signature consisting of TNF-related apoptosis-induced ligand (TRAIL), interferon-γ-induced protein-10 (IP-10), and C-reactive protein (CRP) can accurately differentiate bacterial from viral infections.

Methods: This prospective, multicentre cohort study, entitled AutoPilot-Dx, aimed to validate signature performance and to estimate its potential impact on antibiotic use across a broad paediatric population (>90 days to 18 years) with respiratory tract infections, or fever without source, at emergency departments and wards in Italy and Germany. Infection aetiology was adjudicated by experts based on clinical and laboratory investigations, including multiplex PCR and follow-up data.

Results: In total, 1140 patients were recruited (February 2017–December 2018), of which 1008 met the eligibility criteria (mean age 3.5 years, 41.9% female). Viral and bacterial infections were adjudicated for 628 (85.8%) and 104 (14.2%) children, respectively; 276 patients were assigned an indeterminate reference standard outcome. For the 732 children with reference standard aetiology, the signature discriminated bacterial from viral infections with a sensitivity of 93.7% (95%CI 88.7–98.7), a specificity of 94.2% (92.2–96.1), positive predictive value of 73.0% (65.0–81.0), and negative predictive value of 98.9% (98.0–99.8); in 9.8% the test results were equivocal. The signature performed consistently across different patient subgroups and detected bacterial immune responses in viral PCR-positive patients.

Meeting presentation: The material was presented in part at the 2020 and 2021 virtual meetings of the European Society for Paediatric Infectious Diseases.

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Conclusions: The findings validate the high diagnostic performance of the TRAIL/IP-10/CRP signature in a broad paediatric cohort, and support its potential to reduce antibiotic overuse in children with viral infections. **Cihan Papan, Clin Microbiol Infect 2021;▪:1**

Introduction

Antimicrobial resistance (AMR) is a growing global healthcare problem [1,2]. Antibiotic misuse—e.g. for viral respiratory tract infections (RTIs) [3]—is considered a driver of AMR [4]. Clinical characteristics alone are insufficient for correctly diagnosing the aetiology of infectious diseases, particularly in children [5]. This diagnostic uncertainty leads to antibiotic overuse [6–8], as well as underuse (i.e. withheld or delayed prescription when an antibiotic is warranted [9]).

In a proteomics-based study focusing on the host immune response, Oved and colleagues demonstrated the sensitivity and specificity of a novel signature for differentiating bacterial infections from viral infections. The signature integrates the circulating levels of three proteins: tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), interferon-γ-induced protein-10 (IP-10), and C-reactive protein (CRP) [10–16].

The goals of the present study (AutoPilot-Dx) were to: (a) prospectively validate the performance and generalizability of this host signature in a broad paediatric population with RTI or fever without source (FWS), and (b) retrospectively estimate the potential to impact antibiotic use by assessing discrepancy between the host signature result and recorded antibiotic prescribing practice.

Methods

Study design, setting and population

AutoPilot-Dx was a multicentre, prospective, cohort study conducted at two University Hospitals in Germany and Italy. Eligible subjects were children aged between 90 days and 18 years presenting consecutively to the paediatric emergency department and meeting the following criteria: clinically suspected RTI or FWS, body temperature of ≥38.0°C measured at home or at the ED, and history of illness ≤7 days. Patients who met one or more of the following criteria were excluded: another febrile episode during the previous 2 weeks, antibiotic treatment of over 48 hours, proven/suspected HIV/HBV/HCV infection, primary immunodeficiency, active malignancy, immunosuppressive/immunomodulatory treatment, severe psychomotor retardation, and severe congenital metabolic disorders. The STARD checklist is described in the Supplementary Material (Table S1).

Ethical committee approvals were obtained prior to the study (2016-410M-MA-§ 23b MPG; 10042/17/AV/DM CEAS Umbria). Informed consent was obtained from all patients' parents. The study was registered at clinicaltrials.gov (NCT03052088). The full protocol, including the statistical analysis plan, is available in the Supplementary Material.

Study procedures

In addition to standard-of-care work-up, participants had a study-specific nasopharyngeal swab taken upon enrolment.

Multiplex PCR measurements

Nasopharyngeal swabs underwent multiplex PCR for viral and bacterial pathogens (Allplex™ Respiratory Panel, Seegene, Seoul, Republic of Korea).

Follow-up

A follow-up telephone interview with the participants' parents was conducted 30 days after enrolment, including questions about medical status, relapses, antibiotic use, and school/childcare/parental work absence.

Host signature outcome

Host signature measurements were performed on serum samples according to the manufacturer's instructions (ImmunoXpert™, MeMed, Israel) at the study sites on a Freedom EVO® 75 platform (Tecan, Switzerland); those performing the measurements were blinded to the clinical diagnosis and the result of the reference standard. The signature computationally integrates the blood concentrations of TRAIL, IP-10, and CRP using a locked algorithm, generating a 0–100 score. The predetermined score cut-offs provided by the manufacturer in accordance with its Conformité Européenne In-vitro Diagnostic (CE-IVD) label were used to classify outcomes: <35 for viral infections, >65 for bacterial infections, 35–65 being deemed equivocal. The test is CE marked for use in patients aged ≥90 days with suspicion of acute bacterial or viral infection.

Reference standard outcome

In the absence of a single 'gold standard', a panel of independent adjudicators were employed to generate the reference standard as previously described [15,17]. To ensure quality, adjudicators were paediatricians with >10 years of clinical experience. Adjudicators were blinded to the signature outcome and to their peers to prevent bias, and had access to all data compiled in an electronic case report form for each patient. Adjudicators were provided with a short module explaining the adjudication process (Supplementary Material). Each study patient was assigned a reference standard outcome based on adjudication as follows. Each adjudicator was asked to assign one label, based on the time point of sample collection, out of the following options: bacterial (including bacterial/viral coinfection), viral, indeterminate, or non-infectious aetiology. Unlike previous studies, they additionally indicated their confidence level: high (>90%), moderate (70–90%), or unsure (<70%). For inclusion in the analysis study cohort, a reference standard outcome was required whereby all three adjudicators unanimously assigned viral or bacterial labels with moderate or high confidence level; the remaining cases were considered 'indeterminate'.

Additional analyses were performed on a 'highly confident' subcohort, for which a reference standard outcome required a unanimous label with a high confidence level, on a 'majority' cohort, for

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which a reference standard outcome required only two of the three adjudicators to assign the same label, and on a 'microbiologically confirmed' sub-cohort (Supplementary Material: Methods).

Statistical analysis

The primary endpoint was the diagnostic performance of the signature against the reference standard, based on calculations of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio, negative likelihood ratio, with bacterial infection considered 'positive'. For the secondary endpoint, the diagnostic performance of the signature was compared to other biomarkers at predetermined cut-offs, as follows: CRP at 20 and 80 mg/L, procalcitonin (PCT) at 0.5 ng/mL, white blood cell count (WBC) at 15 000 cells/μL, and absolute neutrophil count (ANC) at 10 000 cells/μL.

The diagnostic performance of the signature against the reference standard was also assessed using a 5-bin analysis, whereby the patients were classified into five bins according to signature score (Supplementary Material). For each bin, likelihood ratio (LR) was defined as the ratio between the bacterial prevalence versus the viral prevalence in the bin. Cochrane Armitage test was used to establish the significance of the increasing bin trend.

Subgroup analyses included analyses by age groups, hospital admission, symptom onset, clinical syndromes, and the subcohorts mentioned above. The intended sample size was calculated as 1142 patients based on the following assumptions: a distribution between bacterial and viral infections within a paediatric cohort of 1:3, reaching a unanimous adjudicator panel consensus in 65% of cases, and the signature yielding an equivocal score in 10% of cases.

Python 3.7.6. was used for statistical analyses. Patient characteristics were compared using Mann–Whitney U test and t test for continuous variables, and χ^2 and Fisher's exact test for categorical data. Clopper–Pearson confidence intervals (CIs) were used when the observed performance was 100%, otherwise normal approximation intervals were used. The statistical significance level was set at 0.05.

Results

Patient characteristics

During the study period (February 2017 to December 2018), 1140 patients were recruited, of whom 1077 met the inclusion and exclusion criteria (Fig. 1). Of these, 69 were excluded due to an

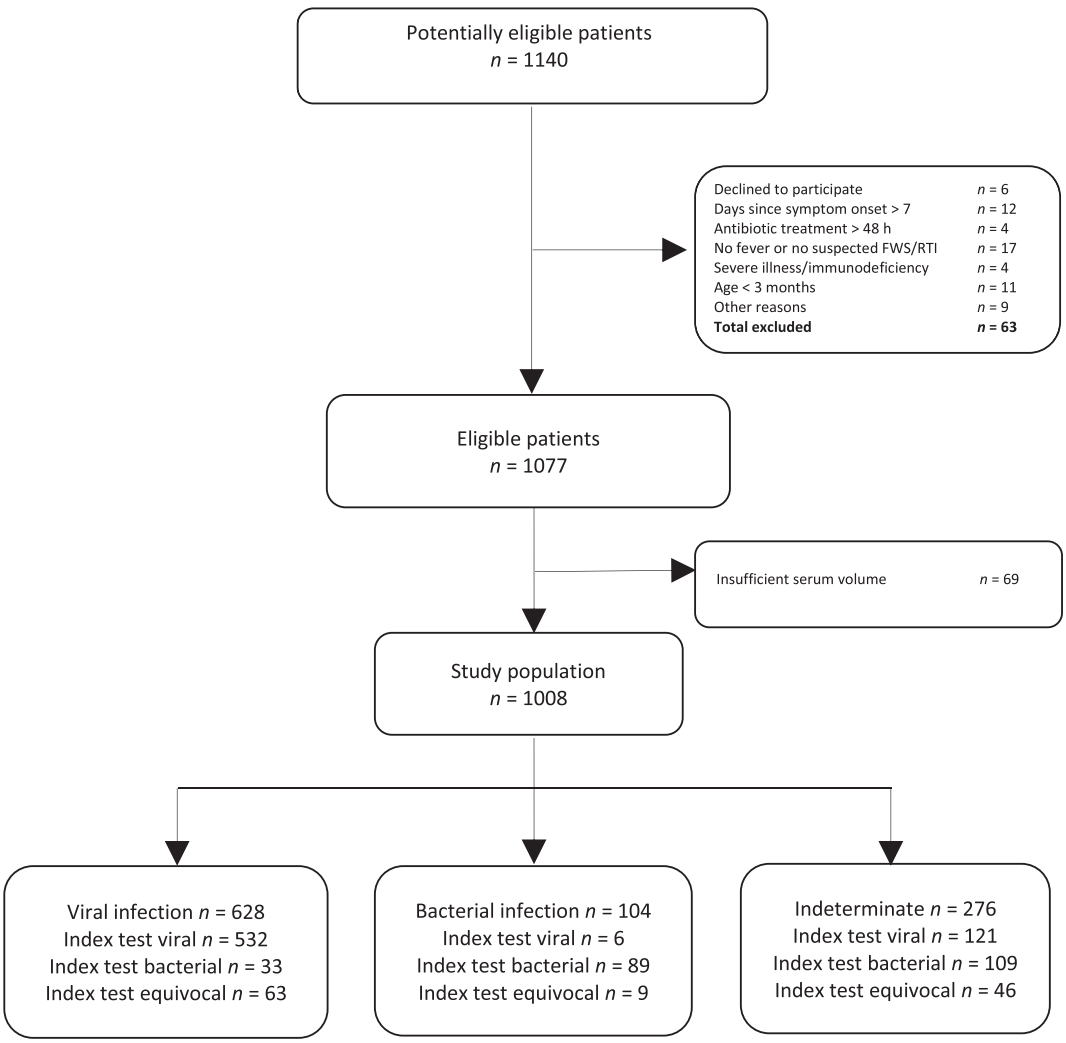

Fig. 1. Flow diagram of the study population.

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insufficient amount of serum, resulting in a study population of 1008. Application of the reference standard led to 628 subjects (62.3%) assigned as viral, 104 (10.3%) as bacterial and 276 (27.4%) as indeterminate cases.

The mean (SD) age of the study population was 3.5 (3.6) years; 41.9% were female (Table 1). Viral PCR detections were observed both in patients with viral (76.9%) and bacterial aetiology (45.2%). Antibiotics were prescribed to 186/628 (29.6%) and 102/104 (98.1%) of the children with viral and bacterial infection, respectively.

Host-protein signature performance

In the cohort for analysis that included the cases assigned bacterial or viral adjudication labels ($n = 732$, equivocal signature result in 9.8%), the signature attained a sensitivity of 93.7% (95% confidence interval 88.7–98.7) and a specificity of 94.2% (92.2–96.1), with negative predictive value of 98.9% (98.0–99.8) and positive predictive value of 73.0% (65.0–81.0). The positive likelihood ratio was 23.1 (15.0–35.5), the negative likelihood ratio was 0.03 (0.001–0.11). There were six children with bacterial reference standard outcomes who received viral signature scores (false negatives); clinical and laboratory data are presented in Supplementary Material Table S2.

Diagnostic performance of the signature based on 5-bin analysis of children with bacterial or viral infection

To examine whether the likelihood of bacterial infection increases with signature score, a bin analysis was performed. The higher the score, the higher was the likelihood of a bacterial infection (Supplementary Material Table S3). Of note, more than 60% of the patients attained high confidence signature results as indicated by either very low scores (0–10) or very high scores (90–100).

Signature performance is consistent across different patient subgroups

The signature performance was evaluated across subgroups of the analysis cohort. Sensitivity and specificity were retained across different age groups, clinical syndromes, and pathogens (Supplementary Material Table S4). Notably, the signature yielded high sensitivity and specificity irrespective of time from symptom onset, demonstrating a clearer discrimination between bacterial and viral cases over illness duration as compared to CRP or PCT (Supplementary Material Figs S1 and S2).

In addition, the signature attained sensitivity of 97.2% (93.4–100.0%) and specificity of 95.8% (94.0–97.6%) across the

Table 1
Characteristics of the study cohort for analysis

	Viral ($n = 628$)	Bacterial ($n = 104$)	Indeterminate ($n = 276$)	All ($n = 1008$)	p
Age in years (mean, SD)	3.3 (3.5)	5.2 (4.1)	4.1 (3.7)	3.5 (3.6)	<0.001
Gender, female (*n*, %)	255.0 (40.6)	52.0 (50.0)	127.0 (46.0)	307.0 (41.9)	0.104
Max temperature in C (mean, SD)	39.2 (0.8)	39.5 (0.8)	39.3 (0.9)	39.2 (0.8)	<0.001
Symptoms:					
Fever duration (mean, SD)	2.1 (1.8)	2.5 (1.7)	2.2 (1.7)	2.2 (1.8)	0.002
Cough (*n*, %)	42.0 (6.7)	7.0 (6.7)	20.0 (7.2)	49.0 (6.7)	0.999
Dyspnoea (*n*, %)	107.0 (17.0)	14.0 (13.5)	54.0 (19.6)	121.0 (16.5)	0.526
Wheezing (*n*, %)	97.0 (15.4)	7.0 (6.7)	42.0 (15.2)	104.0 (14.2)	0.046
Rhinorrhoea (*n*, %)	173.0 (27.5)	12.0 (11.5)	70.0 (25.4)	185.0 (25.3)	<0.001
Pharyngitis (*n*, %)	480.0 (76.4)	75.0 (72.1)	207.0 (75.0)	555.0 (75.8)	0.856
Tonsillitis (*n*, %)	183.0 (29.1)	33.0 (31.7)	112.0 (40.6)	216.0 (29.5)	<0.001
Positive viral PCR result (*n*, %)	483 (76.9)	47 (45.2)	163 (59.1)	693 (68.8)	<0.001
WBC (mean, SD)	10.8 (5.0)	19.5 (8.3)	14.8 (5.9)	12.8 (6.3)	<0.001
ANC (mean, SD)	6.4 (4.1)	14.8 (8.0)	9.6 (4.7)	8.2 (5.5)	<0.001
PCT (median, IQR)	0.2 (0.3)	2.3 (5.4)	0.4 (1.0)	0.3 (0.7)	<0.001
CRP (median, IQR)	11.6 (19.2)	170.0 (145.1)	56.0 (71.5)	20.8 (52.0)	<0.001
Diagnostic workup (*n*, %)					
Urine culture obtained	8 (1.3)	23 (22.1)	22 (8.0)	53 (5.3)	<0.001
Blood culture obtained	223 (35.5)	51 (49.0)	101 (36.6)	375 (37.2)	0.015
Chest radiograph obtained	59 (9.4)	56 (53.8)	91 (33.0)	206 (20.4)	<0.001
Other culture obtained	42 (6.7)	17 (16.3)	31 (11.2)	90 (8.9)	<0.001
Antibiotics initiated	186 (29.6)	102 (98.1)	221 (80.1)	509 (50.5)	<0.001
Disposition (*n*, %)					
Admitted	436 (69.4)	97 (93.3)	218 (79.0)	751 (74.5)	<0.001
Discharged	192 (30.6)	7 (6.7)	58 (21.0)	257 (25.5)	<0.001
Hospital length of stay in days (median, IQR)	2.0 (4.0)	6.0 (3.2)	4.0 (3.5)	3.0 (5.0)	<0.001
Discharge diagnosis (*n*, %)					
LRTI[a]	168 (26.8)	51 (49.0)	101 (36.6)	320 (31.7)	<0.001
URTI[b]	292 (46.5)	17 (16.3)	111 (40.2)	420 (41.7)	<0.001
FWS	14 (2.2)	1 (1.0)	9 (3.3)	24 (2.4)	0.584
Unspecified viral infection[c]	115 (18.3)	0 (0.0)	11 (4.0)	126 (12.5)	<0.001
UTI	2 (0.3)	20 (19.2)	14 (5.1)	36 (3.6)	<0.001
Other[d]	11 (1.8)	8 (7.7)	14 (5.1)	33 (3.3)	<0.001
Child education absence (median days, IQR)	0.0 (5.0)	2.5 (8.0)	1.0 (5.0)	1.0 (5.0)	0.005
Parent work absence (median days, IQR)	0.0 (2.0)	0.0 (3.0)	0.0 (3.0)	0.0 (2.0)	0.112

Values of p are given for the comparisons between viral and bacterial infection.
WBC, white blood cells; ANC, absolute neutrophil count; PCT, procalcitonin; CRP, C-reactive protein; FWS, fever without a source; IQR, interquartile range; LRTI, lower respiratory tract infection; SD, standard deviation; URTI, upper respiratory tract infection; UTI, urinary tract infection.
[a] Including pneumonia, acute bronchitis, and bronchiolitis.
[b] Including pharyngitis, acute otitis media, acute tonsillitis, mastoiditis, laryngitis, herpangina, sinusitis, and otitis externa.
[c] Unspecified viral infection included Influenza.
[d] Including gastroenteritis, central nervous system infections, bone and joint infections, acute appendicitis, mesenteric lymphadenitis, pinworm infection, abscess.

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highly confident cohort ($n = 599$). The 5-bin analysis for the highly confident cohort is shown in the Supplementary Material Table S5. Furthermore, the signature yielded a sensitivity of 81.5% (75.4–87.7%) and 100.0% (84.8–100.0%), and specificity of 88.7% (86.3–91.1%) and 97.1% (95.3–98.8%) across the majority and the microbiologically confirmed cohorts, respectively (Supplementary Material Table S3).

The signature's performance compared favourably with routine biomarkers including PCT, CRP, WBC, and ANC, exhibiting higher sensitivity and/or specificity (Table 2).

Signature detects bacterial immune response in viral PCR-positive patients

We evaluated whether the signature can complement multiplex PCRs by identifying a bacterial immune response in viral PCR-positive patients. Viral positive PCR occurred in 72.3% of patients ($n = 529$), of which 8.9% ($n = 47$) had a bacterial reference standard outcome. The signature correctly identified the bacterial immune response in 42/47 (89.4%) cases, assigned equivocal in 2/47 (4.3%), and misclassified 3/47 as viral (6.4%). Signature detection of a bacterial immune response in viral PCR-positive patients was robust across different viruses (Fig. 2).

Signature's potential reduction of antibiotic misuse

Since host signature results were not provided to the clinicians, the impact of the test on antibiotic prescription cannot be evaluated. It is possible to estimate the host signature's potential to impact antibiotic use by comparing 'current practice', as documented in the medical record, with 'current practice + BV signature', assuming that a contraindicative test result would have triggered a change in practice. In cases where signature result was equivocal, 'current practice' was assumed to be employed. In this model, 'current practice + signature' is estimated to lead to a reduction in overtreatment from 186/628 (29.6%) to 57/628 (9.1%), i.e., a 3.3-fold reduction (relative risk reduction 69.4%, $p < 0.001$), while the proportion of missed bacterial infections would decrease from 9/104 (8.7%) to 7/104 (6.7%), i.e., a 1.3-fold reduction (relative risk reduction 22.2%, p 0.4) (Fig. 3A). Overuse of antibiotics is shown in Fig. 3B.

For indeterminate cases, we assessed the theoretical change in antibiotic prescription. According to the medical record, 221/276 children (80.1%) assigned indeterminate were prescribed antibiotics. If antibiotics were prescribed according to the results of the signature, only 159/276 children (57.6%) would have been prescribed antibiotics and 117/276 (42.4%) would not, representing a 1.4-fold reduction.

Evaluation of the antibiotic prescribing patterns at the two study sites separately is shown in the Supplement Material Fig S3.

Signature dynamics in patients where aetiology is uncertain

The distribution of the signature score across indeterminate cases (27.4%, Table 1) is shown in Supplementary Material Fig. S4. These cases were more likely to receive antibiotics ($p < 0.001$) and be subjected to an x-ray ($p < 0.001$) than those assigned as bacterial or viral. The signature classified 18.5% as high likelihood bacterial ($90 \leq$ score ≤ 100) and 18.5% as high likelihood viral ($0 \leq$ score ≤ 10), in total providing high confidence results for 37.0% of these difficult-to-diagnose cases.

Discussion

In this multinational, prospective, cohort study, we validated the diagnostic performance of a novel host-response-based signature comprising TRAIL, IP-10, and CRP in a broad cohort of paediatric patients with suspicion of RTI or FWS. Importantly, we demonstrated its capacity to complement viral detection by accurately detecting bacterial coinfection. The signature performed highly across all subgroups tested.

This is the first study to assess the capability of the signature to complement viral detection methods. It is well known that direct viral detection does not rule out the possibility of bacterial coinfection, and accordingly antibiotics are often prescribed irrespective of viral detection results [6]. Here we show that patients assigned bacterial adjudication labels yield a bacterial signature score even in the presence of viral detection, confirming that this tool can support appropriate antibiotic use. Of note, the bacterial–viral dichotomy serves as a surrogate for deciding which child would benefit from antibiotics. The capability to aid in appropriate antibiotic use is further supported by our retrospective analysis comparing antibiotic prescription documented in the medical record to potentially altered antibiotic prescription based on the signature result. Notably, the significant impact on antibiotic overuse estimated here represents added value to CRP, as CRP is part of the current practice in Europe.

To date, no single marker of inflammation has proved reliable and accurate enough to differentiate between bacterial and viral aetiologies [18–21]. Tools that incorporate both clinical features and laboratory values have been shown to yield increased diagnostic accuracy; still, these typically attain low specificities [22–24]. Other biomarker combinations are being evaluated for their performance. For example, CRP and myxovirus resistance protein A have been shown to distinguish bacterial from viral infections in patients with RTI [25,26]. Limitations of these studies are

Table 2
Diagnostic performance of the signature compared to single biomarkers

	Cut-off	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV% (95%CI)	NPV% (95%CI)
Signature	<35, >65[a]	94.0 (88.9–99.1)	94.1 (92.0–96.3)	74.3 (65.9–82.6)	98.9 (97.9–99.9)
PCT	0.1	93.4 (88.3–98.5)	20.7 (17.2–24.2)	17.3 (14.0–20.7)	94.6 (90.5–98.8)
	0.25	87.9 (81.2–94.6)	57.6 (53.3–61.9)	26.9 (21.9–32.0)	96.4 (94.3–98.5)
	0.5	80.2 (72.0–88.4)	76.4 (72.7–80.0)	37.6 (30.8–44.4)	95.6 (93.6–97.6)
CRP	20	98.9 (96.8–100.0)	68.9 (64.9–73.0)	36.1 (30.2–42.1)	99.7 (99.2–100.0)
	80	82.4 (74.6–90.2)	97.9 (96.6–99.1)	87.2 (80.2–94.3)	96.9 (95.4–98.4)
WBC	15 000	69.2 (59.7–78.7)	82.8 (79.5–86.1)	41.7 (33.9–49.6)	93.8 (91.6–96.0)
ANC	10 000	75.8 (67.0–84.6)	84.4 (81.2–87.5)	46.3 (38.3–54.3)	95.2 (93.2–97.1)

The analysis included all cases in the main cohort that had each of the biomarker measurements ($n = 603$; 512 with a viral infection, 91 with a bacterial infection).
ANC, absolute neutrophil count; CI, confidence interval; CRP, C-reactive protein; NPV, negative predictive value; PCT, procalcitonin; PPV, positive predictive value; WBC, white blood cells.
[a] Equivocal rate of 10.1%.

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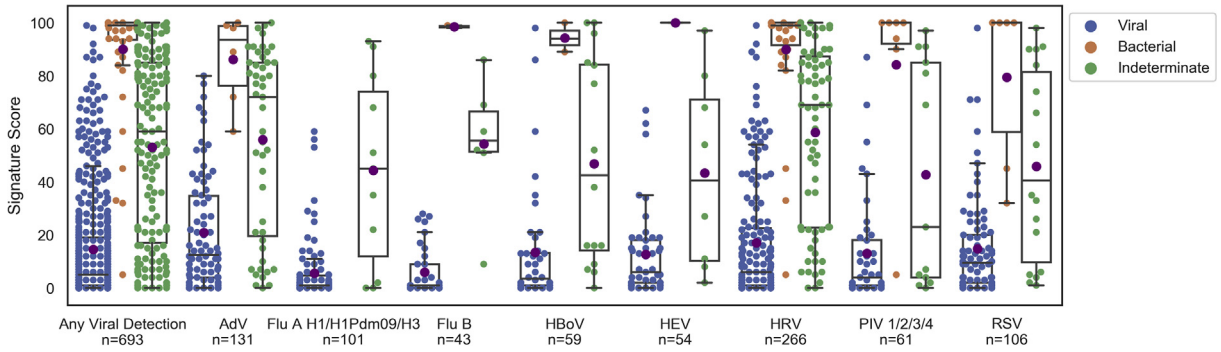

Fig. 2. Signature detects bacterial immune response in viral PCR-positive patients. Each circle represents a patient with at least one virus detected by multiplex PCR. The black line corresponds to the group median and the purple circle corresponds to the group average. The box indicates patients with values between the 25 and 75 percentiles. AdV, adenovirus; Flu, influenza; HBoV, human bocavirus; HEV, human enterovirus; HRV, human rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

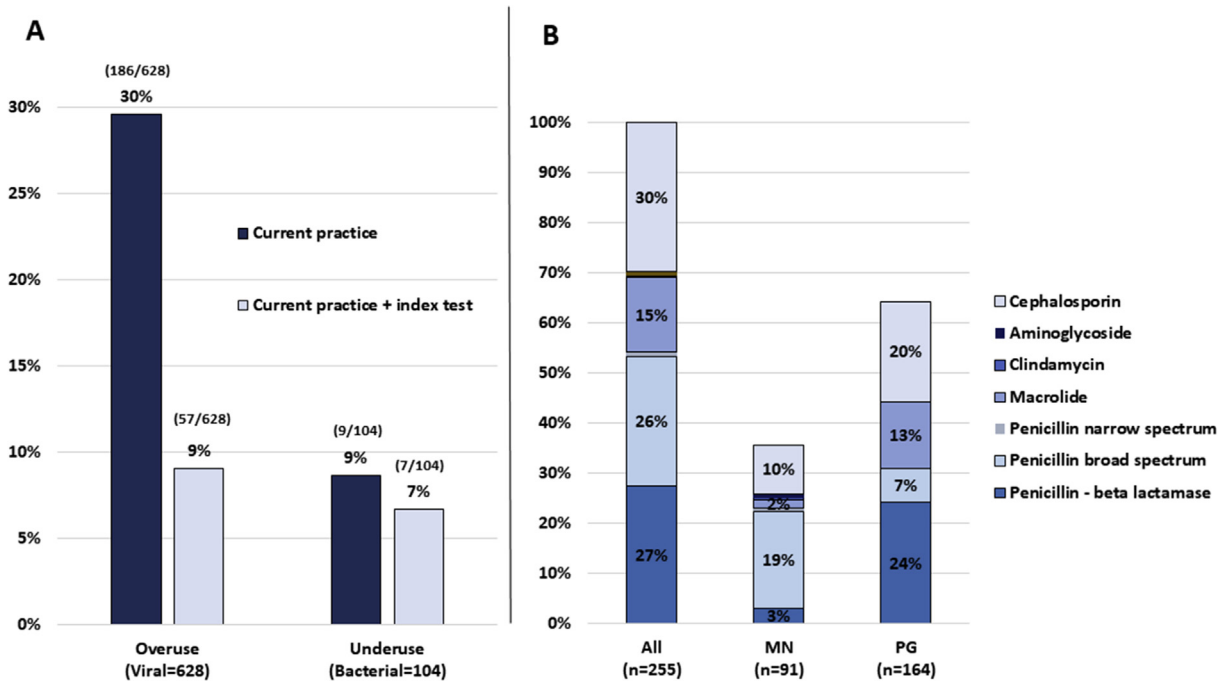

Fig. 3. Estimated impact of the signature on misuse and antibiotic overuse types across different cohorts. (A) Current practice overuse was defined as viral patients receiving antibiotics, underuse as bacterial patients not receiving antibiotics or receiving delayed treatment. The signature's potential misguidance of treatment was defined according to predefined CE-IVD cut-offs: overuse included viral patients with a score over 65, indicative of a bacterial infection (false positives), underuse as bacterial patients with a score under 35, indicative of viral infection (false negatives). Equivocal results corresponding to scores between 35 and 65 do not provide diagnostic information and so potential antibiotic misguidance in these cases was defined according to the ED physician's treatment. (B) Distribution of antibiotic overuse for both study sites (MN, Manheim; PG, Perugia).

the reference standard quality and small cohort size. An alternative approach employs transcriptomics, with several groups reporting high accuracies [27–29]. Transcriptomic tools remain to be fully locked (to assure reproducibility), and their performance validated in large cohorts with a rigorous reference standard.

Our study has several strengths. First, we applied a rigorous reference method that included extensive data collection, and independent expert adjudicators blinded to one another and to the signature result. Second, this was a multinational cohort across all paediatric age groups, supporting the generalizability of the findings.

Our study has also limitations. Focusing on microbiologically confirmed cases tends to overrepresent the easy-to-diagnose cases. On the other hand, using an adjudicator panel approach potentially introduces diagnostic imperfections of real-life clinical practice. Here, this is exemplified by some of the false negatives that may represent viral infections incorrectly assigned by the adjudicators as bacterial. A limitation of our reference standard is that CRP results (obtained during routine care) were available to the adjudicators, raising the possibility of incorporation bias, although the signature notably outperformed CRP alone. Another limitation is that some patients were assigned an indeterminate reference standard outcome and thus excluded from the performance analysis. Of note, the signature provided bacterial or viral result in 43.8% and 39.5% of these cases, respectively (16.7% assigned as equivocal), supporting the idea that it may help with treatment decisions even in difficult-to-diagnose cases. To investigate the impact of these various methodological constraints on performance, especially with regards to indeterminate cases and CRP incorporation bias, we conducted a post-hoc analysis of the signature performance based on reference standards where adjudicators were blinded to CRP and/or forced to label indeterminate cases. This post-hoc analysis of

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270 children randomly selected from the eligible AutoPilot-Dx cohort demonstrates that the sensitivity, specificity, NPV, and PPV may be in the ranges 90–100%, 82.2–94.9%, 98.3–100%, and 34.1–57.1%, respectively (data not shown), with the latter presumably being influenced by the lower prevalence of bacterial infections in the post-hoc sample. Overall, the performance is comparable to that described here.

As noted, the potential impact on antibiotic use was an extrapolation, and does not demonstrate real impact on antibiotic prescribing. Future utility studies are warranted to directly evaluate the signature's utility as an antimicrobial stewardship tool.

In conclusion, we validated the generalizability of a signature comprising TRAIL/IP-10/CRP for accurately differentiating between bacterial and viral infections, especially in RTIs, and demonstrated its capability to complement viral detection. Considering the growing menace of antimicrobial resistance [30], an effective tool for the differentiation between viral and bacterial infection is more urgently needed than ever before.

Author contributions

TT, SE, EE, AC, KO, and TMG conceived the study and its design, had full access to the data, and take responsibility for the integrity of the data and accuracy of the analysis. Funding acquisition was made by TT, SE, EE, AC, KO, and TMG. CP, AA, MP, UH, EF, IT, MBP, DM, KP, LE, NM, EM, TIB, ES, OB, LS, RV, EBar, AS, JGL, MK, MS, RY, EBam, and SS organized and entered data. CP, TMG, NM, RN, EBar, EE, SE, and TT contributed to data analyses. CP, AA, MP, UH, EF, IT, MBP, DM, KP, LE, NM, EM, TIB, ES, OB, LS, TMG, RV, EBar, EE, AS, JGL, MK, MS, RY, EBam, SS and SE had access to the underlying data for verification. CP, AA, LE, NM, TIB, LS, TMG, RN, EBar, SE and TT contributed to data interpretation. CP, AA, TMG, EE, SE and TT wrote the main draft of the manuscript. All authors contributed to the final drafting of the manuscript.

Transparency declaration

AA, MP, UH, EF, IT, MBP, DM, KP, AS, JGL, MK, MS, RY, and SS report no potential conflict of interest relevant to this article. CP has received funding by MeMed Diagnostics for research activities outside this work. SE and TT were co-applicants together with MeMed Diagnostics for grant #701088. LE, NM, EM, TIB, AC, ES, OB, LS, TMG, RN, EBar, KO, EE, and EBam are/were employees of MeMed Diagnostics. This study was supported by the European Commission, Executive Agency for Small and Medium-sized Enterprises, Horizon2020-FTIPilot-2015-1 program (grant number 701088). The funding organization had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.10.019.

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