A novel host-proteome signature to distinguish between acute bacterial and viral infections in febrile children

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Abstract

Bacterial and viral infections are often clinically indistinguishable, leading to inappropriate patient management and antibiotic misuse. Traditional host-proteins such as C-reactive protein, procalcitonin and interleukin-6 can help determine infection etiology[1], but their performance is negatively affected by inter-patient variability.[2-3]

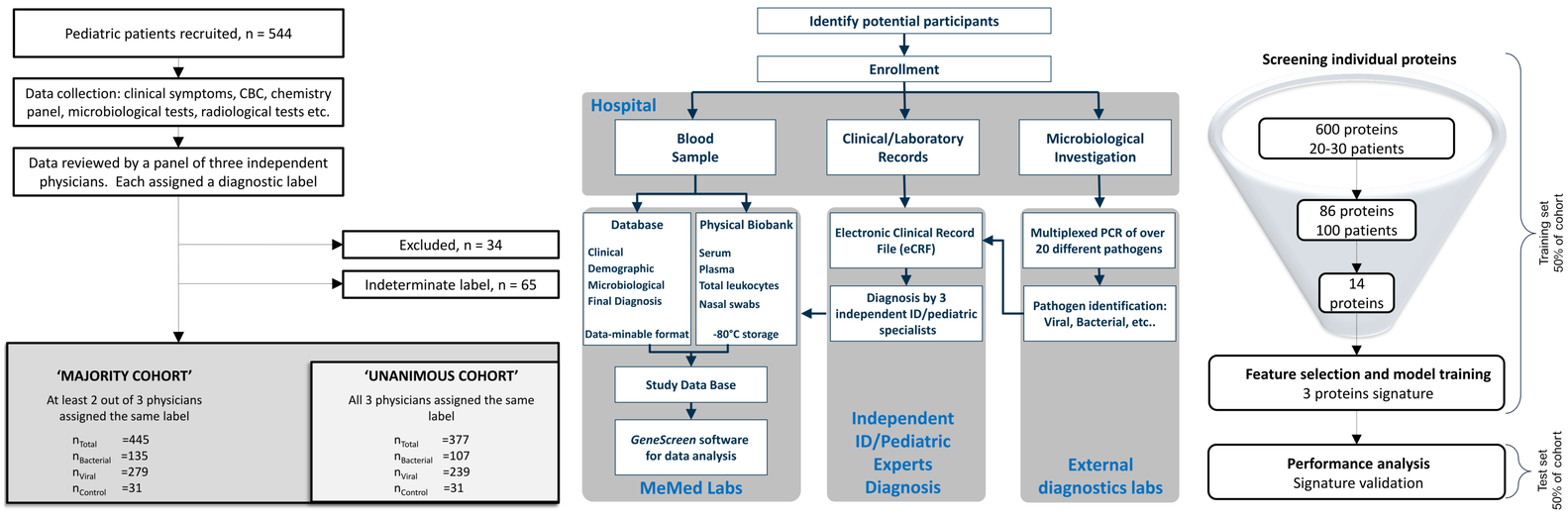
We tested whether a multi-parametric model that combines both traditional bacterial- and novel viral-induced proteins can improve the discrimination between bacterial and viral etiologies.

We prospectively recruited 544 children (≤18 years) with an acute infectious disease and controls with no apparent infectious disease between 2009 and 2013. Final diagnosis was determined by three independent experts. Unanimous diagnosis was attained in 239 viral, 107 bacterial, and 31 control patients. In 133 patients, no unanimous diagnosis was reached and 34 were excluded. We quantitatively screened 600 circulating host-proteins in one of the largest proteome screenings of infectious disease patients to date. Next, we developed a multi-parametric signature using logistic-regression, and validated it using a leave-10%-out cross-validation scheme.

The final signature consisted of bacterial- and viral-induced proteins, yielding an AUC of 0.94 (95% CI, 0.91 to 0.98). The signature was superior to any of the individual proteins (P<0.001), as well as routinely used clinical parameters and their combinations (P<0.001). It remained robust across various clinical syndromes (e.g. respiratory, urinary and systemic), times from symptom onset (0-12 days), and the presence of colonizers (AUCs ranged between 0.89 and 0.99).

The present host-signature provides valuable information over routine clinical variables. This approach has the potential to reduce both antibiotic overuse and underuse in children.

Study Design



We prospectively recruited patients with suspected acute infectious disease and controls with no apparent infection between 2009 and 2013 from Hillel Yaffe and Bnai Zion Medical Centers in Israel (NCT01917461). Patients (≤18 years) were recruited from pediatric emergency departments, pediatric wards and surgical departments.

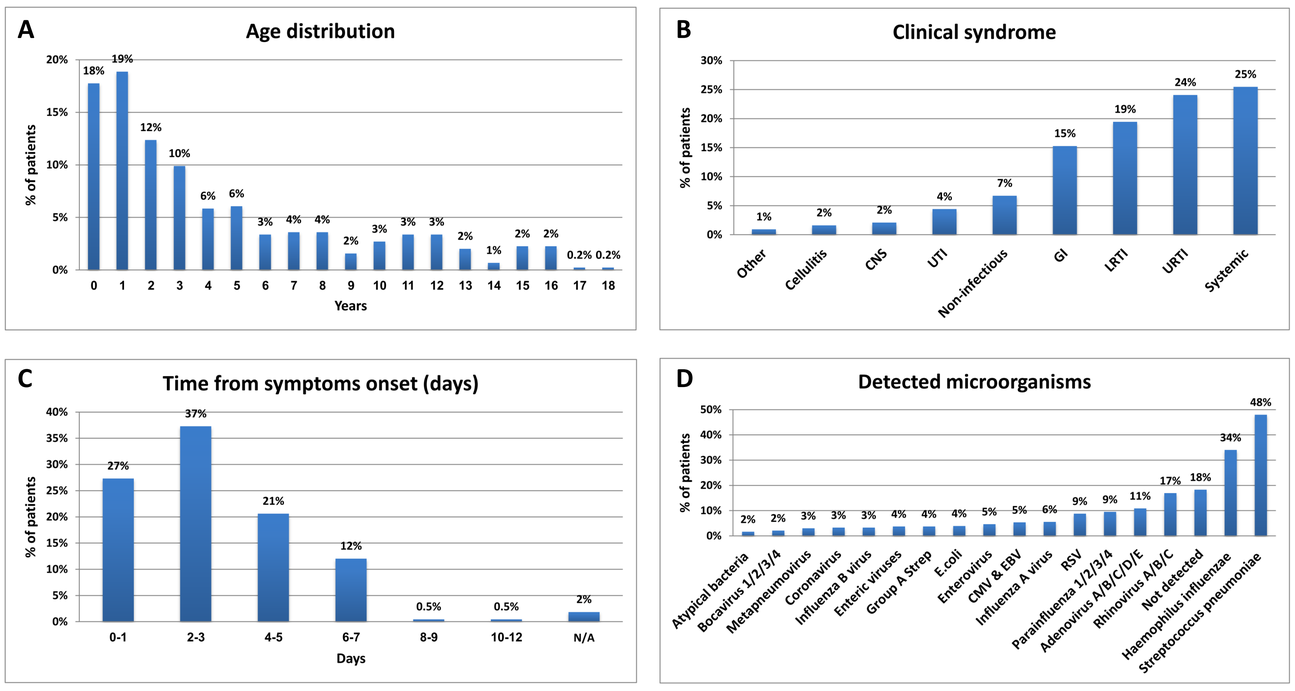
Inclusion criteria for the infectious disease cohort included: clinical suspicion of an acute infectious disease, peak fever >38°C since symptoms onset, and duration of symptoms ≤12 days. Inclusion criteria for the control group included: clinical impression of a non-infectious disease (e.g. trauma), or healthy subjects.

We created a rigorous composite reference standard following recommendations of the Standards for Reporting of Diagnostic Accuracy (STARD).[4] For each patient, three independent physicians assigned a diagnosis based on comprehensive clinical and laboratory investigation including PCR for 21 pathogens. Each panel member assigned one of the following diagnostic labels to each patient: (i) bacterial; (ii) viral; (iii) no apparent infectious disease or healthy (controls); and (iv) indeterminate. Patients with mixed infections (bacteria plus virus) were labeled as bacterial because they are managed similarly (e.g. treated with antibiotics). This process was used to create two cohorts with an increasing level of diagnostic certainty: (i) Majority cohort: Patients were assigned the same label by at least two of the three panel members. (ii) Unanimous cohort (a subgroup of the Majority cohort): Patients were assigned the same label by all three panel members.

We screened 600 circulating host-proteins, developed a multi-parametric signature using logistic-regression on half of the patients and validated it on the remaining half.

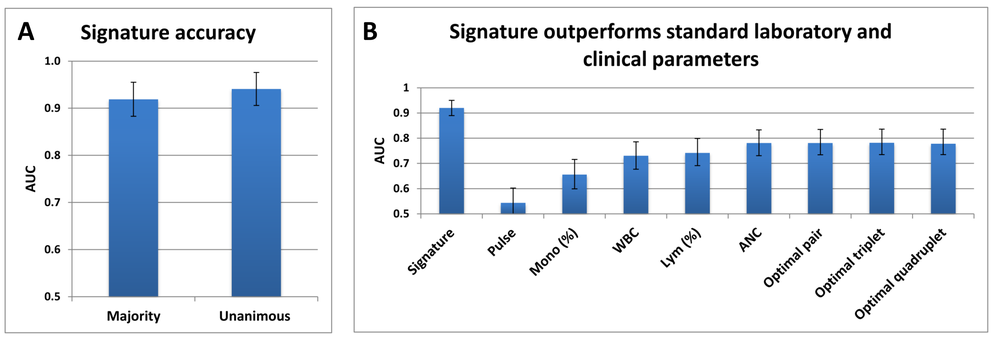
Results

Cohort characteristics



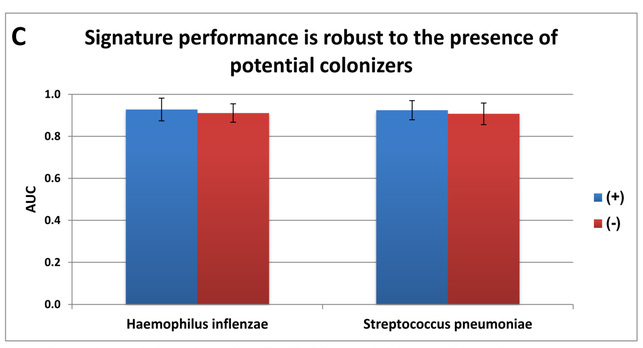
Cohort characteristics. (A) Age distribution. (B) Clinical syndromes. CNS- central nervous system, GI - gastroenteritis , LRTI – lower respiratory tract infection (pneumonia, bronchiolitis, acute bronchitis, and laryngitis), UTRI - upper respiratory tract infection (pharyngitis, acute otitis media, acute sinusitis and acute tonsillitis), UTI - urinary tract infection. (C) Time from symptoms onset. This was defined as the duration (days) from the appearance of the first presenting symptom. (D) Detected microorganisms. We used a wide panel of microbiological tools in order to maximize pathogen detection including: antigen detection, serological investigation, and multiplex PCR (Seeplex® RV15 and Seeplex® PB6 by Seegene, Korea). Influenza A subgroup included H1N1 strains. The atypical bacteria subgroup included *Chlamydophila pneumoniae*, *Mycoplasma pneumonia* and *Legionella pneumophila*. The Enteric viruses subgroup included Rota virus, Astrovirus, Enteric Adenovirus and Norovirus G I/II.

Performance analysis (Bacterial vs Viral)



Signature performance analysis. (A) Signature accuracy (AUC) in the Majority and Unanimous cohorts.

(B) Comparing the signature to lab parameters in diagnosing bacterial from viral patients. Performance of clinical and lab parameters as well as the best performing pair (ANC and lymphocytes % [Lym]), triplet (ANC, Lym % and monocytes % [Mono]), and quadruplets (ANC, Lym %, white blood cells [WBC] and Mono %) of parameters, the values of which were combined using a logistic regression. Comparison was done on the Majority cohort. The signature performed significantly better ($P<10^{-15}$) than the optimal quadruplet.



(C) Signature performance in the presence (+) or absence (-) of *Streptococcus pneumoniae* and *Haemophilus influenzae*. *Streptococcus pneumoniae* and *Haemophilus influenzae* were detected using multiplex-PCR (Seeplex® PB6 by Seegene, Korea) applied on nasopharyngeal wash. Error bars represent 95% CI.

Conclusions

Despite advances in infectious disease diagnosis, timely identification of bacterial infections remains challenging, leading to antibiotic misuse with its profound health and economic consequences. To address the need for better treatment guidance, we developed and validated a signature that combines novel and traditional host-proteins for differentiating between bacterial and viral infections. Our finding in a large sample size of patients is promising, suggesting that this host-signature has the potential to help clinicians manage patients with acute infectious disease and reduce antibiotic misuse in children.

References

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