A Novel Immunoassay that Distinguishes between Bacterial and Viral Infections Based on a Patient's Immune Response

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Abstract

Objectives

Bacterial and viral infections are often clinically indistinguishable, leading to inappropriate patient management and antibiotic misuse. Effective use of infectious disease diagnostics has been hindered by long waits for results, high costs, inaccessible (or unknown) sites of infection, and the presence of non-disease causing colonizing bacteria that can lead to false positive results. An approach that has the potential to address these challenges relies on monitoring the host's immune-response to infection, rather than direct pathogen detection. Our goal was to develop and validate a novel assay that combines blood borne bacterial- and viral-induced host-proteins that can accurately distinguish between bacterial and viral infections.

Methods

We prospectively recruited 1002 hospitalized and emergency department patients with acute infection, and controls with no apparent infection (NCT01917461). For each patient, three independent physicians assigned a diagnosis based on comprehensive clinical and laboratory investigation that included PCR for 21 common pathogens. We quantitatively screened 600 circulating host-proteins and developed a multi-parametric signature using logistic-regression on half of the patients, and validated it on the remaining half.

Results

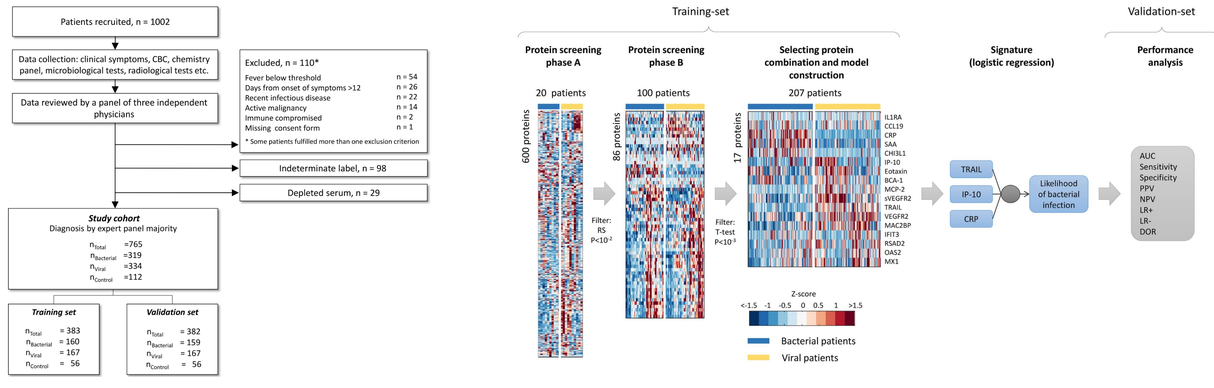
The cohort included 319 bacterial, 334 viral, 112 control and 98 indeterminate patients (139 were excluded based on pre-determined criteria). The cohort was balanced with respect to gender (47% females, 53% males) and included 56% pediatric patients (≤18 years) and 44% adults (>18 years). The best performing host-protein was TNF-related apoptosis-inducing ligand (TRAIL) (area under the ROC curve [AUC] of 0.89; 95% confidence interval [CI], 0.86-0.91), which was consistently up-regulated in viral infected patients. The signature with the highest precision included both viral- and bacterial-induced proteins: TRAIL, Interferon gamma-induced protein-10, and C-reactive protein (AUC of 0.94; 95% CI, 0.92-0.96). The signature outperformed routinely-used clinical parameters, such as white blood cell count (AUC of 0.64±0.04), absolute neutrophil count (AUC of 0.73±0.04), % monocytes (AUC of 0.64±0.04), % lymphocytes (AUC of 0.76±0.04), peak temperature (AUC of 0.51±0.04), pulse (AUC of 0.62±0.04), procalcitonin (AUC of 0.67±0.11), and an algorithm that combines these clinical parameters (AUC of 0.78±0.04). The signature was robust across various physiological systems (respiratory, urinary and systemic), times from symptom onset (0-12 days), and pathogens (56 species), with AUCs between 0.87 and 1.0. Finally, the signature's accuracy was not affected by the presence of potential colonizers and it was able to provide accurate diagnoses even in cases where the infection site was not known or easily accessible. A kit called ImmunoXpert™ was developed, which measures the proteins in 99 minutes using an ELISA format, and computationally integrates the measurements into the final diagnosis.

Conclusions

The present signature combines newly identified viral-induced with traditional bacterial-induced host proteins. It provides valuable information over standard laboratory and clinical parameters, which are routinely used in clinical practice today to facilitate differential diagnosis of infection etiology. Furthermore, assay run time can be further shortened as it is readily translatable to hospital-deployed automated immunoassay machines and point-of-need assay formats. This signature has the potential to significantly improve the management of patients with acute infections and reduce antibiotic misuse.

Concept and Design

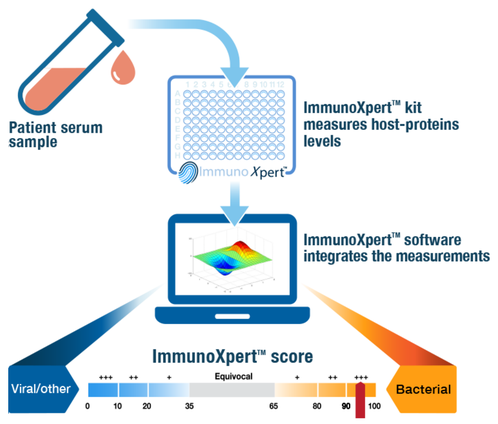
Overview of the study workflow, protein screening, model construction and validation



We prospectively recruited 1002 patients with suspected acute infectious disease and controls with no apparent infection between 2009 and 2013 (Oved et al. *PLOS ONE* 2015). 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. For each patient, the following baseline variables were recorded: demographics, medical history, physical examination, complete blood count (CBC) obtained at enrollment, and chemistry panel. A nasal swab was obtained for microbiological investigation including two multiplex-PCR diagnostic assays (Seeplex® RV15 [n=713; 15 viral strains] and Seeplex® PB6 [n=633; six bacterial strains]), and a blood sample was obtained for host-protein measurements. We created a rigorous expert panel reference standard (Bertens et al., 2013) which follows the broadly accepted recommendations of the Standards for Reporting of Diagnostic Accuracy (STARD; Bossuyt et al., 2003), and the NHS Health Technology Assessment (NHS-HTA) for evaluation of diagnostic tests (Rutjes et al., 2007). Each patient was 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.

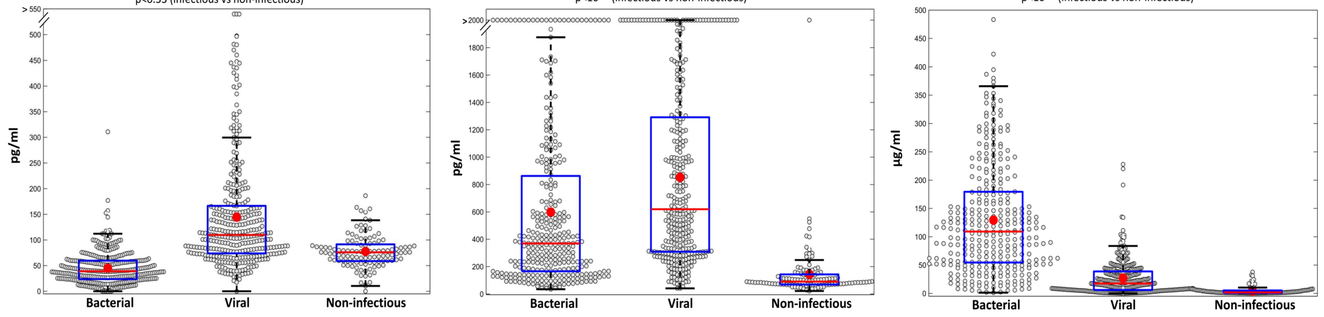
We quantitatively screened 600 circulating host-proteins, developed a multi-parametric signature using logistic-regression on half of the patients and validated it on the remaining half.

Assay principles and graphical user interface



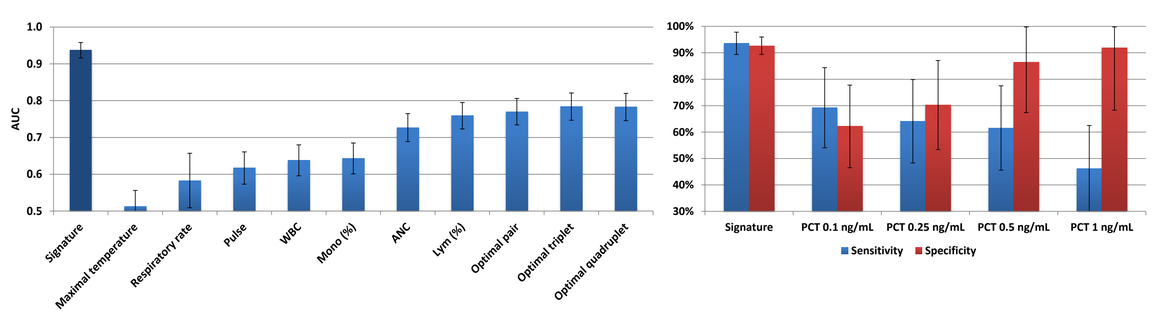
Results and Discussion

The host-proteins comprising the signature show complementary dynamics in response to bacterial, viral and non-infectious diseases

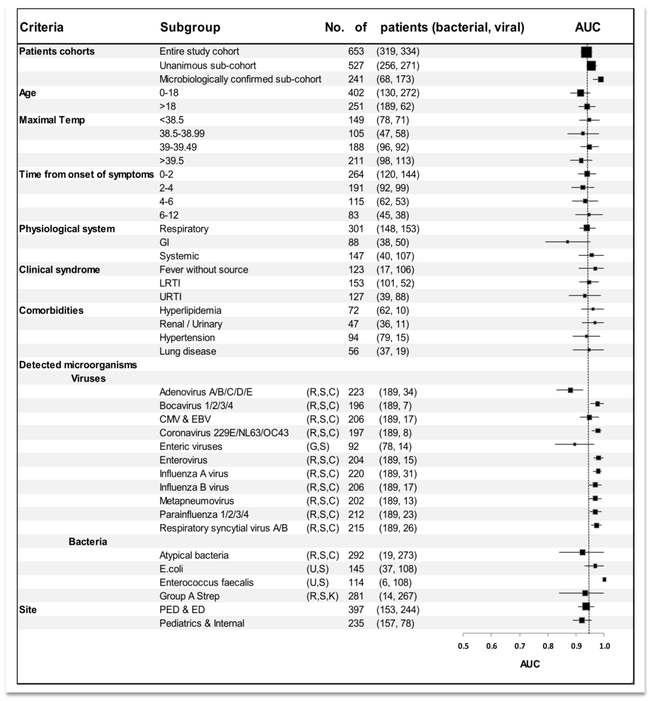


Signature outperformed routine clinical parameters and their combinations

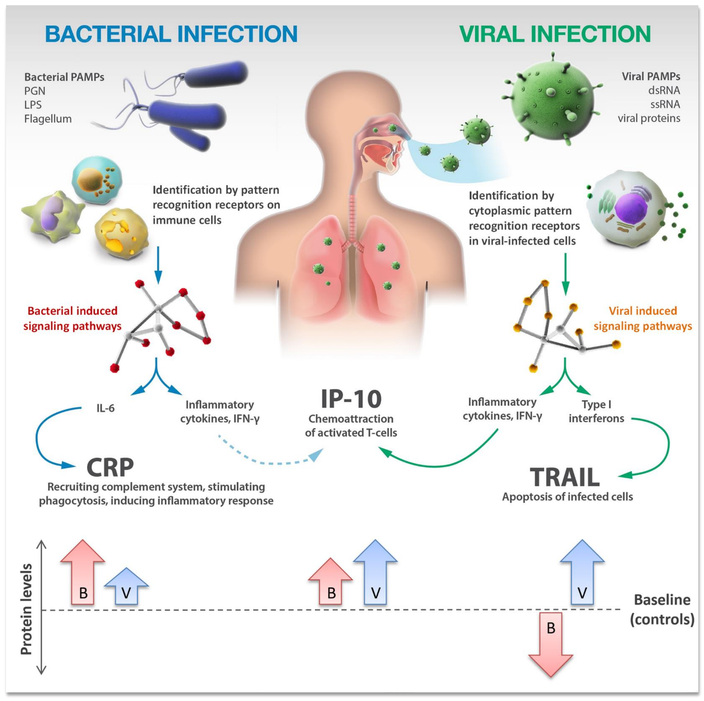
Signature significantly outperformed ($P<10^{-15}$) all clinical parameters and their combinations (best performing pair [ANC and Lym %], triplet [ANC, Lym % and Pulse], and quadruplets [ANC, Lym %, Pulse, Mono %]), as well as procalcitonin ($P<0.001$), using the standard cutoffs routinely applied in the clinical setting.



Signature is robust across patient subgroups



Bacteria and viruses stimulate different immune-pathways



New assay overcomes limitations of current diagnostics