Diagnostic accuracy of a TRAIL, IP-10 and CRP combination for discriminating bacterial and viral etiologies at the Emergency Department

Dear Editor,

We read with interest the review article by ten Oever et al., on immune response-derived biomarkers in the differential diagnosis of inflammatory disorders1 and the related comment by van der Does et al., who reported a retrospective study indicating that TRAIL and IP-10 represent candidates for biomarkers of viral infections in the Emergency Department (ED).2 Here we confirm this finding and show that a signature combining the viral-induced proteins TRAIL and IP-10 with the classical bacterial-induced biomarker CRP is highly discriminatory for ED patients presenting with microbiologically confirmed bacterial and viral infections. Further, using adjudication of an expert panel to expand the reference standard3,4 we provide support that the differential diagnostic performance of this three-biomarker signature is robust across a broad ED population resembling that found in the clinical setting.

A major challenge underlying effective management of febrile ED patients is the difficulty in clinically distinguishing between bacterial and viral etiologies highlighted by ten Oever et al.1 This clinical uncertainty drives antibiotic misuse, both underuse and overuse, with detrimental ramifications for the patient, healthcare system and society, including emergence of antibiotic resistance. While routine diagnostic tests for pathogen detection may aid in determining infection etiology, they often are limited by one or more of the following key limitations: (i) lengthy time to result; (ii) inability to diagnose infections that are not readily accessible (e.g., pneumonia); (iii) uncertainty regarding the clinical interpretation of a viral identification, which does not preclude the possibility of a bacterial co-infection; and (iv) false alarms due to the presence (“carriage”) of potentially pathogenic microbes that are also part of the natural flora (e.g., Streptococcus pneumoniae). Host-based biomarker approaches represent a promising complement to pathogen-based diagnostics, as immune components circulate throughout the body and a distinct response is elicited when a disease-causing pathogen is encountered.

Here we describe a sub-study of the Curiosity study that was conducted prospectively between August 2009 and November 2013 at two Medical Centers in Israel (NCT01917461). The Curiosity study identified and validated a novel host-biomarker signature for differential diagnosis of acute bacterial and viral infections. The heterogeneous study population comprised febrile inpatients and ED arrivals, both children and adults, presenting with diverse clinical syndromes and a variety of pathogens and up to 7 days of symptoms. The optimally performing signature was found to be computational integration of the concentrations of three blood-borne proteins: TRAIL, IP-10 and CRP.5

The aim of this sub-study was to evaluate the diagnostic performance of the same signature and constituent biomarkers in the sub-population of ED patients. The reference
standard was based on microbiological confirmation plus adjudication by an expert panel after review of all participant clinical, laboratory, radiological, microbiological and follow-up data. Each panel member independently assigned one of the following diagnostic labels to a patient: (i) bacterial (including mixed bacterial and viral co-infection); (ii) viral; or (iii) unknown. A "true diagnosis" required positive microbiological confirmation plus a unanimous expert panel, i.e., all three panel members independently assigned bacterial or viral etiology. The inclusion and exclusion criteria, recruitment process, data collection, sample analysis and criteria for microbiological confirmation were described previously. The expert panel was blinded to the test result and test performers were blinded to the reference standard. Predefined cut-offs were applied for each of the biomarkers as follows: TRAIL, 70 pg/ml; IP-10, 500 pg/ml; and CRP, 40 mg/L. For the host-signature test, as defined previously, pre-determined cut-offs were

Figure 1 The host proteins TRAIL, IP-10 and CRP and combinatorial signature are differentially expressed in patients presenting with bacterial and viral infections. Box plots for TRAIL, IP-10, CRP, and host-signature measured over the microbiologically confirmed cohort (n = 155) are presented. Red line and circle correspond to group median and average respectively.

Table 1A Diagnostic performance of the individual biomarkers and a combinatorial signature compared to a microbiologically confirmed reference standard (n<sub>bacterial</sub> = 27, n<sub>viral</sub> = 128). The following cut-offs 70 pg/ml, 500 pg/ml, 40 mg/L were used for TRAIL, IP-10 and CRP respectively. The signature assigned equivocal results to 15% of the bacterial and 9% of the viral patients.

<table>
<thead>
<tr>
<th>Accuracy measure</th>
<th>TRAIL</th>
<th>IP-10</th>
<th>CRP</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>89% [71%, 98%]</td>
<td>74% [54%, 89%]</td>
<td>70% [50%, 86%]</td>
<td>96% [78%, 100%]</td>
</tr>
<tr>
<td>Specificity</td>
<td>84% [76%, 90%]</td>
<td>66% [58%, 75%]</td>
<td>77% [69%, 84%]</td>
<td>93% [87%, 97%]</td>
</tr>
<tr>
<td>LR−</td>
<td>0.13 [0.05, 0.39]</td>
<td>0.39 [0.20, 0.75]</td>
<td>0.38 [0.21, 0.69]</td>
<td>0.05 [0.01, 0.32]</td>
</tr>
<tr>
<td>PPV</td>
<td>53% [38%, 68%]</td>
<td>32% [21%, 45%]</td>
<td>40% [26%, 55%]</td>
<td>73% [54%, 88%]</td>
</tr>
<tr>
<td>NPV</td>
<td>97% [92%, 99%]</td>
<td>92% [85%, 97%]</td>
<td>93% [86%, 97%]</td>
<td>99% [95%, 100%]</td>
</tr>
</tbody>
</table>

Table 1B Diagnostic performance of the individual biomarkers and a combinatorial signature compared to a unanimous expert panel adjudication reference standard (n<sub>bacterial</sub> = 103, n<sub>viral</sub> = 204). The following cut-offs 70 pg/ml, 500 pg/ml, 40 mg/L were used for TRAIL, IP-10 and CRP respectively. The signature assigned equivocal results to 14% of the bacterial and 7% of the viral patients.

<table>
<thead>
<tr>
<th>Accuracy measure</th>
<th>TRAIL</th>
<th>IP-10</th>
<th>CRP</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>86% [78%, 92%]</td>
<td>66% [56%, 75%]</td>
<td>78% [68%, 85%]</td>
<td>90% [82%, 95%]</td>
</tr>
<tr>
<td>Specificity</td>
<td>82% [76%, 87%]</td>
<td>65% [58%, 72%]</td>
<td>80% [74%, 86%]</td>
<td>94% [89%, 97%]</td>
</tr>
<tr>
<td>LR−</td>
<td>0.17 [0.10, 0.27]</td>
<td>0.52 [0.39, 0.69]</td>
<td>0.28 [0.19, 0.40]</td>
<td>0.11 [0.06, 0.20]</td>
</tr>
<tr>
<td>PPV</td>
<td>71% [62%, 78%]</td>
<td>49% [40%, 58%]</td>
<td>67% [57%, 75%]</td>
<td>87% [78%, 93%]</td>
</tr>
<tr>
<td>NPV</td>
<td>92% [87%, 96%]</td>
<td>79% [72%, 85%]</td>
<td>88% [82%, 92%]</td>
<td>95% [91%, 98%]</td>
</tr>
</tbody>
</table>

[] = 95% Confidence interval.
employed such that the test gives three results: viral (score 0–35), equivocal (score 35–65) or bacterial (score 65–100).

Of the 744 participants that met the Curiosity study infectious disease inclusion criteria, 428 participants with suspected infections were recruited at the ED, of which 155 had a confirmed etiology (128 viral and 27 bacterial [or mixed co-infection]). In agreement with the Curiosity study and van der Does et al., all three biomarkers were significantly differentially expressed between bacterial and viral infections: TRAIL (rank-sum P < 10^-11), IP-10 (rank-sum P < 10^-4), and CRP (rank-sum P < 10^-5) (Fig. 1). Notably, the combinatorial signature of all three biomarkers exhibited the greatest diagnostic accuracy, yielding a sensitivity of 96% [95% confidence interval: 78%, 100%] and specificity of 93% [87%, 97%], significantly better than the individual proteins (see Table 1A). Furthermore, the signature outperformed routine lab parameters such as white blood cell count (sensitivity 56% [35%, 75%] and specificity 84% [77%, 90%]; cut-off 15,000 cells/μl) and absolute neutrophil count (sensitivity 59% [39%, 78%] and specificity 88% [81%, 93%]; cut-off 10,000 cells/μl).

Often, the laboratory testing of patients with an infectious etiology does not yield a definitive microbiological confirmation. To examine the signature performance in such cases, we broadened the reference standard by removing the requirement of microbiological confirmation, and based it on unanimous expert panel adjudication. Application of this reference standard resulted in an extended cohort of 307 patients (204 viral and 103 bacterial [or mixed co-infection]). The signature exhibited a sensitivity of 90% [82%, 95%] and specificity of 94% [89%, 97%], again significantly outperforming the individual biomarkers and clinical parameters (see Table 1B), thereby supporting the generalizability of the diagnostic performance results.

In conclusion, the diagnostic performance data reported here support that a host-biomarker signature comprising TRAIL, IP-10 and CRP represents a promising new tool for aiding ED clinicians in determining the bacterial versus viral etiology of infectious disease. The increased diagnostic accuracy may be attributed to the combination of both viral- and bacterial-induced proteins, which complement one another. This actionable information has the potential to support the clinician in deciding whether to prescribe antibiotics. Future clinical studies are required to examine the usefulness of this host-biomarker signature in safely decreasing unnecessary antibiotic prescription at the ED.

Conflict of interest

The Curiosity study was funded by MeMed. EE, TG, RN, OB, AC, EB and KO are employed by MeMed. IS and AK declare no conflict of interests.

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