PART II
ADDITIONAL BIOMARKER STRATEGIES

CHAPTER 6

TRAIL AND IP-10 AS BIOMARKERS OF VIRAL INFECTION IN THE EMERGENCY DEPARTMENT

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ABSTRACT

Introduction

Fever is a common symptom in the Emergency department (ED). If viral infections can be identified more accurately, the overuse of antibiotics, attributed adverse events and antibiotics resistance may be reduced. TNF-related apoptosis-inducing ligand (TRAIL) and interferon-gamma-inducible protein 10 (IP-10) are described as novel biomarkers for viral disease. We investigated if TRAIL and IP-10 can differentiate etiologies of fever in the ED, both as a single biomarker, and in combination with procalcitonin (PCT).

Methods

Adult patients with fever in the ED were included. TRAIL and IP-10 levels were determined in patient cases of confirmed viral, bacterial and non-infectious fever. Confirmed viral and confirmed bacterial infection were defined as laboratory evidence of presence of disease and clinical signs, confirmed non-infectious as absence of laboratory evidence and strong alternative diagnosis. A Kruskal-Wallis test was utilized to determine difference between etiologies of fever for TRAIL and IP-10 levels, and confirmed viral infections were compared with other etiologies of fever using Mann Whitney U tests. The area-under-the-curve (AUC) was calculated for TRAIL and IP-10 and optimal cut-off values were derived. In binary logistic regression analysis, a combined biomarker model including PCT, TRAIL and IP-10 was created and the AUC was calculated.

Results

A total of 54 patients were included, of whom 13 with a confirmed viral disease, 33 with a confirmed bacterial disease and 8 patients with confirmed non-infectious disease. TRAIL levels were significantly higher in patients with confirmed viral infections, compared to patients with a confirmed bacterial infection and non-infectious disease, ($p < 0.001$). IP-10 levels were not significantly different ($p = 0.052$). For the discrimination of confirmed viral infections from other febrile etiologies, levels of both TRAIL ($p = 0.016$) and IP-10 ($p = 0.017$) were significantly higher in confirmed viral disease. AUCs were 0.72 (95%CI 0.56 – 0.88) for TRAIL and 0.72 (95%CI 0.59 – 0.86) for IP-10. The combined biomarker model showed an AUC of 0.84 (95%CI 0.72 – 0.97) for discrimination between confirmed viral and non-viral disease.

Discussion

Levels of TRAIL and IP-10 were significantly elevated in viral infections, compared to bacterial and non-infectious febrile disease in an undifferentiated ED patient cohort. A combined biomarker model with PCT resulted in an even higher diagnostic accuracy of viral disease than single biomarkers individually. The cut-off values of the novel biomarkers require validation, but the results of this study are a proof of principle.

INTRODUCTION

Diagnosing the cause of fever in a patient in the emergency department (ED) is difficult. Fever is a common presenting symptom in both viral and bacterial disease. Also, many non-infectious diseases may cause fever[1-3].
There is a dilemma in the treatment of febrile illness in the ED. On the one hand, patients with a severe bacterial infection have to be treated with antibiotics as soon as possible. Delay is associated with a rise in morbidity and mortality[4]. On the other hand, particularly in this time of antimicrobial stewardship, it is good clinical practice to use antibiotics for treatment of bacterial disease as efficient as possible[5,6]. Antibiotic overtreatment of patients may result in an increase of antibiotic resistance[5,6].
Bacterial cultures and specific viral assays are obtained in the ED, but results often take several days to become available. Due to time restraints, it is not possible to wait for these results in daily ED practice. Treatment has to be started before the definitive diagnosis is available. New strategies for appropriate use of antimicrobial therapy are needed[7,8].
In the standard diagnostic workup of febrile patients, biomarkers are used to focus on predicting the presence of bacteria[2,9,10]. In current practice, C-reactive protein (CRP) and to a lesser extent procalcitonin (PCT) are used as clinical biomarkers for identification of bacterial infection. Although the use of these markers reduces antibiotics in selected populations, diagnostic uncertainty remains[9,10].
Recently, TNF-related apoptosis-inducing ligand (TRAIL) and interferon-gamma-inducible protein 10 (IP-10) were described as novel biomarkers for viral disease[11]. TRAIL is expressed in various cells of the adaptive immune system, and plays a role in the response to viral infections[12]. IP-10 has a role in the inflammation cascade in viral and bacterial disease[13].
Biomarkers focusing on both bacterial and viral disease can be combined. Low levels of PCT have been associated with the absence of bacteria. Therefore, a combination of PCT, which is TRAIL and IP-10 may be valuable for the additional diagnostic accuracy diagnosis of viral infection.

The novel biomarkers for the differentiation of viral disease may be of clinical significance. Therefore, we investigated if TRAIL and IP-10 can differentiate viral and bacterial or non-infectious causes of fever in the ED. Furthermore, we combined the results of TRAIL and IP-10 with PCT in a combined biomarker model.

METHODS

This was was a substudy of a previous study on PCT-guided therapy for febrile patients in the ED at the Slotervaart Hospital, Amsterdam, the Netherlands[14]. The local ethics committee approved the study. From May 2010 to May 2012, a total of 107 adult febrile patients (T > 38.0 °C) were included after written informed consent was obtained. From the cohort of this study, patients with a confirmed diagnosis of bacterial infection, viral infection, or non-infectious disease were selected. The patients were confined to one of the following groups. 1)Confirmed bacterial infection, defined as a positive culture result in concordance with clinical findings. 2) Confirmed viral infection, defined as positive viral PCR in concordance with clinical findings. 3) Non-infectious disease, no evidence of infectious fever despite extensive supplementary diagnostics, and a strong alternative diagnosis. Patients with both a confirmed bacterial and viral infection were excluded. In all patients, levels of TRAIL and IP-10 were determined, using Human TRAIL/TNFSF10 Quantikine ELISA Kit and Human CXCL10/IP-10 Quantikine ELISA Kit of R&D Systems according to the manufacturer's manual.

Data-analysis

A Kruskal-Wallis test was used to determine differences of levels of TRAIL and IP-10 between the three defined groups of patients. Additionally, patients with confirmed viral disease were compared with patients with confirmed non-viral disease (groups of confirmed bacterial infection and non-infectious combined). Furthermore, patients with non-infectious disease and infectious disease (groups confirmed bacterial infection and confirmed viral disease combined), using Mann-Whitney U tests. P-values of <0,05 were considered significant. The area under the ROC for TRAIL and IP-10 was calculated for confirmed viral disease versus non-viral disease. Using the ROC curves, optimal cut-offs of both TRAIL and IP-10 were determined. The value of the square of distance between point (0,1) and the ROC curve (d2) was calculated using d2 = (1-sensitivity)2 + ((1-specificity)2. The lowest value indicated the optimal cut-off. PCT, TRAIL and IP-10 results were combined in a binary logistic regression model. This combined biomarker model for discriminating viral disease used a cut-off of <0.5 μg/L for PCT, and the optimal cut-off values for TRAIL and IP-10. The area under the curve was calculated for the model of the combined biomarkers for confirmed viral disease versus non-viral disease. Data-analysis was performed using statistical package for the social sciences (SPSS) version 21, IBM corporation.

Table 1: Baseline characteristics of study population

		Confirmed viral	Confirmed bacterial	Confirmed non-infectious	p-value (one-way ANOVA)
N		13	33	8	
Female	n (%)	6 (46)	14 (42)	2 (25)	p = 0.616
Age in years	n (%)	53 (36)	65 (30)	56 (37)	p = 0.349
Temperature in ˚C	median [IQR]	38.5 (1.1)	39.0 (0.8)	38.8 (1.7)	p = 0.320
Hospitalization	n (%)	12 (93)	31 (94)	6 (75)	p = 0.257
Hospital length of stay	median [IQR]	6 (5)	7 (9)	7 (15)	p = 0.338
ICU admission	n (%)	2 (15)	6 (18)	1 (11)	p = 0.923
Mortality	n (%)	0 (0)	2 (6)	0 (0)	p = 0.532
Diabetes Mellitus	n (%)	4 (31)	7 (21)	0 (0)	p = 0.241
Immunocompromised	n (%)	1 (8)	5 (12)	1 (13)	p = 0.908
Malignancy	n (%)	1 (8)	5 (15)	4 (50)	p = 0.038
HIV	n (%)	0 (0)	2 (6)	1 (13)	p = 0.484
Steroids	n (%)	2 (15)	7 (21)	2 (25)	p = 0.860
CRP, median mg/mL	median [IQR]	107 (98)	202 (249)	96 (105)	p = 0.060
PCT, median µg/mL	median [IQR]	0.21 (0.38)	1.26 (2.55)	0.30 (0.40)	p = 0.102
TRAIL, median pg/mL	median [IQR]	51 (21)	34 (35)	8 (14)	p = 0.078
IP-10, median pg/mL	median [IQR]	1295 (611)	585 (963)	466 (1234)	p = 0.036
Leukocyte count 10^9 cells/L	median [IQR]	9.7 (5.7)	14.2 (7.0)	6.6 (7.1)	p = 0.002
Respiratory infection	n (%)	11 (85)	12 (36)		
Urinary tract infection	n (%)	0 (0)	13 (39)		
Skin infection	n (%)	1 (8)	3 (9)		
Viremia / bacteriemia only	n (%)	1 (8)	3 (9)		
Cholangitis	n (%)	none	1 (3)		
Meningitis	n (%)	none	1 (3)		

Abbrevations list: N: number. IQR: Inter quartile range. ICU: intensive care unit. HIV: human immunodeficiency virus. CRP: C-reactive protein. PCT: Procalcitonin. TRAIL: TNF-related apoptosis-inducing ligand. IP-10: interferon-γ-inducible protein 10

RESULTS

A total of 54 patients were selected; 13 patients had a confirmed viral infection, 33 a confirmed bacterial infection and 8 patients had confirmed non-infectious disease. Respiratory infection was present in 11 patients with viral disease, and in 12 patients with bacterial disease. Baseline characteristics are reported in table 1, specific pathogens of the defined groups are reported in table 2.

Table 2: Pathogens in the confirmed viral, confirmed bacterial and confirmed non-infectious disease group

Confirmed viral disease		Confirmed bacterial disease		Confirmed non-infectious disease	
Pathogen	N*	Pathogen	N**	Cause	N
Adenovirus	1	E. Coli	11	Fever without known cause, confirmed non-infectious	2
Dengue	1	H. influenzae	2	Gout	1
Herpes simplex virus	1	K. Pneumoniae	4	Hyperthyroidism	1
Influenza A virus	4	M. pneumoniae	2	Malignant neuroleptic syndrome	1
parainfluenza virus	2	P.aegurinosa	8	Tumor fever	3
Rhinovirus	5	S. agalactiae	1		
		S. aureus	2		
		S. pneumoniae	2		
		Enterococcus sp	1		
		Legionella sp	1		
		Micrococcus sp	1		
		Streptococcus sp	3		
Total	14	Total	38	Total	8

N: number. * One patient had both a confirmed adenovirus and rhinovirus infection ** Three patients had confirmed infections with two different bacterial pathogens, one patient had confirmed infection with three different bacterial pathogens.

Figure 1.

Concentration of TRAIL in pg/ml in confirmed viral, bacterial and non-infectious disease

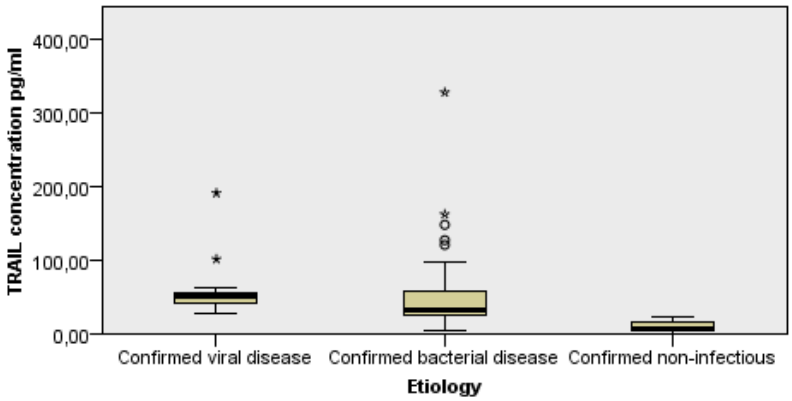
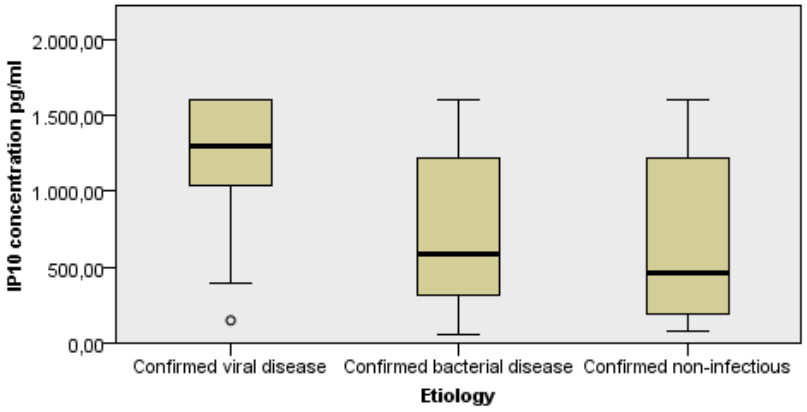


Figure 2.

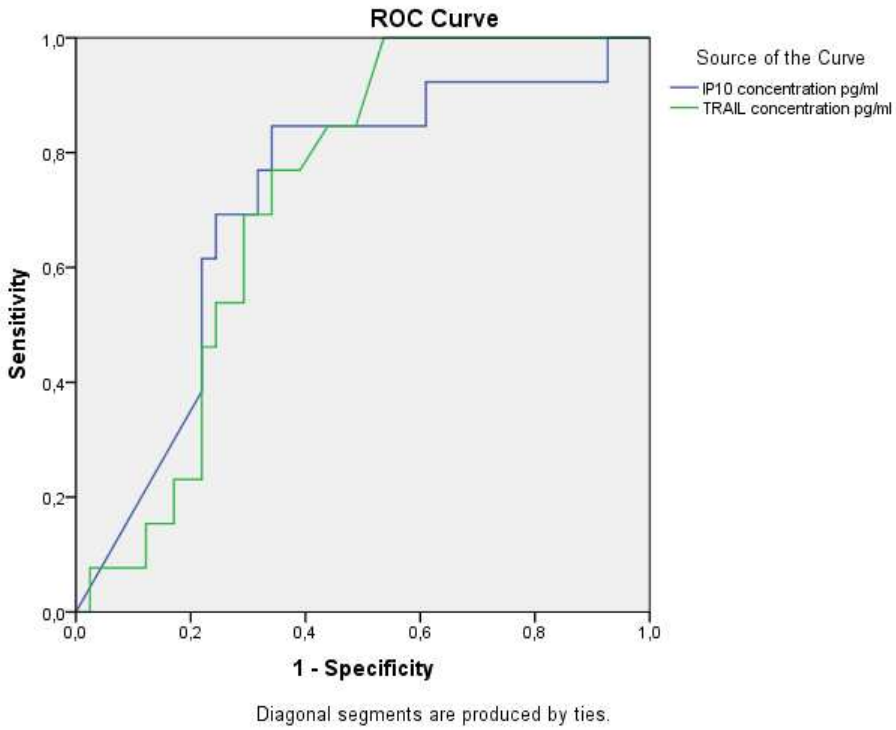
Concentration of IP-10 in pg/ml in confirmed viral, bacterial and non-infectious disease



The levels of TRAIL were significantly higher in patients with a confirmed viral infection, compared to patients with a confirmed bacterial infection and confirmed non-infectious fever ($p > 0.001$). IP-10 did not show a significant difference ($p = 0.052$). Results are shown in figure 1 and 2. TRAIL levels were not significantly different between patients with confirmed viral and confirmed bacterial infections ($p = 0.100$). IP-10 levels were significantly elevated ($p = 0.022$) in confirmed viral versus confirmed bacterial infections.

Figure 3.

Receiver-Operator-Curve of TRAIL and IP-10 in differentiation between confirmed viral disease versus non-viral disease

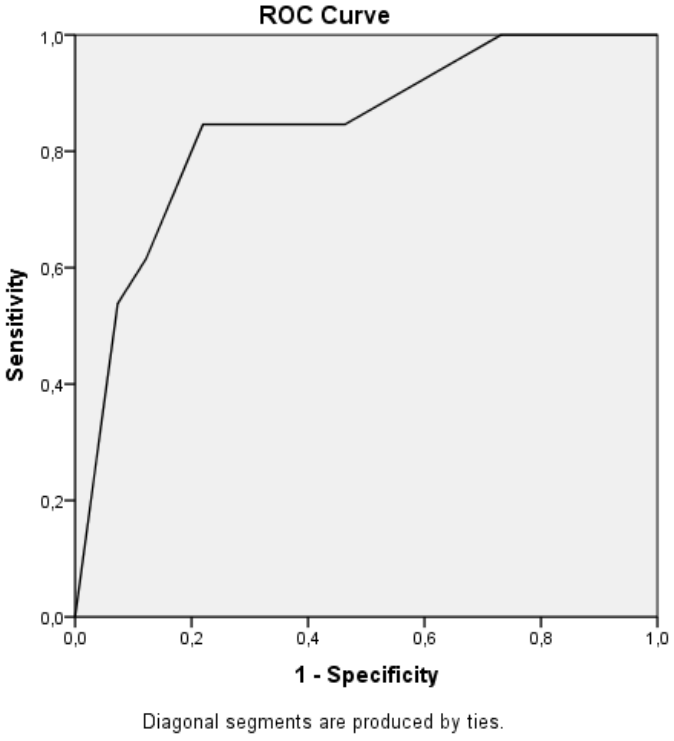


When compared between confirmed viral and confirmed non-viral disease, levels of both TRAIL and IP-10 were significantly higher in confirmed viral disease, ($p = 0.016$, and $p = 0.017$, respectively). TRAIL levels were significantly elevated in patients with confirmed infections, compared to patients with confirmed non-infectious fever, ($p < 0.000$), no significant difference between IP-10 levels was observed ($p = 0.319$). ROC for TRAIL and for IP-10 (figure 3) showed an area under the curve of 0.72 (95%CI 0.56 – 0.88) for TRAIL and 0.72 (95%CI 0.59 – 0.86) for IP-10 respectively, in the discrimination of confirmed viral disease from confirmed non-viral disease (figure 3). The optimal cut-off in differentiating confirmed viral disease from confirmed non-viral disease of TRAIL was 93.83 pg/ml, and 911.43pg/ml for IP-10.

Binary logistic regression analysis for PCT <0.5 µg/L for confirmed viral disease resulted in an odds ratio (OR) of 3.18 (95%CI 0.76 – 13.24). The AUC of PCT <0.5 µg/L for confirmed viral disease was 0.629 (95%CI 0.46 – 0.80). The combined biomarker model for confirmed viral disease versus confirmed non-viral disease consisted of a PCT level of <0.5 µg/L, a TRAIL level of >93.83 pg/ml and an IP-10 level of > 911.43pg/ml. The binary logistic regression analysis of the combined biomarker model resulted in an OR of 5.10 (95%CI 0.96 – 27.04). The AUC of the combined biomarker model was 0.84 (95%CI 0.72 – 0.97). AUC is shown in figure 4.

Figure 4.

Receiver-Operator-Curve of the combined biomarker model, consisting of optimal cut-off values of TRAIL (≥93.83 pg/ml), IP-10 (≥911.49 pg/ml) and procalcitonin (<0.5 µg/L) in differentiation between Confirmed viral disease versus non-viral disease



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DISCUSSION

In this study of febrile ED patients, there was a strong association between elevated levels of TRAIL and IP-10 and the presence of viral infection. The combination of TRAIL and IP-10 with PCT resulted in an even higher accuracy in discriminating confirmed viral disease from confirmed non-viral disease.

Oved et al.[11] reported TRAIL levels to be lower in patients with bacterial infections. In our study, we demonstrated that this finding is reproducible in an undifferentiated cohort of ED patients. This result further strengthens the evidence that TRAIL may be utilized as a biomarker for viral disease in clinical practice. Although a significant difference in IP-10 levels between groups in our population could not be observed, a trend towards significance was shown. Larger validation studies may show the discriminative value of IP-10 in more detail.

This is the first study to report on biological markers for differentiating between confirmed viral and confirmed non-viral infection in an ED setting. These biomarkers may be helpful in ED treatment decision-making. Currently, the initiation of antibiotics in the ED is based on the rule-out of bacterial infections[2,3]. Additional viral rule-in or rule-out may further reduce the over-prescription of antibiotics in the ED. However, the clinical significance of TRAIL and IP-10 in the treatment of febrile patients is still unclear. There is a need for larger validation studies of these novel biomarkers; a larger follow-up study is currently being set-up at our institution. Most importantly, the cut-off values have to be clinically validated in order to use TRAIL and IP-10 to guide antibiotic therapy.

The findings of this study are in line with theoretically favorable characteristics of TRAIL and IP-10. The interferon-gamma (IFN-γ) pathway is activated in reaction to viral infections[15]. TRAIL is in turn upregulated by IFN alpha (IFN-α) and beta (IFN-β), and by IFN-γ, produced autocrinely by T-helper cells. TRAIL binds to the TRAIL receptor and induces apoptosis of the infected cells[12,16-18].

IP-10 is also upregulated by IFN-γ, and less elevated in bacterial infections compared to viral infections[11,19]. This in line with our findings. IP-10 is a CXC chemokine secreted by several cell types including macrophages and is induced in response to diverse stimuli, such as IFN α, β and γ ,but also directly by viruses[20]. IP10 has been shown to play an important role in the recruiting of virus-specific T-cells and viral clearance in simian varicella virus infection[21] and during acute hepatitis C infection[22].

Limitations

A selection of patients of a cohort with undifferentiated febrile patients was used. Of a total of 107 patients, there was a definitive diagnosis in 54 patients. The cut-off values found in our analyses were derived from a small sample size. The cut-off values used in this study show additional accuracy in the diagnosis of confirmed viral disease in a combined biomarker model, and thereby suggest that both TRAIL and

IP-10 are of discriminatory value. However, these cut-off values are not validated in a sufficiently large cohort and can therefore not be utilized in clinical practice yet. Notwithstanding, these findings are a proof of principle for the use of TRAIL and IP-10 in the ED. In this study, patients with either a confirmed bacterial infection, a confirmed viral infection, or confirmed non-infectious disease were included. We excluded patients with both confirmed bacterial and viral infections. In clinical practice, it may be difficult to distinguish a community-acquired pneumonia from a viral upper respiratory infection with bacterial superinfection. Our cohort was a sample of an ED population, consisting of a variety of viral and bacterial pathogens. The patients in the confirmed viral infections group mainly had respiratory tract infections, whereas the patients in the confirmed bacterial infections group had more diverse sites of infection. Besides respiratory tract infections, they also had urinary tract and skin infections. It could well be possible that different inflammatory cascades are activated in different sites of infection. This may account for the differences in TRAIL and IP-10 levels between confirmed viral and confirmed bacterial infections. However, in this clinical study, a difference between markers is shown nonetheless, and these findings advocate further research. At the moment, TRAIL and IP10 are only available as Enzyme Linked Immunosorbent Assay (ELISA), making them less suitable for clinical use in the ED. Further studies in larger ED cohorts are necessary to establish the added value of these viral infection markers in order to motivate diagnostic companies to develop immunoassays on automated immunochemistry platforms with more favorable turn-around times (i.e. < 60 minutes) making these markers more suitable for ED use.

CONCLUSION

Measurement of TRAIL and IP-10 in febrile patients in the ED may be of added value in the diagnostic process, with elevated levels indicating the presence of confirmed viral infection. The addition of TRAIL and IP-10 to PCT in differentiating between confirmed viral and confirmed non-viral disease in a combined biomarker model results in a higher discriminating value than the single biomarkers on their own. These results are a proof of principle. Validation in a larger cohort may determine the clinical value of TRAIL and IP-10 in the ED.

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