PART II
ADDITIONAL BIOMARKER STRATEGIES

CHAPTER 7

IDENTIFYING PATIENTS WITH BACTERIAL INFECTIONS USING A COMBINATION OF BIOMARKERS IN THE EMERGENCY DEPARTMENT

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ABSTRACT

Background

To effectively reduce the unnecessary use of broad spectrum antibiotics in the emergency department(ED), patients with bacterial infections need to be identified accurately. We investigate the diagnostic value of a combination of biomarkers for bacterial infections CRP and PCT, together with biomarkers for viral infections, TRAIL and IP-10, in identifying bacterial infections in a general ED population with fever.

Methods

This is a sub-study in the HiTEMP cohort. Patients with fever were included during ED triage, and blood samples were obtained. Using both diagnostics and expert panel analysis, all patients were classified as having either (suspected or confirmed) bacterial, or non-bacterial disease. Using multivariable logistic regression analysis, three biomarker models were calculated, model 1:(CRP,TRAIL,IP-10), model 2:(PCT,TRAIL,IP-10) and model 3:(CRP, PCT, TRAIL,IP-10).

Results

A total of 315 patients were included, of whom 228 patients had a bacterial infection. The areas under the curve for the combined models were, for model 1: 0.730(95%CI 0.665–0.795), for model 2: 0.748 (95%CI 0.685–0.811), and for model 3: 0.767(95%CI 0.704–0.829).

Discussion

These findings show that a combination of CRP, PCT, TRAIL and IP-10 can identify bacterial infections with higher accuracy than single biomarkers and combinations of a single bacterial biomarkers combined with TRAIL and IP-10.

INTRODUCTION

Antibiotic resistance is a threat to global health[1,2]. The widespread use of broad spectrum antibiotics contributes to the selection pressure of antibiotic resistant bacteria[3,4].

Patients with suspected infections in the emergency department (ED) are often treated with broad spectrum antibiotics, because bacterial infections cannot be ruled out[5]. Currently, the diagnostic workup in EDs consists of clinical assessment and laboratory investigations such as C-reactive protein (CRP) and procalcitonin (PCT). PCT-guided therapy has successfully reduced antibiotics in selected populations of patients with respiratory complaints in the ED[6-8]. However, in a general ED population, PCT-guided therapy proved to be ineffective, due to inaccuracy of PCT in differentiating between bacterial and non-bacterial disease[9]. In order to reduce antibiotics prescriptions in a general ED population, the discrimination of bacterial from non-bacterial disease has to be as accurate as possible. Recently, studies have shown that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and interferon-gamma induced protein-10 (IP-10), two immune response derived biomarkers, can accurately differentiate between viral and bacterial infections in the ED, both as single markers and in combination with CRP or PCT[10-12]. These study populations consisted either of young children, or had highly selected patient populations. Moreover, the combination of both CRP and PCT, together with TRAIL and IP-10, has not been investigated in an adult ED population. Furthermore, the clinical value of the combination of these biomarkers has not been fully elucidated.

The aim of this study is to investigate the predictive value of a combination of CRP, PCT, TRAIL and IP-10 in diagnosing bacterial infections in a general ED population.

METHODS

This was a sub-study of the HiTEMP study cohort, which is described previously[9,13]. In brief, the cohort of this study consisted of adult patients who visited the ED of the Erasmus University Medical Center between August 2014 and June 2016 with a temperature of ≥38.2 ˚C/ ≥100.7 ˚F in ED triage.

Study population

All adult febrile patients were eligible for inclusion. All patients gave written informed consent. Pregnant patients, patients with a solid organ transplant, severe neutropenia, or active chemotherapy, post-operative patients (up to 72 hours), and patients with a confirmed surgical diagnosis before ED triage and patients with a life expectancy of less than 24 hours were excluded. Patients who opted out of participating in additional studies after the HiTEMP study, were excluded.

Study design

In the ED, blood samples were obtained for clinical use and for additional research purposes. In all patients, CRP, PCT, TRAIL and IP-10 were determined. In this study, predictive values of three combined models of multiple biomarkers were investigated, for differentiating between bacterial and non-bacterial disease. All models contained optimal cut-off values of TRAIL and IP-10. Model 1 further included CRP, model 2 included PCT, and model 3 included both CRP and PCT.

Primary outcome

The primary outcome was the presence of either a confirmed or suspected bacterial infection, and defined as “bacterial infection”. Patients were classified in either the confirmed and suspected bacterial infections group, or the non-bacterial infections group, consisting of patients with confirmed and suspected viral infections, patients with non-infectious fever, and patients with undetermined disease, but not suspected of bacterial infection.

Confirmed infections were defined as clinically significant cultures. The presence of a coagulase negative staphylococcus (CNS) in a blood culture was deemed as contamination. Suspected bacterial infections were determined by an expert panel analysis, a structured medical chart review by two independent physicians, using predefined criteria (suppl 1). In case of disagreement, a third expert physician acted as referee. In case of the presence of both a confirmed viral and bacterial infection, the patient was classified in the bacterial infections group, because a bacterial infection was considered clinically relevant.

Data analysis

Differences in baseline characteristics were compared between patients with bac-

terial infections and patients with non-bacterial disease using Fisher's exact test for dichotomous variables and independent samples T-test for continuous variables, and Mann Whitney U test for not normally distributed continuous variables.
Accuracy of CRP, PCT, TRAIL and IP-10 for bacterial infections was reported as sensitivity and specificity and area under receiver operating characteristic curve (AUC) for optimal cut-offs of individual biomarkers. We calculated the optimal cut-off of CRP, PCT, TRAIL and IP-10 using Youden's index. For CRP and PCT, the optimal cut-off was defined as the lowest value that predicted the presence of a bacterial infection. TRAIL and IP-10 were used as a rule-out of bacterial infections. The optimal cut-off was defined as the highest value that still predicted the presence of a bacterial infection. Higher values of TRAIL and IP-10 predicted an absence of a bacterial infection. Sensitivity and specificity for CRP, PCT, TRAIL and IP-10 in diagnosing bacterial infections were reported with binominal proportion confidence intervals (CIs), using the Clopper-Pearson method. We created three multivariable binary logistic regression models to predict combined accuracy of bacterial infections. The models included the optimal cut-offs of the following biomarkers, model 1: CRP, TRAIL, IP-10, model 2: PCT, TRAIL, IP-10, and model 3: CRP, PCT, TRAIL, IP-10. An AUC for each of the models was reported. All statistical tests were two-sided with a significance level of 0.05. Data-analysis was performed with the statistical package for the social sciences (SPSS), version 23, IBM cooperation.

RESULTS

In the HiTEMP study, a total of 449 patients were included in the Erasmus University Medical Center. In this analysis, the total number of patients 315. Nine patients did not consent for additional studies other than the HiTEMP study, and in 125 patients, insufficient additional material for analysis of TRAIL and IP-10 was available. Of these 315 patients, there was no respiratory rate available in 95 patients, and in two patients no blood pressure was available because these variables were not measured in ED triage. Of all patients included in the study, 228 had either a suspected or confirmed bacterial infection. Of these 228 patients, 7 (3%) had a concomitant confirmed viral infection. Another 87 patients were not suspected of having a bacterial infection. Of these 87 patients, 10 (12%) had a confirmed viral infection, 48 (55%) had a suspected viral infection, 23 (26%) had confirmed non-infectious fever, and in 6 (7%) patients the cause of fever was unknown (Table 1).

There were statistically significant differences baseline characteristics between patients with bacterial and non-bacterial disease in age ($p = 0.00$), temperature ($p = 0.02$), malignancy as comorbidity ($p = 0.01$) and diabetes mellitus as comorbidity ($p = 0.00$). The AUC for bacterial infections for CRP was 0.679(95% CI 0.613 – 0.746), for PCT 0.680 (95% CI 0.619 – 0.742), and the ROC for ruling out bacterial infections

Table 1. Baseline characteristics

		All (n = 315)	Non-bacterial infections (n = 87)	Bacterial infections (n = 228)	P-value
Demographic characteristics					
Age	median [IQR]	58 [39 - 69]	47 [27 - 63]	61 [45 - 70]	p < 0.001
Female sex	n (%)	149 (47)	40 (46)	109 (48)	p = 0.80
Vital signs at presentation					
Temperature	median [IQR]	38.7 [38.5 - 39.2]	38.6 [38.4 - 39.1]	38.8 [38.5 - 39.3]	p = 0.15
Heart rate	median [IQR]	105 [95 - 120]	107 [90 - 120]	105 [95 - 120] n = 229	p = 0.96
Systolic bloodpressure	median [IQR] n = 313	130 [118 - 145]	128 [117 - 140] n = 85	130 [119 - 146]	p = 0.18
Diastolic bloodpressure	median [IQR] n = 313	75 [67 - 85]	75 [69 - 85] n = 85	75 [66 - 85]	p = 0.42
Respiratory rate	median [IQR] n = 220	20 [16 - 25]	24 [16 - 24] n = 63	20 [16 - 25] n = 159	p = 0.11
Comorbidity					
Diabetes	n (%)	50 (16)	5 (6)	45 (20)	p = 0.00
Malignancy	n (%)	69 (22)	10 (12)	59 (26)	p = 0.01
HIV	n (%)	16 (5)	6 (7)	10 (4)	p = 0.39
Current medication use					
Current antibiotics use (before ED vis	n (%)	42 (13)	7 (8)	35 (15)	p = 0.10
Corticosteroids	n (%)	45 (14)	16 (18)	29 (13)	p = 0.21
Oral anticoagulants	n (%)	37 (12)	6 (7)	31 (14)	p = 0.12
Acetylsalicylic acid	n (%)	32 (10)	6 (7)	26 (11)	p = 0.30
Biomarkers					
CRP in mg/L	median [IQR]	62 [19 - 142]	24 [13 - 82]	71 [28 - 161]	p < 0.001*
PCT in mcg/L	median [IQR]	0.22 [0.10 - 0.65]	0.13 [0.07 - 0.27]	0.31 [0.11 - 1.12]	p < 0.001*
TRAIL in pg/ml	median [IQR]	28.0 [0.0 - 74.5]	37.6 [0.0 - 145.0]	24.8 [0.0 - 62.4]	p = 0.00*
IP-10 in pg/mL	median [IQR]	470 [197 - 825]	774 [340 - 825]	351 [183 - 723]	p < 0.001
Clinical syndrome at presentation					
Skin	n (%)	31 (10)	1 (1)	30 (13)	
Respiratory	n (%)	120 (38)	45 (52)	75 (33)	
Urogenital	n (%)	65 (21)	0 (0)	65 (28)	
Abdominal	n (%)	35 (11)	4 (5)	31 (14)	
Central nervous system	n (%)	3(1)	0 (0)	3 (1)	
Other	n (%)	4 (1)	0 (0)	4(2)	
Noninfectious	n (%)	25 (8)	24(28)	1(0)	
Unknown	n (%)	34 (11)	13 (15)	21 (9)	
Final diagnosis after expert review					
Suspected bacterial infections	n (%)	113 (36)	0 (0)	113 (50)	
Confirmed bacterial infections	n (%)	115 (37)	0 (0)	115 (50)	
Suspected viral infections	n (%)	48 (15)	48 (55)	0 (0)	
Confirmed viral infections	n (%)	17 (5)	10 (12)	7 (3)	
Confirmed non-infectious fever	n (%)	23 (7)	23 (26)	0 (0)	
Fever of unknown etiology	n (%)	6 (2)	6 (7)	0 (0)	
Additional diagnostics					
Bacteremia	n (%)	58 (18)	1 (1)	57 (18)	

* P-values were calculated with Fisher's exact test for dichotomous variables, and independent samples T-test for continuous variables. Continuous variables that were not normally distributed, were calculated using the Mann-Whitney U test with an *. ** This posititive blood culture was a coagulase negative staphylococcus, and was considered contamination. CRP: C-reactive protein, ED: emergency department, HIV: human immunodeficiency virus, IQR: interquartile range, IP-10: interferon-gamma induced protein-10, PCT: procalcitonin

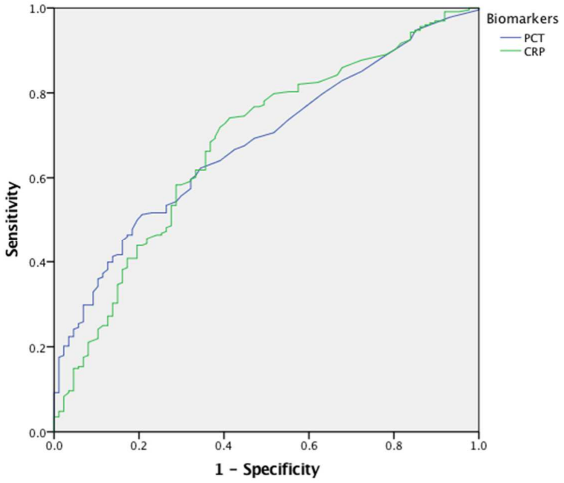
was 0.607 (95% CI 0.532 – 0.683) for TRAIL and 0.665 (0.597 – 0.734) for IP-10. The ROCs are reported in figure 1.

The optimal cut-offs, Youden's index, sensitivity and specificity were reported in table 2. In multivariable logistic regression analysis, the odds ratios (OR) of the optimal cut-offs for biomarkers for bacterial infections in model 1 were: for CRP, OR 3.07 (95% CI 1.78 – 5.31), for TRAIL OR 1.94 (95% CI 1.05 – 3.58) and IP-10 OR 2.58

Figure 1. ROC curves of CRP, PCT, TRAIL and IP10 for suspected and confirmed bacterial infections

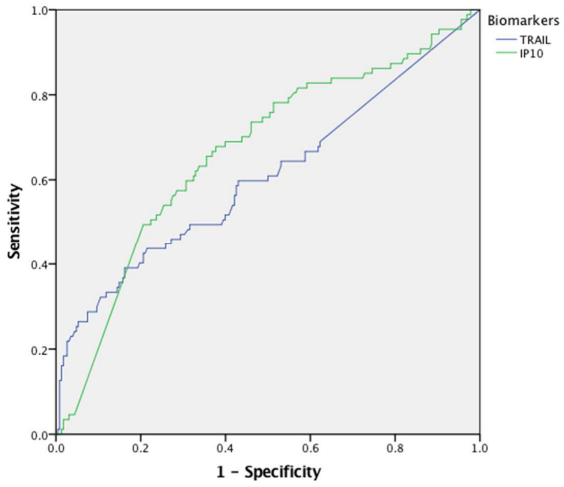
AUC of CRP and PCT for suspected and confirmed bacterial infections

CRP: AUC 0.679 (95% CI 0.613 – 0.746)
PCT: AUC 0.680 (95% CI 0.619 – 0.742)



AUC of TRAIL and IP-10 for ruling out suspected and confirmed bacterial infections

TRAIL: AUC 0.607 (95% CI 0.532 – 0.683)
IP-10: AUC 0.665 (95% CI 0.597 – 0.734)



AUC: Area under curve, CRP: C- reactive protein, IP-10: interferon-gamma induced protein-10 PCT: Procalcitonin, ROC curve: Receiver operator characteristic curve, TRAIL: tumor necrosis factor-related apoptosis-inducing ligand

Table 2. Analyses of predictive values of biomarkers for primary outcome

n = 315

Primary outcome: suspected and confirmed bacterial infections

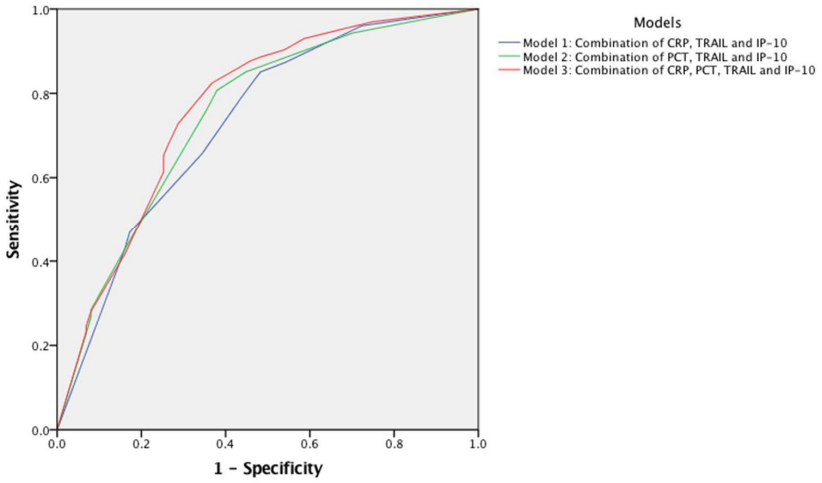
Predictors	AUC	(95% CI)	Youden's index	Optimal cut-off		Sensitivity	(95% CI)	Specificity	(95% CI)
Biomarkers									
CRP	0.679	0.613 – 0.746	0.33	32	mg/ml	0.72	0.66 - 0.78	0.61	0.50 - 0.71
PCT	0.680	0.619 – 0.742	0.31	0.30	mg/ml	0.51	0.45 - 0.58	0.79	0.69 - 0.87
TRAIL*	0.607	0.532 – 0.683	0.23	547	pg/ml	0.83	0.78 - 0.88	0.39	0.29 - 0.50
IP-10*	0.665	0.597 – 0.734	0.30	79.3	pg/ml	0.63	0.56 - 0.69	0.68	0.57 - 0.77

* Results for TRAIL and IP-10 for the abscence of primary outcome. AUC: area under curve, CI: confidence interval, CRP: C-reactive protein, ICU: Intensive care unit, mg/ml: milligrams per milliliter, PCT: procalcitonin, pg/ml: picograms per milliliter, proADM: proadrenomedullin, proET-1: proendothelin-1, qSOFA: quick SOFA score, SIRS: systemic inflammatory response syndrome, suPAR: soluble urokinase-type plasminogen

(95% CI 1.48 – 4.51).
The ORs for model 2 were: for PCT, OR 4.10 (95% CI 2.22 – 7.63), for TRAIL, OR 1.79 (95% CI 0.97 – 3.33) and IP-10, OR 3.45 (95% CI 1.94 – 6.12). The ORs for model 3 were: for CRP, OR 2.33 (95% CI 1.31 – 4.13), for PCT OR 3.30 (95% CI 1.74 -6.28), for TRAIL OR 1.56 (95% CI 0.82 – 2.95) and IP-10 OR 3.09 (95% CI 1.72 – 5.55).

The AUCs of the combined optimal cut-offs of biomarkers models for bacterial infections were, for model 1: AUC of 0.730 (95% CI 0.665 – 0.795), for model 2: 0.748 (95% CI 0.685 – 0.811), and for model 3: 0.767 (95% CI 0.704 – 0.829). The ROCs were reported in figure 2.

Figure 2. ROC curves of combined biomarker models for suspected and confirmed bacterial infections



Model 1: AUC 0.730 (95% CI 0.665 – 0.795)
Model 2: AUC 0.748 (95% CI 0.685 – 0.811)
Model 3: AUC 0.767 (95% CI 0.704 – 0.829)

AUC: Area under curve, CRP: C- reactive protein, IP-10: interferon-gamma induced protein-10 PCT: Procalcitonin, ROC curve: Receiver operator characteristic curve, TRAIL: tumor necrosis factor-related apoptosis-inducing ligand

DISCUSSION

The results of this study showed that a combined model containing optimal cut-offs of CRP, PCT, TRAIL and IP-10 predicted the presence of bacterial infections with higher probability than individual measurements of the currently used biomarkers CRP and PCT. Moreover, the model combining both CRP and PCT, together with TRAIL and IP-10, was more accurate than models with either CRP or PCT as a single marker.

A previous study by van Houten et al. showed that a combination of CRP, TRAIL and IP-10 was superior in diagnosing bacterial infections compared to PCT in young children[11]. Another study, in adult ED patients, showed that PCT in combination both TRAIL and IP-10 was more accurate in ruling in viral infections in patients with confirmed infections than individual measurements of these biomarkers. Our results are in line with these findings. Furthermore, by comparing three combined models, we showed that a combination of both CRP and PCT with TRAIL and IP-10 is superior than either individual biomarker.

In our results, we found a lower AUC than other studies that used a combination of biomarkers in differentiating between bacterial and non-bacterial disease[11,14]. These studies both used a previously described combination, called the "signature test" or "index test"[10]. This test is a logistic regression formula with predefined cut-off levels of CRP (40mg/l), TRAIL (70pg/ml) and IP-10 (500 pg/ml). Furthermore, in the index test, patients were divided into three groups, classified as either having a viral, or equivocal, or bacterial infection. The results presented in these studies showed the accuracy in differentiating bacterial from viral infections, with exclusion of the equivocal group, such as in the study by van Houten et al., who reported a AUC of 0.90 (95% CI 0.86 – 0.95). To effectively reduce antibiotics in patients with infectious diseases in the ED, bacterial infections have to be ruled-out unequivocally. When diagnostic uncertainty remains, biomarker-guided therapy is not effective9. Therefore, future prospective interventional studies should investigate if this approach, with a classification with three categories, or a category which consists of patients with a very low probability of having a bacterial infection, may reduce prescription of antibiotics in patients in this category.

An additional explanation of the differences in diagnostic accuracy between previous studies and our results, is the selection of study populations. Van Houten et al. only included children between 2 and 60 months of age, with either a suspected respiratory tract infection, or fever without source[11]. In pediatric patients, fever is most commonly the result of respiratory infections[15]. In this study, only 38% of patients had respiratory focus of fever. Differences in etiology of fever may account for a lower accuracy in our population.

Limitations

In this study, we used a cohort of the HiTEMP study[9]. The main inclusion criterion was fever. Although this is an objectively measurable variable, it created a selection bias, because patients with suspected infections without fever were excluded from participation. As in similar studies on differentiating between bacterial and non-bacterial disease, the reference standard of suspected and confirmed bacterial infections we used in our study is no gold standard[11,16]. In the structured medical chart review, one of the criteria was “clinical improvement under antibiotics”. Some of the patients who were classified using this criterion, may also have improved without antibiotics. Therefore, there may have been overestimation of the number of patients in the group of suspected bacterial infections, resulting in a lower accuracy of the combination of biomarkers. The multivariable logistic regression model with a combination of biomarkers was calculated using optimal cut-offs. The use of these binary cut-offs made the model user-friendly, at the cost of accuracy. Furthermore, this model was not validated. Therefore, we suggest a validation study of a multivariable model including biomarkers CRP, PCT, TRAIL and IP-10, with the incorporation of a group with intermediate probability of bacterial infections.

CONCLUSION

Using a combination of biomarkers CRP, PCT, TRAIL and IP-10, bacterial infections could be diagnosed with higher accuracy compared to single biomarkers or a combination of either CRP or PCT with TRAIL and IP-10, in adult patients with fever in a general ED. Interventional studies may determine the clinical value of the combination of these biomarkers.

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