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Trends in vital signs and routine biomarkers in sepsis patients during resuscitation in the emergency department: a prospective observational pilot study.

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Keywords

Sepsis; Resuscitation; Vital signs; Routine biomarkers; Response to treatment.

ABSTRACT

Objectives: Sepsis lacks a reliable, readily available, measure of disease activity. Thereby, it remains unclear how to monitor response to treatment. Research on numerous (new) biomarkers associated with sepsis provided disappointing results and little is known about changes in vital signs during sepsis resuscitation. We hypothesized that trends in vital signs together with routine biomarker levels during resuscitation might provide information about the response to treatment at a very early stage of sepsis in the emergency department (ED). We therefore explorer trends in vital signs and routine biomarker levels during sepsis resuscitation in the ED.

Design: Prospective observational pilot study.

Setting: Emergency department of a tertiary care teaching hospital.

Participants: 99 Adult non-trauma patients with suspected infection and two or more SIRS criteria admitted to the ED.

Primary and secondary outcome measures: vital signs and biomarker levels at admittance (T0) and after 3 hours in the ED (T1).

Results: In total, data of 99 patients was analysed. Of these patients, 63 presented with sepsis, 30 with severe sepsis and 6 patients with septic shock. All vital signs decreased, except for peripheral oxygen saturation which increased. Almost all routine biomarker levels decreased during resuscitation, except for CRP, bands, potassium, Troponin T and direct bilirubin which remained stable. Sodium, chloride and NT pro-BNP increased slightly.

Conclusions: Vital signs and biomarker levels showed descending trends during resuscitation, except for parameters directly affected by treatment modalities. Despite these trends most patients clinically improved. Trends in vital signs and routine biomarkers might be helpful in predicting clinical course and response to treatment in sepsis patients during early resuscitation.

Trail registration: N/A

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Our pilot study is the first study that looks at trends in vital signs and biomarker levels during sepsis resuscitation in the emergency department.
- Our pilot study shows that there are significant trends in vital signs and biomarker levels during resuscitation, these trends might potentially serve as a guide for treatment or to measure disease activity.
- Our pilot study was not designed to find the cause of the trends: trends might or might not have evolved as a result of the treatment provided.
- The measurement interval of 3 hours chosen in this study might not be the optimal one, we recommend a follow-up study to find the optimal interval between measurements.

INTRODUCTION

Early and aggressive resuscitation is an important factor to reduce mortality of sepsis.[1, 2] It appears that early recognition of the septic patient and timely and aggressive resuscitation is more important than the specific kind of treatment provided.[1, 3, 4] Sepsis lacks a reliable measure of disease activity, similar to the viral load in HIV or left ventricle function in cardiology.[5, 6] Therefore, it remains unclear how response to treatment can be monitored.[6, 7] One known approach to monitor this is to monitor the patient's vital signs. However, there is little information about changes in vital signs in sepsis and their relation to treatment during early resuscitation in the emergency department (ED). Furthermore, numerous biomarkers associated with sepsis have been studied for this purpose, generally with disappointing results. Their sensitivity and specificity is too low to be of real clinical value and they are often not readily available.[7, 8]

Up to 50% of all patients with sepsis are admitted through the ED.[9] Patients are usually transferred from the ED to either the intensive care unit (ICU) or nursing wards within 4 hours.[10, 11] Within these 4 hours, early resuscitation is initiated, preferably as soon as possible.[6] We hypothesize that trends in vital signs together with routine biomarker levels during the resuscitation of patients with sepsis in the ED might provide information about the response to treatment. This information is useful to guide treatment at a very early stage of sepsis, while the patient is still in the ED. To the best of our knowledge, there is no data available about these trends in the ED. Therefore, we performed a pilot study within the 4-hour timeframe that the patient is in the ED.

METHODS

Study design and setting

We performed a prospective observational pilot study in the ED of the University Medical Center Groningen, a tertiary care teaching hospital with over 34,000 visits to the ED annually. Data was collected between October 2013 and April 2014. The hospital's institutional review board approved the study (METc 2013/297). To prevent selection bias, taking blood samples in patients with an altered mental status due to sepsis was also approved by the review board. In these cases, informed consent was obtained from next of kin or from the patient during their stay in hospital.

Study population and protocol

Adult non-trauma patients visiting the ED with presumed infection or sepsis were screened for inclusion. Inclusion criteria were: age ≥18 years, presumed or confirmed infection, and two or more systemic inflammatory response syndrome (SIRS) criteria as defined by the International Sepsis Definitions Conference.[12]

Patients are usually transferred from the ED to either ICU or nursing ward within 4 hours. To detect trends in vital signs and biomarker levels, we took measurements at two points within this timeframe: at admittance to the ED (T_0) and after 3 hours (T_1). At T_0, a nurse measured the patient's vital signs and took a routine blood sample. Vital signs were measured with a patient monitor (IntelliVue MP30 System with Multi-Measurement Module, Philips, Eindhoven, The Netherlands), except for temperature which was measured using an electronic tympanic ear thermometer (Genius 2; Mountainside Medical Equipment, Marcy, NY, USA). Simultaneously with the routine blood sample, the nurse took a number of additional blood vials for this study. These additional vials were temporarily stored until informed consent was obtained. This procedure ensured that treatment was not delayed for patients participating in the study. The vials for the routine blood sample were immediately sent to the hospital's central laboratory for analysis and were analysed for the routine biomarkers listed in Table 1.

Table 1. Overview of the measured biomarkers and their characteristics.

Name	Unit	CV (%)	Reference values
Routine biomarkers			
Albumin	g/L	1.4	35-50
Alkaline phosphatase (ALP)	U/L	2.2	Male: <115 Female: <98
Aspartate transaminase (AST)	U/L	1.4	Male: <35 Female: <31
Bands	%	n/a	0-3
Bilirubin, direct	umol/L	1.9	<5
Bilirubin, total	umol/L	1.9	<17
Calcium	mmol/L	1.4	2.20-2.60
Chloride	mmol/L	0.8	97-107
Creatinine	umol/L	2.0	Male: 50-110 Female:50-90
C-reactive protein (CRP)	mg/L	3.0	<5
Gamma-glutamyl transferase (Gamma-GT)	U/L	1.9	Male: <55 Female: <38
Glucose	mmol/L	1.5	4.0-5.5 (fastening)
Haemoglobin (Hb)	mmol/L	1.3	Male:8.7-10.6 Female: 7.5-9.9
Lactate	mmol/L	1.5	0.5-2.2
Lactate dehydrogenase (LDH)	U/L	1.3	Male: <248 Female: <247
Leukocytes	10^9/L	1.8	4-10
Potassium	mmol/L	0.8	3.5-5.0
Sodium	mmol/L	0.7	135-145
Thrombocytes	10^9/L	4.4	150-350
Urea	mmol/L	2.5	2.5-7.5
Study specific additional biomarkers			
Cortisol	nmol/L	3.8	08:00: 200-800 16:00: 100-400 22:00: 50-200
D-dimer	ng/ml	4.5	<500
High sensitivity troponin T (hs-Trop T)	ng/L	5.0	<14
N-terminal prohormone of brain natriuretic peptide (NT pro-BNP)	ng/L	2.2	-75 year: <175 >75 year: <450

CV, averaged inter- and intra-essay coefficient of variation during the study inclusion period;

Patients or their healthcare proxies had to provide written informed consent before T_1, otherwise the patient was excluded from the study and the stored vials were destroyed. The stored vials were send to the central laboratory for analysis immediately after obtaining informed consent. The blood in these vials was analysed for 4 additional routinely available biomarkers, as shown in Table 1. These biomarkers were added for the following reasons: NT pro-BNP as a marker for fluid overload, cortisol as a marker for stress response, D-dimer for coagulation status and marker of disseminated intravascular coagulation and Troponin T as marker of myocardial damage.

At T_1 new blood samples were collected and immediately analysed for all biomarkers shown in Table 1. The patient's vital signs were also recorded at T_1 using the same procedures and equipment as at T_0. Furthermore, we recorded the amount of intravenous fluids given to the patient in the ED until T_1. In case a patient was transferred to the ICU or a ward before T_1, a researcher took the T_1 blood samples and vital signs there, according to study protocol.

The attending physician was asked for the suspected focus of sepsis at the moment the patient was transferred out of the ED and was allowed to select multiple options. Demographic data was collected from the patient's electronic medical records. All patients received treatment according to routine sepsis protocol, including fluid resuscitation, antibiotics and supplemental oxygen. The treatment protocol did not change during the inclusion period of the study.

Statistical methods

Continuous data is presented as mean with standard deviation or median with inter-quartile range (IQR) depending on their distribution. Normality was tested using the Shapiro-Wilk test for normality. Categorical data is presented as absolute numbers with percentages. The Wilcoxon related samples signed rank test was used for comparison of biomarker levels and vital signs between T_0 and T_1. Effect sizes are presented as Cohen's *d.*[13] The variance between the sepsis severity groups was tested using the non-parametric Jonckheere-Terpstra test. Missing data was excluded for analysis. All statistical analyses were performed using IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY, USA). A *p*-value of ≤ 0.05 was considered significant; all tests were two-tailed.

RESULTS

In total 101 patients were included. Two patients were excluded, since informed consent could not be obtained from one patient before T_1 and was withdrawn by another patient. The remaining 99 patients were included in the final analysis. Of these patients, 63 presented with sepsis, 30 with severe sepsis and 6 patients with septic shock at ED admission. Patient characteristics, including presumed focus, vital parameters and treatment parameters, are shown in Table 2. The most frequent foci were pulmonic and urogenital. The frequency of these foci did not differ between severity groups. Patients in the septic shock group received more intravenous fluids (3.5L; IQR: 2.9-5.0) compared to severe sepsis and sepsis patients (p=0.009).

Vital signs

Blood pressure at both T_0 and T_1 was inversely related to sepsis severity as blood pressure decreased with increasing severity of sepsis. The results of all vital sign measurements are shown in Table 2 and Table 3. Table 2, shows the vital signs for T_0 and T_1 separated by sepsis severity group. Table 3 includes the deltas between T_0 and T_1 for each vital sign; these are also graphically represented in Fig. 1. We found significant differences for all measured vital signs. As becomes apparent from Fig. 1, all vital signs decreased during the measurement timeframe, except for peripheral oxygen saturation which increased by 1.1%. The heart rate and respiratory rate dropped by more than 10% during resuscitation (p<0.001). At the same time, the systolic blood pressure decreased with 5% and diastolic blood pressure decreased with more than 9% (p<0.001).

Biomarkers

The results for the biomarker levels are shown in Table 3, including deltas between T_0 and T_1 for each biomarker. These deltas are also shown in Fig. 2. Almost all routine biomarkers levels decreased during resuscitation in the ED, except for CRP, bands, potassium and direct bilirubin which remained stable. Levels of sodium and chloride increased slightly with respectively 0.8 and 2.1% (p<0.001). The levels of NT pro-BNP increased with 3.0% (p=0.039) during resuscitation. Cortisol and D-dimer levels decreased by respectively 20.1% (p<0.001) and 3.7% (p=0.039). The high sensitivity Troponin T levels did not show a significant trend.

Biomarker levels were below the laboratory's lower detection limit in several instances. In these instances their value was set to half the lower detection limit. Direct bilirubin levels were below the detection limit (1.0 umol/L) in cases 5 at T_0 and 3 at T_1, D-dimer levels (detection limit: 150 ng/ml) in cases 5 at T_0 and 7 at T_1, hs-Trop T levels (detection limit: 3.0 ng/L) in 3 cases at T_0 and 5 cases at T_1. During the calculation of the deltas, the T_0 values of the biomarkers were zero in a few cases. To avoid division by zero problems during the calculation of the percentual different, these values were handled as missing data. Band levels were zero at T_0 in 5 instances and thrombocyte levels in one instance, these values have been excluded from analysis.

Table 2. Patient characteristics, vital signs and treatment parameters in the emergency department.

	N	Overall	Sepsis	Severe sepsis	Septic Shock	*p*
	99	99 (100.0%)	63 (63.6%)	30 (30.3%)	6 (6.1%)	
Demographics						
Age [b]	99	59 (47-70)	60 (49-70)	56 (44.5-73.3)	56.5 (47-68.8)	0.50
Gender						
Male [a]	99	57 (57.6%)	29 (46.0%)	23 (76.7%)	5 (83.3%)	1.00
Female [a]	99	42 (42.4%)	34 (54.0%)	7 (23.3%)	1 (16.7%)	1.00
Vital signs						
T_0: Heart rate (bpm) [b]	99	110 (100-120)	110 (100-120)	112.5 (104.5-120.8)	113.5 (93.5-136.8)	0.66
T_1: Heart rate (bpm) [b]	93	98 (90-108.5)	98 (89-110)	100 (94.3-105)	100 (88.8-138)	0.71
T_0: Syst. blood pressure (mmHg) [c]	99	124.1 ± 21.87	128.9 ± 18.96	123.3 ± 17.63	78.2 ± 17.00	0.002*
T_1: Syst. blood pressure (mmHg) [c]	91	115.4 ± 19.09	119.7 ± 17.84	112.3 ± 17.41	89.8 ± 17.66	0.002*
T_0: Diast. blood pressure (mmHg) [c]	99	71.5 ± 15.58	73.4 ± 14.48	71.7 ± 15.63	50.2 ± 12.22	0.03*
T_1: Diast. blood pressure (mmHg) [c]	91	64.6 ± 13.32	65.3 ± 12.50	64.9 ± 15.46	55.5 ± 7.01	0.27
T_0: MAP (mmHg) [c]	99	89.2 ± 15.98	91.9 ± 14.01	89.4 ± 14.71	59.67 ± 13.32	0.02*
T_1: MAP (mmHg) [c]	91	81.5 ± 14.12	83.5 ± 13.05	80.3 ± 15.38	67.2 ± 10.23	0.02*
T_0: Respiration rate (rpm) [b]	93	23 (18-28)	23 (18-27.3)	23 (18-27.5)	29 (21-34.8)	0.31
T_1: Respiration rate (rpm) [b]	86	20 (17.8-24)	20 (18-27)	20 (18.8-24)	24 (14.3-34.3)	0.27
T_0: Oxygen saturation (%) [b]	98	96 (93-98)	95 (93-98)	96 (92.8-98)	94 (86.5-98)	0.88
T_1: Oxygen saturation (%) [b]	89	97 (95-98.5)	97 (96-98)	97 (95.3-99)	96 (93.5-97.3)	0.72
T_0: Temperature (°C) [b]	99	38.4 (37.5-38.9)	38.4 (37.7-38.9)	38.6 (37.8-39.0)	36.9 (34.5-38.8)	0.58
T_1: Temperature (°C) [b]	91	37.7 (36.8-38.6)	37.7 (37.1-38.5)	37.7 (36.6-38. 8)	36.6 (36.6-39.2)	0.60
Presumed focus						
Respiratory [a]	99	49 (49.5%)	31 (49.2%)	13 (43.3%)	5 (83.3%)	0.71
Urogenital [a]	99	31 (31.3%)	21 (33.3%)	8 (26.7%)	2 (33.3%)	0.61
Skin/soft-tissue/wound [a]	99	6 (6.1%)	4 (6.3%)	2 (6.7%)	0 (0.0%)	0.80
Intra-abdominal [a]	99	21 (21.2%)	13 (20.6%)	6 (20.0%)	2 (33.3%)	0.76
Catheter/tube/implant [a]	99	3 (3.0%)	2 (3.2%)	1 (3.3%)	0 (0.0%)	0.86
Meningitis [a]	99	1 (1.0%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	0.46
Other or unknown focus [a]	99	15 (15.2%)	10 (15.9%)	4 (13.3%)	1 (16.7%)	0.82
Treatment parameters						
Intravenous fluids (L) [b]	98	1.0 (0.5-2.0)	1.0 (0.5-1.5)	1.0 (0.9-2.0)	3.5 (2.9-5.0)	0.009*
T_0: Supplemental oxygen (L) [b]	99	0.0 (0.0-2.0)	0.0(0.0-2.0)	0.0(0.0-2.0)	2.0(0.0-15.0)	0.74
T_1: Supplemental oxygen (L) [b]	87	2.0 (0.0-3.0)	2.0(0.0-3.0)	0.0(0.0-2.5)	13.5(1.5-15.0)	0.25

Data is presented as:
[a] absolute number and percentage (%).
[b] median and inter quartile range (IQR).
[c] mean ± standard deviation.
*, significant result; bpm, beats per minute; ED, emergency department; MAP, mean arterial pressure; rpm, respirations per minute;

Table 3. Delta in vital signs and biomarker levels between T_0 and T_1.

	N	T0	N	T1	Delta (T1-T0)	*p*	*d*
Vital signs							
Heart rate (bpm) [b]	99	110 (100-120)	93	98 (90-108.5)	-10 (-17.5;-4.0)	<0.001*	-0.75
Syst. blood pressure (mmHg) [a]	99	124.1 ± 21.87	91	115.4 ± 19.09	-7.5 ± 19.02	<0.001*	-0.38
Diast. blood pressure (mmHg) [a]	99	71.5 ± 15.58	91	64.6 ± 13.32	-6.4 ± 13.37	<0.001*	-0.44
MAP (mmHg) [a]	99	89.2 ± 15.98	91	81.5 ± 14.12	7.0 ± 13.86	<0.001*	-0.46
Respiration rate (rpm) [b]	93	23 (18-28)	86	20 (17.8-24)	-2 (-6;-2)	0.003*	-0.32
Oxygen saturation (%) [b]	98	96 (93-98)	89	97 (95-98.5)	1.0 (-1.0;5.0)	0.001*	-0.35
Supplemental oxygen (L) [b]	99	0.0 (0.0-2.0)	87	0.0 (0.0-3.0)	0.0 (0.0;2.0)	<0.001*	-0.40
Temperature (°C) [b]	99	38.4 (37.5-38.9)	91	37.7 (36.8-38.6)	-0.4(-1.0;0.3)	<0.001*	-0.41
Routine biomarkers							
Albumin (g/L) [b]	93	37 (35-40.5)	98	34 (31.8-37)	-3.0 (-5.0;-1.0)	<0.001*	-0.78
ALP (U/L) [b]	97	83 (55-136)	98	71.5 (48.8-115.5)	-9.0 (-14.8;-3.0)	<0.001*	-0.77
AST (U/L) [b]	98	26 (20-38.3)	98	24 (18-36.3)	-2.0 (-4.5;-0.0)	<0.001*	-0.56
Bands (%)[b]	82	0.0 (0.0-2.3)	97	0.0 (0.0-3.5)	0.0 (0.0;0.0)	0.72	-0.04
Bilirubin, direct (umol/L) [b]	97	4 (3-8)	98	4 (3-9)	0.0 (-1.0;1.0)	0.36	-0.09
Bilirubin, total (umol/L) [b]	97	12 (8-18)	98	11 (8-16)	-1.0 (-2.0;0.0)	<0.001*	-0.41
Calcium (mmol/L) [b]	92	2.23 (2.14-2.30)	98	2.09 (1.98-2.20)	-0.1 (-0.2;-0.08)	<0.001*	-0.78
Chloride (mmol/L) [b]	92	100 (96-102)	98	102 (99-106)	2.0 (1.0;4.0)	<0.001*	-0.78
Creatinine (umol/L) [b]	99	85 (64-123)	98	83.5 (63.5-128.3)	-1.5 (-8.0;4.0)	0.02*	-0.24
CRP (mg/L) [b]	99	93 (36-201)	98	99.5 (45.8-184.3)	0.0 (-12.3;8.5)	0.45	-0.08
Gamma-GT (U/L) [b]	96	46 (26.5-106.5)	98	39.5 (24.8-92.8)	-4.0 (-12.0;-1.0)	<0.001*	-0.70
Glucose (mmol/L) [b]	99	7.1 (6.1-8.6)	97	6.7 (5.9-7.7)	-0.5 (-1.2;0.3)	0.001*	-0.34
Hemoglobin (mmol/L) [b]	99	7.9 (6.9-8.7)	99	7.3 (6.5-8.2)	-0.6 (-0.8;-0.2)	<0.001*	-0.79
Lactate (mmol/L) [b]	86	1.6 (1.08-2.1)	96	1.2 (0.9-1.7)	-0.2 (-0.8;0.1)	<0.001*	-0.39
LDH (U/L) [b]	98	212 (163-257.5)	98	177 (142.5-232.8)	-21.0 (-41.0;-8.0)	<0.001*	-0.69
Leukocytes (10E9/L) [b]	99	12.1 (8.4-20.4)	99	11.9 (7.9-17.0)	-0.6 (-1.6;0.4)	0.005*	-0.28
Potassium (mmol/L) [b]	98	3.9 (3.5-4.3)	97	3.8 (3.5-4.3)	0.0 (-0.3;0.2)	0.28	-0.11
Sodium (mmol/L) [b]	98	137 (133-139)	98	138 (134-140.3)	1.0 (0.0;3.0)	<0.001*	-0.59
Thrombocytes (10E9/L) [b]	99	208 (163-284)	99	188 (143-259)	-15.0 (-29.0;-3.0)	<0.001*	-0.63
Urea (mmol/L) [b]	99	7.2 (4.8-12.2)	98	6.8 (4.1-12.1)	-0.4 (-0.8;-0.1)	<0.001*	-0.64
Study specific additional biomarkers							
Cortisol (nmol/L) [b]	91	860 (505-1245)	90	765 (366.3-1150)	-127.5 (-352.5;-20.0)	<0.001*	-0.43
D-dimer (ng/ml) [b]	92	735 (354-2274)	90	779 (357-2403)	-44 (-171;23)	0.003*	-0.32
hs-Trop T (ng/L) [b]	92	20 (8.3-39)	92	23 (8.3-40.8)	-0.5 (-3.0;3.0)	0.68	-0.04
NT pro-BNP (ng/L) [b]	93	409 (143-2036)	91	483 (155-2788)	10.0 (-21.0;169.0)	0.04*	-0.22

Data is presented as:
[a] absolute number and percentage (%).
[b] median and inter quartile range (IQR).
[c] mean ± standard deviation.
*, significant result; bpm, beats per minute; MAP, mean arterial pressure; rpm, respirations per minute; CI, confidence interval;

DISCUSSION

We performed a pilot study aimed to detect trends in vital signs and biomarker levels during the early resuscitation of patients with sepsis in the ED. To the best of our knowledge, no other studies analysed trends in vital signs and routine biomarker levels during resuscitation in the ED. Nowak et al. recently report the registration of vital sign data in the first 4 hours in the ED, but this data was neither analysed nor reported.[14]

We found a generally descending trend in most of the vital signs and biomarker levels during the patient's resuscitation in the ED. We specifically noticed descending trends in blood pressure, despite volume therapy. We observed this trend also in other (yet unpublished) studies in our ED. Paradoxically, the patients seem to improve despite this descending trend. This is supported by the relatively low in-hospital and 28-day mortality in our study of respectively 5.1 and 3.0% (Two patients died after more than 28 days in the hospital). We can only speculate on the mechanism behind this seemingly paradoxical trend in blood pressure, but it would be an interesting topic for further research.

We found only a few ascending trends; we speculate that these ascending trends might be a direct result of the treatment modalities. The only vital sign that showed an ascending trend was the peripheral oxygen saturation, which is most likely caused by supplementation of oxygen, reflected by a higher amount of supplemental oxygen at T_1. The biomarkers that showed an ascending trend were sodium, chloride and NT pro-BNP. The increase of sodium and chloride levels can easily be explained by the patients receiving intravenous saline solution. This might also explain the increase in NT pro-BNP caused by increased ventricular volume expansion of the heart.[15] On the other hand, there might also be a direct association between NT pro-BNP and the systemic inflammatory response.[15]

Limitations and recommendations

The main limitation of our pilot study is that it was not designed to detect the cause of the trends: trends might or might not have evolved as a result of the treatment provided. The detected trends could be influenced by several factors, like treatment parameters, dilution effects (by intravenous fluids), variation in laboratory analyses or circadian rhythms. Dilution might play a role, but we would expect a more even distribution over the different biomarkers when the effects were mainly caused by dilution. Of the measured biomarkers, only cortisol has a well-known circadian rhythm. The variance in laboratory analyses is unlikely to entirely explain the trends, as reflected by the average coefficient of variance during the study's inclusion period shown in Table 1. All factors mentioned above need to be taken into account in further research. Once the clinical value of the trends has been analysed, they can potentially serve as a guide for treatment or to measure disease activity.

In our pilot study design, we chose an arbitrary interval for the vital sign measurements and repeated blood draw of 3 hours. Although trends became apparent during this timeframe, the interval might not be the optimal one. We recommend that follow-up studies should determine the optimal interval, with either shorter or longer intervals between repeated measurements. We are currently running a follow-up study to detect trends in vital signs of patients with sepsis during the first hour in our ED. In that study, the interval between vital sign measurements is 5 minutes. Furthermore, we are in the process of designing a new study in which we will continuously record the patient's vital signs beat-to-beat during the first 48 hours in the hospital. The latter study should

provide valuable insight in the trends and variability of vital signs in sepsis patients and potentially provide an early warning of patient deterioration.[16]

Most vital signs and biomarker levels showed descending trends during the resuscitation of patients with sepsis in the ED, except for those parameters directly affected by treatment modalities. Despite the generally descending trends the patients clinically improved. Although the causes of these trends were not analysed in this pilot study, they might convey valuable information about the response to treatment. Therefore, the results of this pilot study ask for a new line of research that analyses trends during early resuscitation in the quest for a response to treatment parameter in patients with sepsis.

COMPETING INTERSTS AND FUNDING

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We thank the nurses and physicians in our emergency department for their assistance during the acquisition of the data. We thank the clinical chemists of our central laboratory for providing us with the coefficient of variation values for each biomarker.

AUTHOR'S CONTRIBUTIONS

VMQ designed the study, assisted with data acquisition, carried out data analysis and drafted the manuscript. MvM participated in the study design, assisted with data interpretation and revised the manuscript. JCtM participated in the study design, assisted with data interpretation and revised the manuscript. JJML participated in the study design, assisted with data interpretation, revised the manuscript and has given final approval of the version to be published.

DATA SHARING

No additional data available.

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LEGENDS TO THE FIGURES

Figure 1: Delta in vital signs between T_0 and T_1.

SaO2, peripheral oxygen saturation.

Figure 2: Delta in biomarker levels between T_0 and T_1.

ALP, alkaline phosphatase; AST, aspartate transaminase; CRP, C-reactive protein; Gamma-GT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; hs-Trop T, high sensitivity troponin T; NT pro-BNP, N-terminal prohormone of brain natriuretic peptid

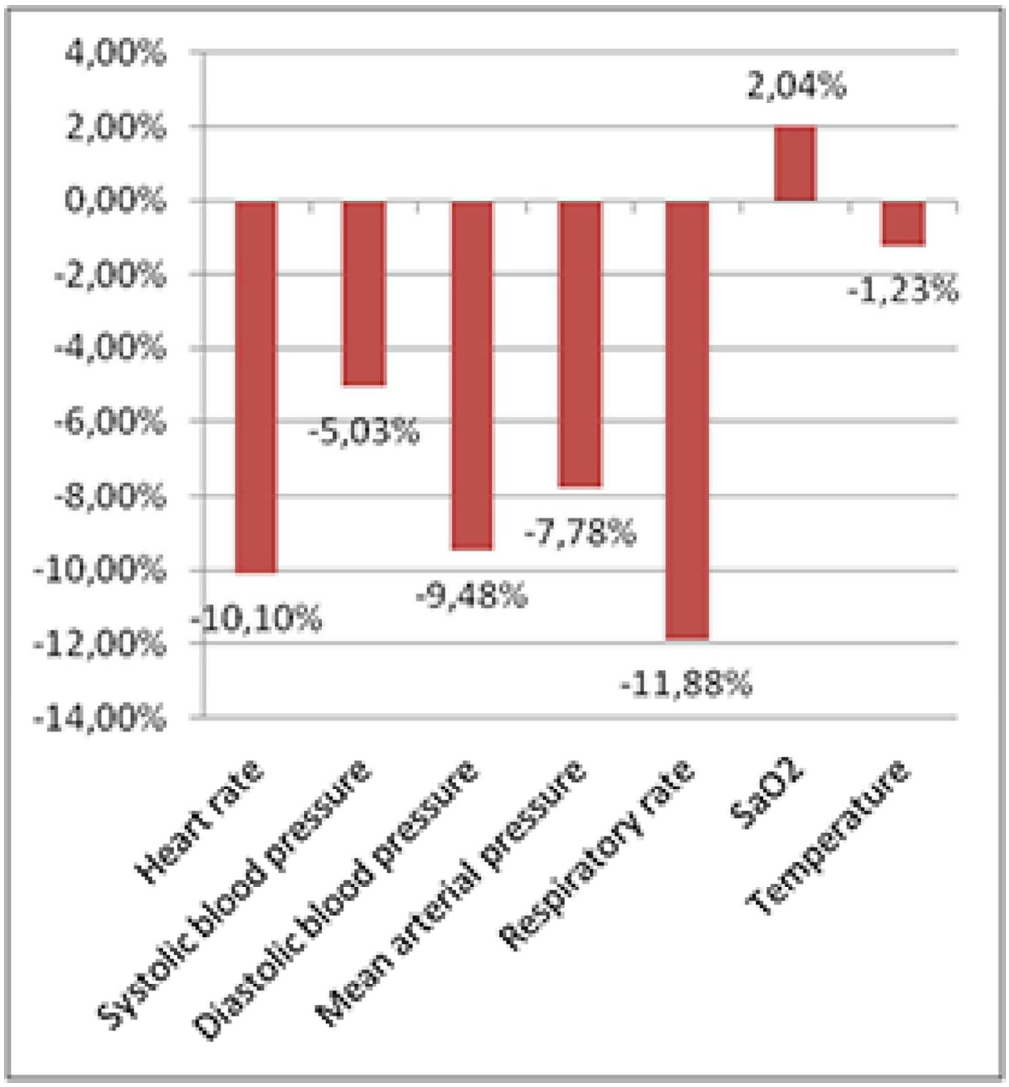

Figure 1: Delta in vital signs between T0 and T1.
SaO2, peripheral oxygen saturation.

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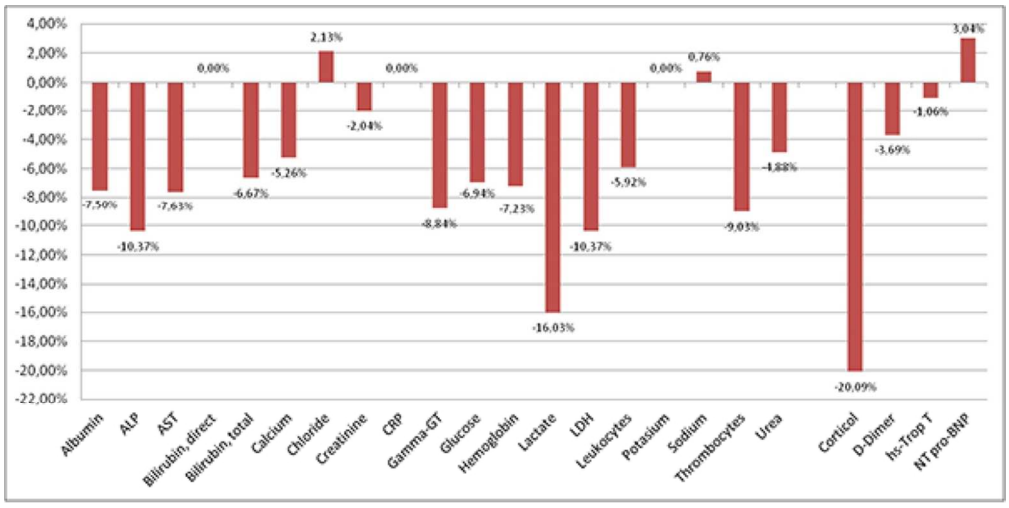

Delta in biomarker levels between T0 and T1.
ALP, alkaline phosphatase; AST, aspartate transaminase; CRP, C-reactive protein; Gamma-GT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; hs-Trop T, high sensitivity troponin T; NT pro-BNP, N-terminal prohormone of brain natriuretic peptid

105x52mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3-4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-5
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	3-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4-5
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	5-6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	n/a

		(*e*) Describe any sensitivity analyses	n/a
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	5
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	5-7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	n/a
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	n/a
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*n/a*
		Cross-sectional study—Report numbers of outcome events or summary measures	*5-7*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-7
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	8-9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8-9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-9
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Trends in vital signs and routine biomarkers in sepsis patients during resuscitation in the emergency department: a prospective observational pilot study.

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Trends in vital signs and routine biomarkers in sepsis patients during resuscitation in the emergency department: a prospective observational pilot study.

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Word count: 3214 words

Keywords

Sepsis; Resuscitation; Vital signs; Routine biomarkers; Response to treatment.

ABSTRACT

Objectives: Sepsis lacks a reliable, readily available, measure of disease activity. Thereby, it remains unclear how to monitor response to treatment. Research on numerous (new) biomarkers associated with sepsis provided disappointing results and little is known about changes in vital signs during sepsis resuscitation. We hypothesized that trends in vital signs together with routine biomarker levels during resuscitation might provide information about the response to treatment at a very early stage of sepsis in the emergency department (ED). We therefore explorer trends in vital signs and routine biomarker levels during sepsis resuscitation in the ED.

Design: Prospective observational pilot study.

Setting: Emergency department of a tertiary care teaching hospital.

Participants: 99 Adult non-trauma patients with suspected infection and two or more SIRS criteria admitted to the ED.

Primary and secondary outcome measures: vital signs and biomarker levels at admittance (T0) and after 3 hours in the ED (T1).

Results: In total, data of 99 patients was analysed. Of these patients, 63 presented with sepsis, 30 with severe sepsis and 6 patients with septic shock. All vital signs decreased, except for peripheral oxygen saturation which increased. Almost all routine biomarker levels decreased during resuscitation, except for CRP, bands, potassium, Troponin T and direct bilirubin which remained stable. Sodium, chloride and NT pro-BNP increased slightly.

Conclusions: Vital signs and biomarker levels showed descending trends during resuscitation, except for parameters directly affected by treatment modalities. Despite these trends most patients clinically improved. Trends in vital signs and routine biomarkers might be helpful in predicting clinical course and response to treatment in sepsis patients during early resuscitation.

Trail registration: N/A

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Our pilot study is the first study that looks at trends in vital signs and biomarker levels during sepsis resuscitation in the emergency department.
- Our pilot study shows that there are significant trends in vital signs and biomarker levels during resuscitation, these trends might potentially serve as a guide for treatment or to measure disease activity.
- Our pilot study was not designed to find the cause of the trends: trends might or might not have evolved as a result of the treatment provided.
- The measurement interval of 3 hours chosen in this study might not be the optimal one, we recommend a follow-up study to find the optimal interval between measurements.

INTRODUCTION

Early and aggressive resuscitation is an important factor to reduce mortality of sepsis.[1, 2] It appears that early recognition of the septic patient and timely and aggressive resuscitation is more important than the specific kind of treatment provided.[1, 3, 4] Sepsis lacks a reliable measure of disease activity, similar to the viral load in HIV or left ventricle function in cardiology.[5, 6] Therefore, it remains unclear how response to treatment can be monitored.[6, 7] One known approach to monitor this, is to monitor the patient's vital signs. However, there is little information about changes in vital signs in sepsis and their relation to treatment during early resuscitation in the emergency department (ED). Furthermore, numerous biomarkers associated with sepsis have been studied for this purpose, generally with disappointing results. Their sensitivity and specificity is too low to be of real clinical value and they are often not readily available.[7, 8]

Up to 50% of all patients with sepsis are admitted through the ED.[9] Patients are usually transferred from the ED to either the intensive care unit (ICU) or nursing wards within 4 hours.[10, 11] Within these 4 hours, early resuscitation is initiated, preferably as soon as possible.[6] We hypothesize that trends in vital signs together with routine biomarker levels during the resuscitation of patients with sepsis in the ED might provide information about the response to treatment. This information is useful to guide treatment at a very early stage of sepsis, while the patient is still in the ED. The response to treatment could be used to tailor the patient's treatment and monitoring, and – at the same time – prevent doing harm to the mildly septic patient with too aggressive treatment. It could furthermore serve as a feasible and accurate way recognize the patients with a great chance to deteriorate and potentially provide an early warning of deterioration.[12] To the best of our knowledge, there is no data available about trends in vital signs and biomarkers during resuscitation in the ED. Therefore, we performed a pilot study within the 4-hour timeframe that the patient is in the ED.

METHODS

Study design and setting

We performed a prospective observational pilot study in the ED of the University Medical Center Groningen, a tertiary care teaching hospital with over 34,000 visits to the ED annually. The pilot study was aimed to establish power calculations for and the feasibility of a full-scale study on the use of trends in vital signs and biomarkers as response to treatment parameter. The pilot aimed to include a convenience sample of 100 patients within a limited 6 month timeframe. Data was collected between October 2013 and April 2014. The hospital's institutional review board approved the study (METc 2013/297). To prevent selection bias, taking blood samples in patients with an altered mental status due to sepsis was also approved by the review board. In these cases, informed consent was obtained from next of kin or from the patient during their stay in hospital.

Study population and protocol

Adult non-trauma patients visiting the ED with presumed infection or sepsis were screened for inclusion. Inclusion criteria were: age ≥18 years, presumed or confirmed infection, and two or more systemic inflammatory response syndrome (SIRS) criteria as defined by the International Sepsis Definitions Conference.[13]

Patients are usually transferred from the ED to either ICU or nursing ward within 4 hours. To detect trends in vital signs and biomarker levels, we took measurements at two points within this timeframe: at admittance to the ED (T_0) and after 3 hours (T_1). At T_0, a nurse measured the patient's vital signs and took a routine blood sample. Vital signs were measured with a patient monitor (IntelliVue MP30 System with Multi-Measurement Module, Philips, Eindhoven, The Netherlands), except for temperature which was measured using an electronic tympanic ear thermometer (Genius

2; Mountainside Medical Equipment, Marcy, NY, USA). Simultaneously with the routine blood sample, the nurse took a number of additional blood vials for this study. These additional vials were temporarily stored until informed consent was obtained. This procedure ensured that treatment was not delayed for patients participating in the study. The vials for the routine blood sample were immediately sent to the hospital's central laboratory for analysis and were analysed for the routine biomarkers listed in Table 1.

Table 1. Overview of the measured biomarkers and their characteristics.

Name	Unit	CV (%)	Reference values
Routine biomarkers			
Albumin	g/L	1.4	35-50
Alkaline phosphatase (ALP)	U/L	2.2	Male: <115 Female: <98
Aspartate transaminase (AST)	U/L	1.4	Male: <35 Female: <31
Bands	%	n/a	0-3
Bilirubin, direct	umol/L	1.9	<5
Bilirubin, total	umol/L	1.9	<17
Calcium	mmol/L	1.4	2.20-2.60
Chloride	mmol/L	0.8	97-107
Creatinine	umol/L	2.0	Male: 50-110 Female:50-90
C-reactive protein (CRP)	mg/L	3.0	<5
Gamma-glutamyl transferase (Gamma-GT)	U/L	1.9	Male: <55 Female: <38
Glucose	mmol/L	1.5	4.0-5.5 (fastening)
Haemoglobin (Hb)	mmol/L	1.3	Male:8.7-10.6 Female: 7.5-9.9
Lactate	mmol/L	1.5	0.5-2.2
Lactate dehydrogenase (LDH)	U/L	1.3	Male: <248 Female: <247
Leukocytes	10^9/L	1.8	4-10
Potassium	mmol/L	0.8	3.5-5.0
Sodium	mmol/L	0.7	135-145
Thrombocytes	10^9/L	4.4	150-350
Urea	mmol/L	2.5	2.5-7.5
Study specific additional biomarkers			
Cortisol	nmol/L	3.8	08:00: 200-800 16:00: 100-400 22:00: 50-200
D-dimer	ng/ml	4.5	<500
High sensitivity troponin T (hs-Trop T)	ng/L	5.0	<14
N-terminal prohormone of brain natriuretic peptide (NT pro-BNP)	ng/L	2.2	-75 year: <175 >75 year: <450

CV, averaged inter- and intra-essay coefficient of variation during the study inclusion period;

Patients or their healthcare proxies had to provide written informed consent before T_1, otherwise the patient was excluded from the study and the stored vials were destroyed. The stored vials were send to the central laboratory for analysis immediately after obtaining informed consent. The blood in these vials was analysed for 4 additional routinely available biomarkers, as shown in Table 1. These biomarkers were added for the following reasons: NT pro-BNP as a marker for fluid overload, cortisol as a marker for stress response, D-dimer for coagulation status and marker of disseminated intravascular coagulation and Troponin T as marker of myocardial damage.

At T_1 new blood samples were collected and immediately analysed for all biomarkers shown in Table 1. The patient's vital signs were also recorded at T_1 using the same procedures and equipment as at T_0. Furthermore, we recorded the amount of intravenous fluids given to the patient in the ED until T_1. In case a patient was transferred to the ICU or a ward before T_1, a researcher took the T_1 blood samples and vital signs there, according to study protocol.

The attending physician was asked for the suspected focus of sepsis at the moment the patient was transferred out of the ED and was allowed to select multiple options. Demographic data was collected from the patient's electronic medical records. All patients received treatment according to routine sepsis protocol, including fluid resuscitation, antibiotics and supplemental oxygen. According to protocol, fluid resuscitation was performed by an initial fluid challenge of 500 ml saline solution (NaCl 0.9%) in 10 minutes, followed by 500 ml every 15 minutes until a mean arterial pressure (MAP)

of ≥65 mmHg was reached.[6] When the MAP was still <65 mmHg after 2L of saline, an intensivist was consulted to transfer the patient to the ICU and start inotropic medication. From previous studies in our department, we know that the median time to start fluid resuscitation was 21 minutes (sepsis 26, severe sepsis 15 and septic shock 4 minutes). Antibiotics were given in accordance with the guidelines provided by the Dutch Working Party on Antibiotic Policy (SWAB).[14] The median time to intravenous antibiotics was 61 minutes (sepsis 75, severe sepsis 54 and septic shock 45 minutes) from ED entrance in previous sepsis studies in our department. Supplemental oxygen was given to maintain a SaO2 between 94-98%. The treatment protocol did not change during the inclusion period of the study.

Statistical methods

Continuous data is presented as mean with standard deviation or median with inter-quartile range (IQR) depending on their distribution. Normality was tested using the Shapiro-Wilk test for normality. Categorical data is presented as absolute numbers with percentages. The Wilcoxon related samples signed rank test was used for comparison of biomarker levels and vital signs between T_0 and T_1. Effect sizes are presented as Cohen's *d*.[15] The variance between the sepsis severity groups and effect of medication or comorbidities on the response to treatment was tested using the non-parametric Jonckheere-Terpstra test. Missing data was excluded for analysis. All statistical analyses were performed using IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY, USA). A *p*-value of ≤0.05 was considered significant; all tests were two-tailed.

RESULTS

In total 101 patients were included. Two patients were excluded, since informed consent could not be obtained from one patient before T_1 and was withdrawn by another patient. The remaining 99 patients were included in the final analysis. Of these patients, 63 presented with sepsis, 30 with severe sepsis and 6 patients with septic shock at ED admission. Patient characteristics, including comorbidities and medication use prior to ED presentation are shown in Table 2. Patients with severe sepsis more frequently had a history of mild liver disease (p=0.02). Patients with sepsis used diuretics more often (p=0.02). The presumed focus of infection, vital signs and treatment parameters, are shown in Table 3. The most frequent foci were pulmonic and urogenital. The frequency of these foci did not differ between severity groups. Patients in the septic shock group received more intravenous fluids (3.5L; IQR: 2.9-5.0) compared to severe sepsis and sepsis patients (p=0.009).

Vital signs

Blood pressure at both T_0 and T_1 was inversely related to sepsis severity as blood pressure decreased with increasing severity of sepsis. The results of all vital sign measurements are shown in Table 3 and Table 4. Table 2, shows the vital signs for T_0 and T_1 separated by sepsis severity group. Table 4 includes the deltas between T_0 and T_1 for each vital sign; these are also graphically represented in Fig. 1. We found significant differences for all measured vital signs. As becomes apparent from Fig. 1, all vital signs decreased during the measurement timeframe, except for peripheral oxygen saturation which increased by 1.1%. The heart rate and respiratory rate dropped by more than 10% during resuscitation (p<0.001). At the same time, the systolic blood pressure decreased with 5% and diastolic blood pressure decreased with more than 9% (p<0.001).

Biomarkers

The results for the biomarker levels are shown in Table 4, including deltas between T_0 and T_1 for each biomarker. These deltas are also shown in Fig. 2. Almost all routine biomarkers levels decreased

during resuscitation in the ED, except for CRP, bands, potassium and direct bilirubin which remained stable. Levels of sodium and chloride increased slightly with respectively 0.8 and 2.1% (p<0.001). The levels of NT pro-BNP increased with 3.0% (p=0.039) during resuscitation. Cortisol and D-dimer levels decreased by respectively 20.1% (p<0.001) and 3.7% (p=0.039). The high sensitivity Troponin T levels did not show a significant trend.

Biomarker levels were below the laboratory's lower detection limit in several instances. In these instances their value was set to half the lower detection limit. Direct bilirubin levels were below the detection limit (1.0 umol/L) in cases 5 at T_0 and 3 at T_1, D-dimer levels (detection limit: 150 ng/ml) in cases 5 at T_0 and 7 at T_1, hs-Trop T levels (detection limit: 3.0 ng/L) in 3 cases at T_0 and 5 cases at T_1. During the calculation of the deltas, the T_0 values of the biomarkers were zero in a few cases. To avoid division by zero problems during the calculation of the percentual different, these values were handled as missing data. Band levels were zero at T_0 in 5 instances and thrombocyte levels in one instance, these values have been excluded from analysis.

Medication and comorbidity

To explore confounding factors that might have affected the response to treatment, we analysed associations between medication use at ED presentation, comorbidity and the measured vital signs and biomarker levels. The use of anti-hypertensive medication did not have a significant effect on the changes in vital signs. Although trends in NT pro-BNP levels were more ascending in patients using renin angiotensin system (RAS) inhibitors (median: 8.7%, IQR: 3.1%; 37.2%) compared to patients that did not use RAS-inhibitors (median: 0.2%; IQR: -10.5%; 14.1%; p=0.024). NT pro-BNP levels showed also higher ascending trends in patients with congestive heart disease (median: 23.1%, IQR: 5.2%; 37.1%) compared to patients without (median: 1.0%, IQR: -10.2%; 13.0%; p=0.006). Patients using diuretics also had higher ascending trends in NT pro-BNP levels (median: 14.8%, IQR: 1.7%; 41.2%) compared to patients without diuretics (median: 0.2%, IQR: -11.3%; 10.9%; p=0.004). However, patients using diuretics (median: 1.0L, IQR: 0.5L; 1.0L) received less fluid resuscitation (median: 1.5L, IQR: 1.0L; 2.1L; p=0.004). The change in body temperature was not affected by the antipyretic effect of NSAIDs. However, in patients using paracetamol an ascending trend in body temperature (median: 0.8%, IQR: -1.8%; 1.8%) was observed, while patients without paracetamol showed a descending trend in body temperature (median: -1.4%, IQR: -2.8%; 0.3%; p=0.021).

The use of antibiotics prior to ED presentation did not affect the response to treatment of the infection parameters (leukocytes, C-reactive protein), neither was the leukocyte response associated with the use of immunosuppressive medication. However, users of immunosuppressive medication (median: 3.7%, IQR: -4.8%; 43.0%) showed a tendency of ascending C-reactive protein levels (median: -1.4%, IQR: -40.1%; -5.8%; p=0.017). An effect of immunosuppressive or other medication on cortisol levels was not found.

Patients with congestive heart failure showed less decrease in heart rate (median: 1.4%, IQR: -3.8%; 7.8%; vs median: -10.3%, IQR: -16.5%; -4.2%; p=0.006) and had an increasing requirement for supplemental oxygen (median: 2.0L, IQR: 2.0L; 3.0L; vs median: 0.0L, IQR: 0.0L; 2.0L; p=0.011). We did not find an association between chronic obstructive pulmonary disease (COPD) and the response in oxygen saturation or need for supplemental oxygen. Patients with metastasised tumours tended to have increasing D-dimer levels (median: 4.4%, IQR: -1.7%; 19.4%; vs median: -4.7%, IQR: -16.2%; 3.0%; p=0.023).

Table 2. Patient characteristics, comorbidity, medication at presentation in the emergency department.

	N	Overall	Sepsis	Severe sepsis	Septic Shock	*p*
	99	99 (100.0%)	63 (63.6%)	30 (30.3%)	6 (6.1%)	
Demographics						
Age [b]	99	59 (47-70)	60 (49-70)	56 (44.5-73.3)	56.5 (47-68.8)	0.50
Gender						
Male [a]	99	57 (57.6%)	29 (46.0%)	23 (76.7%)	5 (83.3%)	1.00
Female [a]	99	42 (42.4%)	34 (54.0%)	7 (23.3%)	1 (16.7%)	1.00
Comorbidity						
Myocardial infarction [a]	99	13 (13.1%)	11 (17.5%)	2 (6.7%)	0 (0%)	0.08
Congestive heart failure [a]	99	6 (6.1%)	5 (7.9%)	1 (3.3%)	0 (0%)	0.29
Peripheral vascular disease [a]	99	6 (6.1%)	4 (6.3%)	2 (6.7%)	0 (0%)	0.80
Cerebrovascular disease [a]	98	12 (12.1%)	9 (14.3%)	2 (6.7%)	1 (16.7%)	0.44
Dementia [a]	99	3 (3.0%)	2 (3.2%)	1 (3.3%)	0 (0%)	0.86
Chronic pulmonary disease [a]	99	23 (23.2%)	17 (27.0%)	14 (13.3%)	2 (33.3%)	0.33
Connective tissue disease [a]	99	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.00
Ulcer disease [a]	99	2 (2.0%)	1 (1.6%)	1 (3.3%)	0 (0%)	0.76
Mild liver disease [a]	99	11 (11.1%)	3 (4.8%)	8 (26.7%)	0 (0%)	0.02*
Diabetes [a]	99	16 (16.2%)	12 (19.0%)	4 (13.3%)	0 (0%)	0.25
Hemiplegia [a]	99	2 (2.0%)	1 (1.6%)	1 (3.3%)	0 (0%)	0.76
Moderate or severe renal disease [a]	99	23 (23.2%)	11 (17.5%)	10 (33.3%)	2 (33.3%	0.08
Diabetes with end-organ damage [a]	99	3 (3.0%)	2 (3.2%)	1 (3.3%)	0 (0%)	0.86
Any tumour [a]	99	30 (30.3%)	20 (31.7%)	9 (30.0%)	1 (16.7%)	0.60
Leukaemia [a]	99	5 (5.1%)	3 (4.8%)	2 (6.7%)	1 (16.7%)	0.92
Lymphoma [a]	99	7 (7.1%)	6 (9.5%)	1 (3.3%)	0 (0%)	0.20
Moderate or severe liver disease [a]	99	4 (4.0%)	1 (1.6%)	3 (10.0%)	0 (0%)	0.16
Metastatic solid tumour [a]	99	8 (8.1%)	6 (9.5%)	2 (6.7%)	0 (0%)	0.44
AIDS [a]	99	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.00
Charlson index [b]	99	2 (1-4)	2 (1-4)	2 (2-4)	1 (1-2)	0.80
Medication at emergency department presentation						
RAS inhibitor [a]	99	25 (25.3%)	16 (25.4%)	5 (16.7%)	4 (66.7%)	0.70
β-blocker [a]	99	34 (34.3%)	21 (33.3%)	10 (33.3%)	3 (50.0%)	0.69
Calcium-channel blocker [a]	99	15 (15.2%)	12 (19.0%)	2 (6.7%)	1 (16.7%)	0.19
Antibiotic [a]	99	30 (30.3%)	19 (30.2%)	10 (33.3%)	1 (16.7%)	0.93
Immunosuppressive medication [a]	99	33 (33.3%)	19 (30.2%)	13 (43.3%)	1 (16.7%)	0.51
Diuretic [a]	99	24 (24.2%)	20 (31.7%)	4 (13.3%)	0 (0%)	0.02*
Non-steroid anti-inflammatory drug [a]	99	32 (32.3%)	23 (36.5%)	9 (30.0%)	0 (0%)	0.16
Paracetamol [a]	99	18 (18.2%)	11 (17.5%)	7 (23.3%)	0 (0%)	0.99
Anti-diabetic medication [a]	99	17 (17.2%)	13 (20.6%)	4 (13.3%)	0 (0%)	0.19

Data is presented as:
[a] absolute number and percentage (%).
[b] median and inter quartile range (IQR).
*, significant result;

Table 3. Presumed focus, vital signs and treatment parameters in the emergency department.

	N	Overall	Sepsis	Severe sepsis	Septic Shock	*p*
	99	99 (100.0%)	63 (63.6%)	30 (30.3%)	6 (6.1%)	
Presumed focus						
Respiratory [a]	99	49 (49.5%)	31 (49.2%)	13 (43.3%)	5 (83.3%)	0.71
Urogenital [a]	99	31 (31.3%)	21 (33.3%)	8 (26.7%)	2 (33.3%)	0.61
Skin/soft-tissue/wound [a]	99	6 (6.1%)	4 (6.3%)	2 (6.7%)	0 (0.0%)	0.80
Intra-abdominal [a]	99	21 (21.2%)	13 (20.6%)	6 (20.0%)	2 (33.3%)	0.76
Catheter/tube/implant [a]	99	3 (3.0%)	2 (3.2%)	1 (3.3%)	0 (0.0%)	0.86
Meningitis [a]	99	1 (1.0%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	0.46
Other or unknown focus [a]	99	15 (15.2%)	10 (15.9%)	4 (13.3%)	1 (16.7%)	0.82
Vital signs						
T_0: Heart rate (bpm) [b]	99	110 (100-120)	110 (100-120)	112.5 (104.5-120.8)	113.5 (93.5-136.8)	0.66
T_1: Heart rate (bpm) [b]	93	98 (90-108.5)	98 (89-110)	100 (94.3-105)	100 (88.8-138)	0.71
T_0: Syst. blood pressure (mmHg) [c]	99	124.1 ± 21.87	128.9 ± 18.96	123.3 ± 17.63	78.2 ± 17.00	0.002*
T_1: Syst. blood pressure (mmHg) [c]	91	115.4 ± 19.09	119.7 ± 17.84	112.3 ± 17.41	89.8 ± 17.66	0.002*
T_0: Diast. blood pressure (mmHg) [c]	99	71.5 ± 15.58	73.4 ± 14.48	71.7 ± 15.63	50.2 ± 12.22	0.03*
T_1: Diast. blood pressure (mmHg) [c]	91	64.6 ± 13.32	65.3 ± 12.50	64.9 ± 15.46	55.5 ± 7.01	0.27
T_0: MAP (mmHg) [c]	99	89.2 ± 15.98	91.9 ± 14.01	89.4 ± 14.71	59.67 ± 13.32	0.02*
T_1: MAP (mmHg) [c]	91	81.5 ± 14.12	83.5 ± 13.05	80.3 ± 15.38	67.2 ± 10.23	0.02*
T_0: Respiration rate (rpm) [b]	93	23 (18-28)	23 (18-27.3)	23 (18-27.5)	29 (21-34.8)	0.31
T_1: Respiration rate (rpm) [b]	86	20 (17.8-24)	20 (18-27)	20 (18.8-24)	24 (14.3-34.3)	0.27
T_0: Oxygen saturation (%) [b]	98	96 (93-98)	95 (93-98)	96 (92.8-98)	94 (86.5-98)	0.88
T_1: Oxygen saturation (%) [b]	89	97 (95-98.5)	97 (96-98)	97 (95.3-99)	96 (93.5-97.3)	0.72
T_0: Temperature (°C) [b]	99	38.4 (37.5-38.9)	38.4 (37.7-38.9)	38.6 (37.8-39.0)	36.9 (34.5-38.8)	0.58
T_1: Temperature (°C) [b]	91	37.7 (36.8-38.6)	37.7 (37.1-38.5)	37.7 (36.6-38. 8)	36.6 (36.6-39.2)	0.60
Treatment parameters						
Intravenous fluids (L) [b]	98	1.0 (0.5-2.0)	1.0 (0.5-1.5)	1.0 (0.9-2.0)	3.5 (2.9-5.0)	0.009*
T_0: Supplemental oxygen (L) [b]	99	0.0 (0.0-2.0)	0.0(0.0-2.0)	0.0(0.0-2.0)	2.0(0.0-15.0)	0.74
T_1: Supplemental oxygen (L) [b]	87	2.0 (0.0-3.0)	2.0(0.0-3.0)	0.0(0.0-2.5)	13.5(1.5-15.0)	0.25

Data is presented as:
[b] median and inter quartile range (IQR).
[c] mean ± standard deviation.
*, significant result; bpm, beats per minute; MAP, mean arterial pressure; rpm, respirations per minute; syst, systolic; diast, diastolic.

Table 4. Delta in vital signs and biomarker levels between T_0 and T_1.

	N	T0	N	T1	Delta (T1-T0)	*p*	*d*
Vital signs							
Heart rate (bpm) [b]	99	110 (100-120)	93	98 (90-108.5)	-10 (-17.5;-4.0)	<0.001*	-0.75
Syst. blood pressure (mmHg) [a]	99	124.1 ± 21.87	91	115.4 ± 19.09	-7.5 ± 19.02	<0.001*	-0.38
Diast. blood pressure (mmHg) [a]	99	71.5 ± 15.58	91	64.6 ± 13.32	-6.4 ± 13.37	<0.001*	-0.44
MAP (mmHg) [a]	99	89.2 ± 15.98	91	81.5 ± 14.12	7.0 ± 13.86	<0.001*	-0.46
Respiration rate (rpm) [b]	93	23 (18-28)	86	20 (17.8-24)	-2 (-6;-2)	0.003*	-0.32
Oxygen saturation (%) [b]	98	96 (93-98)	89	97 (95-98.5)	1.0 (-1.0;5.0)	0.001*	-0.35
Supplemental oxygen (L) [b]	99	0.0 (0.0-2.0)	87	0.0 (0.0-3.0)	0.0 (0.0;2.0)	<0.001*	-0.40
Temperature (°C) [b]	99	38.4 (37.5-38.9)	91	37.7 (36.8-38.6)	-0.4(-1.0;0.3)	<0.001*	-0.41
Routine biomarkers							
Albumin (g/L) [b]	93	37 (35-40.5)	98	34 (31.8-37)	-3.0 (-5.0;-1.0)	<0.001*	-0.78
ALP (U/L) [b]	97	83 (55-136)	98	71.5 (48.8-115.5)	-9.0 (-14.8;-3.0)	<0.001*	-0.77
AST (U/L) [b]	98	26 (20-38.3)	98	24 (18-36.3)	-2.0 (-4.5;-0.0)	<0.001*	-0.56
Bands (%)[b]	82	0.0 (0.0-2.3)	97	0.0 (0.0-3.5)	0.0 (0.0;0.0)	0.72	-0.04
Bilirubin, direct (umol/L) [b]	97	4 (3-8)	98	4 (3-9)	0.0 (-1.0;1.0)	0.36	-0.09
Bilirubin, total (umol/L) [b]	97	12 (8-18)	98	11 (8-16)	-1.0 (-2.0;0.0)	<0.001*	-0.41
Calcium (mmol/L) [b]	92	2.23 (2.14-2.30)	98	2.09 (1.98-2.20)	-0.1 (-0.2;-0.08)	<0.001*	-0.78
Chloride (mmol/L) [b]	92	100 (96-102)	98	102 (99-106)	2.0 (1.0;4.0)	<0.001*	-0.78
Creatinine (umol/L) [b]	99	85 (64-123)	98	83.5 (63.5-128.3)	-1.5 (-8.0;4.0)	0.02*	-0.24
CRP (mg/L) [b]	99	93 (36-201)	98	99.5 (45.8-184.3)	0.0 (-12.3;8.5)	0.45	-0.08
Gamma-GT (U/L) [b]	96	46 (26.5-106.5)	98	39.5 (24.8-92.8)	-4.0 (-12.0;-1.0)	<0.001*	-0.70
Glucose (mmol/L) [b]	99	7.1 (6.1-8.6)	97	6.7 (5.9-7.7)	-0.5 (-1.2;0.3)	0.001*	-0.34
Hemoglobin (mmol/L) [b]	99	7.9 (6.9-8.7)	99	7.3 (6.5-8.2)	-0.6 (-0.8;-0.2)	<0.001*	-0.79
Lactate (mmol/L) [b]	86	1.6 (1.08-2.1)	96	1.2 (0.9-1.7)	-0.2 (-0.8;0.1)	<0.001*	-0.39
LDH (U/L) [b]	98	212 (163-257.5)	98	177 (142.5-232.8)	-21.0 (-41.0;-8.0)	<0.001*	-0.69
Leukocytes (10E9/L) [b]	99	12.1 (8.4-20.4)	99	11.9 (7.9-17.0)	-0.6 (-1.6;0.4)	0.005*	-0.28
Potassium (mmol/L) [b]	98	3.9 (3.5-4.3)	97	3.8 (3.5-4.3)	0.0 (-0.3;0.2)	0.28	-0.11
Sodium (mmol/L) [b]	98	137 (133-139)	98	138 (134-140.3)	1.0 (0.0;3.0)	<0.001*	-0.59
Thrombocytes (10E9/L) [b]	99	208 (163-284)	99	188 (143-259)	-15.0 (-29.0;-3.0)	<0.001*	-0.63
Urea (mmol/L) [b]	99	7.2 (4.8-12.2)	98	6.8 (4.1-12.1)	-0.4 (-0.8;-0.1)	<0.001*	-0.64
Study specific additional biomarkers							
Cortisol (nmol/L) [b]	91	860 (505-1245)	90	765 (366.3-1150)	-127.5 (-352.5;-20.0)	<0.001*	-0.43
D-dimer (ng/ml) [b]	92	735 (354-2274)	90	779 (357-2403)	-44 (-171;23)	0.003*	-0.32
hs-Trop T (ng/L) [b]	92	20 (8.3-39)	92	23 (8.3-40.8)	-0.5 (-3.0;3.0)	0.68	-0.04
NT pro-BNP (ng/L) [b]	93	409 (143-2036)	91	483 (155-2788)	10.0 (-21.0;169.0)	0.04*	-0.22

Data is presented as:
[a] absolute number and percentage (%).
[b] median and inter quartile range (IQR).
[c] mean ± standard deviation.
*, significant result; bpm, beats per minute; MAP, mean arterial pressure; rpm, respirations per minute; CI, confidence interval; syst, systolic; diast, diastolic.

DISCUSSION

We performed a pilot study aimed to detect trends in vital signs and biomarker levels during the early resuscitation of patients with sepsis in the ED. To the best of our knowledge, no other studies analysed trends in vital signs and routine biomarker levels during resuscitation in the ED. Nowak et al. recently report the registration of vital sign data in the first 4 hours in the ED, but this data was neither analysed nor reported.[16]

We found a generally descending trend in most of the vital signs and biomarker levels during the patient's resuscitation in the ED. We specifically noticed descending trends in blood pressure, despite volume therapy. We observed this trend also in other (yet unpublished) studies in our ED. Paradoxically, the patients seem to improve despite this descending trend. This is supported by the relatively low in-hospital and 28-day mortality in our study of respectively 5.1 and 3.0% (Two patients died after more than 28 days in the hospital). We can only speculate on the mechanism behind this seemingly paradoxical trend in blood pressure. During further analysis we found that, the use of anti-hypertensive or diuretic medication prior to ED admittance, a history of congestive heart failure or myocardial infarction did not explain decrease in blood pressure. Patients with congestive heart failure did however show less decrease in heart rate, the use of β-blockers did not affect the change in heart rate. The use of paracetamol prior to ED presentation leaded to an increasing trend in body temperature, perhaps while the antipyretic effect of paracetamol has worn off during the patient's stay in the ED. The descending trend in cortisol levels could not be explained by comorbidities or medication use prior to ED presentation, therefore it is likely that it is partly influenced by its circadian rhythm und partly by the reduction of bodily stress as a response to treatment.

We found only a few ascending trends; we speculate that these ascending trends might be a direct result of the treatment modalities. The only vital sign that showed an ascending trend was the peripheral oxygen saturation, which is most likely caused by supplementation of oxygen, reflected by higher amounts of supplemental oxygen at T_1. Patients with a history of congestive heart failure showed an increasing oxygen need, while a history of COPD did not explain the additional oxygen requirement. The biomarkers that showed an ascending trend were sodium, chloride and NT pro-BNP. The increase of sodium and chloride levels can easily be explained by the patients receiving intravenous saline solution (NaCl 0.9%). This might also explain the increase in NT pro-BNP caused by increased ventricular volume expansion of the heart. On the other hand, there might also be a direct association between NT pro-BNP and the systemic inflammatory response.[17] Furthermore, we found ascending trends in NT pro-BNP levels in patients using RAS-inhibitors or diuretics, although patients using diuretics received less fluid resuscitation, which might suggest that they had earlier volume expansion of the heart.

Limitations

The main limitation of our pilot study is that it was not designed to detect the cause of the trends: trends might or might not have evolved as a result of the treatment provided. Detected trends could be influenced by several factors, like comorbidity, medication use prior to ED presentation, treatment parameters, dilution effects (by intravenous fluids), variation in laboratory analyses or circadian rhythms. We performed post-hoc tests to explore influences of comorbidities and medication use prior to ED presentation in our pilot population, as described above. Dilution might play a role, but we would expect a more even distribution over the different biomarkers when the

effects were mainly caused by dilution. Of the measured biomarkers, only cortisol has a well-known circadian rhythm. The variance in laboratory analyses is unlikely to entirely explain the trends, as reflected by the average coefficient of variance during the study's inclusion period shown in Table 1. All factors mentioned above need to be taken into account in further research. Once the clinical value of the trends has been analysed, they can potentially serve as a guide for treatment or to measure disease activity.

Recommendations

In our pilot study design, we chose an arbitrary interval for the vital sign measurements and repeated blood draw of 3 hours. Although trends became apparent during this timeframe, the interval might not be the optimal one. We recommend that follow-up studies should determine the optimal interval, with either shorter or longer intervals between repeated measurements. We are currently running a follow-up study in septic patients to detect trends in vital signs measured in 5 minute intervals during their stay in our ED. In this follow-up study we explore the course of vital sign changes in more detail. Furthermore, we are in the process of designing a new study, using the results of this pilot study, in which we will continuously record the patient's vital signs beat-to-beat during the first 48 hours in the hospital. The latter study should provide valuable insight in the trends and variability of vital signs in sepsis patients and potentially provide an early warning of patient deterioration.[12] Vital signs could also potentially be used to titrate the amount of fluid resuscitation and supplemental oxygen.

The routine biomarkers measured in this pilot study did, in general, only show relatively minor changes during the measurement interval. This makes them less suitable as response to treatment parameter. The measured study specific biomarkers, except hs-Trop T, showed larger changes during the measurement interval. We recommend further research to explore their specific responses to treatment. We expect that NT pro-BNP could be a parameter to measure response to fluid resuscitation and might in the future be used to titrate the amount of fluids given. Furthermore, cortisol could be a parameter to measure the body's stress level in response to treatment. The levels of D-dimer could provide information about the status of the coagulation system and disease activity, especially in patients with metastasised tumours.

Based on our results, we recommend further exploration of the use of vital signs as a response to treatment parameter in sepsis. They show relatively the largest changes within the measurement interval and are furthermore easily, cheaply and non-invasively measurable.

We expect that trends with a decrease in heart rate, respiratory rate and temperature, an increase in oxygen saturation and blood pressure could be valued as a positive response to treatment in sepsis patients, although this pilot study could not (yet) confirm this assumption.

COMPETING INTERSTS AND FUNDING

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AUTHOR'S CONTRIBUTIONS

VMQ designed the study, assisted with data acquisition, carried out data analysis and drafted the manuscript. MvM participated in the study design, assisted with data interpretation and revised the manuscript. JCtM participated in the study design, assisted with data interpretation and revised the manuscript. JJML

participated in the study design, assisted with data interpretation, revised the manuscript and has given final approval of the version to be published.

DATA SHARING

No additional data available.

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LEGENDS TO THE FIGURES

Figure 1: Delta in vital signs between T_0 and T_1.

SaO2, peripheral oxygen saturation.

Figure 2: Delta in biomarker levels between T_0 and T_1.

ALP, alkaline phosphatase; AST, aspartate transaminase; CRP, C-reactive protein; Gamma-GT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; hs-Trop T, high sensitivity troponin T; NT pro-BNP, N-terminal prohormone of brain natriuretic peptid

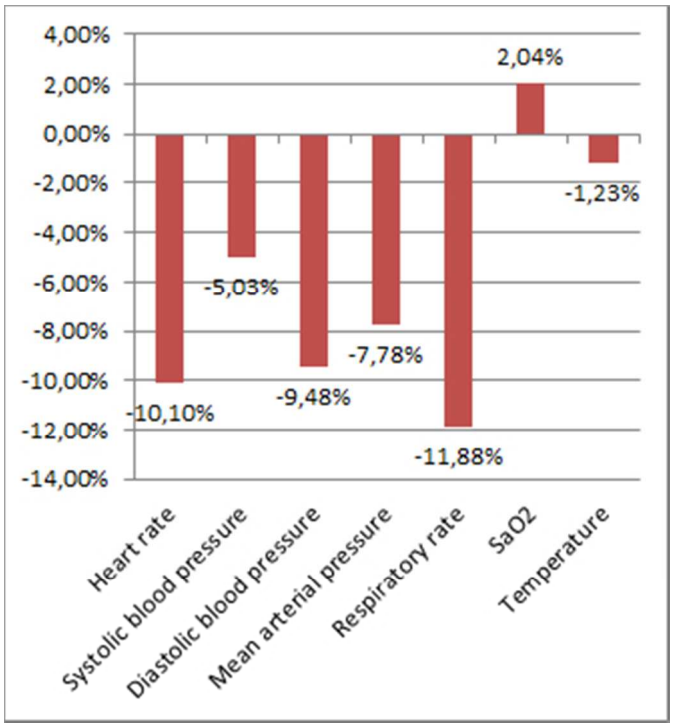

Figure 1: Delta in vital signs between T0 and T1.
SaO2, peripheral oxygen saturation.
28x30mm (300 x 300 DPI)

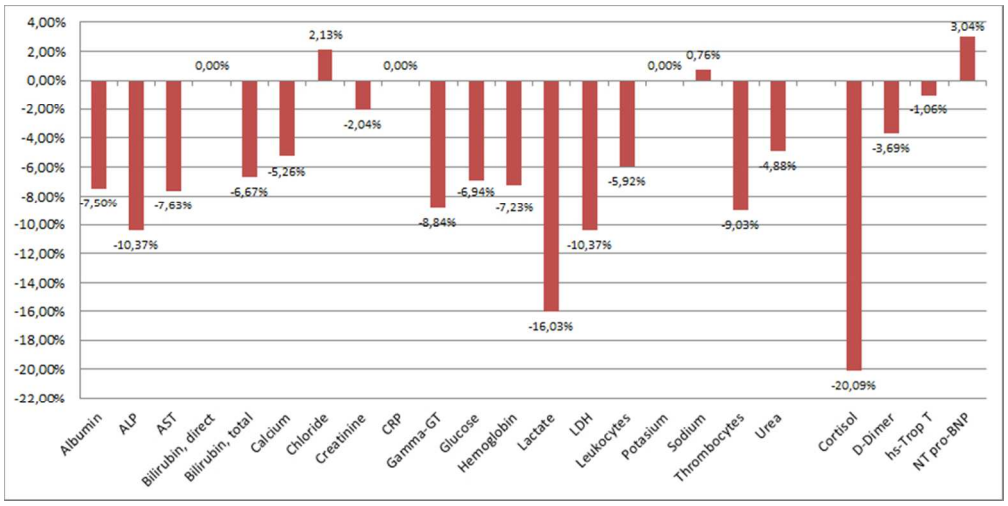

Figure 2: Delta in biomarker levels between T0 and T1.
ALP, alkaline phosphatase; AST, aspartate transaminase; CRP, C-reactive protein; Gamma-GT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; hs-Trop T, high sensitivity troponin T; NT pro-BNP, N-terminal prohormone of brain natriuretic peptid.
72x35mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-5
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	3
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	5-6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	n/a

		(*e*) Describe any sensitivity analyses	n/a
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	5
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-9
		(b) Indicate number of participants with missing data for each variable of interest	5-9
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	n/a
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	n/a
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*n/a*
		Cross-sectional study—Report numbers of outcome events or summary measures	*5-9*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-9
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n/a
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10-11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	10-11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.