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|  |
| --- |
| **≥ 1 of the following criteria must be fulfilled:** |
| * Airway obstruction * Respiratory arrest * Respiratory rate < 8 pr minute * Respiratory rate > 40 pr minute * SpO2 < 85% with > 9 L O2 treatment * Non-invasive ventilation initiated in ambulance * Systolic blood pressure < 90 mmHg and symptoms\* * Vasopressor initiated in ambulance * Heart rate / pulse < 35 or > 130 and symptoms\* * Glasgow Coma Scale < 9 * Persistent seizures * Core temperature < 32 degrees Celsius * qSOFA ≥ 2 and infection suspected * Ambulance request * Nurse in ED request * Medical Doctor in ED request |
| \*Sweaty, clammy, pale, dizzy, altered mentation, uneasy. |

### Supplementary table 1. Inclusion criteria for Medical and Sepsis Rapid Response Teams in the Emergency Department at Oslo University Hospital.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Analyte** | **Collection tube (Vacuette)** | **Instrument** | **Reagent** | **Calibrator** | **Reference Interval** |
| CRP | Serum with gel (Ref: 456073R) | Roche Cobas 8000 c702 | CRPL4 Tina-quant (Ref: 07876424) | CFAS Proteins Traceability: ERM-DA474 | ≤4 mg/L1 |
| Procalcitonin | Li-heparin plasma with gel (Ref: 456087) | Roche Cobas 8000 e801 | Elecsys BRAHMS PCT (Termo Fisher) (Ref: 07301715190) | PCT Cal1 / PCT Cal2 Traceability: Roche | <0.1 µg/L2 |
| Calprotectin | Li-heparin plasma with gel (Ref: 456087) | Roche Cobas 8000 c502 | GCAL (Gentian) (Ref: 1201) | Gentian Calprotectin calibrator Traceability: Gentian | 1.69 mg/L3 |
| Interleukin-6 | Li-heparin plasma with gel (Ref: 456087) | Roche Cobas 8000 e801 | Interleukin 6 (Ref: 07027532190) | IL-6 CalSet Traceability:1st Int. Standard (NIBSC) 89/548 | ≤7 pg/mL4 |

### Supplementary table 2. Collection tubes, instruments, reagents and calibrators used in the measuring of biomarkers CRP, Procalcitonin, Calprotectin and Interleukin 6.

All reagents and calibrators were purchased from Roche unless otherwise stated. 1Reference interval established locally at Oslo University Hospital with samples from healthy donors. 2Morgenthaler, N. G., et al. (2002). "Detection of procalcitonin (PCT) in healthy controls and patients with local infection by a sensitive ILMA." Clin Lab 48(5-6): 263-270.3. 3Gentian. Gentian Calprotectin Reagent Kit Instructions for use. Date of issue 2022-01-24. Moss, Norway 2022. 4Roche Cobas Elecsys IL-6 package insert 07027535200V2.0, 2019

|  |  |  |
| --- | --- | --- |
| **Diagnosis** | **Number** | **Percent of total** |
| Intoxication | 22 | 5.3 % |
| Non-infectious COPD exacerbation | 22 | 5.3 % |
| Heart failure | 20 | 4.8 % |
| Seizures and epilepsy | 19 | 4.6 % |
| Cerebral stroke | 16 | 3.9 % |
| Heart arrhythmia | 11 | 2.7 % |
| Other neurologic disease | 5 | 1.2 % |
| Pulmonary embolism | 5 | 1.2 % |
| Drug reaction | 4 | 1.0 % |
| Psychosis | 4 | 1.0 % |
| Haemorrhagic shock | 3 | 0.7 % |
| Myocardial infarction | 3 | 0.7 % |
| Cancer | 3 | 0.7 % |
| Anaemia | 2 | 0.5 % |
| Acute rheumatic or immunological disease | 2 | 0.5 % |
| Drowning/Near-drowning | 2 | 0.5 % |
| Acute functional decline | 2 | 0.5 % |
| Gastric ulcer | 2 | 0.5 % |
| Other | 17 | 4 % |

### Supplementary table 3. List of non-infectious diagnoses.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **All admissions** | **Infection likelihood** | | | **p-value** |
|  | **Not likely** | **Probable** | **Definite** |
| Total Admissions | 391 | 154 (39) | 135 (35) | 102 (26) |  |
|  |  |  |  |  |  |
| **Baseline Demographic** |  |  |  |  |  |
| Age, mean (SD) | 68 (19) | 64 (19) | 71 (18) | 69 (19) | 0.005# |
| Male, n (%) | 222 (57) | 86 (56) | 80 (59) | 57 (56) | 0.880‡ |
| Charlson Comorbidity Index, mean (SD) | 2.7 (2.4) | 2.2 (2.3) | 3.1 (2.4) | 3.1 (2.4) | 0.003# |
|  |  |  |  |  |  |
| **Clinical characteristics in ED** |  |  |  |  |  |
| SOFA increase, mean (SD) | 3.0 (2.1) | 3.1 (2.3) | 2.6 (1.7) | 3.3 (2.4) | 0.028# |
| SOFA increase ≥ 2, n (%) | 285 (73) | 114 (74) | 93 (69) | 80 (76) | 0.062‡ |
| qSOFA, mean (SD) | 1.4 (0.72) | 1.3 (0.7) | 1.5 (0.8) | 1.4 (0.8) | 0.064# |
| qSOFA ≥ 2, n (%) | 163 (42) | 53 (34) | 62 (46) | 48 (47) | 0.395‡ |
| SIRS, mean (SD) | 2.4 (1.1) | 2.0 (1.1) | 2.6 (1.0) | 2.7 (0.9) | <0.001# |
| NEWS, mean (SD) | 8.4 (3.6) | 8.2 (3.4) | 8.4 (3.6) | 8.8 (3.8) | 0.442# |
| Blood culture drawn (%) | 318 (81) | 87 (56) | 131 (97) | 100 (98) | <0.001‡ |
| Antibiotics in ED, n (%) | 259 (66) | 43 (28) | 123 (91) | 93 (91) | <0.001‡ |
|  |  |  |  |  |  |
| **Laboratory parameters in ED** |  |  |  |  |  |
| Calprotectin (mg/L), median (IQR) 9 | 2.48 (2.58) | 1.53 (1.61) | 2.72 (2.36) | 3.8 (4.0) | <0.001§ |
| CRP (mg/L), median (IQR) 11 | 37 (109) | 5 (16) | 84 (118) | 88 (146) | <0.001§ |
| IL-6 (pg/mL), median (IQR) 14 | 89 (382) | 18 (34) | 156 (381) | 460 (4339) | <0.001§ |
| PCT (µg/L), median (IQR) 13 | 0.20 (0.85) | 0.10 (0.02) | 0.3 (0.98) | 1.2 (13.3) | <0.001§ |
| Leucocytes (×109/L), median (IQR) | 11.0 (7.3) | 9.6 (6.2) | 12.3 (8.0) | 12.3 (7.9) | 0.001§ |
| Platelets (×109/L), mean (SD) | 253 (112) | 258 (108) | 252 (108) | 248 (122) | 0.738# |
| Creatinine (mmol/L), median (IQR) | 89 (56) | 86 (56) | 88 (50) | 97 (81) | 0.038§ |
| Bilirubin, total (µmol/L), median (IQR) | 10 (9) | 7 (9) | 10 (9) | 11 (10) | 0.004§ |
| Lactate (mmol/L), median (IQR) | 1.9 (1.7) | 2.0 (3.2) | 1.6 (1.3) | 2.0 (1.5) | 0.006§ |
|  |  |  |  |  |  |
| **Outcome measures** |  |  |  |  |  |
| Hospital mortality, n (%) | 32 (9) | 15 (10) | 8 (6) | 9 (9) | 0.502‡ |
| 30 days mortality, n (%) | 46 (13) | 16 (11) | 20 (16) | 10 (10) | 0.328‡ |
| Hospital stay, median days (IQR) | 6 (6) | 4 (7) | 6 (4) | 7 (11) | <0.001§ |
| ICU treatment, n (%) | 148 (38) | 81 (52) | 24 (18) | 43 (42) | <0.001‡ |
| Mechanical ventilation, n (%) | 21 (5) | 12 (8) | 1 (1) | 8 (8) | 0.013‡ |
| Max SOFA increase during hospitalization, mean (SD) | 3.3 (2.6) | 3.4 (2.6) | 2.7 (1.8) | 3.9 (3.2) | 0.001# |
| Sepsis (%) | 177 (45) | N/A | 96 (71) | 81 (79) | N/A |

### Supplementary table 4. Patient characteristics.

P-values computed using #One-way ANOVA, ‡Pearson Chi-squared, §Kruskal-Wallis tests. If multiple admissions per patient, mortality was analysed including only first admission. Sepsis during hospitalization is defined as an increase in SOFA ≥ 2 and infection probable or definite. The number of missing values is marked in superscript for the four biomarkers studied.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **All** | **Infection «Not likely»** | **«Infection»** |
| **Procalcitonin**,*t* (p-value) | 0,670 (0,503) | 0,094 (0,925) | 0,392 (0,695) |
| **Calprotectin**,*t* (p-value) | 0,465 (0,642) | -0,166 (0,869) | 0,371 (0,711) |
| **CRP**,*t* (p-value) | 1,706 (0,089) | 0,913 (0,363) | 1,628 (0,105) |
| **Interleukin 6**,*t* (p-value) | 0,549 (0,583) | 1,459 (0,147) | -0,464 (0,643) |

### Supplementary table 5. Biomarker and mortality association.

Student t-test comparing mean log-transformed biomarker values in patients that survived 30 days and those that did not. For all patients and stratified on infection likelihood.

|  |  |  |
| --- | --- | --- |
|  | **AUC (95% CI)** | |
|  | **qSOFA ≥ 2** | **qSOFA < 2** |
| **Calprotectin** | 0,729 (0,643- 0,815) | 0,801 (0,744-0,859) |
| **CRP** | 0,881 (0,825-0,937) | 0,924 (0,890-0,958) |
| **IL6** | 0,901 (0,849-0,953) | 0,888 (0,845-0,932) |
| **PCT** | 0,844 (0,777-0,910) | 0,819 (0,765-0,874) |

### Supplementary table 6a. Biomarker AUCs stratified by qSOFA ≥ 2 and < 2.

AUC and 95% confidence interval for biomarkers Calprotectin, CRP, IL6 and PCT in discriminating patients with and without infection in the ED.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **N (%)** | **Sensitivity (%)** | **Specificity (%)** | **PPV, % (95% CI)** | **NPV, % (95% CI)** |
| **CRP ≥ 31 mg/L and IL-6 ≥ 52 pg/mL** |  |  |  |  |  |
| **All** | 178 (46) | 72 (66-77) | 94 (89-97) | 95 (91-97) | 68 (64-73) |
| **qSOFA < 2** | 89 (39) | 68 (59-76) | 97 (91-99) | 97 (92-99) | 66 (60-72) |
| **qSOFA ≥ 2** | 89 (55) | 76 (67-84) | 89 (76-96) | 91 (83-96) | 71 (63-78) |
|  |  |  |  |  |  |
| **CRP ≥ 31 mg/L or IL-6 ≥ 52 pg/mL** |  |  |  |  |  |
| **All** | 265 (68) | 95 (91-97) | 74 (66-81) | 85 (81-88) | 90 (85-94) |
| **qSOFA < 2** | 136 (60) | 91 (85-96) | 80 (71-87) | 88 (83-91) | 86 (77-91) |
| **qSOFA ≥ 2** | 129 (79) | 99 (95-100) | 62 (48-75) | 80 (74-85) | 98 (86-100) |

### Supplementary table 6b. Test abilities for combinations of CRP and IL-6 optimal cut-off values.

Sensitivity, specificity and positive and negative predictive values for CRP and IL-6 optimal cut-off values in different combinations (both or at least one above optimal cut-off value), for all patients and stratified by qSOFA.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **All** | **qSOFA ≥ 2** | **qSOFA < 2** |
| **AUC (95% CI)** | 0.952 (0.932-0.972) | 0.958 (0.930-0.986) | 0.950 (0.922-0.977) |
| **Sensitivity (95% CI)** | 93 (89-96) | 95 (88-98) | 92 86-96 |
| **Specificity (95% CI)** | 82 (75-88) | 79 (66-89) | 88 80-93 |
| **PPV (95% CI)** | 89 (84-93) | 90 (83-95) | 91 (84-95)) |
| **NPV (95% CI)** | 89 (84-93) | 88 (74-95) | 90 (82-95) |

### Supplementary table 7. AUCs for the best regression model, stratified by qSOFA ≥ 2 and < 2.

AUC, sensitivity, specificity and positive and negative likelihood ratios for the best regression model predicting presence of infection (IL6, CRP and ED clinical judgement) stratified by qSOFA.



### Supplementary figure 1. Scatter plots comparing log-transformed biomarker values.

Linear regression was performed on log-transformed biomarker values to assess association. R2 and p-values in adjacent boxes (pink = “Infection, black = infection “Not likely” and blue dashed = all patients).



### Supplementary figure 2. Biomarker levels grouped according the causative agent group.

Virus (n=17), Gram positive bacteria (n=45), Gram-negative bacteria (n=71), mixed infection (viral and/or Gram positive and/or Gram-negative infections, n=9), unknown etiology, but infection probable (n=95) and no infection (n=154). Asterisks indicates significance by Kruskal-Wallis multiple comparisons test, \*\*\* p < 0.001, \*\* p <0.01. Grey dashed line indicates optimal cut-off value in our cohort (Youden’s index). No statistics was performed on the group Infection “Not likely”.

|  |  |  |
| --- | --- | --- |
| **Causative agent** | **Number** | **Percent** |
| *Escherichia coli* | 42 | 30 |
| *Streptococcus* species | 21 | 15 |
| *Klebsiella* species | 10 | 7 |
| SARS-CoV-2 | 10 | 7 |
| *Staphylococcus aureus* | 10 | 7 |
| Polymicrobial bacterial infection | 9 | 6 |
| Other Enterobacteriales | 8 | 6 |
| Other virus | 5 | 4 |
| *Clostridium* species | 4 | 3 |
| Combined viral and bacterial infection | 4 | 3 |
| *Haemophilus influenza* | 3 | 2 |
| Influenza A | 2 | 1 |
| *Streptococcus pneumoniae* | 2 | 1 |
| *Pseudomonas* species | 2 | 1 |
| Other bacteria | 10 | 7 |

### Supplementary table 8. List of all identified causative agents.



### Supplementary figure 3. Sampling of follow-up cohort.

Each dot indicates sampling and no missing biomarker values. Grey means excluded patients.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **All** | **Infection "Not likely"** | **Infection** |
| No (%) | 45 | 15 (33) | 30 (67) |
| Male (%) | 31 (69) | 10 (67) | 21 (70) |
| Age, median (IQR) | 66 (25) | 69 (18) | 66 (24) |
| Antibiotics in ED, n (%) | 39 (87) | 11 (73) | 28 (93) |
| qSOFA in ED, mean (SD) | 1.3 (0.8) | 1.2 (0.9) | 1.4 (0.8) |
| SIRS in ED, mean (SD) | 2.5 (1.0) | 2.4 (0.8) | 2.6 (1.0) |
| NEWS in ED, mean (SD) | 8.5 (3.8) | 8.7 (3.7) | 8.3 (4.0) |
| ICU admission, n (%) | 35 (78) | 14 (93) | 21 (70) |
| 30 day mortality, n (%) | 3 (7) | 0 (0) | 2 (6) |
| Causative agent identified, n (%) | 23 (51) | 0 (0) | 23 (77) |
| SOFA increase | 4.5 (3.1) | 4.5 (3.2) | 4.5(3.1) |

### Supplementary table 9. Patient characteristics of the patients included for follow-up sampling.



### Supplementary figure 4. Scatter plots comparing CRP and IL-6 values on different days in patients in the group “Infection” in the follow-up cohort.

Axes are on a logarithmic scale. Correlations estimated with Spearman’s Rho, asterisks indicate significance; \*\* p < 0.01, \*\*\*\* p < 0.0001.