qS-Ne2Mo Score – A New Risk Stratification Tool For Early Detection of Septic Shock in The Field of Emergency Medicine

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**Title Page**

**qS-Ne2Mo score – a new risk stratification tool for early detection of septic shock in the field of emergency medicine**

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Abstract

Background
Sepsis is one of the most significant healthcare concerns of the 21st century. In the United States sepsis affect 1.7 million adults, with 270,000 fatal cases, according to the estimation of Centers for Disease Control and Prevention. The management of sepsis relies on early recognition, therefore the emergency departments have distinctive role in sepsis care, hence the need for early reliable risk stratification tools.

Methods
A retrospective, quantitative study was performed in Department of Emergency, University of Szeged, Hungary. Patients with suspected infection were enrolled to four subgroups based on the results of patient examination and laboratory results. In all cases (N=276), cell population data markers were analyzed along with ordinary infection biomarkers, such as CRP, PCT and WBC. Performance of cell population data parameters were investigated with ROC (Receiver Operating Curve) analysis.

Results
Almost all cell population biomarkers showed significant differences in the subgroup analysis. Remarkable performance was found in three markers (NE-SFL/M, MO-X/M and NE-WY/M) in patients having septic shock. Combining quick SOFA with these biomarkers (qS-Ne2Mo score) resulted in excellent diagnostic ability for septic shock (AUC 0.914, p<0.001), with good sensitivity (73.9%) and excellent specificity (89%).

Conclusions
Since determination of cell population data requires complete blood count analysis, turn-around time of this novel indicator is significantly lower than other methods. qS-Ne2Mo score might be used as an initial screening tool to select only those patients that need more extensive laboratory investigations for their proper treatment and spare inadequate, time and money consuming laboratory requests.

Trial Registration: University of Szeged, Ethical Committee ref. nr. 25/2016-SZTE

Keywords: sepsis, septic shock, biomarkers, emergency medical services
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Introduction

Sepsis is one of the most significant healthcare concerns of the 21st century. In the United States sepsis affect 1.7 million adults, with 270,000 fatal cases, according to the estimation of Centers for Disease Control and Prevention [4]. The management of sepsis relies on early recognition, therefore the emergency departments have distinctive role in sepsis care, hence the need for early reliable risk stratification tools.

According to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) infectious diseases can rapidly progress to life-threatening conditions such as sepsis and septic shock. Therefore, an early and reliable screening tool could potentially improve outcomes [16]. In suspected cases the ‘one-hour bundle’ is recommended [7]. The bundle consists of fluid-, and vasopressor therapy, serum lactate measurements, microbiologic sampling, and commencement of broad spectrum antibiotics. Time zero is defined as the time of the triage, after which treatment should be started immediately if sepsis or septic shock is suspected in order to enable the emergency team to complete all tasks of the bundle within this relatively short time frame.

According to Sepsis-3, sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. In patients with suspected infection, quick Sequential Organ Failure Assessment score (qSOFA score) is recommended as an initial screening tool for sepsis. This is a simple bedside risk stratification method assessing the mental status, respiratory rate, and systolic blood pressure. In cases having qSOFA score two or three points, sepsis or septic shock is suspected, and the one-hour bundle is to be completed. If qSOFA score indicates low risk, then the recommendation is to use clinical judgement. In cases with positive qSOFA
score or in cases where sepsis is suspected clinically, the Sequential Organ Failure Assessment
(SOFA) score is to be calculated. SOFA is a 0–24-point scale, and sepsis-related organ
failure is confirmed by an increase of two or more points. Septic shock is confirmed if
vasopressor therapy is needed to maintain mean arterial pressure over 65 mmHg and the serum
lactate level is above 2 mmol/l [4]. Cases having SOFA less than two points, have significantly
lower chance for poor outcome, we considered them as infection without organ failure.
Although, Sepsis-3 definitions are clear but to complete them is time-consuming and there are
several concerns specially with the very early phase of screening for suspected sepsis. qSOFA
score is neither specific nor sensitive for the poor outcome [8,11]. Using clinical judgement
(without any solid recommendation) brings too much subjectivity into the clinical practice. On
one hand, if clinical suspicion is used overzealously, then milder cases go through extensive
laboratory testing, additional painful blood works (ABG) and hasty administration of wide
spectrum antibiotics with all the adverse consequences without any real benefit. On the other
hand, if clinical sepsis awareness is low, consequences can be disastrous. This necessitates to
find objective biomarkers, which have a good additional value to the clinical parameters
provided by qSOFA score and reinforce the clinical decision making in this crucial, initial phase
of sepsis management.

For an emergency physician the earliest laboratory result to get is the complete blood count
(CBC) including white blood cell (WBC) count. Although WBC count is cheap, widely
available, fast, and requested in almost all cases with suspected infection, in itself not reliable
to detect sepsis [5]. With novel methods, analyzers can provide quantitative measurements of
morphological and functional features in neutrophil leukocytes, lymphocytes, and monocytes
with practically the same turn-around-time as WBC count. Sysmex XN analyzers use optical
measurements of light scattering and fluorescence to quantify different cellular characteristics
(i.e., internal complexity, nucleic acid content and cell size). These descriptive data are known as cell population data (CPD).

Although CPD provides huge amount of data on white blood cells, clinical applicability in the emergency setting is yet to be determined. Therefore, the aim of this retrospective study was to analyze the performance of CPD parameters in the diagnosis of sepsis. We also tried to find CPD parameters that could be combined with qSOFA for risk stratification benchmarked against the calculated SOFA score, and the final Sepsis-3 category.

Materials and methods

This retrospective study was conducted at the Emergency Department (ED) of the University of Szeged, Hungary. All data were collected from September 2019 until January 2020 from patients who received emergency care in the ED because of a suspected infection.

Patient data were obtained from the hospital’s electronic medical record system, based on the approval of the University Ethical Committee (ref nr. 25/2016-SZTE). Basic descriptive data (such as age, gender, date of service) were collected, along with data from the first medical examination (blood pressure, heart rate, respiratory rate, and level of consciousness). Detailed laboratory results were collected based on the initial laboratory tests, such as organ function tests, coagulation tests, and CBC. Appropriate specimen were taken from all patients for microbiology assessment.

Four subgroups were created: No-infection, Infection, Sepsis, Septic shock. For the presence of infection the following criteria had to be fulfilled: a) confirmed infection either by microbiology or imaging techniques such as chest X-ray / CT scan; b) SOFA score less than 2 points. As a confirmation method for infection criteria (a), all analyses were performed both in the whole cohort (as described in the criteria) and in the microbiology-positive subgroup (“final sample”). Sepsis and septic shock were diagnosed according to the Sepsis-3 definitions [4].
The blood samples were collected in 3 ml Vacutainer Plastic K$_3$EDTA tubes (Ref.# 368857, Becton-Dickinson, Franklin Lakes, NJ, USA) and were analysed using automated haematology analyser Sysmex XN20 (Sysmex Corporation, Kobe, Japan) within 2 hours of sample collection. The measured parameters included total blood count and cell population data (CPD) of neutrophils, lymphocytes and monocytes on white blood cell differential (WDF) channel. The precision of white blood cell (WBC) count determination was 3% or less according the manufacturer, when the WBC count was 4.00 G/L or more. The precision of immature granulocyte ratio (IGR) was 2% at the WBC count of 4.00 G/L or more.

WDF channel of the Sysmex XN20 haematology analysers uses optical side scatter along X axis to assess the granularity and internal structure (complexity) of the cells. Fluorescence intensity, which corresponds to RNA/DNA cell content, is plotted on the y-axis and is an indicator for increased RNA activity. The forward scatter along the Z axis was in accordance with the cell size. The parameters on X axis include neutrophils cell complexity (NE-SSC), lymphocytes cell complexity (LY-X), monocytes cells complexity (MO-X), neutrophils complexity and width of dispersion (NE-WX), lymphocytes complexity and width of dispersion (LY-WX) and monocytes complexity and width of dispersion (MO-WX). The parameters reported on the Y-axis include neutrophils fluorescence intensity (NE-SFL), lymphocytes fluorescence intensity (LY-Y), monocytes fluorescence intensity (MO-Y), neutrophils fluorescence intensity and the width of dispersion (NE-WY), lymphocytes fluorescence intensity and the width of dispersion (LY-WY) and monocyte fluorescent intensity and width of dispersion (MO-WY). The following parameters were reported on the Z-axis, neutrophils cell size (NE-FSC), lymphocytes cell size (LY-Z), monocytes cell size (MO-Z), neutrophils cell size and the width of dispersion (NE-WZ), lymphocytes cell size and the width of dispersion (LY-WZ) and monocytes cell size and the width of dispersion (MO-WZ). The
The precision of measured and calculated data as stated by the manufacturer are presented in Table 1.

|                  | mean | CV% | mean | CV% | mean | CV% |
|------------------|------|-----|------|-----|------|-----|
| **WBC# (G/L)**   | 32.5 | 1.19| 4.8  | 1.38| 1.5  | 4.72|
| **NEU# (G/L)**   | 29.6 | 1.13| 2.8  | 1.85| 0.77 | 6.39|
| [NE-SSC(ch)]     | 152.7| 0.23| 153.3| 0.50| 1.4  | 0.83|
| [NE-SFL(ch)]     | 42.5 | 0.74| 48.9 | 1.10| 1.1  | 1.97|
| [NE-FSC(ch)]     | 83.2 | 1.55| 95.0 | 0.98| 1.4  | 1.47|
| [NE-WX]          | 318.6| 1.76| 308.0| 3.96| 23.8 | 8.05|
| [NE-WY]          | 827.0| 1.65| 644.6| 5.85| 110.9| 14.85|
| [NE-WZ]          | 709.7| 1.77| 650.1| 4.14| 54.9 | 8.23|
| **LY# (G/L)**    | 1.06 | 4.18| 1.2  | 3.14| 0.49 | 5.86|
| [LY-X(ch)]       | 81.5 | 0.76| 85.7 | 0.57| 0.5  | 0.57|
| [LY-Y(ch)]       | 53.6 | 1.12| 74.8 | 1.64| 1.5  | 2.37|
| [LY-Z(ch)]       | 56.5 | 1.40| 60.5 | 1.30| 0.8  | 1.24|
| [LY-WX]          | 452.0| 5.46| 421.9| 6.82| 47.8 | 10.71|
| [LY-WY]          | 1036.0| 6.98| 912.5| 5.85| 81.5 | 6.74|
| [LY-WZ]          | 646.5| 6.14| 541.2| 3.02| 36.3 | 6.17|
| **MO# (G/L)**    | 1.75 | 3.44| 0.6  | 5.13| 0.16 | 12.73|
| [MO-X(ch)]       | 121.2| 0.68| 119.4| 0.95| 2.3  | 1.86|
| [MO-Y(ch)]       | 95.9 | 1.69| 108.3| 4.34| 12.4 | 10.43|
| [MO-Z(ch)]       | 65.4 | 1.78| 69.9 | 2.75| 2.9  | 4.08|
| [MO-WX]          | 263.4| 6.39| 252.1|10.05| 33.2 | 12.01|
| [MO-WY]          | 865.4| 5.69| 731.1| 7.12| 143.0| 31.52|
| [MO-WZ]          | 661.0| 4.06| 607.2| 6.55| 67.4 | 10.55|
Table 1. Within-run imprecision of cell counts and cell population data (CPD). The mean values and %CVs were obtained from 20 replicates of three patients at different concentrations of white blood cells (WBC). NEU: Neutrophil granulocytes; LY: Lymphocytes; MO: Monocytes

Determination of C-Reactive Protein and Procalcitonin

The blood samples were collected in 5 ml vacutainer tubes (Plastic STT II Advance tubes; Ref.#367955, Becton-Dickinson, Franklin Lakes, NJ, USA) for measurement of CRP and PCT. The specimens were allowed to clot for 30 minutes and centrifuged for 10 minutes at 2000xg and the serum was subjected for analysis. The assays were performed within 2 hours of specimen collection.

CRP was measured by immunturbidimetric method (3. generation C-reactive protein kit, Roche, Mannheim, Germany) on Roche Cobas c501 analyzer (Roche, Mannheim, Germany). The detection limit was <0.3 mg/L and the measuring range was 0.3–350 mg/L. The interassay CVs at 2.36 and 157 mg/L concentrations were 1.3% and 11%, respectively.

PCT was measured by electrochemiluminescence immunoassay method (Elecsys BRAHMS PCT kit, Roche, Mannheim, Germany) on an automated Cobas e601 analyzer (Roche, Mannheim, Germany). The detection limit was ≤0.02 μg/L and the measuring range was 0.02-100 μg/L. The interassay CVs at 0.431 μg/L and 54.4 μg/L were 2.6% and 1.6%, respectively.

Statistical analysis

Distribution of data was analyzed with standard Kolmogorov-Smirnov (K-S) test. Interestingly, blood pressure showed normal distribution, while all other descriptive parameters were not normally distributed. Descriptive statistics were made with means and standard deviations or medians and interquartile ranges (25 and 75 %), respectively. Analysis of differences between groups were calculated using one-way ANOVA (Levene’ F) or Kruskal – Wallis test with post
hoc Bonferroni correction, based on the distribution of the data. Any difference was considered significant above p=0.05 level, and confidence intervals were set to 95%.

Performance of cell population data (CPD) parameters were investigated with ROC (Receiver Operating Curve) analysis. Cutoff values were set using Youden’s method, based on AUC (area under the curve) analysis. Data performance was considered good above 0.8, and excellent above 0.9 AUC, respectively.

Results

From September 2019 to January 2020 overall 452 patients were enrolled to the study, who arrived at the emergency department with a suspected infection. Data were collected from the electronic patient records. After clearing the raw data, in 176 cases SOFA score was incomplete, therefore these cases were excluded from the study.

The baseline and descriptive characteristics of the whole cohort are summarized in Table 2 and 3.

| Baseline demographics | no infection (N=86) | infection (N=37) | sepsis (N=129) | septic shock (N=24) |
|-----------------------|--------------------|-----------------|----------------|---------------------|
| Age (years)           | 63 (50.7-80)       | 67 (56.5-76)    | 74 (66.5-84)   | 80.5 (72.2-84.7)    |
| Females               | 35 (40.7%)         | 18 (48.6%)      | 72 (55.8%)     | 7 (29.2%)           |
| Source of infection   | n/a                |                 |                |                     |
| lower respiratory tract | n/a | 14 (37.8%) | 54 (41.9%) | 6 (25 %) |
| upper respiratory tract | n/a | 2 (5.4%) | 3 (2.3%) | 2 (8.3%) |
| gastrointestinal | n/a | 2 (5.4%) | 4 (3.1%) | 0 (0%) |
| intraabdominal | n/a | 3 (8.1%) | 17 (13.2%) | 2 (8.3%) |
| Skin/soft tissue | n/a | 5 (13.5%) | 10 (7.8%) | 1 (4.2%) |
Table 2. Baseline characteristics of the whole sample (N=276)

| Variables                  | no infection (N=86) | infection (N=37) | sepsis (N=129) | septic shock (N=24) | significance* |
|----------------------------|---------------------|------------------|-----------------|---------------------|---------------|
| systolic RR                | 141.66 (26.82)      | 134.49 (19.12)   | 121.67 (30.13)  | 96.63 (29.62)       | p=0.000       |
| diastolic RR               | 82.29 (17.46)       | 78.35 (13.78)    | 67.43 (18.26)   | 52.92 (19.75)       | p=0.000       |
| mean arterial pressure (MAP)| 102.08 (18.98)      | 97.06 (14.45)    | 85.51 (20.77)   | 67.48 (22.14)       | p=0.000       |
| Respiratory rate (RR)      | 17 (16-20)          | 19 (17-22)       | 20 (17-24)      | 24 (18-36)          | p=0.000       |
| Glasgow Coma Scale (GCS)   | 15 (15)             | 15 (15)          | 15 (14-15)      | 10 (5-14)           | p=0.000-0.012 |
| SOFA                       | 1 (0-3)             | 1 (1)            | 3 (2-4)         | 8 (7-10)            | p=0.000-0.033 |
| Microbiology positivity rate| 1/86 (1.1%)        | 15/37 (40.54%)   | 76/129 (58.9 %) | 19/24 (79.2%)       | p=0.000-0.002 |

Data are summarized as mean (standard deviation), median (quartiles 25%-75%) or frequency (percentage) as appropriate

Table 3. Descriptive parameters of the whole sample (N=276)

In 66 cases, microbiology results were not available at the time of the data collection, and the confirmation of the initial diagnosis was based on imaging results. These cases were excluded from the final sample, and data of the remaining 210 patients were analyzed separately (Table 3 and 4).
In the “No infection” group (N=82) although the initial impression was suspected infection, non-infective underlying conditions were proven, and infection was ruled out. In patients with proven infection SOFA score was calculated and need for noradrenalin and lactate elevation were taken into consideration in forming the other three groups: “Infection”: SOFA<2 (N=16), “Sepsis”: SOFA ≥ 2 (N=89) and “Septic shock”: SOFA ≥ 2 + need for noradrenalin and lactate level > 2mmol/L (N=23).

Baseline and descriptive characteristics of the final sample can be seen in Table 4 and 5.

| Baseline demographics | no infection (N=82) | infection (N=16) | sepsis (N=89) | septic shock (N=23) |
|-----------------------|--------------------|-----------------|--------------|-------------------|
| Age (years)           | 63 (49-79.2)       | 65 (55.7-78)    | 74 (68-84)   | 81 (73-85)        |
| Females               | 33 (40.2%)         | 9 (56.3%)       | 50 (56.2%)   | 7 (30.4%)         |
| Source of infection   | n/a                | n/a             | n/a          | n/a               |
| lower respiratory tract | n/a              | 3 (18.8%)       | 36 (40.4%)   | 5 (21.7%)         |
| upper respiratory tract | n/a              | 0 (0%)          | 0 (0%)       | 2 (8.7%)          |
| gastrointestinal      | n/a                | 2 (12.5%)       | 3 (3.4%)     | 0 (0%)            |
| intraabdominal        | n/a                | 0 (0%)          | 10 (11.2%)   | 2 (8.7%)          |
| Skin/soft tissue      | n/a                | 1 (6.3%)        | 6 (6.7%)     | 1 (4.3%)          |
| central nervous system | n/a               | 1 (6.3%)        | 0 (0%)       | 0 (0%)            |
| urinary tract         | n/a                | 8 (50%)         | 26 (29.2%)   | 10 (43.5%)        |
| bone                  | n/a                | 0 (0%)          | 2 (2.2%)     | 0 (0%)            |
| unknown               | n/a                | 1 (6.3%)        | 6 (6.7%)     | 3 (13%)           |

Data are summarized as mean (min-max) or frequency (percentage) as appropriate

Table 4. Baseline characteristics of the final sample (N=210)
Variables | no infection (N=82) | infection (N=16) | sepsis (N=89) | septic shock (N=23) | significance*
--- | --- | --- | --- | --- | ---
systolic RR | 142.5* (26.54) | 129.6* (17.49) | 118.5 (30.7) | 93.78* (26.7) | p=0.000*-1.000


diastolic RR | 82.93* (17.1) | 78.56* (11.8) | 65.04 (17.84) | 51.04* (17.9) | p=0.000*-1.000


mean arterial pressure (MAP) | 102.7* (18.54) | 95.58* (12.94) | 82.88 (20.65) | 65.29* (19.78) | p=0.000*-1.000


Respiratory rate (RR) | 17* (16-20) | 18 (17-21) | 20 (18-25) | 24* (18-35) | p=0.000*-1.000


Glasgow Coma Scale (GCS) | 15 (15) | 15 (15) | 15 (14-15) | 10* (5-15) | p=0.000*-1.000


SOFA | 1 (0-2) | 1 (1) | 4 (3-5) | 8* (7-10) | p=0.000*-0.898


Microbiology positivity rate | 0/82 (0%) | 16/16 (100 %) | 89/89 (100 %) | 23/23 (100%) | p<0.001

*: One-way ANOVA (Levene’s F) or Kruskal – Wallis test with post hoc Bonferroni correction, based on the distribution of the data

Table 5. Descriptive parameters of the final sample (N=210)

All statistical tests were performed in both samples (ie. the whole cohort and final sample), and interestingly, the same pattern can be seen in almost every examined variable (Table 3 and 5.) Although this was not among the main reasons behind our study, in our interpretation, this finding confirms the validity of the clinical decision in cases when no microbiology results are available.

CRP, PCT and WBC performance

Standard infection markers were also compared in all groups. White blood cell count (WBC) and C-reactive Protein (CRP) was significantly lower in non-infected than in every other group.
(10.55 vs 81.35-131.3, p<0.001). These parameters showed no statistical difference between
the other three groups of infection, sepsis and septic shock. Procalcitonin (PCT) showed
significant difference between almost all groups, respectively (0.068 – 4.73, p=0.000-0.024);
no significant difference could be measured between infection and sepsis group (0.16 vs 0.756,
p=0.074) (Table 6.).

| Parameters | no infection (N=82) | infection (N=16) | sepsis (N=89) | septic shock (N=23) | significance |
|------------|---------------------|------------------|----------------|----------------------|--------------|
| CRP        | 10.55* (3.52-27.82) | 81.35 (34.8-173.6) | 118.25 (55.7-212.3) | 131.3 (82-223.7) | p<0.001*-1.000 |
| PCT        | 0.068 (0.05-0.126)  | 0.16 (0.091-0.5)  | 0.756 (0.28-5.03)  | 4.73* (0.54-20.92) | p=0.000*-0.965 |
| WBC        | 9.49* (8.12-11.25)  | 15.03 (9.1-17.24) | 11.95 (9.24-17.96) | 13.58 (9.03-20.48) | p=0.000*-1.000 |

Table 6. Standard biomarkers in Sepsis-3 cohorts

Performance of CPD parameters

As a second step cell population data were analyzed. Ne-SSC, NeFSC/M, MoZ/M and Mo-
WZ/M showed normal distribution, all the other parameters were not normally distributed.
According to the descriptive analysis multiple parameters showed significant changes. The
highlighted NE-SFL/M, MO-X/M and NE-WY/M parameters showed significant changes
between septic shock and the other groups (Table 7.)

| Parameters | no infection (N=82) | infection (N=16) | sepsis (N=89) | septic shock (N=23) | significance |
|------------|---------------------|------------------|----------------|----------------------|--------------|
| NEUT #     | 6.595* (5.29-8.51)  | 11.94 (7.38-15.23) | 9.92 (7.27-14.07) | 12.79 (8.51-21.01) | p=0.000*-1.000 |
| NEUT %     | 73.25* (62.55-79.65) | 86.15 (77.55-89.2) | 86.8 (79.55-91.45) | 87.2 (83-94.4) | p=0.000*-1.000 |
|    | IGR                | NE-SSC               | NE-SFL/M              | NE-FSC/M              | LY-X/M               | LY-Y/M               | LY-Z/M               | MO-X/M               | MO-Y/M               | MO-Z/M               | NE-WX/M              | NE-WY/M              | NE-WZ/M              | LY-WX/M              | LY-WY/M              | LY-WZ/M              | MO-WX/M              |
|----|--------------------|----------------------|-----------------------|-----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|    | 0.4 (0.3-0.6)      | 0.65 (0.4 – 1.05)    | 0.7 (0.5 – 1.35)      | 1* (0.4 – 3.4)       | p=0.000*-1.000       |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| NE-SSC | 149.59 (4.45)      | 152.91 (3.86)        | 150.49 (5.12)         | 150.2 (4.18)          | p=0.068              |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| NE-SFL/M | 39.5 (37.9-41.2)  | 40.6 (39.6 – 41.52)  | 43.8 (41.5 – 47.5)    | 46.6* (42.5 – 54.2)  | p=0.000*-1.000       |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| NE-FSC/M | 81.6 (3.78)       | 82.03 (3.35)         | 80.04 (4.66)          | 80.9 (4.4)            | p=0.053              |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| LY-X/M | 78.9 (77.7-80.6)   | 78.45 (76.85 – 80.4) | 79.6 (77.9 – 81.1)    | 81.7* (78.8 – 83.1)  | p=0.005*-1.000       |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| LY-Y/M | 61.4 (58.6-63.3)   | 57.95 (55.5 – 60.72) | 59.7 (56.25 – 63.8)   | 61.6 (56.8 – 65.8)   | p=0.052-1.000        |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| LY-Z/M | 55.9 (55-56.4)     | 55.5 (54.7-56.3)     | 55.6 (54.3 – 56.75)   | 56.6 (54.8 – 58.7)   | p=0.062              |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| MO-X/M | 117.4* (115.9-118.8) | 120.1 (119.17 – 121.22) | 120.8 (118.5 – 122.8) | 123.1 (120.8 – 127.9) | p=0.000*-1.000       |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| MO-Y/M | 92.6* (88.3-96.2)  | 95.45 (93.95 – 102.6) | 96.2 (91.05-103.3)    | 100.1 (95.1 – 107.9) | p=0.000*-1.000       |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| MO-Z/M | 61.54 (2.4)        | 61.36 (2.62)         | 61.38 (3.03)          | 63.05 (2.72)          | p=0.097              |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| NE-WX/M | 320 (310.5-330)   | 322.5 (305 – 333)    | 323 (313.5 – 334)     | 330 (315 – 338)      | p=0.205              |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| NE-WY/M | 658 (626-688.5)   | 678 (658.75 – 736)   | 726 (678 – 796)       | 831* (753 – 945)     | p=0.000*-0.364       |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| NE-WZ/M | 712 (691.5-746)   | 712.5 (683.5 – 753.7) | 723 (699 – 747.5)     | 747 (698 – 774)      | p=0.129              |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| LY-WX/M | 469* (444.5-504.5) | 505.1 (437.25 – 551) | 508 (450.5 – 546.5)   | 500 (447 – 531)      | p=0.013*-1.000       |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| LY-WY/M | 874 (836-916)     | 883 (804.5 – 1101.2) | 941* (840.5 – 1068.5) | 931 (842 – 1084)     | p=0.009*-1.000       |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| LY-WZ/M | 577 (556.5-588.5) | 568 (564 – 609.7)    | 586 (561 – 629.5)     | 592 (524 – 645)      | p=0.140              |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
| MO-WX/M | 261 (249-276)     | 252 (242 – 267.5)    | 272 (250.5 – 290)     | 266 (257 – 296)      | p=0.055-1.000        |                     |                      |                      |                      |                      |                     |                     |                     |                     |                      |                     |
Table 7. Performance of CPD parameters in Sepsis-3 cohorts

| Parameter  | Mean (Range)       | | | | | |
|------------|--------------------|---|---|---|---|---|
| MO-WY/M    | 715 (678.5–759.5)  | 759.5 (677.5–836.5) | 748 (682–825.5) | 754 (714–818) | p=0.08 |
| MO-WZ/M    | 671.77 (67.37)    | 661.1 (48.76)        | 667.79 (84.4)    | 689.83 (72.24) | p=0.769 |

As a third step, ROC analyses aiming to validate the performance of all CPD parameters were carried out in each subgroup. As seen in Figures 1-4, NE-SFL/M, MO-X/M and NE-WY/M emerged from the dataset with clinical progression of the inflammatory response. These parameters showed no notable performance in earlier stages, but in septic shock, AUC’s became significantly remarkable (NE-SFL/M=0.745; MO-X/M=0.77; NE-WY/M=0.826; all p<0.001).

Figure 1. ROC analysis of no infection group
Figure 2. ROC analysis of infection group

Figure 3. ROC analysis of sepsis group
Figure 4. ROC analysis of septic shock group

Cell population data markers in septic shock

Using Youden’s method to determine the best cutoff values, NE-SFL/M, MO-X/M and NE-WY/M emerged from CPD dataset. These group of variables were set as a new index, Ne²Mo. Sensitivity and specificity measurements showed acceptable performance. (Table 8.)

|             | suggested cutoff value | sensitivity | specificity |
|-------------|------------------------|-------------|-------------|
| NE-SFL/M    | 44.45                  | 0.696       | 0.780       |
| MO-X/M      | 120.75                 | 0.826       | 0.710       |
| NE WY/M     | 751.5                  | 0.783       | 0.770       |

Table 8. Ne²Mo suggested cutoff performances

Using the above cutoff values, ROC analysis was carried out using positive Ne²Mo against septic shock as a grouping variable. ROC analysis showed AUC of 0.828, which means good but not excellent diagnostic accuracy for septic shock. (data not shown)
Quick SOFA and CPD combined performance as an early marker of septic shock

Since Ne$_2$Mo score is completely based on the laboratory results, an idea of incorporating the clinical presentation emerged. Quick SOFA (qSOFA) is a widely used scoring system for identifying high-risk patients for in-hospital mortality with suspected infection outside the ICU [14]. A new ordinal variable (qS-Ne$_2$Mo) was formed by forging qSOFA and Ne$_2$Mo. qS-Ne$_2$Mo is a 6-point scale, scoring 1 point each for the positive qSOFA items or Ne$_2$Mo values above the suggested cutoffs. As seen in Figure 5, this new score enhances the individual performance of the single items, with remarkable diagnostic ability for septic shock (AUC of 0.914, p<0.001). Setting the cutoff for 4 points for this score results in good sensitivity (73.9%) and excellent specificity (89%).

Figure 5. ROC analysis of qS-Ne$_2$Mo score in septic shock

After setting the cutoff to 4 points in qSNe$_2$Mo, risk estimates were calculated for each group of patients. Odds ratio (OR) for infection was 0.839; sepsis 1.681 and septic shock 22.56.
According to this estimation, having 4 or more points in the score is signaling elevated risk of either being septic or in septic shock.

**Discussion**

In this retrospective study we aimed to assess the possible application of CPD parameters in sepsis recognition comparing them to regular infection markers. Also, we aimed to find the possible role of CPD parameters when combined with the initial recommended screening tool, qSOFA.

Recently several articles were published about possible use of CPD parameters measured by Sysmex XN analyzers in sepsis management. In Park’s study NE-SFL and NE-WY (two CPD parameters represent neutrophil immaturity or activation) were found useful in detection of sepsis, but no strong correlation with severity was found [10]. In pediatric population, Biban and his team found significantly higher values of NE-SFL, MO-WX, and MO-Y CPD parameters among children with sepsis or septic shock on admission to intensive care unit [2]. Urrechaga and her team suggested the use of NEMO score as a risk stratification scale in sepsis using two CPD parameters: MO-X and NE-SFL [17]. Urrechaga in a following article found that among septic cases those CPD parameters were higher which represented the monocyte complexity and the neutrophil leucocyte activation (NE-SFL, NE-WY, MO-X, MO-WX and MO-Z) [18]. Buoro and her team reached similar conclusions that MO-X and NE-SFL showed the best performance in detecting sepsis, though almost all the parameters were significantly higher in patients admitted to ICU compared to healthy subjects [3].

Our results suggested that although almost all CPD parameters showed significant changes in the different groups, but no single parameter showed a convincing correlation with the final sepsis diagnosis (SOFA≥2). In comparison with the regular parameters no superior CPD value was identified.
From our initial results, where CPD parameters were compared in each study groups, three parameters (NE-SFL/M, MO-X/M and Ne-WY/M) showed significant results in distinguishing septic shock from sepsis, infection and no infection and we also achieved good results with ROC analysis comparing these parameters with the diagnosis of septic shock. Determining the cutoff values for these parameters and combining them with the qSOFA score we formed a six-point scale – qS-Ne2Mo – which can predict the presence of septic shock with a cutoff value of 4 points.

To complete qS-Ne2Mo score, simple vital signs and no additional blood samples are needed. With the fast turn-around-time of CPD, qS-Ne2Mo score can be completed within a short period of time starting from triage and can identify septic shock patients way before the completion of SOFA score or before the initial resuscitation attempts would have results.

Possible fields of application of qS-Ne2Mo score

Final septic shock diagnosis can be made only after the initial treatment attempt, and only the therapy refractory cases need vasopressors and have constantly high lactate levels. During the initial management of septic patients, it is unclear how vehement treatment approach is the right choice. In 2001, Rivers et al. found that early goal directed therapy reduced the mortality in severe sepsis and septic shock, and this type of treatment came with higher fluid doses more transfusions and with earlier dobutamine treatment. In the Rivers study the initial marker of severe sepsis and septic shock was at least two of possible four SIRS criteria with systolic blood pressure no higher than 90 mmHg after initial fluid challenge (20-30mL/bodyweight infusion under 30 minutes) or a serum lactate level 4mmol/L or more [13]. The current 2016 SSC guidelines do not recommend against the early goal-directed therapy (EGDT) but advise a more relaxed approach in fluid challenge 30mL/kg over three hours, and a sort of a wait-and-see approach is promoted to decide how the patient’s hemodynamic status changes during this 3-hour period. Although EGDT showed breakthrough results in reduction of severe sepsis and
septic shock related mortality compared to standard therapy, now this approach has been challenged by many recent randomized controlled trials, that failed to reproduce those excellent results in the Rivers study [1, 9, 12]. Maybe, this controversy lies in the fact that only the most severe cases can profit from EGDT (i.e. septic shock), but on arrival no certain marker distinguishes those patients and the initial indicators for early septic shock were insufficient. With qS-Ne2Mo score, septic shock can be identified early and reliably and maybe more aggressive treatment strategy like EGDT can be justified by positive qS-Ne2Mo score.

In the current updated Surviving Sepsis Campaign (SSC) guidelines a 1-hour time-window is recommended to complete lactate measurement, start fluid resuscitation, obtain blood cultures, and commence broad-spectrum antibiotics in suspected sepsis or septic shock cases [7]. This early antibiotic therapy is highly debated. Many articles question the advantages of a rushed antibiotic administration in all septic cases, but it is generally accepted that in septic shock one of the cornerstones of the successful therapy is the prompt initiation of antibiotics [6, 15]. With qS-Ne2Mo score septic shock can be identified very early. qS-Ne2Mo may be a good clinical tool for selecting patients who are eligible for immediate (within 1 hour) broad spectrum antibiotic treatment, while others can wait for more thorough investigations, and can receive a more targeted antibiotic treatment which might lower the current extreme rise of antibiotic resistant strains.

Finally, we must mention the possible economic role of using CPD parameters and the qS-Ne2MO score. CPD comes with an additional 50% price on top of a regular CBC measurement, still not reaching the price of a single CRP measurement not to mention PCT which costs approximately 20 times more than a CBC and CPD measurement together. Therefore qS-Ne2Mo score might be used as an initial screening tool to select only those patients that need more extensive laboratory investigations for their proper treatment and spare inadequate, time and money consuming laboratory requests. In places where CBC is used solely with CRP and
PCT is not available, CPD parameters and qS-Ne2Mo score could be a very useful tool to enhance performance of sepsis management.

**Limitations**

Our study has certain limitations. The sample size was relatively small to come to firm conclusions. Furthermore, we cannot make any comments on the efficacy and outcomes of a qS-Ne2Mo based approach in the management of this patient population.

**Conclusion**

In this retrospective observational study, we examined the possible role of CPD parameters in sepsis and septic shock diagnosis. According to our results combining the qSOFA score with three CPD parameters, a new risk stratification score was developed to identify septic shock early. qS-Ne2MO score showed promising prognostic value for the final diagnosis of septic shock using the current Sepsis-3 criteria. For the suggested applications of qS-Ne2Mo score, further studies are required to provide the sufficient evidence for safe clinical use.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the University of Szeged Ethical Committee (ref nr. 25/2016-SZTE).

**Consent for publication**

Not applicable.

**Availability of data and materials**

Research data is available upon reasonable request to the corresponding author.

**Competing interests**
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Authors’ contributions**

PE: participated in design of the study and in patient selection, drafted the manuscript

LP: created data-analysis framework, provided the data analysis, wrote the original and revised draft

IF: created the original idea and conceptualization, provided laboratory data, wrote original and revised draft

KF: carried out hematology analysis, provided laboratory data and consultancy

ZM: supervised the manuscript, participated in data analysis

ZP: created the original idea and conceptualization, supervised the draft writing process

All authors read and approved the final manuscript

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**List of abbreviations**

ABG: additional painful blood works
AUC: area under the curve
CBC: complete blood count
CPD: cell population data
CRP: c-reactive protein
EGDT: early goal-directed therapy
GCS: Glasgow coma scale
MAP: mean arterial pressure
PCT: procalcitonin
qSOFA: quick Sequential Organ Failure Assessment
qS-Ne2Mo: quick SOFA combined with NE-SFL/M, MO-X/M and NE-WY/M
ROC analysis: receiver operating curve analysis
SIRS: systemic inflammatory response syndrome
SOFA: Sequential Organ Failure Assessment
SSC: surviving sepsis campaign
WBC: white blood cell

References

1. ARISE Investigators, ANZICS Clinical Trials Group, Peake, S. L., Delaney, A., Bailey, M., Bellomo, R., Cameron, P. A., Cooper, D. J., Higgins, A. M., Holdgate, A., Howe, B. D., Webb, S. A., & Williams, P. Goal-directed resuscitation for patients with early septic shock. N Engl J Med 2014; 371:1496-1506. DOI: 10.1056/NEJMoal404380
2. Biban P, Teggi M, Gaffuri M, Santuz P, Onorato D, Carpenè G, Gregori D and Lippi G. Cell Population Data (CPD) for Early Recognition of Sepsis and Septic Shock in Children: A Pilot Study. Front. Pediatr. 2021; 9:642377. doi: 10.3389/fped.2021.642377
3. Buoro S, Seghezzi M, Vavassori M, Dominoni P, Apassiti Esposito S, Manenti B, Mecca T, Marchesi G, Castellucci E, Azzarà G, Ottomano C, Lippi G. Clinical significance of cell population data (CPD) on Sysmex XN-9000 in septic patients with or without liver impairment. Ann Transl Med 2016; 4(21):418. doi: 10.21037/atm.2016.10.73

4. Centers for Disease Control and Prevention. Sepsis. Clinical Information. (online) Page last reviewed. December 7, 2020. Accessed April 14, 2021.

5. Karon BS, Tolan NV, Wockenfus AM, Block, DR, Baumann NA, Bryant SC, Clements CM. Evaluation of lactate, white blood cell count, neutrophil count, procalcitonin and immature granulocyte count as biomarkers for sepsis in emergency department patients. Clin Biochem. 2017; 50(16-17):956-958. doi:10.1016/j.clinbiochem.2017.05.014

6. Kumar, A., Roberts, D., Wood, K. E., Light, B., Parrillo, J. E., Sharma, S., Suppes, R., Feinstein, D., Zanotti, S., Taiberg, L., Gurka, D., Kumar, A., & Cheang, M. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Critical care medicine, 2006; 34(6):1589–1596. https://doi.org/10.1097/01.CCM.0000217961.75225.E9

7. Levy, M. M., Evans, L. E., & Rhodes, A. The surviving sepsis campaign bundle: 2018 update. Intensive Care Med 2018 Jun;44(6):925-928. doi: 10.1007/s00134-018-5085-0.

8. Maitra S, Som A, Bhattacharjee S. Accuracy of quick Sequential Organ Failure Assessment (qSOFA) score and systemic inflammatory response syndrome (SIRS) criteria for predicting mortality in hospitalized patients with suspected infection: a meta-analysis of observational studies. Clin Microbiol Infect. 2018; 24(11):1123-1129. doi:10.1016/j.cmi.2018.03.032

9. Mouncey, P. R., Osborn, T. M., Power, G. S., Harrison, D. A., Sadique, M. Z., Grieve, R. D., Jahan, R., Harvey, S. E., Bell, D., Bion, J. F., Coats, T. J., Singer, M., Young, J. D., Rowan, K. M., & ProMISe Trial Investigators. Trial of early, goal-directed resuscitation for septic shock. N Engl J Med 2015; 372:1301-1311 DOI: 10.1056/NEJMoal500896

10. Park, S.H., Park, C.-J., Lee, B.-R., Nam, K.-S., Kim, M.-J., Han, M.-Y., Kim, Y.J., Cho, Y.-U. and Jang, S. Sepsis affects most routine and cell population data (CPD) obtained using the Sysmex XN-2000 blood cell analyzer: neutrophil-related CPD NE-SFL and NE-WY provide useful information for detecting sepsis. Int. Jnl. Lab. Hem., 2015; 37: 190-198. https://doi.org/10.1111/ijlh.12261
11. Perman, S.M., Mikkelsen, M.E., Goyal, M., Ginde, A., Bhardwaj, A., Drumheller, B., Cham Sante, S., Agarwal A.K., Gaieski, D.F. The sensitivity of qSOFA calculated at triage and during emergency department treatment to rapidly identify sepsis patients. Sci Rep 2020; 10:20395 https://doi.org/10.1038/s41598-020-77438-8

12. ProCESS Investigators, Yealy, D. M., Kellum, J. A., Huang, D. T., Barnato, A. E., Weissfeld, L. A., Pike, F., Terndrup, T., Wang, H. E., Hou, P. C., LoVecchio, F., Filbin, M. R., Shapiro, N. I., & Angus, D. C. A randomized trial of protocol-based care for early septic shock. N Engl J Med 2014; 370:1683-1693 DOI: 10.1056/NEJMoa1401602

13. Rivers, E., Nguyen, B., Havstad, S., Ressler, J., Muzzin, A., Knoblich, B., Peterson, E., Tomlanovich, M., & Early Goal-Directed Therapy Collaborative Group. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med 2001; 345:1368-1377 DOI: 10.1056/NEJMoa010307

14. Seymour CW, Liu VX, Iwashyna TJ, Brunckhorst FM, Rea TD Scherag A, Rubenfeld G, Kahn JM, Shankar-Hari M, Singer M, Deutschman CS, Escobar GJ, Angus DC. Assessment of Clinical Criteria for Sepsis For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):762-774. doi:10.1001/jama.2016.0288.

15. Sherwin, R., Winters, M. E., Vilke, G. M., & Wardi, G. Does Early and Appropriate Antibiotic Administration Improve Mortality in Emergency Department Patients with Severe Sepsis or Septic Shock? The Journal of Emergency Medicine, 2017; 53(4), 588–595. https://doi.org/10.1016/j.jemermed.2016.12.009

16. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016; 315(8):801–810. doi:10.1001/jama.2016.0287

17. Urrechaga, E., Bóveda, O., & Aguirre, U. Role of leucocytes cell population data in the early detection of sepsis. Journal of Clinical Pathology, 2018; 71(3), 259–266. https://doi.org/10.1136/jclinpath-2017-204524

18. Urrechaga E, Bóveda O, Aguirre U. Improvement in detecting sepsis using leukocyte cell population data (CPD). Clin Chem Lab Med. 2019; 57(6):918-926. doi:10.1515/cclm-2018-0979. PMID: 30838839.