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

Peter Erdelyi
University of Szeged Albert Szent-Gyorgyi Health Centre: Szegedi Tudomanyegyetem Szent-Gyorgyi Albert Klinikai Kozpont

Laszlo Papp (papp.laszlo@med.u-szeged.hu)
University of Szeged https://orcid.org/0000-0001-7038-5215

Imre Foldesi
University of Szeged Albert Szent-Gyorgyi Health Centre: Szegedi Tudomanyegyetem Szent-Gyorgyi Albert Klinikai Kozpont

Katalin Farkas
University of Szeged Albert Szent-Gyorgyi Health Centre: Szegedi Tudomanyegyetem Szent-Gyorgyi Albert Klinikai Kozpont

Zsolt Molnar
University of Pécs Clinical Center: Pecsi Tudomanyegyetem Klinikai Kozpont

Zoltan Peto
University of Szeged Albert Szent-Gyorgyi Health Centre: Szegedi Tudomanyegyetem Szent-Gyorgyi Albert Klinikai Kozpont

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Title Page

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Peter Erdelyi, MD (first author), University of Szeged, Department of Emergency, 6725, Szeged, Semmelweis str. 6, Hungary mail: erdelyi.peter@med.u-szeged.hu

Laszlo Papp, PhD, University of Szeged, Department of Emergency, 6725, Szeged, Semmelweis str. 6, Hungary mail: papp.laszlo@med.u-szeged.hu

Imre Foldesi, PharmD, PhD, University of Szeged, Institute of Laboratory Medicine, 6725, Szeged, Semmelweis str. 6, Hungary mail: foldesi.imre@med.u-szeged.hu

Katalin Farkas, University of Szeged, Institute of Laboratory Medicine, 6725, Szeged, Semmelweis str. 6, Hungary mail: lokine.farkas.katalin@med.u-szeged.hu

Zsolt Molnar, MD, PhD, DEEA, University of Pécs, Institute for Translational Medicine, 7624 Pécs, Szigeti str. 12. mail: zsoltmolna@gmail.com

Zoltan Peto, MD, PhD, University of Szeged, Department of Emergency, 6725, Szeged, Semmelweis str. 6, Hungary mail: peto.zoltan@med.u-szeged.hu

correspondence to: Laszlo Papp, PhD, papp.laszlo@med.u-szeged.hu, +36/30/433-0302

University of Szeged, Department of Emergency, 6725, Szeged, Semmelweis str. 6, Hungary

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 score (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].

Determination of White blood cell count, Immature granulocyte ratio and Cell population data

The blood samples were collected in 3 ml Vacutainer Plastic K_3EDTA 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

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

155 *Table 1.* Within-run imprecision of cell counts and cell population data (CPD). The mean
156 values and %CVs were obtained from 20 replicates of three patients at different concentrations
157 of white blood cells (WBC). NEU: Neutrophil granulocytes; LY: Lymphocytes; MO:
158 Monocytes

159 *Determination of C-Reactive Protein and Procalcitonin*

160 The blood samples were collected in 5 ml vacutainer tubes (Plastic STT II Advance tubes;
161 Ref.#367955, Becton-Dickinson, Franklin Lakes, NJ, USA) for measurement of CRP and PCT.
162 The specimens were allowed to clot for 30 minutes and centrifuged for 10 minutes at 2000xg
163 and the serum was subjected for analysis. The assays were performed within 2 hours of
164 specimen collection.
165 CRP was measured by immunturbidimetric method (3. generation C-reactive protein kit,
166 Roche, Mannheim, Germany) on Roche Cobas c501 analyzer (Roche, Mannheim, Germany).
167 The detection limit was <0.3 mg/L and the measuring range was 0.3–350 mg/L. The interassay
168 CVs at 2.36 and 157 mg/L concentrations were 1.3% and 11%, respectively.
169 PCT was measured by electrochemiluminescence immunoassay method (Elecsys BRAHMS
170 PCT kit, Roche, Mannheim, Germany) on an automated Cobas e601 analyzer (Roche,
171 Mannheim, Germany). The detection limit was ≤0.02 µg/L and the measuring range was 0.02-
172 100 µg/L. The interassay CVs at 0.431 µg/L and 54.4 µg/L were 2.6% and 1.6%, respectively.

173 *Statistical analysis*

174 Distribution of data was analyzed with standard Kolmogorov-Smirnov (K-S) test. Interestingly,
175 blood pressure showed normal distribution, while all other descriptive parameters were not
176 normally distributed. Descriptive statistics were made with means and standard deviations or
177 medians and interquartile ranges (25 and 75 %), respectively. Analysis of differences between
178 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%)*

central nervous system	*n/a*	*1 (2.7%)*	*1 (0.8%)*	*0 (0%)*
urinary tract	*n/a*	*8 (21.6%)*	*30 (23.3%)*	*10 (41.7%)*
bone	*n/a*	*0 (0%)*	*2 (1.6%)*	*0 (0%)*
unknown	*n/a*	*2 (5.4%)*	*8 (6.2%)*	*3 (12.5%)*

192 *Data are summarized as median (with quartiles 25%-75%) or frequency (percentage) as appropriate*

193 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

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

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

197 In 66 cases, microbiology results were not available at the time of the data collection, and the
198 confirmation of the initial diagnosis was based on imaging results. These cases were excluded
199 from the final sample, and data of the remaining 210 patients were analyzed separately (Table
200 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			
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' 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

222 (10.55 vs 81.35-131.3, p<0.001). These parameters showed no statistical difference between
223 the other three groups of infection, sepsis and septic shock. Procalcitonin (PCT) showed
224 significant difference between almost all groups, respectively (0.068 – 4.73, p=0.000-0.024);
225 no significant difference could be measured between infection and sepsis group (0.16 vs 0.756,
226 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

227 Table 6. Standard biomarkers in Sepsis-3 cohorts

228 *Performance of CPD parameters*

229 As a second step cell population data were analyzed. Ne-SSC, NeFSC/M, MoZ/M and Mo-
230 WZ/M showed normal distribution, all the other parameters were not normally distributed.
231 According to the descriptive analysis multiple parameters showed significant changes. The
232 highlighted NE-SFL/M, MO-X/M and NE-WY/M parameters showed significant changes
233 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	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

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

Table 7. Performance of CPD parameters in Sepsis-3 cohorts

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).

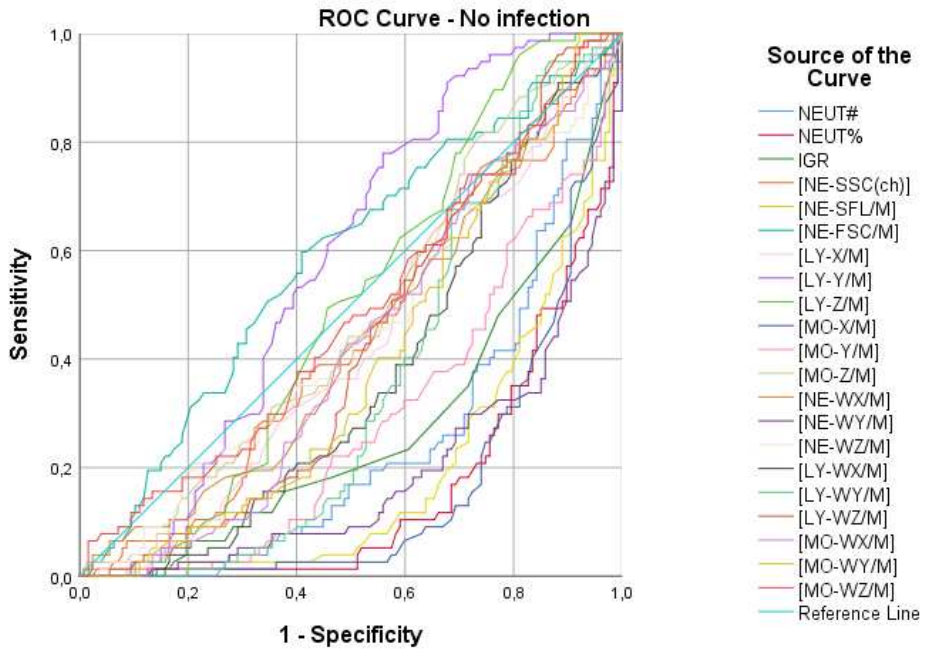
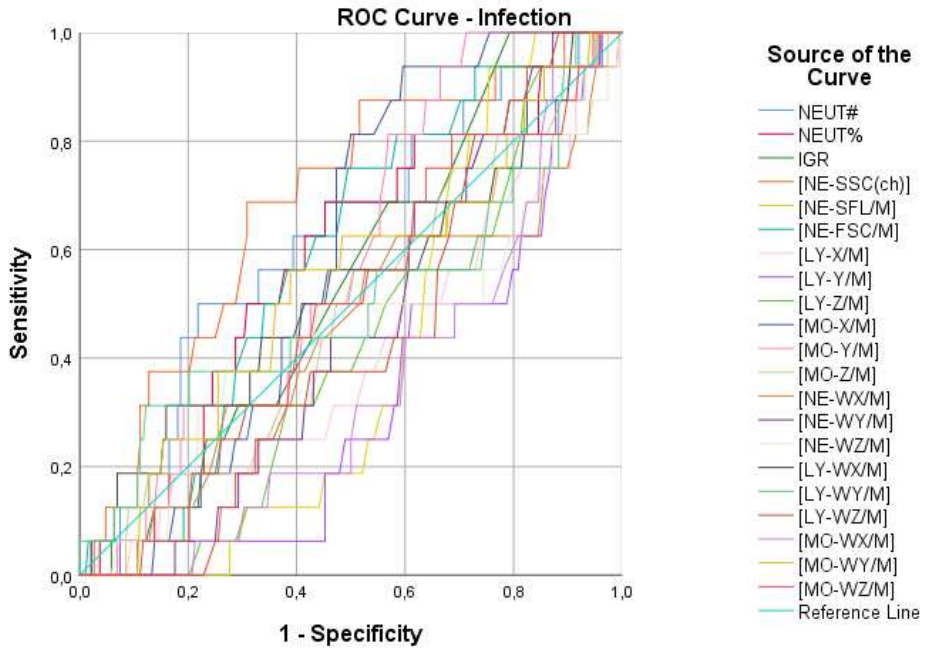


Figure 1. ROC analysis of no infection group



243

244 Figure 2. ROC analysis of infection group

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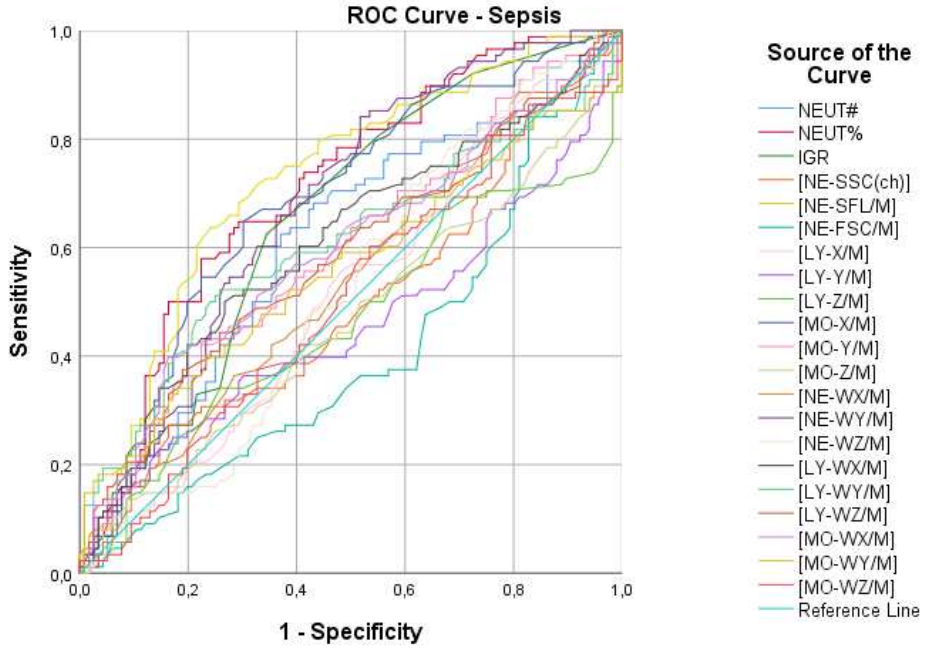

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247 Figure 3. ROC analysis of sepsis group

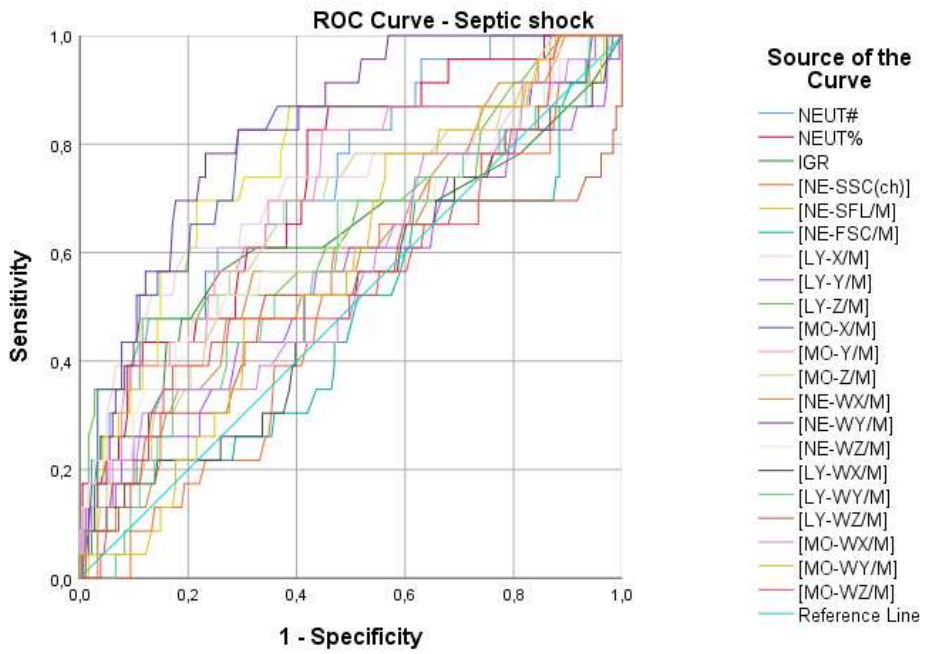

Figure 4. ROC analysis of septic shock group

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, Ne_2Mo. 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. Ne_2Mo suggested cutoff performances

Using the above cutoff values, ROC analysis was carried out using positive Ne_2Mo 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_2Mo 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-Ne2Mo) was formed by forging qSOFA and Ne_2Mo. qS-Ne2Mo is a 6-point scale, scoring 1 point each for the positive qSOFA items or Ne_2Mo 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 %).

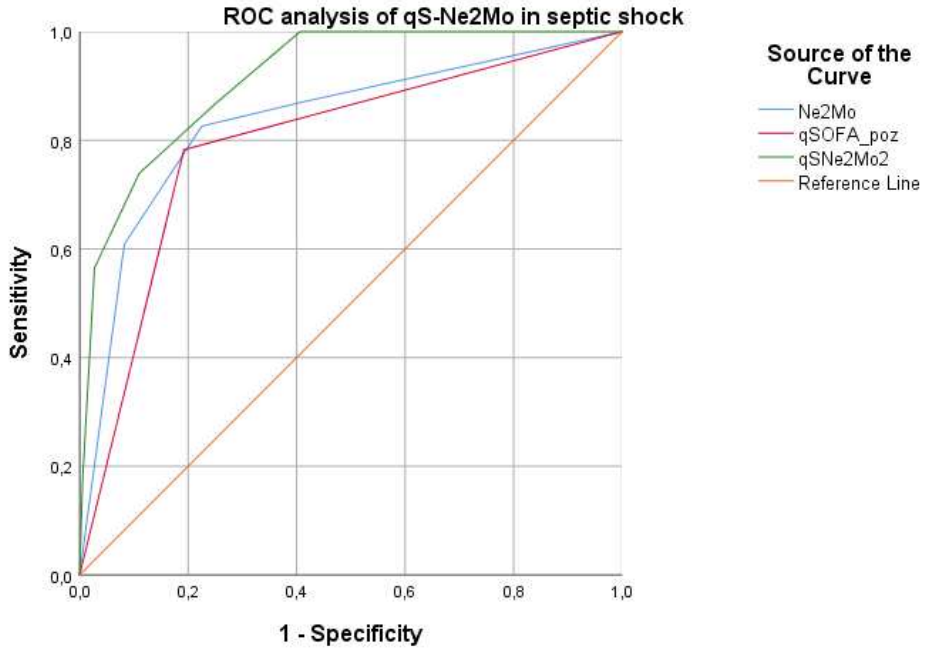

Figure 5. ROC analysis of qS-Ne_2Mo score in septic shock

After setting the cutoff to 4 points in qSNe2Mo, 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: procaltitonin

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

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