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Original article

Neutrophil count, lymphocyte count and neutrophil-to-lymphocyte ratio in relation to response to adjunctive dexamethasone treatment in community-acquired pneumonia

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ABSTRACT

Background: It is hypothesised that community-acquired pneumonia (CAP) patients with more severe disease or inflammation might benefit more from adjunctive corticosteroid treatment. Neutrophil count, lymphocyte count and neutrophil-lymphocyte ratio (NLR) have been associated with inflammation and disease severity in CAP. We investigated the interaction between these parameters and adjunctive dexamethasone effects on clinical outcomes in CAP.
Methods: We conducted a post hoc analysis of the randomised placebo-controlled Santeon-CAP trial ($n = 401$), which showed a positive effect of adjunctive oral dexamethasone on length of stay (LOS) in CAP patients. White blood cell (WBC) count, neutrophil count, NLR (highest tertile vs. lowest two tertiles) and lymphocyte count (lowest tertile vs. highest two tertiles) were examined as potential effect modifiers of treatment with dexamethasone on LOS (primary outcome) and ICU-admission, 30-day mortality and hospital readmission.
Results: WBC differential counts were available for 354 patients. The effect of dexamethasone on LOS was more pronounced in high WBC count, high neutrophil count or high NLR subgroups (difference in median LOS of 2 days versus zero days in the reference subgroups, p for interaction < 0.05). There was no effect modification for the other outcomes. Patients with low WBC and low neutrophil counts did not benefit from dexamethasone, while hospital readmission rate was higher in those treated with dexamethasone (6% vs. 11%).
Conclusions: WBC count and/or neutrophil might be easily available biomarkers to guide selection of CAP patients who are more likely to benefit from adjunctive dexamethasone treatment. Future prospective trials are needed to confirm this predictive potential.

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1. Introduction

The cornerstones of community-acquired pneumonia (CAP) treatment are early diagnosis and timely initiation of appropriate antibiotic treatment [1]. Despite advances in antibiotic treatment, CAP remains a leading cause of morbidity and mortality worldwide [2]. Adjunctive therapies, such as corticosteroids, might improve clinical outcomes.

In CAP, invading pathogens trigger a host immune response essential for controlling and eliminating pathogens in the lung. However, dysregulation of the initial inflammatory response can lead to tissue damage and excessive systemic inflammation resulting in severe disease and ultimately unfavourable clinical outcomes [3].

Adjunctive treatment with corticosteroids, potent inhibitors of the host immune response, has shown to improve outcomes for CAP patients by reducing length of hospital stay (LOS) and time to clinical stability [4]. In addition, we recently showed that adjunctive corticosteroids reduced ICU-admission rate [5]. However, because CAP is a heterogeneous disease, it is unlikely that all patients benefit equally from adjunctive corticosteroid treatment [6]. Furthermore, a higher risk of hospital readmission in patients treated with adjunctive corticosteroids remains a concern [4,5]. Therefore, identifying a subset of patients who are more likely to benefit from corticosteroid treatment is necessary.

It is hypothesised that patients with an excessive inflammatory response and thus more severe disease would benefit most from adjunctive corticosteroid treatment. However, stratification of CAP patients by parameters indicative of more inflammation or more severe disease, such as C-reactive protein levels, pneumonia severity index (PSI) score, inflammatory status based on cytokine levels, initial ICU-admission or the presence of systemic inflammatory response criteria, have not yielded a clear-cut definition of a CAP subgroup benefiting from corticosteroid treatment [4,5,7,8].

White blood cells (WBCs) populations play a key role in the local and systemic inflammatory response in CAP [3]. Neutrophilia is a widely used and recognised infection marker in CAP and more recently, lymphocytopenia has been associated with more severe disease and higher levels of systemic inflammatory cytokines in CAP [9]. In acute respiratory distress syndrome, lymphocyte depletion correlated with severity of lung injury [10]. A combination of both, the neutrophil-lymphocyte count ratio (NLR), has been recognized as an indicator of systemic inflammation and predictor of clinical outcomes in sepsis, cardiovascular and oncological disease [11–13]. In CAP, NLR has also shown to be associated with more severe disease and has been identified as a predictor of mortality [14,15]. So far, no studies have investigated the interaction between WBC differential cell counts and adjunctive corticosteroid treatment on clinical outcomes in patients with CAP.

We performed a post hoc analysis of a randomised trial investigating adjunctive oral dexamethasone in non-ICU patients with CAP to assess if neutrophil count, lymphocyte count and/or NLR modified the response to adjunctive oral dexamethasone treatment in adults hospitalised with CAP.

2. Methods

2.1. Population and study design

We performed a post hoc analysis of the multicentre Santeon-CAP study (n = 401; NCT01743755) [5]. In short, the Santeon-CAP study investigated the effect of adjunctive treatment with 6 mg oral dexamethasone for four days vs. placebo on the primary outcome LOS in non-ICU hospitalised CAP patients. Randomisation was stratified by disease severity defined by PSI risk class (PSI risk class I-III vs. PSI risk class IV-V) [16]. In the Santeon-CAP study, dexamethasone reduced LOS by 0.5 days and decreased the risk of secondary ICU-admission. CAP was defined as a new opacity on chest x-ray combined with at least two of the following signs and symptoms: cough, sputum production, body temperature $>$ 38.0 °C or $<$ 36.0 °C, findings at chest auscultation consistent with pneumonia, C-reactive protein concentration (CRP) $>$ 15 mg/l, and/or white blood cell count $> 10 \times 10^9$ cells per litre or $< 4 \times 10^9$ cells per litre. Immunocompromised patients, patients for whom corticosteroid treatment was indicated or patients who used corticosteroids prior to admission were excluded. Further information on inclusion criteria and study procedures is reported elsewhere [5]. For this post hoc analysis, we included those patients for whom a full WBC differential was available at emergency department presentation.

2.2. Data collection

We retrospectively searched the medical records of all patients enrolled in the Santeon-CAP study for the availability of a WBC differential (not part of the original study protocol) at time of presentation to the emergency department. We collected WBC counts, neutrophil counts and lymphocyte counts. NLR was calculated by dividing neutrophil count by lymphocyte count. Baseline patient characteristics, baseline laboratory test results and clinical outcomes were available as part of the original study protocol.

2.3. Definition of subgroups and outcomes

Patients were stratified based on WBC count, neutrophil count, lymphocyte count and NLR values. For each parameter, patients were divided in a “high” group and a “low” group. Because there are no earlier studies assessing the relationship between WBC count, neutrophil count, lymphocyte count, and NLR and the effect of adjunctive corticosteroid treatment on clinical outcomes in CAP, there was no clear guidance for choosing cut-off values for stratification into subgroups. Based on the hypothesis that patients with more extreme values would benefit most from corticosteroid treatment, we stratified patients into high or low groups according to tertiles. Thereby selecting a reference group with more extreme values while minimising the risk of too small numbers in subgroups, as might be the case when using quartiles. Based on the hypothesis that patients with the highest WBC count, neutrophil count, and NLR would have more severe disease and thus would benefit most from dexamethasone, the high subgroup for these parameters was defined as a count or ratio equal to or higher than the third tertile value. The low subgroup was defined as a count or ratio below the third tertile value. For lymphocyte count, we hypothesised that patients with the lowest lymphocyte count would have more severe disease. Therefore, the low lymphocyte subgroup was defined as a lymphocyte count below the first tertile value and the high lymphocyte count subgroup was defined as a lymphocyte count equal to or higher than the first tertile value.

The primary outcome was LOS. LOS was measured in days and was calculated from day of hospital admission to day of hospital discharge or day of in-hospital death. Rules for discharge were that patients needed to be clinically stable (improvement of shortness of breath, absence of hyperthermia or hypothermia, consistent decrease of C-reactive protein concentrations and adequate oral intake and gastrointestinal absorption) and be in a condition to leave the hospital. Secondary outcomes were ICU-admission after initial admission to the general ward, all-cause 30-day mortality, and hospital readmission within 30 days of initial hospital admission.

2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS 26.0. After stratifying patients into subgroups, differences in baseline characteristics between the high and low subgroups of each parameter were analysed using the Chi-squared test for categorical variables, and a Student’s T-test or Mann-Whitney U-test for continuous variables. Multivariate binary logistic regression analysis was performed to further analyse the association between baseline characteristics and WBC count parameter subgroups. The multivariate model was adjusted for baseline

characteristics with a statistically significant difference between high and low subgroups upon univariate analysis. Next, time to discharge was plotted in a Kaplan-Meier curve for the placebo and dexamethasone group in each WBC differential subgroup. Finally a Poisson regression model, including treatment allocation, WBC differential parameter subgroup and their interaction as covariates, was used to test for interaction between randomly assigned treatment with dexamethasone and WBC differential parameters on LOS. For secondary categorical outcomes, a binary logistic regression analysis was used. Because LOS is cut short for patients who died in hospital, these patients might incorrectly count as having a shorter LOS. Therefore, a sensitivity analysis was performed for LOS excluding patients who died in hospital.

Unless noted otherwise, data are presented as mean (SD, standard deviation) or median [IQR, interquartile range] for continuous variables, and as count (%) for categorical variables.

3. Results

3.1. Population characteristics

A full blood count differentiation at time of hospital admission was available for 354 out of 401 Santeon-CAP study participants. Patient characteristics are shown in Table 1. There were no differences in baseline characteristics between the placebo group ($n = 169$) and the dexamethasone group ($n = 185$). Clinical outcomes (Table 1) showed a trend towards similar results as observed for the total Santeon-CAP study population with a statistically significant difference in LOS and a trend towards a reduction in secondary ICU-admissions.

3.2. Subgroups based on differential blood count values

For WBC count, neutrophil count, and NLR the high subgroups were defined as a count or ratio $\geq 15.6\ 10^9$ cells/l, $\geq 13.2\ 10^9$ cells/l, and ≥ 15.5, respectively. For lymphocyte count, the cut-off value for the low subgroup was $\leq 0.71\ 10^9$ cells/l. Patient characteristics at baseline for each subgroup are shown in Table 2. Multivariate analysis showed that COPD (OR 1.91 (95% CI (1.07–3.39)), heart rate (OR 1.02 (95% CI 1.01–1.03)), and no antibiotic treatment prior to admission (OR 1.99 (95% CI 1.12–3.53)) were associated with a neutrophil count $\geq 13.2\ 10^9$ cells/l. Similar results were found for WBC count, where COPD (OR 2.15 (95%CI 1.20–3.85)), heart rate (OR 1.02 (95% CI 1.00–1.03)), no antibiotic treatment prior to admission (OR 1.83 (95% CI 1.03–3.24)) and female gender (OR 1.73 (95% CI 1.07–2.80)) were associated a WBC count $\geq 15.6\ 10^9$ cells/l. The high NLR subgroup had a higher mean PSI score and more signs of systemic inflammation compared to the low NLR subgroup (Table 2). On multivariate analysis a NLR ≥ 15.5 was associated with higher body temperature at presentation (OR 1.40 (95%CI 1.11–1.76)), infection with *S. pneumoniae* (OR 2.17 (95%CI 1.19–3.98)), COPD (OR 1.86 95%CI (1.02–3.40)) and no antibiotic treatment prior to admission (OR 2.10 (95% CI 1.12 - 3.75)). The low lymphocyte subgroup also had a higher mean PSI score than the high lymphocyte subgroup. On multivariate analysis a low lymphocyte count $< 0.71\ 10^9$ cells/l was only associated with higher body temperature at presentation (OR 1.42 (95% CI 1.14–1.77)).

Except for a lower ICU-admission rate in the low lymphocyte count subgroup compared to the high lymphocyte subgroup (10 (9%) vs. 6 (3%); $p = 0.010$), there was no statistically significant differences in clinical outcomes between WBC differential parameter subgroups for the whole study population (Table 2). Selecting only patients who received placebo, thus excluding any effect of dexamethasone on clinical outcomes, we found that NLR ≥ 15.5 was associated with a significantly longer median LOS compared to NLR ≥ 15.5 (5.0 [4.0–7.0] vs 6.0 [4.0–8.0]; $p = 0.023$). Similar to the analysis in the whole cohort, ICU-admission rate was higher in the low lymphocyte count subgroup compared to the high lymphocyte count subgroup (7 (13%) vs 4 (4%); $p = 0.026$).

Table 1
Baseline characteristics and clinical outcomes for the whole study population.

	All patients $N = 354$	Placebo $N = 169$	Dexamethasone $N = 185$	P*
Male	209 (59)	101 (60)	108 (58)	0.79
Age (years)	64.7 (15.9)	63.7 (16)	65.6 (15)	0.25
PSI score	80.8 (28.1)	80.5 (28.5)	81.1 (27.8)	0.86
CURB65 score	1 [0–2]	1 [0–2]	1 [0–2]	0.91
Antibiotic treatment prior to hospital admission	101 (29)	51 (30)	50 (27)	0.49
Altered mental status	20 (6)	10 (6)	10 (5)	0.84
Current smoker	87 (25)	39 (24)	48 (27)	0.44
Comorbidities				
COPD	67 (19)	31 (18)	36 (20)	0.79
Diabetes	74 (21)	37 (22)	37 (20)	0.66
Congestive heart failure	31 (9)	12 (7)	19 (10)	0.29
Liver disease	4 (1)	2 (1)	2 (1)	0.93
Neoplastic disease	14 (4)	6 (4)	8 (4)	0.71
Renal disease	51 (14)	21 (12)	30 (16)	0.31
Heart rate (bpm)	99.5 (20.2)	98.0 (18.8)	100.0 (21.4)	0.60
Body temperature (°C)	38.3 (1.1)	38.3 (1.2)	38.4 (1.1)	0.37
Respiratory rate (breaths/min)	21.7 (6.0)	21.9 (6.0)	21.4 (6.0)	0.49
Oxygen saturation (%)	93.7 (4.1)	93.7 (4.1)	93.6 (4.1)	0.83
c-reactive protein (mg/l)	210 [84–319]	201 [80–309]	215 [91–330]	0.22
leukocyte count(10^9 cells/l)	13.0 [9.7–17.8]	12.5 [9.4–17.4]	13.7 [10.2–18.2]	0.21
Neutrophil count (10^9 cells/l)	10.7 [7.8–15.1]	10.4 [7.5–14.9]	11.0 [8.0–15.3]	0.30
Lymphocyte count (10^9 cells/l)	0.95 [0.63–1.4]	0.99 [0.63–1.4]	0.94 [0.36–1.3]	0.68
Identified microorganism				
Legionella spp.	24 (7)	13 (8)	11 (6)	0.51
Influenza virus A/B	23 (7)	11 (7)	12 (7)	0.99
Streptococcus pneumoniae	64 (18)	28 (17)	36 (20)	0.48
Clinical outcomes				
LOS (days)	5.0 [4.0–7.0]	5.0 [4.0–8.0]	5.0 [3.0–7.0]	0.029
ICU-admission	16 (5)	11 (7)	5 (3)	0.085
30-day mortality	11 (3)	7 (4)	4 (2)	0.28
Readmission < 30 days	28 (8)	9 (6)	19 (10)	0.10

Data are presented as mean (SD), median [IQR], or *n* (%). *P*-value for Students-*T* test, Whitney-Mann *U* or Chi-squared test as appropriate. **P* for difference between placebo and dexamethasone group.

3.3. Effect modification by subgroup

Although scatterplots show a large spread in WBC count differential parameter values, we observed more placebo patients compared to dexamethasone patients in the upper right quadrant (LOS longer than 3rd quartile and high count/ratio) for WBC count ($n = 13$ vs $n = 10$), Neutrophil count ($n = 13$ vs $n = 7$) and NLR ($n = 20$ vs $n = 9$), and in the lower right quadrant (LOS longer than 3rd quartile and lowest count) for lymphocyte count ($n = 21$ vs $n = 11$) (Supplementary Fig. 1). Kaplan Meier curves of time to discharge per subgroup showed shorter time to discharge for patients receiving dexamethasone compared to placebo in the high WBC count, neutrophil count and NLR subgroup and the low lymphocyte subgroup. This was not seen in the other subgroups (Fig. 1).

There was a statistically significant interaction between treatment allocation and WBC count, neutrophil count and NLR subgroups on LOS (Table 3). In the high subgroups of these parameters, median LOS was 2 days shorter in patients who received dexamethasone compared to those who received a placebo. In the low subgroups of these parameters, there was no difference in LOS between the placebo and dexamethasone

Table 2
Comparison of baseline characteristics and clinical outcomes between high and low subgroups for each WBC differential parameter.

	White blood cell count			Neutrophil count			Lymphocyte count			Neutrophil/lymphocyte ratio		
	Low (n = 235)	High (n = 119)	P	Low (n = 236)	High (n = 118)	P	Low (n = 117)	High (n = 237)	P	Low (n = 235)	High (n = 119)	P
Baseline characteristics												
Male	150 (64)	60 (50)	.010	147 (62)	62 (53)	.079	79 (68)	130 (55)	.023	134 (57)	75 (63)	.28
Age (years)	64 (16)	66 (15)	.17	64 (16)	67 (15)	.13	68 (15)	63 (16)	.002	63 (16)	68 (14)	.014
PSI score	81 (28)	81 (27)	.90	80 (28)	82 (27)	.56	89 (26)	77 (28)	< 0.001	76 (27)	90 (27)	< 0.001
CURB65 score	1.0 [0–2]	2.0 [1–2]	.12	1.0 [0–2]	2.0 [1–2]	.039	2.0 [1–2]	1.0 [0–2]	< 0.001	1.0 [0–2]	2.0 [1–2]	< 0.001
Antibiotic treatment prior to hospital admission	80 (34)	21 (18)	.001	81 (35)	20 (17)	.001	25 (21)	76 (32)	.034	82 (35)	19 (16)	< 0.001
Altered mental status	13 (6)	7 (6)	.89	12 (5)	8 (7)	.52	8 (7)	12 (5)	.50	8 (3)	12 (10)	.010
Current smoker	48 (21)	39 (34)	.010	50 (22)	37 (33)	.033	26 (23)	61 (27)	.44	52 (23)	35 (30)	.13
COPD	34 (15)	33 (28)	.003	34 (14)	33 (28)	.002	19 (16)	48 (20)	.36	36 (15)	31 (26)	.015
Diabetes	48 (20)	26 (22)	.76	47 (20)	27 (23)	.52	29 (25)	45 (19)	.21	49 (21)	25 (21)	.97
Congestive heart failure	23 (10)	8 (7)	.34	25 (11)	7 (6)	.084	15 (13)	16 (7)	.057	22 (9)	9 (8)	.57
Liver disease	4 (2)	0 (0)	.15	4 (2)	0 (0)	.16	2 (2)	2 (1)	.47	2 (1)	2 (2)	.49
Neoplastic disease	9 (4)	5 (4)	.87	9 (4)	5 (4)	.85	7 (6)	7 (3)	.17	7 (3)	7 (6)	.19
Renal disease	36 (15)	15 (13)	.49	35 (15)	16 (14)	.75	21 (18)	30 (13)	.18	35 (15)	16 (13)	.71
Heart rate (bpm)	97 (18)	104 (23)	.002	97 (18)	104 (23)	.001	100 (18)	99 (21)	.94	98 (20)	102 (21)	.08
Body temperature (°C)	38.4 (1.2)	38.3 (1.1)	.78	38.3 (1.2)	38.4 (1.1)	.47	38.6 (1.1)	38.2 (1.2)	.001	38.2 (1.1)	38.6 (1.1)	.001
Respiratory rate (Breaths/min)	22 (6)	21 (6)	.61	22 (6)	22 (6)	.92	22 (6)	21 (6)	.14	21 (6)	23 (6)	.010
Oxygen saturation (%)	94 (4)	94 (4)	.33	94 (4)	94 (4)	.47	93 (4)	94 (4)	.016	94 (4)	93 (4)	.023
C-reactive protein (mg/L)	206 [82–313]	234 [88–329]	.26	198 [82–311]	238 [95–335]	.13	210 [63–331]	210 [96–310]	.98	193 [88–300]	253 [81–345]	.051
Pathogens												
Legionella spp.	18 (8)	6 (5)	.36	18 (8)	6 (5)	.37	9 (8)	15 (6)	.63	13 (6)	11 (9)	.19
Influenza virus A/B	19 (8)	4 (3)	.088	18 (8)	5 (4)	.22	11 (9)	12 (5)	.12	17 (7)	6 (5)	.43
S. pneumoniae	34 (15)	30 (25)	.013	36 (15)	28 (24)	.051	29 (25)	35 (15)	.021	32 (14)	32 (27)	.002
Length of stay (days)	5.0 [4.0–7.0]	5.0 [4.0–7.0]	.90	5.0 [4.0–7.8]	5.0 [4.0–7.0]	0.59	5.0 [3.5–8.0]	5.0 [4.0–7.0]	0.61	5.0 [3.0–7.0]	5.0 [4.0–7.0]	0.11
ICU-admission	12 (5)	4 (3)	.46	12 (5)	4 (3)	0.47	10 (9)	6 (3)	0.010	9 (4)	7 (6)	0.38
30 day mortality	8 (3)	3 (3)	.65	8 (3)	3 (3)	0.67	5 (4)	6 (3)	0.37	8 (3)	3 (3)	0.65
Readmission <30 days	20 (9)	8 (7)	.53	20 (9)	8 (7)	0.56	12 (11)	16 (7)	0.24	16 (7)	12 (10)	0.30

Data are presented as mean (SD), median [IQR], or n (%). P-value for Students-T test, Whitney-Mann U or Chi-squared test as appropriate.

group. The interaction term between lymphocyte count subgroups and treatment allocation was not statistically significant. Nine (2.5%) patients died in hospital. In the sensitivity analysis excluding these patients, results were similar to those of the primary analysis (Table 3).

Because the high neutrophil count, high WBC count and high NLR subgroups were all associated with a history of COPD and no antibiotic treatment prior to hospital admission, we also examined whether antibiotic treatment status prior to admission or COPD status were driving factors behind the observed response to dexamethasone in these subgroups. We therefore tested for effect modification of dexamethasone by COPD status and antibiotic treatment prior to admission on LOS. There was no interaction between treatment allocation and COPD status or between treatment allocation and antibiotic treatment prior to hospital admission on LOS. Results of this analysis are shown in Supplementary Tables 1 and 2. There was also no interaction between presence of pneumococcal pneumonia and treatment allocation on LOS (p for interaction 0.16).

In the high WBC and neutrophil count subgroups, no patients in the dexamethasone group were admitted to the ICU. Therefore, logistic regression analysis to test the statistical significance of the interaction between WBC and neutrophil count subgroups and treatment allocation for ICU-admission was not possible. There was no further statistically significant interaction between WBC differential parameter subgroups and treatment allocation on secondary outcomes (Table 4).

Frequency of adverse events per subgroup are shown in Supplementary Table 3.

4. Discussion

In this secondary analysis of the Santeon-CAP cohort, we observed that the effect of adjunctive oral dexamethasone treatment on LOS was modified by WBC count $\geq 15.6\ 10^9$ cells/l, neutrophil count $\geq 13.2\ 10^9$ cells/l, NLR ≥ 15.5. In these subgroups dexamethasone reduced LOS by two days compared to no reduction in the reference groups. We did not observe differences in treatment response between subgroups for secondary outcomes.

In line with our hypothesis and similar to other reports, we observed that both patients with a high NLR and a low lymphocyte count had more severe disease [9,14,15]. Furthermore, secondary ICU-admission rate was three times higher in patients with a lymphocyte count $< 0.71\ 10^9$ cells/l compared to those with a lymphocyte count $\geq 0.71\ 10^9$ cells/l (9% vs 3%, $p = 0.010$). These findings are similar to Mendez et al. [9] who defined a subgroup of patients with lymphocytopenic CAP (lymphocyte count $< 0.724\ 10^9$ cells/l) with more severe disease. Compared to the high NLR or low lymphocyte count subgroup, the high WBC count and high neutrophil count subgroup constituted of a different type of patient. A neutrophil count $\geq 13.2\ 10^9$ cells/l and WBC count $\geq 15.6\ 10^9$ cells/l were both associated with a history of COPD. Yet, regarding PSI score, clinical signs and clinical outcomes, there was no difference between the high and low neutrophil count/WBC count subgroups.

Contrary to our hypothesis and despite the fact that patients with low lymphocyte count showed more severe disease, we did not find a statistically significant interaction between lymphocyte count and adjunctive treatment with dexamethasone for the clinical outcomes studied. Because we did find an interaction between neutrophil count

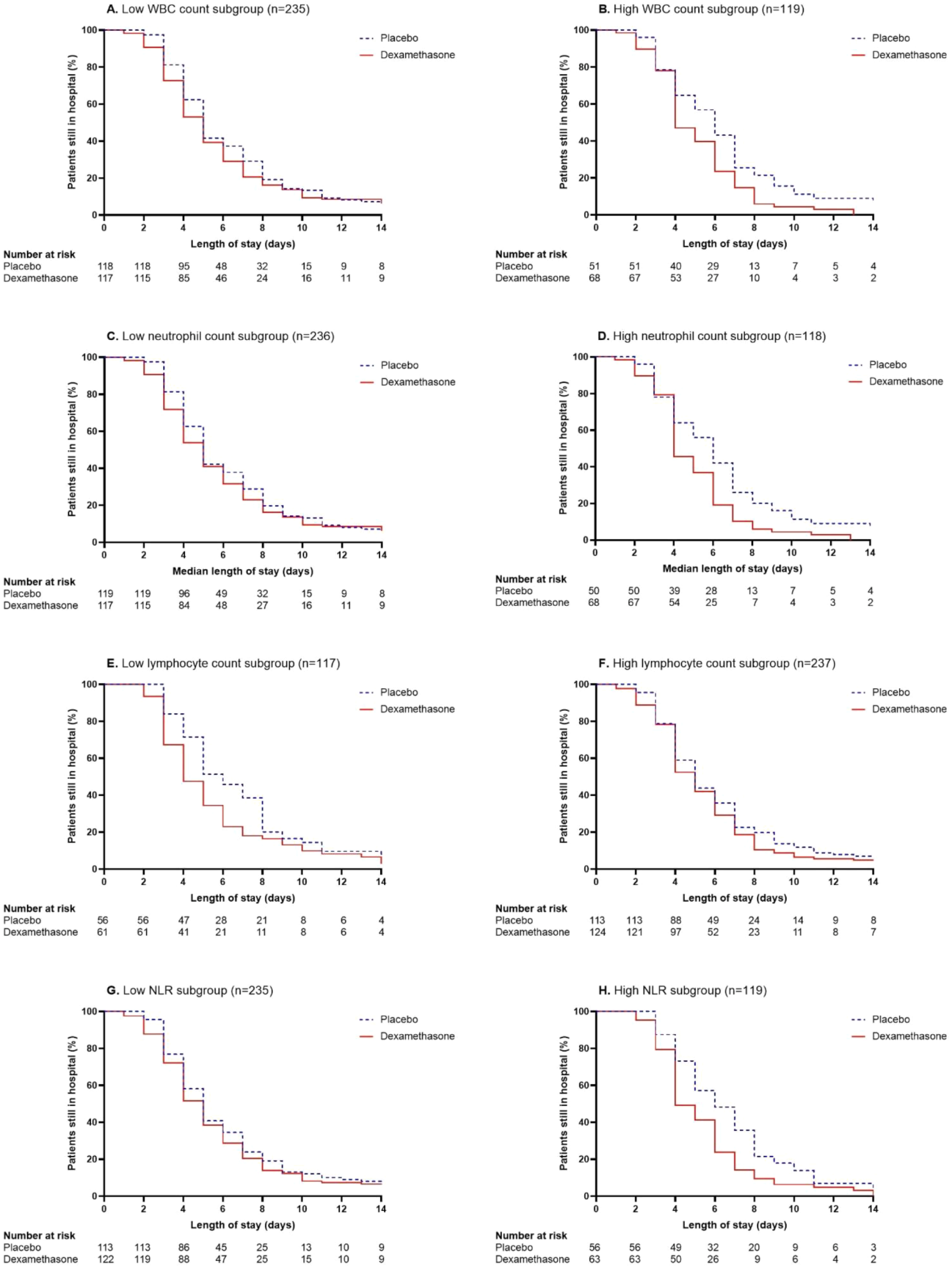

Fig. 1. Kaplan Meier curves for time to discharge comparing dexamethasone group and placebo group within each white blood count differential subgroup. 1A Low WBC count subgroup. 1B High WBC count subgroup. 1C Low neutrophil count subgroup. 1D High neutrophil count subgroup. 1E Low lymphocyte count subgroup. 1F High lymphocyte count subgroup. 1G Low neutrophil-lymphocyte ratio subgroup. 1H High neutrophil-lymphocyte ratio subgroup.

Table 3
Differences in response to dexamethasone on median length of stay by WBC differential parameter subgroups for the whole cohort and for patients who did not die in hospital.

	Low		High		
	Placebo	Dexamethasone	Placebo	Dexamethasone	P*
White blood cell count					
All patients	5.0 [4.0–8.0]	5.0 [3.0–7.0]	6.0 [4.0–8.0]	4.0 [4.0–6.0]	0.002
Patients who did not die in hospital	5.0 [4.0–8.0]	5.0 [3.0–7.0]	6.0 [4.0–7.0]	4.0 [4.0–6.0]	0.035
Neutrophil count					
All patients	5.0 [4.0–8.0]	5.0 [3.0–7.0]	6.0 [4.0–8.0]	4.0 [4.0–6.0]	0.001
Patients who did not die in hospital	5.0 [4.0–8.0]	5.0 [3.0–7.0]	6.0 [4.0–7.0]	4.0 [4.0–6.0]	0.018
Lymphocyte count					
All patients	5.5 [4.0–8.0]	4.0 [3.0–6.0]	5.0 [4.0–7.0]	5.0 [4.0–7.0]	0.52
Patients who did not die in hospital	5.0 [4.0–8.0]	4.0 [3.0–6.0]	5.0 [4.0–7.0]	5.0 [4.0–7.0]	0.15
NLR					
All patients	5.0 [4.0–7.0]	5.0 [3.0–7.0]	6.0 [4.0–8.0]	4.0 [4.0–6.0]	0.007
Patients who did not die in hospital	5.0 [3.3–7.0]	5.0 [3.0–7.0]	6.0 [4.0–8.0]	4.0 [4.0–6.0]	0.031

*_P_-value for interaction between randomly assigned treatment allocation and low/high subgroup membership.

Table 4
Differences in response to dexamethasone on secondary outcomes by WBC differential parameter subgroups.

	Low		High		
	Placebo	Dexamethasone	Placebo	Dexamethasone	P*
White blood cell count					
ICU-admission	7 (6)	5 (4)	4 (8)	0 (0)	–
30-day mortality	5 (4)	3 (3)	2 (4)	1 (2)	0.74
Readmission < 30 days	7 (6)	13 (11)	2 (4)	6 (9)	0.87
Neutrophil count					
ICU-admission	7 (6)	5 (4)	4 (8)	0 (0)	–
30-day mortality	5 (4)	3 (3)	2 (4)	1 (2)	0.72
Readmission < 30 days	7 (6)	13 (11)	2 (4)	6 (9)	0.89
Lymphocyte count					
ICU-admission	7 (13)	3 (5)	4 (4)	2 (2)	0.85
30-day mortality	4 (7)	1 (2)	3 (3)	3 (2)	0.31
Readmission < 30 days	4 (8)	8 (13)	5 (5)	11 (9)	0.93
Neutrophil-lymphocyte ratio					
ICU-admission	6 (5)	3 (3)	5 (9)	2 (3)	0.79
30-day mortality	5 (4)	3 (3)	2 (4)	1 (2)	0.88
Readmission < 30 days	6 (6)	10 (8)	3 (6)	9 (14)	0.49

*_P_-value for interaction between randomly assigned treatment allocation and low/high subgroup membership.

and dexamethasone treatment but not between lymphocyte count and dexamethasone treatment, the effect modification by NLR subgroup is more likely to be driven by the high neutrophil count than by low lymphocyte count.

To our knowledge, this is the first study investigating the interaction between WBC differential parameters and the effect of dexamethasone on clinical outcomes in patients with CAP. Other parameters indicative of more inflammation or more severe disease such as PSI score and CRP have been studied previously. Subgroups analyses by PSI score and CRP were conducted as part of the primary analysis of the Santeon-CAP study [5]. Stratification by PSI score did not yield a subgroup benefitting more from adjunctive dexamethasone. In the subgroups with a CRP concentration above the median, LOS was shorter and ICU admission rate was lower for patients who received dexamethasone compared those who received placebo, this was not seen in patients with a CRP below median. However, in an individual patient data meta-analysis of six trials investigating adjunctive corticosteroid treatment, there was no effect modification by CRP concentration > 188 mg/L. Furthermore, there was also no effect modification by PSI score on LOS [4]. The uncertain role of PSI score and CRP in identifying patients who benefit from corticosteroid treatment makes it interesting to further explore the role of white blood cell differential parameters.

Neutrophils are the first immune cells to infiltrate the lung in response to microorganisms invading the lung. Neutrophils use several mechanisms to eliminate invading pathogens including the formation of neutrophil extracellular traps (NETs) [3]. In a secondary analysis of a randomised trial investigating adjunctive prednisone in CAP, Ebrahimi et al. [17] found that CAP is accompanied by pronounced NET formation and that the degree of NETosis was correlated with peripheral WBC and neutrophil count. Furthermore, the authors found that prednisone modulated NETosis and they noted significant effect modification of the effect of adjunctive prednisone treatment by NET levels on time to clinical stability. Thus it was postulated that the beneficial effects of corticosteroids in CAP might be caused by modulation of NET formation or pre-activation of neutrophils. These findings may be a possible explanation for the fact that, in the present study, the beneficial effect of dexamethasone seemed to be stronger in patients with higher neutrophil counts.

We also found an association between high neutrophil count and history of COPD. Only patients with COPD who did not have clinical signs of an exacerbation COPD at hospital admission were enrolled in the Santeon-CAP study, therefore we do not believe that the positive effects of dexamethasone on LOS in the high neutrophil group were due to treatment of COPD exacerbations. Furthermore, similar to an individual patients data meta-analysis of six trials investigating adjunctive corticosteroids in CAP, we did not find effect modification of the effect of dexamethasone by COPD on LOS [4]. Moreover, we found that high neutrophil count, WBC count and NLR were more frequent in patients without prior outpatient antibiotic treatment. A possible explanation might be that these patients had more fulminant disease and thus were sent to hospital in an earlier stage of disease. Patients pre-treated with antibiotics at home might have had less fulminant disease and might

have had some treatment effect leading to a decrease in WBC counts and thus lower WBC counts at admission. This is supported by the fact that mean PSI score (83 (28) vs. 75 (28); $p = 0.012$) was higher in patients who did not receive antibiotics prior to admission. Nevertheless, we did not find interaction of the effect of dexamethasone on LOS by antibiotic treatment prior to admission.

The aim of this study was to search for subgroups of patients who are more likely to benefit from corticosteroid treatment. When it comes to balancing benefits and harms regarding adjunctive corticosteroid treatment in CAP, risk of hospital readmission is an important concern. In the original analysis of the Santeon-CAP study, readmission rate was twice as high in the dexamethasone group compared to the placebo group (10% vs. 5%; $p = 0.051$) [5]. Briel et al. reported similar findings in their individual patient data meta-analysis of six trials investigating adjunctive corticosteroid treatment in CAP [4]. In the present study, we did not observe that the effect of adjunctive dexamethasone on hospital readmission rate was modified by WBC differential parameters. For WBC count and neutrophil count, differences in readmission rates between patients treated with dexamethasone and those treated with placebo were similar in both the high and low subgroups. Because a 2 day (33%) reduction in LOS can be quite significant for a patient, the risk of readmission should be weighed against the significant gains of an earlier discharge. An additional finding, which might be equally important in clinical practice, is that in this study low WBC count and low neutrophil count subgroups constituted of a subgroup of patients who did not benefit from corticosteroid treatment but did have a higher risk of hospital readmission due to corticosteroid treatment. We might have identified a subgroup with no benefits but just the harms of corticosteroid treatment. This might be as important as the identification of a subgroup with benefits and not harms of corticosteroids.

There are several limitations to the present study. First and most importantly, this is a secondary analysis of a single study and our results need to be verified in a separate cohort, and would need validation in a prospective study before these findings can be implemented in clinical practice. Second, we could not include all patients from the initial Santeon-CAP study due to missing WBC differential counts thus some selection bias cannot be excluded. However, baseline characteristics were very similar to those of the whole Santeon-CAP population reported in the original analysis [5]. Third, the cut-off point for stratification into subgroups was based on the distribution of our data rather than predefined cut-off points. Since our study is the first to investigate if the effect of adjunctive corticosteroid treatment on clinical outcomes was modified by WBC differential parameters, there were no clear cut-off points available in literature. Furthermore, our patient population consisted of non-ICU patients with CAP. Our results cannot be generalised to patients admitted to the ICU with CAP. Finally, in our population, 30-day mortality rate was lower compared to the population in similar trials investigating corticosteroids in CAP [18–20], if there were effect modification for 30 day mortality we might not have enough statistical power to show those differences.

Even though further confirmatory research is required, neutrophil count or WBC appear a promising parameter in guiding corticosteroid treatment in non-ICU patients with CAP. This study can been seen as one in many for identifying a subgroup of CAP who should, or should not, be enrolled in future clinical trials. A leukogram is easy to perform and is often already part of the initial patient work-up in the emergency department.

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Declaration of Competing Interest

The authors declare they have no conflict of interest

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejim.2021.10.030.

References

[1] Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, Cooley LA, Dean NC, Fine MJ, Flanders SA, Griffin MR, Metersky ML, Musher DM, Restrepo MI, Whitney CG. Diagnosis and treatment of adults with community-acquired pneumonia. an official clinical practice guideline of the American thoracic society and infectious diseases society of America. Am J Respir Crit Care Med 2019;200:e45–67.

[2] GBD 2016 Lower Respiratory Infections Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the global burden of disease study 2016. Lancet Infect Dis 2018;18:1191–210. https://doi.org/10.1016/S1473-3099(18)30310-4.

[3] Pechous RD. With friends like these: the complex role of neutrophils in the progression of severe pneumonia. Front Cell Infect Microbiol 2017;7:160.

[4] Briel M, Spoorenberg SMC, Snijders D, Torres A, Fernandez-Serrano S, Meduri GU, Gabarrús A, Blum CA, Confalonieri M, Kasenda B, Siemieniuk RAC, Boersma W, Bos WJW, Christ-Crain M. Ovidius study group CSG and SSG. Corticosteroids in patients hospitalized with community-acquired pneumonia: systematic review and individual patient data metaanalysis. Clin Infect Dis 2017;66:346–54.

[5] Wittermans E, Vestjens SM, Spoorenberg SM, Blok WL, Grutters JC, Janssen R, et al. Adjunctive treatment with oral dexamethasone in non-ICU patients hospitalised with community-acquired pneumonia: a randomised clinical trial. Eur Respir J 2021;58:2002535 [Epub ahead of print].

[6] Chalmers JD. Corticosteroids for community-acquired pneumonia: a critical view of the evidence. Eur Respir J 2016;48:984–6.

[7] Urwyler SA, Blum CA, Coslovsky M, Mueller B, Schuetz P, Christ-Crain M. Cytokines and cortisol – predictors of treatment response to corticosteroids in community-acquired pneumonia? J Intern Med 2019;286:75–87.

[8] Remmelts HHF, Meijvis SCA, Heijligenberg R, Rijkers GT, Oosterheert JJ, Bos WJW, Endeman H, Grutters JC, Hoepelman AIM, Biesma DH. Biomarkers define the clinical response to dexamethasone in community-acquired pneumonia. J Infect 2012;65:25–31.

[9] Méndez R, Menéndez R, Amara-Elori I, Feced L, Piró A, Ramírez P, Sempere A, Ortega A, Bermejo-Martín JF, Torres A. Lymphopenic community-acquired pneumonia is associated with a dysregulated immune response and increased severity and mortality. J Infect 2019;78:423–31.

[10] Lee KY. Pneumonia, acute respiratory distress syndrome, and early immune-modulator therapy. Int J Mol Sci 2017;18:388.

[11] Huang Z, Fu Z, Huang W, Huang K. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: a meta-analysis. Am J Emerg Med 2020;38:641–7.

[12] Guthrie GJK, Charles KA, Roxburgh CSD, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. Crit Rev Oncol Hemato 2013;88:218–30.

[13] Angkananard T, Anothaisintawee T, McEvoy M, Attia J, Thakkinstian A. Neutrophil lymphocyte ratio and cardiovascular disease risk: a systematic review and meta-analysis. Biomed Res Int 2018:2018.

[14] de Jager CPC, Wever PC, Gemen EFA, Kusters R, van Gageldonk-Lafeber AB, van der Poll T, Laheij RJF. The neutrophil-lymphocyte count ratio in patients with community-acquired pneumonia. PLoS One 2012;7:4–11.

[15] Cataudella E, Giraffa CM, Di Marca S, Pulvirenti A, Alaimo S, Pisano M, Terranova V, Corriere T, Ronsisvalle ML, Di Quattro R, Stancanelli B, Giordano M, Vancheri C, Malatino L. Neutrophil-to-lymphocyte ratio: an emerging marker predicting prognosis in elderly adults with community-acquired pneumonia. J Am Geriatr Soc 2017;65:1796–801.

[16] Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE, Coley CM, Marrie TJ, Kapoor WN. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med 1997;336:243–50.

[17] Ebrahimi F, Giaglis S, Hahn S, Blum CA, Baumgartner C, Kutz A, Van Breda SV, Mueller B, Schuetz P, Christ-Crain M, Hasler P. Markers of neutrophil extracellular traps predict adverse outcome in communityacquired pneumonia: secondary analysis of a randomised controlled trial. Eur Respir J 2018:51.

[18] Snijders D, Daniels JMA, de Graaff CS, van der Werf TS, Boersma WG. Efficacy of corticosteroids in community-acquired pneumonia. Am J Respir Crit Care Med 2010;181:975–82.

[19] Meijvis SCA, Hardeman H, Remmelts HHF, Heijligenberg R, Rijkers GT, van Velzen-Blad H, Voorn GP, van de Garde EMW, Endeman H, Grutters JC, Bos WJW, Biesma DH. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. Lancet 2011;377:2023–30.

[20] Blum CA, Nigro N, Briel M, Schuetz P, Ullmer E, Suter-Widmer I, Winzeler B, Bingisser R, Elsaesser H, Drozdov D, Arici B, Urwyler SA, Refardt J, Tarr P, Wirz S, Thomann R, Baumgartner C, Duplain H, Burki D, Zimmerli W, Rodondi N, Mueller B, Christ-Crain M. Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet 2015;385:1511–8.