Aus der Klinik für Pneumologie

Geschäftsführender Direktor: Prof. Dr. med. Claus Franz Vogelmeier

des Fachbereichs Medizin der Philipps-Universität Marburg

High eosinophil blood counts are associated with a shorter length of hospital stay in exacerbated COPD patients – a retrospective analysis

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Julia Sophia Tüffers aus Wiesbaden

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1.Korreferent/in: Herr Prof. Dr. H. Garn

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1. Abbreviations, Figures and Tables

1.1 Abbreviations

AECOPD	acute exacerbation of chronic obstructive pulmonary disease
CBC	complete blood count
CMI	Case Mix Index
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CXCL10	C-X-C motif chemokine ligand 10
DRG	Diagnosis Related Groups
eos	eosinophils
FEV_1	forced expiratory volume in one second
GOLD	Global Initiative for Chronic Obstructive Lung Disease
ICS	inhaled corticosteroids
ICU	intensive care unit
IL	interleukin
IQR	interquartile range
LOS	length of hospital stay
NLR	Neutrophil-to-lymphocyte ratio
PCT	Procalcitonin
PRIND	Prolonged reversible ischemic neurologic deficit
RCT	randomized controlled trial
VC	vital capacity

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2. Introduction

Morbidity and mortality of chronic obstructive pulmonary disease (COPD) is strongly associated with severe exacerbations (Hurst, Vestbo et al. 2010). In severely exacerbated COPD patients, the courses of disease are strongly varying. In order to understand the nature of this, it is necessary to take account of the different biologic clusters of COPD exacerbation, as described by Bafadhel et al.: In a prospective clinical cohort study of 2011, she presented four different exacerbation phenotypes (bacterial, viral, sputum-eosinophil and pauci-inflammatory) and the possibility of predicting two of them from stable state with the use of biomarkers (sputum IL-1ß for bacteria- and peripheral blood eosinophils (eos) for eosinophil-associated exacerbations) (Bafadhel, McKenna et al. 2011). The best effective treatment options for the above mentioned exacerbation phenotypes, possibly leading to a personalized management of COPD-exacerbations, are a highly discussed topic (Zhou, Zhou et al. 2017).

The current GOLD recommendations emphasize blood eosinophils as a marker for responsiveness to inhaled corticosteroids (ICS). The recommended thresholds are < 100cells/µl and > 300cells/µl (Singh, Agusti et al. 2019, Global Initiative for Chronic Obstructive Lung Disease 2021, Venkatesan 2022). These recommendations are based on a high number of analyses from both population-based cohort studies and secondary analyses from randomized clinical trials: In two randomized controlled trials comparing treatment with ICS/LABA to LABA, significant treatment effects could be recorded among patients with eosinophil counts > 100/µl (Siddiqui, Guasconi et al. 2015, Pavord, Lettis et al. 2016). Similar findings have also been recognized in a post-hoc-analysis of the WISDOM-study: Watz et al. found that higher blood eosinophils are associated with an increased exacerbation rate after steroid withdrawal in patients pre-treated with triple therapy (Watz, Tetzlaff et al. 2016). Further evidence comes from pharmaceutical parallel-groups randomized clinical trials, in which inhaled triple therapy has been compared to different dual combinations. Higher blood eosinophils were usually associated with higher ICS efficacy (Ferguson, Rabe et al. 2018, Lipson, Barnhart et al. 2018, Papi, Vestbo et al. 2018).

The therapeutic and prognostic implications of blood eosinophils during an exacerbation are less clear: In a post-hoc analysis Bafadhel et al. evaluated the

rate of recovery of eosinophilic (blood eos ≥ 200/µl and/or ≥ 2%) and non-eosinophilic exacerbations of COPD subjects (n=243) who participated in a multi-centre randomised control trial. In the eosinophilic exacerbation population, the length of hospital stay was shorter than in patients with non-eosinophilic exacerbations following treatment with oral corticosteroids. The CRP tended to be normal (Bafadhel, Greening et al. 2016).

Standard care guidelines recommend severe acute exacerbations of COPD to be treated with 40mg of prednisone per day for five days. As studies have shown, treatment with systemic glucocorticoids improves the patients' clinical outcome in terms of a better lung function, oxygenation and a reduction of hospital length of stay (Singh, Agusti et al. 2019, Global Initiative for Chronic Obstructive Lung Disease 2021, Venkatesan 2022). At the same time treatment with corticosteroids goes along with many side effects such as hyperglycaemia, muscle catabolism and a higher risk for osteoporotic fractures (Niewoehner, Erbland et al. 1999, Walsh, Lewis et al. 2002, Jagoe and Engelen 2003). Bafadhel et al. have indicated that patients with low eosinophil blood counts may be less responsive to corticosteroid treatment (Bafadhel, McKenna et al. 2012). In order to prevent overuse of systemic corticosteroids, Sivapalan et al. compared the effects of eosinophil-guided corticosteroid treatment of exacerbated COPD patients to standard care in a multicentre, controlled trial in Denmark. They showed that an eosinophil-guided treatment seems to be non-inferior when compared to treatment according to current guidelines (Sivapalan, Lapperre et al. 2019).

Besides the eosinophil blood counts, there are other inflammatory parameters which may also have impact on the patients' course of disease and may in fact be helpful in guiding treatment decisions. In a multicentre, randomized, controlled clinical trial, Butler et al. analyzed CRP-guided antibiotic treatment in 653 exacerbated COPD patients who consulted a clinician at a general medical care practice in England and Wales. While patients in the CRP-guided group received fewer antibiotics than the control group, no difference in the clinical outcome could be detected between the two groups (Butler, Gillespie et al. 2019).

Furthermore, in a retrospective observational cohort study in Istanbul, Turkey, Duman et al. compared the outcomes of different eosinophilic groups of hospitalized COPD patients (n=1704) and analyzed inflammatory markers associated with the patients' outcome. Besides the CRP, they identified the Neutrophil-to-lymphocyte ratio (NLR) as being significantly higher in non-eosinophilic patients. Lu et al. conducted a retrospective study of 604 subjects categorizing patients in frequent and non-frequent exacerbators and comparing clinical characteristics during acute exacerbations of COPD. Both Duman, as well as Lu et al. found the NLR to be associated with a worse outcome (Duman, Aksoy et al. 2015, Lu, Chen et al. 2021).

Because data from real life settings confirming these RCT-based findings are scarce, we retrospectively evaluated the association between baseline eosinophil count and both short- and long-term clinical outcomes, as well as different inflammatory parameters and markers of disease severity in patients hospitalized due to an exacerbation of their underlying COPD.

3. Methods

Patient Population

In this analysis, we present retrospectively collected data of 1007 exacerbations which were treated at the University Medical Center Marburg between 01/2013 and 12/2018. All patients had been diagnosed with an acute exacerbation of COPD according to the ICD code (J44.0/J44.1). According to national guidelines valid at the time of data collection, there were no standardized criteria for hospital admission due to acute exacerbation. The decision was based on the patients' clinical impression, reflected by the metabolic status (assessed by blood gas analysis), breathing frequency and oxygen saturation.

Patients were predominantly male (65%), had a median age of 74 years (IQR 65 years – 83 years) and a median FEV_1 of 1.03l (42.6% predicted). Our analysis was based on a subgroup of 417 cases of patients in whom a full blood cell count was obtained at the day of admission. These patients exhibited similar baseline characteristics (63.3% male, 73.3 years of age, FEV_1 1.15l, 43.98% predicted) (table 1). No significant differences were detected between the groups.

Tables 1.1 and 1.2 – Baseline characteristics

Tables 1.1 and 1.2 display the baseline characteristics depending on the blood eosinophils on the day of admission. Continuous parameters are expressed as Median and (IQR).

	All	**< 2 [%]**	**≥ 2 [%]**
N	417	276	141
Age [years]	74.0 (65.0 - 83.0)	72.0 (64.0 - 82.0)	76.0 (67.0 - 83.0)
Male sex [%]	63.3	61.2	67.4
Packyears	40.0 (30.0 - 59.0)	40.0 (25.0 - 50.0)	40.0 (30.0 - 60.0)
FEV_1[l]	1.03 (0.74-1.44)	1.00 (0.71 - 1.33)	1.11 (0.78 -1.50)
FEV_1 [pred. %]	42.6 (30.6 - 54.8)	41.8 (29.1 - 53.0)	42.6 (33.9 - 57.6)
FEV_1/VC	46.0 (37.1 - 57.2)	44.8 (35.5 - 56.0)	47.5 (38.5 - 58.5)

	< 100 [eos/µl]	**100-300 [eos/µl]**	**> 300 [eos/µl]**
N	219	130	68
Age [years]	73.0 (63.0 - 83.0)	73.0 (65.0 - 81.3)	76.0 (67.0 - 83.0)
Male sex [%]	60.3	67.4.	65.2
Packyears	40.0 (22.0 - 50.0)	40.0 (30.0 - 50.0)	45.0 (30.0 - 60.0)
FEV_1 [l]	0.98 (0.71 - 1.30)	1.06 (0.74 - 1.50)	1.10 (0.81 - 1.54)
FEV_1 [pred. %]	38.3 (29.5 - 51.9)	45.3 (30.8 - 58.2)	42.6 (34.4 - 58.8)
FEV_1/VC	45.2 (35.6 - 54.5)	44.4 (36.4 - 62.3)	50.7 (41.4 - 55.5)

Accompanying comorbidities appeared frequently in all strata, whereas a clear correlation between the occurrence of comorbidities and blood eosinophils could not be established (table 2).

Table 2 – Comorbidities

Table 2 displays the comorbidities according to the blood eosinophils of patients at the day of admission.

	All	< 2 [%]	≥ 2 [%]	< 100 [eos/µl]	100-300 [eos/µl]	> 300 [eos/µl]
N	417	276	141	219	130	68
Hypertension [%]	64.3	62.3	68.1	60.7	70.8	63.2
Heart failure [%]	26.9	31.2	18.4	32	23.8	16.2
Ischaemic heart disease [%]	29.3	25.7	36.2	26	30.8	36.8
Stroke/PRIND [%]	9.8	10.5	8.5	9.1	11.5	8.8
Acute renal failure [%]	12	14.9	6.4	16	8.5	5.9
Chronic renal failure [%]	19.7	19.9	19.1	21.9	16.2	19.1
Depression [%]	9.6	9.1	10.6	10	8.5	10.3
Diabetes mellitus [%]	25.9	23.6	30.5	21.5	32.3	27.9
Anemia [%]	11.8	12.3	10.6	13.7	9.2	10.3
Gastro-oesophageal reflux [%]	4.6	5.1	3.5	4.6	3.8	5.9
Asthma [%]	6.7	6.2	7.8	5.5	8.5	7.4
Cancer currently [%]	9.6	8	12.8	9.6	10	8.8

Chart reviews were done to describe further characteristics of these patients and to evaluate their clinical outcomes. From the routine medical records we transferred and analyzed the following parameters: Case Mix Index (CMI) - a formula of the Diagnosis Related Groups (DRG) system for the calculation of patients' case severity, which is used as a controlling instrument of hospitals relevant for financial reimbursement - as a rough marker of disease severity, inflammatory markers and other basic laboratory values. Spirometry records were used for the evaluation of the patients' respiratory status. This lung function data had either been conducted during the period of the hospital stay or up to three months in advance at stable state.

As primary endpoint, we compared the length of hospital stay. Further analyses described other markers of disease severity and long-term outcome (re-hospitalization and mortality within our hospital as well as the annual severe exacerbation rate within our hospital) according to the above-mentioned eosinophil blood cell count strata (100 and 300 eosinophils/µl and 2%). As this was a purely retrospective analysis from clinical routine data, we obtained a waiver by the ethics committee because a formal review was considered as not being mandatory (EM_MR_greulich_130320).

Subgroup analyses

Three separated subgroup analyses were performed: Firstly, we analyzed a subgroup of 243 cases of patients, who - according to their medical records - did not receive systemic steroids before the full blood count was obtained. Baseline characteristics of these patients were similar to the primary analysis population (tables 3.1 and 3.2).

Tables 3.1 and 3.2 – Baseline Characteristics in cases of patients naïve for systemic steroids (before blood collection)

Tables 3.1 and 3.2 display the baseline characteristics in patients naïve for systemic steroids (n=243). Continuous parameters are displayed as Median and (IQR); § significant between < 2% and ≥ 2%, * significant between < 100 and 100-300; # significant between < 100 and > 300; $ significant between 100-300 and > 300.

	All	**< 2 [%]**	**≥ 2 [%]**	**p-value**
N	243	148	95	
Age [years]	73.0 (65.0 - 82.0)	71.0 (64.0 - 80.0)	78.0 (68.0 - 83.0)	§
Male sex [%]	65.4	64.9	66.3	n.s.
Packyears	40.0 (30.0 - 60.0)	40.0 (28.8 - 60.0)	40.0 (30.0 - 60.0)	n.s.
FEV_1 [l]	1.05 (0.73 - 1.54)	0.98 (0.68 - 1.31)	1.25 (0.79 - 1.69)	§
FEV_1 [pred. %]	42.5 (30.1 - 56.0)	36.8 (24.7 - 52.7)	46.0 (35.2 - 62.0)	§§
FEV_1/VC	45.4 (36.7 - 55.4)	43.0 (35.1 - 50.1)	47.5 (38.5 - 56.8)	§

	< 100 [eos/µl]	**100-300 [eos/µl]**	**> 300 [eos/µl]**	**p-value**
N	114	76	53	
Age [years]	72.0 (62.0 - 82.0)	72.5 (66.3 - 81.0)	76.0 (67.5 - 83.0)	n.s.
Male sex [%]	64.9	64.5	67.9	n.s.
Packyears	40.0 (25.0 - 50.0)	40.0 (30.0 - 57.5)	45.0 (30.0 - 60.0)	n.s.
FEV_1 [l]	0.97 (0.70 - 1.30)	1.05 (0.69 - 1.57)	1.26 (0.89 - 1.69)	* #
FEV_1 [pred. %]	34.4 (24.9 - 51.8)	45.2 (32.4 - 57.2)	45.8 (34.8 - 64.4)	##
FEV_1/VC	43.7 (35.2 - 49.7)	43.7 (36.0 - 56.8)	50.7 (41.2 - 55.9)	n.s.

Secondly: For the vast majority of cases admitted to our hospital, chest x-rays were performed as part of clinical routine (> 95%). As exacerbations of COPD and pneumonia may overlap in some cases, we performed additional analyses. Including CT-scans (if available) as well, we analyzed respiratory pathologies of all subjects. By excluding patients with radiological signs of pneumonia (n=89), a study population of 322 exacerbations remained. The evaluation was based on the medical report of the respective responsible radiologist. Baseline characteristics of this subgroup were similar to the others and can be seen in tables 4.1 and 4.2.

Tables 4.1 and 4.2 - Baseline characteristics in cases of patients without radiological signs of pneumonia

Tables 4.1 and 4.2 display the baseline characteristics of cases of patients without signs of pneumonia (n=322). Continuous parameters are displayed as Median and (IQR); § significant between < 2% and ≥ 2%, * significant between < 100 and 100-300; # significant between < 100 and > 300; $ significant between 100-300 and > 300, & indicates a significantly different gender distribution.

	All	< 2 [%]	≥ 2 [%]	p-value
N	322	206	116	
Age [years]	72.5 (64 - 83)	72 (62 - 82)	76 (67 - 83)	n.s.
Male sex [%]	60.6	56.8	67.2	n.s.
Packyears	40.0 (30.0 - 55.5)	40.0 (25.0 - 50.0)	40.0 (30.0 - 60.0)	n.s.
FEV_1 [l]	1.00 (0.73 - 1.41)	0.95 (0.69 -1.26)	1.13 (0.81 - 1.51)	§§
FEV_1 [pred. %]	41.3 (30.0 - 54.0)	37.2 (28.0 - 52.0)	44.1 (34.0 - 57.5)	§
FEV_1/VC	45.2 (36.0 - 55.5)	43.0 (34.7 - 52.4)	47.1 (38.7 - 58.5)	§

	< 100 [eos/µl]	100-300 [eos/µl]	> 300 [eos/µl]	p-value
N	163	102	57	
Age [years]	72 (62 - 82)	72 (65 - 81)	78 (67 - 84.5)	n.s.
Male sex [%]	54.0	70.6	61.4	&
Packyears	40.0 (20.0 - 50.0)	40.0 (30.0 - 50.00)	45.0 (30.0 - 60.0)	n.s.
FEV_1 [l]	0.94 (0.70 - 1.24)	1.07 (0.73 - 1.49)	1.13 (0.89 - 1.55)	##
FEV_1 [pred. %]	36.8 (28.8 - 51.0)	43.7 (26.8 - 55.7)	45.5 (34.8 - 61.5)	#
FEV_1/VC	43.0 (34.5 - 51.8)	44.4 (35.6 - 58.5)	49.5 (41.6 - 55.9)	#

Thirdly: Of the 417 cases of patients in whom a full blood cell count was obtained at the day of admission, we additionally selected a number of 371 cases, which all had received systemic corticosteroids (according to their medical records) either before their hospital stay and/or during admission. Slight changes in baseline characteristics could be observed and can be seen in tables 5.1 and 5.2.

Tables 5.1 and 5.2 - Baseline characteristics in cases of patients with corticosteroids

Tables 5.1 and 5.2 display the baseline characteristics of patients with corticosteroids and only include cases in which patients had received systemic corticosteroids according to their medical records either before and/or during hospital admission. Continuous parameters are displayed as Median and (IQR); § significant between < 2% and ≥ 2%, * significant between < 100 and 100-300; # significant between < 100 and > 300; $ significant between 100-300 and > 300.

	All	< 2 [%]	≥ 2 [%]	p-value
N	371	250	121	
Age [years]	73.0 (64.0 - 82.0)	72.0 (62.8 - 81.3)	76.0 (67.0 - 83.0)	n.s.
Male sex [%]	61.7	58.8	67.8	n.s.
Packyears	40.0 (30.0 - 60.0)	40.0 (25.0 - 50.0)	45.0 (30.0 - 60.0)	n.s.
FEV_1 [l]	1.00 (0.73-1.41)	0.97 (0.70 - 1.29)	1.12 (0.78 -1.54)	§
FEV_1 [pred. %]	41.7 (29.9 - 54.0)	38.5 (28.3 - 52.7)	42.7 (32.3 - 58.0)	§
FEV_1/VC	45.2 (36.4 - 55.4)	44.1 (35.4 - 54.9)	47.0 (38.5 - 55.9)	n.s.

	< 100 [eos/µl]	100-300 [eos/µl]	> 300 [eos/µl]	p-value
N	195	112	64	
Age [years]	72.0 (62.0 - 82.0)	73.0 (65.0 - 81.8)	76.0 (67.0 - 83.0)	n.s.
Male sex [%]	57.4	67.0	65.6	n.s.
Packyears	40.0 (20.0 - 50.0)	40.0 (30.0 - 50.0)	47.5 (30.0 - 60.0)	n.s.
FEV_1 [l]	0.95 (0.70 - 1.25)	1.05 (0.74 - 1.55)	1.12 (0.81 - 1.55)	#
FEV_1 [pred. %]	37.1 (28.7 – 50.8)	45.3 (28.8 – 58.5)	42.7 (34.5 - 60.3)	n.s.
FEV_1/VC	44.6 (35.3 – 53.9)	44.4 (36.2 – 60.2)	48.5 (40.6 – 54.6)	n.s.

Additional subgroup analyses

Of the 417 cases of patients in whom complete blood counts (CBC) were obtained at the day of admission, we defined and analyzed two more subgroups.

Firstly, we compared both short- and long-term outcomes (length of hospital stay and re-hospitalization and survival rates) between a) patients with low numbers of blood eosinophils (< 2%) and high levels of CRP (≥ 30mg/dl) and b) patients with high numbers of blood eosinophils (≥ 2%) and low levels of CRP (< 30mg/dl). For the different thresholds, we estimated a cut-off value of 30mg/dl for CRP, following the clinical cohort study of Butler et al., who established thresholds of 20mg/dl and 40mg/dl when investigating the non-inferiority of CRP-guided treatment with antibiotics in patients with AECOPD (Butler, Gillespie et al. 2019).

Secondly, we compared the above-mentioned parameters between another subgroup of patients, replacing the CRP with the Neutrophil-to-lymphocyte ratio. Duman et al. analyzed the utility of inflammatory markers in exacerbated COPD patients and applied a cut-off value of 7 for the NLR (Duman, Aksoy et al. 2015). Following this estimation, our two patient groups were defined as a) patients with blood eosinophils < 2% and a NLR ≥ 7 and b) patients with blood eosinophils ≥ 2% and a NLR < 7.

Statistical analyses

Metric outcome variables were compared between the different eosinophil strata. As data was not normally distributed, for continuous parameters Mann-Whitney test was used for two group comparisons. Kruskal-Wallis test with Dunn's test was applied for three group comparisons. The exact fisher test was applied to compare categorical variables. Chi-Square test was performed to compare gender between the groups. For patients that were re-hospitalized in our hospital or died in our hospital during the observational time period, Kaplan-Meier curves were used to visualize the results and Wilcoxon log rank test was applied to compare the data between the strata. IBM SPSS statistics version 24 (IBM, Armonk, New York, USA) was used for all calculation, graphs were constructed using IBM SPSS or GraphPad version 7 (GraphPad, San Diego, CA, USA).

4. Results

Of the 417 cases of patients that were included in the study, 276 (66.2%) had a peripheral blood eosinophil count of < 2%, whereas 141 cases (33.8%) had ≥ 2% eosinophils of the total leucocyte count. In the absolute strata, there were 219 exacerbations (52.5%) with eosinophil counts < 100/µl, 130 (31.2%) with eosinophils between 100-300/µl and 68 (16.3%) that exhibited absolute eosinophilic cell counts > 300/µl.

Length of hospital stay

Patients with low eosinophils (< 2%, < 100/µl) had a longer median time in hospital (length of hospital stay – LOS) as compared to patients with high eosinophils (figure 1). This was true for both relative and absolute values.

Figure 1 - Hospital length of stay according to the blood eosinophils at the day of admission

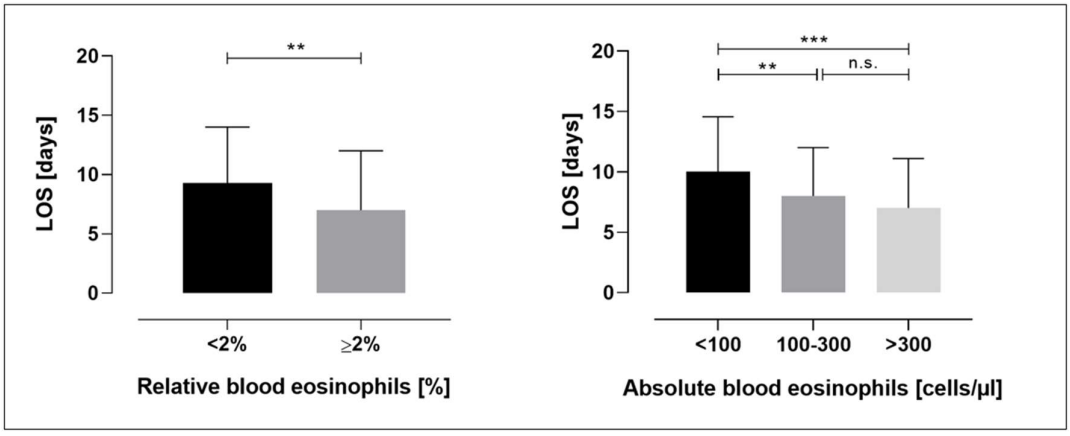

Figure 1 displays the median (+ IQR) hospital length of stay according to the blood eosinophils at the day of admission. Patients with a low relative eosinophil count experienced a longer hospital stay as compared to patients with a high relative eosinophil count (left diagram; $p < 0{,}005$; Mann-Whitney test). Patients with low absolute number of blood eosinophils also exhibited longer length of hospital stay as compared to patients with an intermediate or high number of blood eos (right diagram; $p < 0.005$; $p < 0.001$; Kruskal-Wallis test with Dunn's test).

Restricting the analysis to patients naïve for systemic steroids (before blood collection) yielded similar outcomes: The median LOS in patients with low eosinophils was longer than in their respective control group (< 2%: 9 vs. ≥ 2%: 7 days, $p < 0.05$). The same applied to the absolute eosinophilic groups (< 100/µl: 9.18 vs. 100-300/µl: 7 days, n.s.; 100-300/µl: 7 vs. > 300/µl: 6.54 days, n.s.; < 100/µl: 9.18 vs. > 300/µl: 6.54 days, $p < 0.05$).

Restricting the analysis to patients without consolidation on chest x-ray yielded similar results, as well: The median LOS in patients with low eosinophils was longer than in patients with a higher relative number of blood eosinophils (< 2%: 9 vs. ≥ 2%: 7 days, $p < 0.05$). Similar trends could be observed regarding strata derived from absolute numbers of eosinophils (< 100/µl: 9.96 vs. 100-300/µl: 8 n.s.; 100-300/µl: 8 vs. > 300/µl: 6.54 days, n.s.; < 100/µl:9.96 vs. > 300/µl: 6.54 days, $p < 0.01$).

Additionally, analyzing the 371 cases of patients which had received systemic corticosteroids according to their medical records either before their hospital stay and/or during admission, also yielded similar outcomes: The median LOS in patients with low eosinophils was longer than in patients with a higher relative number of blood eosinophils (< 2%: 9.16 vs. ≥ 2%: 7 days, $p < 0.001$). This applied to the absolute numbers of eosinophils, too (< 100/µl: 10 vs. 100-300/µl: 8 days, $p < 0.01$.; 100-300/µl: 8 vs. > 300/µl: 7 days, n.s.; < 100/µl: 10 vs. > 300/µl: 7 days, $p < 0.001$).

Inflammatory markers

Patients with low eosinophil blood counts (< 2%, < 100/µl) had higher inflammatory markers when compared to their respective control groups. Both the median CRP and the blood neutrophils were significantly higher in patients with low eosinophils as compared to patients with high eosinophils (figure 2). The Neutrophil-to-lymphocyte ratio (NLR) in patients with non-eosinophilic exacerbations was more than double the median of patients with eosinophilic exacerbations. This was true for both absolute and relative values. The same trend could be observed for Procalcitonin (PCT) and a number of further inflammatory markers (tables 6.1 and 6.2).

Figure 2 - Inflammatory markers according to the blood eosinophils at the day of admission

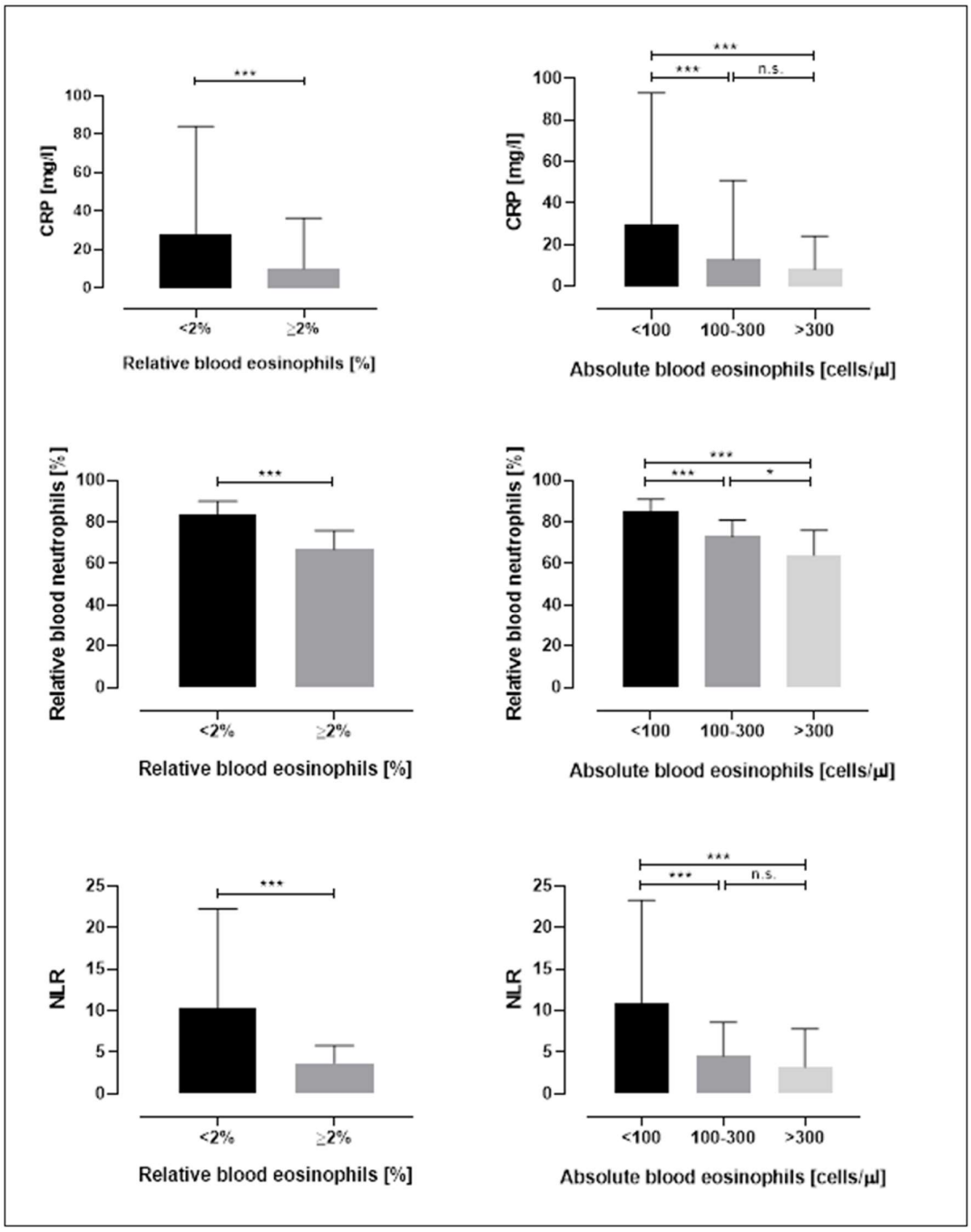

Figure 2 shows the inflammatory markers CRP, Neutrophils and NLR, defined as median (+ IQR) according to the blood eosinophils at the day of admission.

Tables 6.1 and 6.2 – Inflammatory markers in cases of patients with a full cell blood count at the day of admission

Tables 6.1 and 6.2 display the subgroup analyses of median and IQR of inflammatory markers according to the blood eosinophils at the day of admission. Continuous parameters are displayed as Median and (IQR); § significant between < 2% and ≥ 2%, * significant between < 100 and 100-300; # significant between < 100 and > 300; $ significant between 100-300 and > 300, n=413 for CRP, n=417 neutrophils, n=416 for NLR, n=417 for leucocytes, n=156 for Procalcitonin, n=406 for Fibrinogen.

	All	**< 2 [%]**	**≥ 2 [%]**	**p-value**
n	417	276	141	
CRP [mg/l]	20.0 (6.00 - 66.5)	28.0 (10.0 - 83.9)	10.0 (2.50 - 36.6)	§§§
Neutrophils [%]	78.0 (68.0 - 88.0)	83.5 (76.0 - 90.0)	67.0 (57.5 - 76.0)	§§§
NLR	7.18 (3.61 - 15.25)	10.25 (5.62 - 22.25)	3.69 (2.23 - 5.81)	§§§
Leucocytes [g/l]	11.2 (8.32 - 14.8)	12.5 (9.06 -16.1)	9.38 (7.67 - 12.2)	§§§
Procalcitonin [µg/l]	0.05 (0.05 - 0.20)	0.10 (0.05 - 0.25)	0.05 (0.05 - 0.10)	§
Fibrinogen [g/l]	5.05 (4.00 - 6.80)	5.14 (4.00 - 14.0)	4.70 (3.65 - 6.08)	§

	< 100 [eos/µl]	**100-300 [eos/µl]**	**> 300 [eos/µl]**	**p-value**
n	219	130	68	
CRP [mg/l]	30.0 (12.0 - 93.0)	13.0 (2.50 - 51.0)	8.50 (2.50 - 25.0)	*** ###
Neutrophils [%]	85.0 (76.0 – 91.0)	73.0 (65.0 - 81.0)	64.0 (56.0 - 76.0)	*** ### $$
NLR	10.88 (6.17 - 23.25)	4.56 (2.94 - 8.57)	3.24 (2.04 - 7.80)	*** ###
Leucocytes [g/l]	12.0 (8.41 -16.5)	11.2 (8.51 - 13.5)	9.98 (7.99 -14.5)	n.s.
Procalcitonin [µg/l]	0.10 (0.05 - 0.33)	0.05 (0.05 - 0.20)	0.05 (0.05 - 0.05)	##
Fibrinogen [g/l]	5.30 (4.10 -14.0)	5.00 (3.80 – 6.50)	4.50 (3.50 - 5.65)	##

Subgroup analyses regarding inflammatory markers (firstly, excluding cases of exacerbations in non-systemic-steroid-naïve patients; secondly ruling out cases with signs of pneumonia in the chest x-ray and thirdly, restricting the analysis to cases in which steroids were administered to patients before and/or during their stay at hospital) confirmed our analyses in trends at similar significant levels (tables 7 to 9).

Tables 7.1 and 7.2 – Inflammatory markers in cases of patients naïve for systemic steroids (before blood collection)

Tables 7.1 and 7.2 display the subgroup analyses of inflammatory markers according to the blood eosinophils in cases of patients that were administered systemic steroids only after the blood samples had been taken. Continuous parameters are displayed as Median and (IQR); § significant between < 2% and ≥ 2%, * significant between < 100 and 100-300; # significant between < 100 and > 300; $ significant between 100-300 and > 300; n=240 for CRP, n=238 for neutrophils, n= 237 for NLR, n=243 for leucocytes, n=86 for Procalcitonin, n=234 for Fibrinogen.

	All	**< 2 [%]**	**≥ 2 [%]**	**p-value**
n	243	148	95	
CRP [mg/l]	16.0 (2.60 - 59.0)	24.0 (8.75 - 74.5)	7.55 (2.50 - 26.0)	§§§
Neutrophils [%]	76.0 (63.0 - 85.3)	82.0 (73.0 - 90.00)	64.5 (55.8 - 73.5)	§§§
NLR	5.85 (2.87 - 12.29)	9.11 (4.63 - 18)	2.98 (2 - 5.42)	§§§
Leucocytes [g/l]	10.5 (8.16 -13.4)	11.2 (8.53 -14.7)	9.38 (7.66 - 11.8)	§§§
Procalcitonin [µg/l]	0.05 (0.05 - 0.20)	0.10 (0.05 - 0.20)	0.05 (0.05 - 0.05)	§
Fibrinogen [g/l]	5.00 (3.88 - 6.53)	5.14 (3.90 - 14.0)	4.60 (3.50 - 5.90)	§

	< 100 [eos/µl]	**100-300 [eos/µl]**	**> 300 [eos/µl]**	**p-value**
n	114	76	53	
CRP [mg/l]	25.0 (11.0 - 80.8)	9.00 (2.50 - 45.3)	7.55 (2.50 - 24.3)	*** ###
Neutrophils [%]	83.0 (74.0 - 91.0)	70.0 (60.0 - 78.0)	61.0 (54.5 - 74.5)	*** ### $
NLR	9.84 (5.6 - 22.75)	3.94 (2.3 - 6.72)	2.83 (2 - 5.85)	*** ###
Leucocytes [g/l]	10.6 (8.05 -15.5)	10.7 (8.37 - 12.5)	9.98 (7.99 - 13.9)	n.s.
Procalcitonin [µg/l]	0.10 (0.05 - 0.28)	0.05 (0.05 - 0.10)	0.05 (0.05 - 0.05)	#
Fibrinogen [g/l]	5.14 (4.12 - 6.90)	5.00 (3.48 - 6.43)	4.20 (3.50 - 5.30)	*** #

Tables 8.1 and 8.2 - Inflammatory markers in cases of patients without radiological signs of pneumonia

Tables 8.1 and 8.2 display the subgroup analyses of inflammatory markers according to the blood eosinophils in patients without any signs of pneumonia. Continuous parameters are displayed as Median and (IQR); § significant between < 2% and ≥ 2%, * significant between < 100 and 100-300; # significant between < 100 and > 300; $ significant between 100-300 and > 300; n= 319 for CRP, n=322 for neutrophils, n=321 for NLR, n=322 for leucocytes, n=86 for Procalcitonin, n=234 for Fibrinogen.

	All	**< 2 [%]**	**≥ 2 [%]**	**p-value**
n	322	206	116	
CRP [mg/l]	16.0 (4.95 - 60.3)	22.7 (8.00 - 65.9)	9.00 (2.50 - 42.2)	§§§
Neutrophils [%]	76.0 (66.8 - 86.0)	82.0 (74.0 - 89.0)	66.5 (57.0 - 75.8)	§§§
NLR	6.33 (3.34 - 12.71)	9 (5.07 - 17.7)	3.62 (2.07 - 5.48)	§§§
Leucocytes [g/l]	10.9 (8.16 - 14.4)	12.1 (8.67 - 15.3)	9.37 (7.67 - 11.9)	§§§
Procalcitonin [µg/l]	0.05 (0.05 - 0.20)	0.05 (0.05 - 0.20)	0.05 (0.05 - 0.15)	n.s.
Fibrinogen [g/l]	4.80 (3.88 - 6.33)	4.90 (3.90 - 6.65)	4.55 (3.65 - 5.98)	n.s.

	< 100 [eos/µl]	**100-300 [eos/µl]**	**> 300 [eos/µl]**	**p-value**
n	163	102	57	
CRP [mg/l]	25.0 (10.0 - 67.5)	13.0 (2.50 - 47.0)	7.10 (2.50 - 32.0)	** ###
Neutrophils [%]	82.0 (75.0 - 90.0)	73.0 (64.0 - 79.3)	63.0 (55.5 - 76.0)	*** ### $$
NLR	9.56 (5.5 - 22)	4.63 (2.91 - 7.09)	3 (2 - 7.09)	*** ###
Leucocytes [g/l]	11.3 (8.16 - 15.5)	11.2 (8.33 - 13.3)	9.70 (7.79 - 14.5)	n.s.
Procalcitonin [µg/l]	0.10 (0.05 - 0.20)	0.10 (0.05 - 0.20)	0.05 (0.05 - 0.05)	#
Fibrinogen [g/l]	4.90 (4.00 - 6.50)	4.95 (3.65 - 6.48)	4.20 (3.50 - 5.40)	n.s.

Tables 9.1 and 9.2 – Inflammatory markers in cases of patients with corticosteroids

Tables 9.1 and 9.2 display the inflammatory markers in cases of patients with corticosteroids and only include cases in which patients had received systemic corticosteroids according to their medical records either before and/or during hospital admission. Continuous parameters are displayed as Median and (IQR); § significant between < 2% and ≥ 2%, * significant between < 100 and 100-300; # significant between < 100 and > 300; $ significant between 100-300 and > 300; n=367 for CRP, n=371 for neutrophils, n=370 for NLR, n=371 for leucocytes, n=145 for Procalcitonin, n=361 for Fibrinogen.

	All	< 2 [%]	≥ 2 [%]	p-value
n	371	250	121	
CRP [mg/l]	17.1 (6.00 – 63.0)	27.0 (10.0 – 77.9)	9.50 (2.50 – 25.0)	§§§
Neutrophils [%]	79.0 (67.0 – 88.0)	83.0 (75.0 - 90.0)	65.0 (56.5 - 76.0)	§§§
NLR	7.36 (3.4 - 15.73)	10.13 (5.58 - 22)	3.33 (2.04 - 6.3)	§§§
Leucocytes [g/l]	11.3 (8.46 – 14.8)	12.5 (9.02 -15.7)	9.59 (7.93 – 12.3)	§§§
Procalcitonin [µg/l]	0.05 (0.05 - 0.20)	0.10 (0.05 - 0.23)	0.05 (0.05 - 0.10)	§
Fibrinogen [g/l]	5.00 (3.90 - 6.45)	5.10 (4.00 – 6.90)	4.60 (3.50 – 5.90)	§

	< 100 [eos/µl]	100-300 [eos/µl]	> 300 [eos/µl]	p-value
n	195	112	64	
CRP [mg/l]	28.0 (11.2 – 80.5)	14.0 (2.50 - 50.0)	8.00 (2.50 –22.0)	*** ###
Neutrophils [%]	85.0 (76.0 - 91.0)	73.0 (64.0 - 81.0)	62.5 (55.3 –75.5)	*** ### $$
NLR	11.38 (6.33 - 23.25)	4.69 (2.63 - 8.87)	3.14 (2 - 7.7)	*** ###
Leucocytes [g/l]	12.0 (8.36 -16.0)	11.5 (9.27 – 13.5)	10.0 (8.00 -14.5)	n.s.
Procalcitonin [µg/l]	0.10 (0.05 - 0.30)	0.05 (0.05 - 0.20)	0.05 (0.05 - 0.05)	#
Fibrinogen [g/l]	5.14 (4.10 - 6.85)	5.00 (3.80 - 6.43)	4.35 (3.50 –5.60)	#

Case Mix Index

A significant difference could be established between the Case Mix Index and the different strata of eosinophil blood counts. The analysis showed that patients with a lower relative eosinophilic count recorded a higher median CMI than those with a relative eosinophilic count ≥ 2% (< 2%: 0.91 vs. ≥ 2%: 0.74, $p < 0.001$). The same applied to the absolute eosinophilic counts and the particular thresholds (< 100/µl: 0.91 vs. 100-300/µl: 0.74, $p < 0.05$; 100-300/µl: 0.74 vs. > 300: 0.74, n.s.; < 100/µl: 0.91 vs. > 300/µl: 0.74, $p < 0.01$).

Longitudinal analyses

Overall 72.66% of the 417 included exacerbated patients experienced a re-hospitalization to our hospital (median follow-up of 254 days) while 28.78% died during follow-up in our hospital (median follow-up of 1469 days). Time to hospital readmission (figure 3 a) and b)) or time to death (in-hospital-mortality only, figure 3 c) and d)) did not differ between the groups. Repeating the analysis in the above defined subgroups did not alter the results (data not shown).

Figure 3 – Rehospitalization and survival rates according to the blood eosinophils at the day of admission

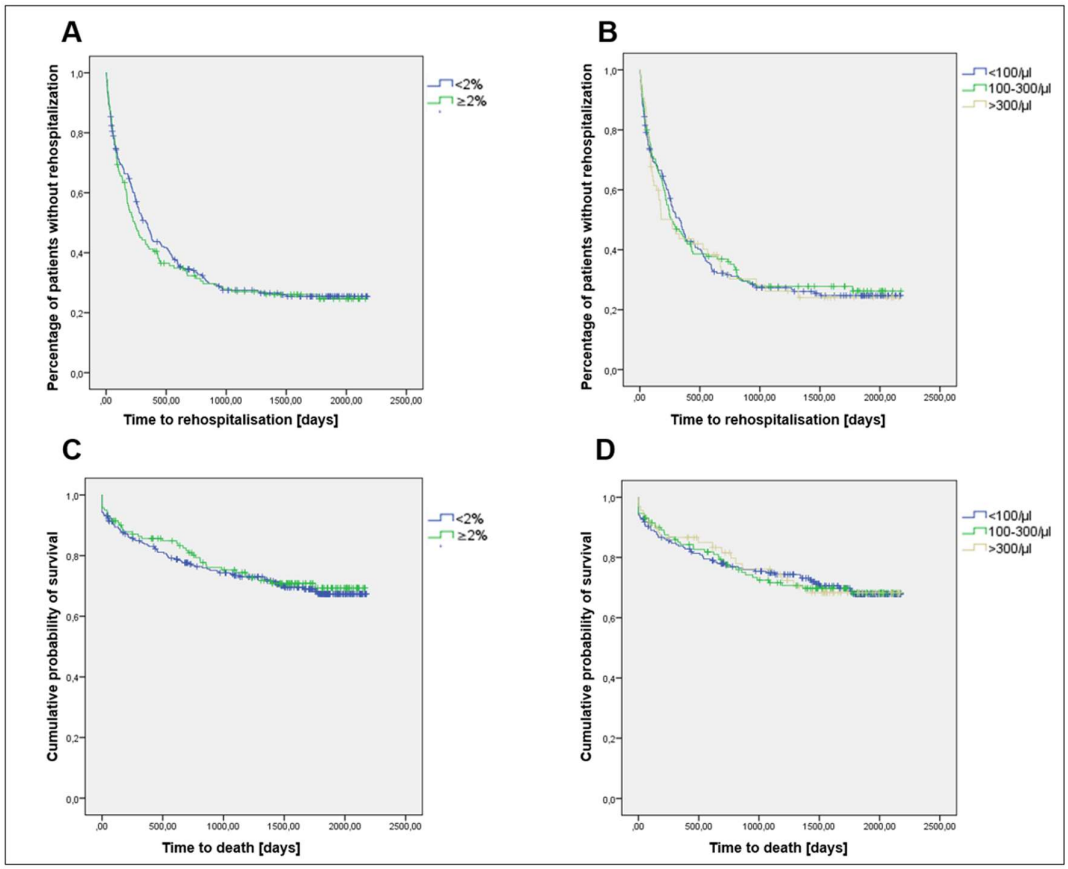

Figure 3: Time to re-hospitalization (A and B) or death (C and D)) according to the blood eosinophils at the day of admission. No significant differences were detected (Log-Rank Mantel-Cox) between the groups.

Of all 417 cases of patients with a full cell blood count at the day of admission, we observed the annual exacerbation rate until the date of death or endpoint of this study (31.12.2018) according to the levels of blood eosinophil counts. This calculation only included severe hospitalized exacerbations. In both relative and absolute strata, the differences in median exacerbation rate were not significant (< 2%: 0.27 vs. ≥ 2%: 0.25 annual exacerbations in the relative and < 100/µl: 0.27 vs. 100-300/µl: 0.26 vs. > 300/µl: 0.29 in the absolute strata).

Additional subgroup analyses

Hospital length of stay was compared between a) patients with low numbers of blood eosinophils (< 2%) and high levels of CRP (≥ 30mg/dl) and b) patients with high numbers of blood eosinophils (≥ 2%) and low levels of CRP (< 30mg/dl). This subgroup consisted of 232 subjects, out of which 132 belonged to group a) and 101 to group b). Patients with low eosinophil blood counts and high levels of CRP exhibited longer hospital admission rates, defined as median value, than their respective control group (< 2% eos and ≥ 30mg/dl CRP: 9.94 days vs. ≥ 2% eos and < 30mg/dl CRP: 7 days, $p < 0.0001$).

Hospital readmission rates and time-to-death were not significant between the different strata, but a tendency was clearly visible. Patients with low eosinophil blood counts and high levels of CRP tended to be readmitted to hospital less often than their control group ($p=0.14$, Log Rank Mantel-Cox), whereas survival rates showed a trend towards the opposite: Survival among patients with high eosinophil blood counts and low CRP levels seemed to be better than in the other group ($p=0.21$, Log Rank Mantel-Cox) (figure 4, A and B).

Secondly, we compared the above-mentioned parameters between a) patients with blood eosinophils < 2% and NLR of ≥ 7 and b) patients with blood eosinophils ≥ 2% and NLR < 7. The number of patients included in this subgroup analysis was 293 (182 subjects in group a) and 111 in group b)).

Again, there was a significant difference in hospital length of stay between the two groups. Patients with low eosinophil blood counts and a high NLR exhibited a longer median time of hospital admission than their control group (< 2% eos and ≥ 7 NLR: 10.1 days vs. ≥ 2% eos and < 7 NLR: 7 days, $p < 0.001$).

Time to re-hospitalization was not significantly different between the two groups. Parallel to the latter subgroup, as can be seen in figure 4, patients with low eosinophil counts and a high NLR showed trends towards being readmitted to the hospital less often than patients with high eosinophilic counts and a NLR below 7 ($p=0.22$, Log Rank (Mantel-Cox) test). Time-to-death yielded similar trends as in the eos- and CRP-guided subgroup. However, the results were of no significance, either ($p=0.11$, Log Rank (Mantel-Cox) test).

There was a weak to moderate positive correlation between the inflammatory markers CRP and NLR (r=0.276, p < 0.001).

Figure 4 – Re-hospitalization and survival rates in cases of patients with low eos and high CRP/NLR or high eos and low CRP/NLR levels

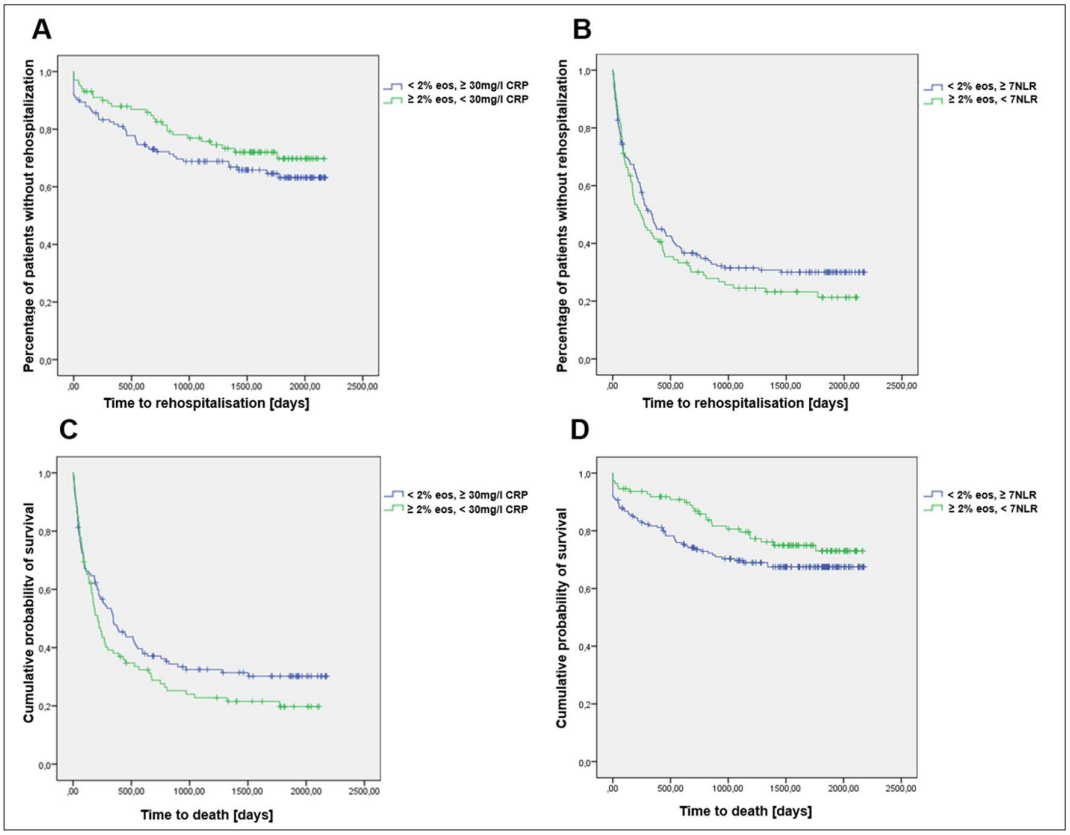

Figure 4: Time to re-hospitalization (A and B) or death (C and D)) according to the blood eosinophils at the day of admission and the levels of blood CRP or NLR. Significant differences could not be detected (Log-Rank Mantel-Cox).

5. Discussion

In our retrospective analysis, patients suffering from a non-eosinophilic exacerbation (< 2% or < 100/µl) stayed in the hospital for a longer period of time as compared to patients exhibiting a higher eosinophil count at the day of admission. These patients tended to demonstrate a stronger general inflammatory reaction, based on their levels and numbers of CRP, leucocytes, neutrophil fraction, NLR, PCT, and fibrinogen. The nature of the exacerbation was not relevant with regard to re-hospitalization (due to COPD) and/or death (in our hospital). Our real world data confirms and enhances existing data from clinical trials and other (mainly smaller) cohorts.

Eosinophil blood counts in COPD exacerbation

Comparing the percentage of eosinophilic exacerbations as well as clinical outcome parameters of our study to published results from other studies, at least four important manuscripts have to be taken into account. Bafadhel et al. analyzed 243 COPD subjects at presentation to hospital with an exacerbation that participated in a multi-center randomized controlled trial in Great Britain evaluating early rehabilitation (Bafadhel, Greening et al. 2016). Duman et al. performed a retrospective chart review in a tertiary teaching hospital, recruiting 1704 patients hospitalized with COPD exacerbation in a large hospital in Turkey (Duman, Aksoy et al. 2015). MacDonald et al. conducted two cohort studies (n=242 for the retrospectively collected derivation cohort, n=99 for the prospectively collected validation cohort) in patients hospitalized for an acute exacerbation of COPD in Melbourne (MacDonald, Osadnik et al. 2019). Ko et al. performed a prospective observational study, recruiting 346 exacerbated COPD patients in a tertiary hospital in Hong Kong with the objective of calculating different cut-off values of peripheral blood eosinophils for the assessment of hospital length of stay (Ko, Chan et al. 2020).

In the above-mentioned studies, the percentage of eosinophilic exacerbations was roughly comparable. It was around 20% (threshold: ≥ 2%) in the British and Turkish studies and 33.8% in our study. The same was true if absolute eosinophils were used to stratify the groups (> 300/µl defined as eosinophilic):

We found 16.3% to be eosinophilic while the colleagues in Melbourne found 25.2%. Only the Hong Kong study of Ko et al. recorded a higher proportion of subjects with an eosinophil count of ≥ 2% (43.4%). Taken together, eosinophilic exacerbations seem to range from one quarter up to almost one half regardless of the setting or continent in which the studies have been performed.

Regarding outcome parameters, again we find our results in line with the other observations: Although assessed with different methods (comparison of means, comparison of median values, Kaplan-Meyer analysis) in every study, the length of hospital stay was significantly longer in patients with low eosinophils as compared to patients experiencing an eosinophilic exacerbation.

In a prospective clinical cohort study of 2011, Bafadhel et al. described different clusters of exacerbations, leading to four different exacerbation phenotypes: bacterial, viral, eosinophilic and pauci-inflammatory. By investigating the immunopathogenesis, she came to the conclusion that COPD related exacerbations can be classified into their particular phenotype by the use of specific biomarkers (sputum IL-1ß, serum CXCL10 and peripheral eosinophils). Two of these phenotypes can be predicted from stable state (sputum IL-1ß for bacterial- and peripheral blood eosinophils for eosinophil-associated exacerbations) (Bafadhel, McKenna et al. 2011).

In relation to our research, the existence of specific exacerbation phenotypes that can be linked to different biological clusters in COPD exacerbations indicates an explanation to the strong variations in courses of disease in the respective groups of patients. This finding also provides a logical explanation for the differential responsiveness to ICS-treatment of COPD patients, dependent on the number of blood eosinophils (Pascoe, Locantore et al. 2015, Siddiqui, Guasconi et al. 2015, Greulich and Vogelmeier 2018). Stable COPD patients with high sputum and blood eosinophils, who received ICS-treatment, exhibit (as shown by various studies) a decrease in the number of exacerbations when compared to patients with low eosinophilic counts (Pascoe, Lipson et al. 2016, Ferguson, Tashkin et al. 2017, Pascoe, Barnes et al. 2019). As shown by Watz et al. in the WISDOM-trial, these patients also tend to be more sensitive to ICS-withdrawal from an

ICS/LABA/LAMA combination, recognizable by an increase in exacerbation rates (Watz, Tetzlaff et al. 2016).

In addition to the above-mentioned studies, in the CORTICO-COP trial, Sivapalan et al. investigated the eosinophil-guided therapy of severely exacerbated COPD patients with systemic corticosteroids in three university hospitals in Denmark. All 318 patients received one dose of 80mg methylprednisolone shortly after being admitted to the hospital. Subsequently from days 2 to 5, patients in the eosinophil-guided group were given corticosteroid treatment depending on their blood eosinophil counts (threshold ≥ 300/µl), whereas patients in the control group received daily corticosteroid treatment continuously throughout the treatment period. Using the primary endpoints of days alive and out of hospital within 14 days or treatment failure after 30 days, no difference between the eosinophil-guided and the control-group was found. Thus, Sivapalan et al. conclude that the omission of corticosteroids in exacerbated patients, whenever their blood eosinophil counts are below 300/µl, seems to be non-inferior compared with treatment according to the current GOLD guidelines (Singh, Agusti et al. 2019, Sivapalan, Lapperre et al. 2019, Global Initiative for Chronic Obstructive Lung Disease 2021, Venkatesan 2022).

Based on this data and the results of our study, one may speculate that the association between acute severe exacerbations with eosinophilic inflammation and a shorter length of hospital stay may be due to a rapid response to steroid treatment.

To further reinforce the generalizability of our results, we conducted subgroup analyses. By doing so, we aimed to exclude the most important avoidable influences on the relevant clinical parameters.

It is well recognized that systemic steroids work against eosinophilic inflammation and can lower the amount of eosinophilic blood cells (Meagher, Cousin et al. 1996, Prazma, Bel et al. 2019). In order to clear out any bias regarding the eosinophilic thresholds due to medically induced changes to the blood work, we analyzed a subgroup of patients, who – according to the medical records – did not receive systemic steroids before the full blood count was obtained. This analysis did not change our results.

In patients with low eosinophil blood counts, consolidation in the x-ray was found more often than in their respective control group (< 2%: 26.8% vs ≥ 2%: 15.6%). The thought stands to reason, that these patients may have just suffered from community-acquired pneumonia instead of a combination with an acute exacerbation of COPD, and one might consider that this could be the reason for the stronger inflammatory reaction. With regard to the sometimes challenging differential diagnosis between a bacterial exacerbation of COPD and a community-acquired pneumonia, both Bafadhel et al. and we tried to account for that by repeating our analysis after having excluded patients with signs of consolidation on chest x-ray (28% in Bafadhel's study and 20% in ours). This did not change our results. A similar outcome in hospital length of stay could be shown in both studies, whereas the difference was only significant in ours (this might be due to the number of cases included in the two studies (without pneumonia Bafadhel et. al. 174 vs. 322 in our study)) (Bafadhel, Greening et al. 2016).

In another subgroup analysis, we evaluated baseline characteristics, hospital length of stay and inflammatory markers in cases of patients that all received corticosteroid treatment either before and/or during hospital admission. This did not alter our data, either - patients with low eosinophil blood counts still exhibited a longer median length of stay at the hospital than their respective control group.

Inflammatory markers in COPD exacerbation

Regarding parameters of inflammation, we present similar results to the studies of Bafadhel, MacDonald and Duman. In all three studies, CRP values appeared to be higher in non-eosinophilic exacerbations. We confirm their findings that lower eosinophils are associated with higher levels of inflammation (Duman, Aksoy et al. 2015, Bafadhel, Greening et al. 2016, MacDonald, Osadnik et al. 2019). As the median PCT was higher in this group, this might reflect a more bacterial inflammation (Riedel 2012, Huang, Yealy et al. 2018).

We enhance the number of parameters by demonstrating that this is not only true for CRP and PCT, but also for neutrophils, NLR, leucocytes, and fibrinogen.

Besides the CRP blood level, one of the most conclusive inflammatory markers in our research was the Neutrophil-to-lymphocyte ratio, being significantly higher in non-eosinophilic patients. The NLR can be used as a diagnostic parameter for the assessment of a subclinical systemic inflammation (Al-Halawani, Kyung et al. 2020). As other studies have shown, increased levels of NLR can be associated with acute exacerbations of COPD (Lee, Lee et al. 2016, Farah, Ibrahim et al. 2017).

In a retrospective study of Lu et al., patients with acute severe COPD exacerbation were categorized in frequent and non-frequent exacerbators. Significantly higher levels of NLR were found in frequent exacerbators. These patients exhibited lower levels of absolute blood eosinophil counts, too. The NLR was positively correlated with a worse outcome (defined as invasive ventilation, admission to ICU or death) in frequent exacerbators (Lu, Chen et al. 2021).

Adding to this, in a systematic review of 22 studies, Gonzalez et al. reported higher levels of NLR in both stable and exacerbated COPD patients with low eosinophil blood counts (< 2%). They suggested the NLR as a marker for acute exacerbation. Their research included the previously mentioned study of Duman et al. and goes in line with the results of Aksoy and colleagues, who conducted a retrospective observational study with two groups of exacerbated COPD patients (eosinophilic and non-eosinophilic, cut-off at 2%, n=2727). Aksoy analyzed inflammatory markers between the two eosinophilic and non-eosinophilic groups and detected significantly higher CRP and NLR-levels in non-eosinophilic patients as well (both $p < 0.001$ at baseline) (Duman, Aksoy et al. 2015, Aksoy, Gungor et al. 2018, Pascual-Gonzalez, Lopez-Sanchez et al. 2018).

In our subgroup analyses the CRP, neutrophils, NLR and other inflammatory markers remained higher in non-eosinophilic patients, too. These results go in line with our findings in the additional subgroup analyses: The analysis of hospital length of stay according to a combination of low/high relative eosinophil blood counts and low/high parameters of inflammation (demonstrated by CRP and NLR-blood levels) resulted in an even more significant difference in duration of hospital administration than it did when patients were analyzed on the basis of their blood eosinophil counts only ($p < 0.001$ in eos- and CRP-guided subgroup,

$p < 0.001$ in eos- and NLR-guided subgroup vs. $p < 0.01$ in the analysis of LOS in patients with relative blood eos < 2%/ ≥ 2%).

Our findings support Duman's hypothesis that eosinophil blood counts in connection with low levels of CRP and NLR might mainly appear in accordance with non-infectious exacerbations (Duman, Aksoy et al. 2015).

The association of lower eosinophil blood counts with higher levels of inflammation, taken together with the longer hospital stay, once more supports the existence of different exacerbation phenotypes and the notion that a more non-eosinophilic phenotype (which may include bacterial or viral infections) may need a longer time to clinical improvement.

Deriving from the above mentioned, the possibility of a more targeted therapy should be taken into account as well. By using inflammation parameters to guide treatment decisions, antibiotic overuse could be prevented. In a controlled trial of 653 randomized subjects, Butler et al. analyzed CRP-guided antibiotic treatment in exacerbated COPD patients who consulted a general medical practice in England and Wales. In the CRP-guided group, a smaller amount of antibiotics was prescribed and the outcome, based on primary care consultation during six months of follow-up, was non-inferior in comparison to the usual-care group (Butler, Gillespie et al. 2019). Especially in ambulant settings, inflammation parameters such as the CRP are essential. They should remain an integral part within the decision-making process of treatment options of acute COPD exacerbations.

Besides the CRP, we think that the Neutrophil-to-lymphocyte is becoming increasingly important, since it is cheap and easy to determine as part of a complete blood cell count. Whether it has the potential as a biomarker for a more bacterial type of exacerbation, or whether it could play a role in the prediction of long-term risk of COPD and which is the most diagnostically conclusive cut-off value, still needs to be further investigated.

Case Mix Index

A high CMI was associated with low eosinophil blood counts, whereas the opposite applied to patients with high numbers of eosinophils. These findings go along with the previously discussed results (longer LOS and higher level of inflammation in patients with low eosinophil blood counts) and support our hypothesis of a more rapid response to corticosteroid treatment in patients with eosinophilic exacerbations. However, the possibility has to be taken into account that a high disease severity itself, reflected by a high CMI, might also be the reason for the strongly varying courses of disease in patients with acute exacerbations of COPD. Due to the retrospective nature of this study, we do not see a way to further examine this matter.

Long term outcome

With regard to long-term outcome Duman and colleagues recorded no significant differences in terms of survival among the non-eosinophilic and eosinophilic patients, but higher hospital readmission rates in the non-eosinophilic group. In a cohort study of 133 COPD patients, Prudente et al. analyzed the connection between eosinophil blood counts and risk of death over a period of nine years. They identified an increased risk of death in patients with eosinophil blood counts below 2% and 150/µl. A similar result was obtained by MacDonald, who detected a lower 12 months survival in patients with eosinophil blood counts < 150/µl (Duman, Aksoy et al. 2015, MacDonald, Osadnik et al. 2019, Prudente, Ferrari et al. 2021) .

In our analysis, we did not find any correlation between exacerbation phenotype and re-hospitalization or death based on the level of eosinophil blood counts. This confirms the results of Bafadhel and Ko but contradicts what has been found by Duman, Prudente and MacDonald (Duman, Aksoy et al. 2015, Bafadhel, Greening et al. 2016, MacDonald, Osadnik et al. 2019, Ko, Chan et al. 2020, Prudente, Ferrari et al. 2021).

A number of explanations are possible: As our study is based on chart reviews only and no formal assessment of survival has been accomplished, we can only

report re-hospitalization and/or death that occurred in our hospital. Furthermore, long- term health outcomes in COPD may be more closely related to disease severity, number of exacerbations, or comorbidities (Soler-Cataluna, Martinez-Garcia et al. 2005, Celli, Cote et al. 2008, Divo, Cote et al. 2012, Agusti, Edwards et al. 2013).

The impact of inflammatory parameters in eosinophilic and non-eosinophilic patients on long-term outcome has been investigated in several studies: In the study of Duman et al., non-eosinophilic patients exhibited higher levels of CRP and NLR. Also, these parameters were found to be associated with higher readmission rates and worse survival during the 6-month follow-up period (Duman, Aksoy et al. 2015). Regarding long-term outcome, various cohort studies have shown an elevated NLR in COPD patients at stable phase to be associated with a higher risk of future exacerbations in the respective period of follow up (between 1 and 3 years) (Lee, Lee et al. 2016, Sakurai, Chubachi et al. 2018, Ellingsen, Janson et al. 2021).

In the additional subgroup analysis, we did not detect a significant connection between re-hospitalization and different kinds of exacerbations, being categorized by the eosinophil blood count and level of CRP/NLR. Nevertheless, patients with high eosinophil blood counts and low inflammatory levels clearly tended to being readmitted to the hospital more often than their respective control groups. Time-to-death analysis in these subgroups was not significant, either, but the results showed trends towards the opposite (patients with low eosinophil counts and high NLR levels tended to exhibit greater mortality rates than their control group).

One could argue that patients of our additional subgroup analysis should have been divided into four different groups, instead of two, each group reflecting one combination of high/low eos and high/low inflammation markers. On the basis of these markers, only two combinations (patients with low eos and high inflammation markers and patients with high eos and low inflammation markers) can logically be linked to two of the exacerbation phenotypes described by Bafadhel (eosinophil and neutrophil phenotype) (Bafadhel, McKenna et al. 2011). For reasons of clarification, we remained with these two groups in our analysis.

Based on the results of our analysis, we cannot make a definite statement about the impact of eosinophil blood levels and/ or bacterial inflammation parameters on long term outcome. We think that both eosinophil blood counts, as well as bacterial inflammation parameters may play an important role in the estimation of long-term risk factors in patients with COPD in the future. Due to the retrospective design of our study, we were unable to further investigate this matter. In order to gain better knowledge of what influences the course of disease in COPD, observational studies with a systematical assessment of the exacerbation phenotype are needed.

Lung function in COPD exacerbation

A new finding of the current study is that lung function was worse in patients with a non-eosinophilic exacerbation (significant only in subgroup analysis). In two prospective clinical studies, Crisafulli et al. analyzed clinical predictors of a) treatment failure within 7 days and b) prolonged hospital stay in patients with severe exacerbations of COPD. In the first study they found that treatment failure was associated with worse lung function, while in the second study there was no difference in lung function between patients with a hospital stay ≤ 7 and > 7 days (Crisafulli, Torres et al. 2016, Crisafulli, Ielpo et al. 2018).

Furthermore, in a retrospective study, Tang et al. investigated the relationship of blood eosinophilia with pulmonary function parameters in 247 exacerbated COPD patients. They also found that patients with high levels of blood eosinophil counts (≥ 2% eos) had better lung function than patients in the non-eosinophilic group (< 2% eos) (Tang, Huang et al. 2020). This goes in line with the already mentioned study of Ko et al., who detected an improvement of FEV_1 in patients with eosinophil blood counts ≥ 2% after 12 months, when compared to the spirometry records at baseline (Ko, Chan et al. 2020).

Since we did not differentiate whether spirometry was performed in the beginning of admission or towards the end of the hospital stay (after treatment response), we could not systematically assess whether lung function was a confounding factor or a treatment result. As our data are in line with other studies, we think both explanations may be possible.

Limitations of this study

There are a number of limitations to our study: Firstly, the retrospective study design limits the interpretation of our data. Secondly, no standardized discharge criteria have been used. Thirdly, we could not assess re-hospitalization or mortality that occurred outside our hospital. Fourthly, of the 1007 cases of patients included in this research, 590 cases had to be excluded, because a full cell blood count was not obtained at the day of admission. Since there is no standardized protocol for the sampling of a complete cell blood count in exacerbated patients admitted to the hospital, the possibility has to be mentioned that patients with a missing CBC might have had fewer symptoms relating to a less severe exacerbation. In a recently published multicenter, cross-sectional, epidemiological study, Miravitles et al. analyzed determinants of blood eosinophil levels between COPD and non-COPD subjects. Mainly mild forms of COPD were included in this study and no association between blood eosinophil counts and frequency of exacerbation could be determined (Miravitlles, Soler-Cataluña et al. 2022). Fifthly, although recommended by guidelines and hospital standard operating procedures, in some cases systemic steroids may not have been administered. However, repeating the analysis without cases in which systemic steroid treatment had not been documented did not change the results significantly. Sixthly, we did not assess the exacerbation phenotype systematically.

Also taking these limitations into account, the present study adds to the growing body of evidence that blood eosinophils may serve as a biomarker not only for ICS-responsiveness with regard to the prevention of exacerbations but also for responsiveness towards systemic steroids during an acute exacerbation of COPD. Furthermore, the use of eosinophil blood counts as a biomarker for steroid treatment might be an important instrument in the prevention of corticosteroid overuse in the future. As shown by Sivapalan et al., it will potentially lead to a decline in the administration of steroids and consequently result in a reduction of both long- and short-term side effects (Sivapalan, Lapperre et al. 2019).

Overall, we enhance the generalizability of pre-existing data by introducing real-world data (as compared to RCT data) from a high-standard Western European

country (as compared to the other available studies) (Duman, Aksoy et al. 2015, Bafadhel, Greening et al. 2016, MacDonald, Osadnik et al. 2019, Ko, Chan et al. 2020).

Conclusion

In summary, our data supports the hypothesis that patients with low eosinophil blood counts may be less responsive to systemic corticosteroids when compared to patients with high levels of eosinophils. This may translate into a longer hospital length of stay. Thus, the data adds to the growing body of evidence that blood eosinophils may serve as a biomarker not only for inhaled corticosteroid-responsiveness with regard to the prevention of exacerbations but also for responsiveness towards systemic steroids during an acute exacerbation of chronic obstructive pulmonary disease.

In addition, our data supports the existence of different exacerbation phenotypes during an acute exacerbation of COPD. The association of lower eosinophil blood counts with higher levels of inflammation, taken together with the longer hospital stay, once more supports the notion that a more non-eosinophilic phenotype (which may include bacterial or viral infections) may need a longer time to clinical improvement.

6.1 Summary (English)

Morbidity and mortality of chronic obstructive pulmonary disease is associated with severe exacerbations. In severely exacerbated patients, the courses of disease are strongly varying. This might be due to the existence of different exacerbation phenotypes and their respective respond to the chosen treatment.

As a marker for the responsiveness to inhaled corticosteroids, current recommendations emphasize blood eosinophil cell counts. For the treatment of moderate to severe exacerbations, the administration of systemic steroids is recommended. Analyses from randomized clinical trials indicate a favorable response to systemic corticosteroids in exacerbated patients with blood eosinophils >2%, however data outside clinical trials are scarce.

We retrospectively evaluated the association between baseline eosinophil blood count and both short- and long-term clinical outcomes, as well as different inflammatory parameters and markers of disease severity in patients hospitalized due to an exacerbation of their underlying chronic obstructive pulmonary disease.

We evaluated data from 1007 cases of patients who were admitted to the University Medical Center Marburg between 01/2013 and 12/2018. All patients had been diagnosed with an acute exacerbation of chronic obstructive pulmonary disease. Patients were predominantly male (65%), had a median age of 74 years and a median forced expiratory volume in one second of 1.03l (42.6% predicted). Our analysis was based on a subgroup of 417 patients in whom a full blood cell count was obtained at the day of admission. We compared the hospital length of stay, inflammatory parameters and long-term outcome using established thresholds for the eosinophil blood cell count (100 and 300/µl and 2%). For patients that were re-hospitalized or died in our hospital during the observational time period, Kaplan-Meier curves were used to evaluate the long-term outcome. Patients with low eosinophils (< 2%, < 100/µl) had a longer median time in hospital (length of hospital stay) as compared to patients with high eosinophils (< 2%: 9.31 vs. ≥ 2%: 7 days, and < 100/µl: 10 vs. 100-300/µl: 8 vs. > 300/µl: 7 days). The median C-reactive protein and more inflammatory markers (Procalcitonin, Neutrophil-to-lymphocyte ratio, neutrophils, leucocytes and fibrinogen) were higher in patients with low eosinophils as compared to the other

groups (< 2%: 22.7 vs. ≥ 2%: 9 mg/dl and < 100/µl: 25 vs. 100-300/µl: 13.5 vs. > 300/µl: 7.1 mg/dl). Time to re-hospitalization or time to death did not differ between the strata of eosinophils.

To further reinforce the generalizability of our results and to prevent avoidable influences, we did three subgroup analyses, excluding a) patients that had received systemic steroids before blood collection, b) patients with radiological signs of pneumonia and c) patients who did not receive any systemic steroids either before their hospital stay and/or during admission. These restricted analyses did not alter the results significantly. In an additional subgroup analysis, we compared both short- and long-term outcome between patients with low eosinophil blood counts and high parameters of inflammation (demonstrated by C-reactive protein and Neutrophil-to-lymphocyte ratio) and those with high eosinophils and low parameters of inflammation. Hospital length of stay was significantly higher in patients with low eosinophils and high C-reactive protein/ Neutrophil-to-lymphocyte ratio. Time to re-hospitalization and time to death did not differ significantly among the groups.

The shorter length of hospital stay and the lower levels of inflammation in eosinophilic patients can be interpreted as an indication for the existence of different exacerbation phenotypes. This could be an explanation for the better responsiveness to steroid treatment in eosinophilic patients and the notion that a more non-eosinophilic phenotype (which may include bacterial or viral infections) may need a longer time to clinical improvement.

Our data confirms the results of other clinical studies and add to the growing body of evidence that blood eosinophils may serve as a biomarker not only for inhaled corticosteroid-responsiveness with regard to the prevention of exacerbations but also for responsiveness towards systemic steroids during an acute exacerbation of chronic obstructive pulmonary disease.

6.2 Summary (German)

Das Auftreten akuter Exazerbationen im Rahmen der chronisch obstruktiven Lungenerkrankung ist mit einer Erhöhung der Morbidität und Mortalität assoziiert. Entscheidend für den Schweregrad und Verlauf der Exazerbation ist das Ansprechen auf die medikamentöse Therapie, welche bei moderaten und schweren Exazerbationen leitliniengerecht in Form von systemischen Steroiden erfolgen soll. Aus Analysen anderer klinischer Studien geht hervor, dass an chronisch obstruktiver Lungenerkrankung leidende Patienten während einer Exazerbation besser auf systemische Kortikosteroide ansprechen, wenn ihre Eosinophilenzahl im peripheren Blut > 2% beträgt. Aktuelle Leitlinien empfehlen die Anwendung der Eosinophilenzahl als Biomarker für das Ansprechen auf inhalative Kortikosteroide.

Die vorliegende Statistik beruht auf einer retrospektiven Datenanalyse von 1007 Fällen von Patienten, die im Zeitraum von 01/2013 bis 12/2018 im Universitätsklinikum Marburg behandelt worden sind. Es wurden all diejenigen Patienten in die Analyse mit eingeschlossen, welche die ICD-10-Diagnose einer akuten Exazerbation der chronisch obstruktiven Lungenerkrankung erhalten hatten. Das klinische Ergebnis der exazerbierten Patienten mit hohen Eosinophilenwerten wurde dem der Patienten mit niedrigen Eosinophilenwerten gegenübergestellt: Hierbei verglichen wir den Zusammenhang der Blut-Eosinophilen mit der Verweildauer im Krankenhaus und mit unterschiedlichen Entzündungsmarkern. Zur Beurteilung von Langzeitauswirkungen wurden Kaplan-Meier-Überlebenszeitanalysen (Zeit bis zur Rehospitalisierung und zum Tod der Patienten) durchgeführt. Wir zogen hierfür unterschiedliche, in der Literatur bereits verwendete, Grenzwerte für die Zahl der Eosinophilen im Blut heran (100 und 300/µl und 2%).

Die Patienten waren überwiegend männlich (65%), hatten ein medianes Alter von 74 Jahren und eine mediane Einsekundenkapazität von 1.03l (42.6% des Sollwerts). Die Analyse selbst bezieht sich auf 417 Fälle von Patienten, bei denen zu Beginn des Krankenhausaufenthalts ein Differentialblutbild abgenommen wurde.

Patienten mit wenig Eosinophilen im peripheren Blut (< 2%, < 100/µl) verbrachten im Median eine längere Zeit im Krankenhaus als Patienten mit einer hohen Zahl an Eosinophilen (> 2%, > 300/µl). Dies gilt sowohl für die Relativ- als auch für die Absolutwerte (< 2%: 9.31 vs. ≥ 2%: 7 Tage und <100/µl: 10 vs. 100-300/µl: 8 vs. >300/µl: 7 Tage). Der mediane Wert des C-reaktiven Proteins, sowie weitere inflammatorische Marker (Procalcitonin, Neutrophilen-Lymphozyten-Ratio, Neutrophile, Leukozyten und Fibrinogen) waren bei Patienten mit niedriger Eosinophilenzahl im Vergleich zur anderen Gruppe signifikant erhöht (< 2%: 22.7 vs. ≥ 2%: 9 mg/dl und < 100/µl: 25 vs. 100-300/µl: 13.5 vs. > 300/µl: 7.1 mg/dl). In Bezug auf die Zeit bis zur Rehospitalisierung und das Überleben waren keine signifikanten Unterschiede zwischen den jeweiligen Gruppen nachzuweisen.

Zur besseren Generalisierbarkeit der Ergebnisse wurden weitere Subgruppenanalysen durchgeführt, in denen erstens Patienten, die bereits vor der ersten Blutentnahme Kortison bekommen hatten, zweitens, Patienten mit radiologischen Zeichen einer Pneumonie und drittens, Patienten, die gar kein Kortison erhalten hatten, ausgeschlossen wurden. Die Auswertung der jeweiligen Subgruppenanalysen führte zu keiner signifikanten Veränderung der Ergebnisse. In einer zusätzlichen Analyse wurden Kurz- und Langzeitergebnisse von Patienten mit niedrigen Eosinophilen und hohen Entzündungswrten (dargestellt anhand von C-reaktivem Protein und Neutrophilen-Lymphozyten-Ratio) und jenen mit hohen Eosinophilen und niedrigen Entzündungswerten verglichen. Die stationäre Verweildauer war bei Patienten mit niedrigen Eosinophilen und hohem C-reaktivem Protein/ Neutrophilen-Lymphozyten-Ratio signifikant erhöht, wohingegen die Langzeitergebnisse keine signifikanten Unterschiede zeigten.

Die kürzere Krankenhausverweildauer und die geringeren Entzündungswerte unter schwer exazerbierten Patienten mit chronisch obstruktiver Lungenerkrankung und hohen Werten von Blut-Eosinophilen (> 2% oder > 300/µl) kann sowohl als Hinweis für ein besseres Ansprechen auf systemische Steroide als auch für das Existieren spezifischer Exazerbationstypen gedeutet werden. Unsere Ergebnisse bestätigen die Daten anderer klinischer Studien und sind damit ein wichtiges Kriterium für die Anwendbarkeit der Blut-Eosinophilen als Biomarker für das Ansprechen auf systemische Steroide auch im klinischen Alltag.

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8.1 List of academic teachers

Meine akademischen Lehrenden waren in Marburg:

Vorklinischer Studienabschnitt:

Basler, Baranovski, Bauer, Bette, Bertoune, Braun, Brehm, Bonaterra, Cetin, Daut, Decher, del Rey, Eickmann, Feuser, Grundmann, Hildebrandt, Hobiger, Koolman, Lill, Löffler, Mey, Milani, Mueller, Neumüller, Oberwinkler, Oliver, Preisig-Müller, Reese, Rost, Röhm, Rust, Schäfer, Schütz, Schwarz, Seitz, Steiniger, Schratt, Suske, Thieme, Weihe, Wertenbruch, Westermann, Westermann, Wilhelm, Wrocklage

Klinischer Studienabschnitt:

Aigner, Al-Fakhri, Arenz, Barth, Bartsch, Bauer, Baum, Baumann, Becker, Becker, Bender, Best, Bien, Bliemel, Bohlander, Bösner, Burchert, Carl, Czubayko, Damanakis, Dettmeyer, Divchev, Donner-Banzhoff, Duda, Ehlenz, Eming, Eschbach, Fendrich, Frink, Fritz, Fuchs-Winkelmann, Gebhardt, Geks, Geraedts, Görg, Gress, Greulich, Grikscheit, Grimm, Grosse, Grzeschik, Hertl, Hoch, Höffken, Hofmann, Holland, Holzer, Hoyer, Jansen, Jerrentrup, Josephs, Kampmann, Kann, Keber, Kill, Kirschbaum, Klemmer, Klose, Knipper, Koczulla, Köhler, König, Kühnert, Lohoff, Lüsebrink, Mahnken, Maier, Maisner, Maurer, Menzler, Moll, Morin, Mossdorf, Müller, Mutters, Neubauer, Nimsky, Oberkircher, Oertel, Opitz, Pagenstecher, Parahuleva, Peterlein, Pfützner, Portig, Pöttgen, Plant, Rastan, Renke, Renz, Richter, Riera-Knorrenschild, Rothmund, Ruchholtz, Rüsch, Schäfer, Schieffer, Schmeck, Schmidt, Schneider, Schu, Seifert, Seitz, Sekundo, Sevinc, Sieveking, Sommer, Stuck, Strik, Tackenberg, Thum, Timmermann, Timmesfeld, Vogelmeier, Vogt, Vojnar, Wächter, Wagner, Werner, Wiesmann, Wissniowski, Wittig, Worzfeld, Wulf, Zavorotny, Zemlin, Ziller, Zimmer, Zwiorek

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