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# Significance of peripheral blood morphology with granulocyte distribution and spirometry in evaluation of asthma and COPD treatment

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**Abstract:**

**Background**

The two main chronic obstructive diseases are asthma – affecting 1-18% of adult population – and chronic obstructive pulmonary disease (COPD) prevalent in up to 6% of adults. In both cases the treatment depends on diseases' severity. In management of these conditions, spirometry and complete blood count are two major monitoring tests. Our aim was to compare blood morphology results and spirometry values between patients in groups of different treatment intensity in asthma and COPD. By measuring that, we expected to study whether asthma/COPD patients have a need of stepping-up their treatment steps.

**Methods**

A retrospective analysis of patients admitted in 2013-2019 to an outpatient pulmonology clinic in Łódź (Poland). Spirometry values, complete blood count and information on pharmacological treatment were obtained from archival data. Patients were assigned with disease severity according to present GINA/GOLD recommendations. The study included 125 patients – 47 with COPD (22 females) and 78 with asthma (57 females).

**Results**

Among patients with asthma, a positive correlation in white blood cell count (WBC) (r=0,236, p=0.038) and ascending GINA treatment steps was found. Significant negative correlations were shown between ascending GINA treatment steps and FEV1, FEV1%, FVC%, MEF50, MEF50, PEF%. In COPD patients, positive correlations between ascending GOLD treatment groups and white blood cell count, neutrophil count, basophil percentage, platelet count (r=0.346; 0.309; 0.321; 0.401 respectively) were found. Negative correlations were shown between ascending GOLD treatment groups and FEV1, FEV1%, FVC, FVC%, MEF50, MEF50%, PEF, PEF% (r=-0.732; -0.575; -0.705; -0.498; -0.632; -0.558; -0.688; -0.597 respectively).

**Conclusions**

Negative correlations between ascending GINA and GOLD treatment steps and spirometry values may suggest that asthma and COPD patients may benefit from stepping-up the treatment steps earlier. Potential advantages of that more intensive treatment needs to be examined in the future.

**Keywords: Asthma, COPD, blood morphology, eosinophils, spirometry, BMI**

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## 1. Introduction.

The two main chronic obstructive diseases are asthma and chronic obstructive pulmonary disease (COPD). Both diseases are mainly characterized by chronic inflammation of the respiratory tract. Although the two diseases share some similarities regarding their clinical features, the underlying pathomechanisms responsible for inflammation in either of the diseases are quite distinct [1].

Asthma is a heterogeneous pulmonary disease with a wide range of phenotypes. Current evidence and expert opinions indicate that it is not one simple disease with a common pathomechanism for all patients, but rather a set of phenotypes with a similar, but clearly different clinical picture and pathomechanism [2]. The range of differences in asthma phenotypes is due to: immunological factors (which is partially reflected in such terms as "allergic asthma" and "non-allergic asthma" ), patient-related (early-onset vs. late-onset asthma, obesity-associated asthma, smoking-associated asthma) and the disease severity itself. In allergic asthma (also called "extrinsic asthma" [3]) inhaled allergens bind to IgE receptors present on surface of mast cells. In response to that mast cells release broncho-constricting mediators such as histamine, cysteinyl-leukotrienes (Cys-LT) and prostaglandin D2 (PGD2). Dendritic cells release CCL17 and CCL22 chemokines in response to allergens, which attract Th-2 cells. Th-2 cells play a major role in modulation of immune response by releasing various cytokines, including IL-4 and IL-13 (responsible for Ig-E production by B-cells), IL-5 (crucial in eosinophilic inflammation) and IL-9 (which stimulates mast cells)[4]. Another approach is the characteristic of asthma as either eosinophilic (EA) or non-eosinophilic (NEA) [5], however these two subgroups do not translate into clinical phenotypes. Both EA and NEA can be caused by different underlying molecular mechanism and can lead to different outcomes. EA refers to phenotype of asthma, where significant number of eosinophils is present either in airway, sputum and/or blood, while NEA is characterized by low number of eosinophils, but still with dominant number of inflammatory cells, such as neutrophils or mixed granulocytes inflammatory cells. Reduction of eosinophilia can improve control of disease and reduce exacerbation risk in EA patients. IL-5 and IL-5R inhibitors – such as mepolizumab or benralizumab [6],[7] – can be used in patients with severe EA, with peripheral eosinophil counts over 300 cells/μL and non-responding to treatment. Additionally, blood or sputum eosinophilia is a marker of response to corticosteroids. NEA patients respond poorly to corticosteroids and anti-inflammatory drugs used in EA [8]. The underlying inflammatory process is a complex chain of cellular interactions, which lead to asthma symptoms: cough, wheezing, chest tightness and shortness of breath, which are resulting from bronchospasm and airway remodeling [9]. Regardless to the factors that trigger patient's asthma, the symptoms generally remain the same [10].

COPD is a disease of the lungs that affects mostly middle-aged or elderly patients and is one of the major global causes of death. According to the World Health Organization, it is now the third leading cause of death worldwide [11]. COPD is characterized by symptoms such as dyspnea and cough with or without sputum. The main risk factor of this disease is tobacco smoking, but air pollution or fuel exposure also seem to have an influence on evolution of the disease [12]. The exposure to the risk factors indicates inflammatory process in the lungs and airways. In COPD various cytokines are released from bronchial epithelial cells and macrophages in response to various inhalable irritants, mostly to cigarette smoke. Secretion of TGF-β by epithelial cells stimulates fibroblasts and leads to fibrosis of small airways. Macrophages release chemotactic factors, which attract monocytes, neutrophils, cytotoxic T-cells and Th-1 cells. Proteases released by neutrophils and macrophages leads to destruction of alveolar wall and to hypersecretion of mucus [4].Although eosinophilic inflammation is rather a characteristic of allergic asthma than of COPD, the Evaluation of COPD Longitudinally to Identify Predictive Surrogate (ECLIPSE) study has shown that 37,4% of patients with COPD have consistently elevated blood eosinophil count (>150 cells/μL) [13]. Evidence also suggest that the

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higher blood eosinophil count, the higher is the associated with exacerbation risk in patients with a pervious history of exacerbations. Furthermore, available evidence shows that high blood eosinophil count predicts response to inhaled corticosteroids (ICS) therapy in patients with COPD [14]. An important factor influencing asthma and COPD clinical course is the patient's body weight, particularly in obese patients. Studies show that in patients with asthma, increased body weight correlates with more frequent and severe exacerbations and with reduced response to corticosteroids [15]. Contrary to that, in patients with COPD obesity can reduce the risk of mortality [16]. However, as most studies define obesity as increased body mass index (BMI). other suggest that resisting on BMI is unreliable, mostly due to increased BMI in athletic patients [15]. Nevertheless, the BMI is acknowledged as an easy and effective measure of patient's body constitution.

Regardless of inflammatory phenotypes, both asthma and COPD patients are treated based on their clinical presentation. According to GINA 2020 guidelines there are 5 steps of asthma treatment , each with increasing intensity of treatment. The newest GINA report do not recommend using short-acting-beta agonists (SABA) as the only treatment due to higher risk of exacerbations and recommends usage of inhaled corticosteroids (ICS) + long-acting beta2agonist as the basis of asthma treatment. Patients with severe asthma on the 5$^{th}$ step of the treatment may benefit from biological treatment[17]. COPD patients are assigned to one of four groups (A-D), according to GOLD guidelines. Treatment in the GOLD 2020 recommendations include using long-acting-beta agonists (LABA)s, long-acting muscarinic antagonists (LAMAs), short-acting-beta agonists (SABAs), inhaled corticosteroids (ICS) and their combinations[12].

The aim of this study was to compare blood morphology results and spirometry values between patients in groups of different treatment intensity in asthma and COPD and assess the possible correlations between patients' Body Mass Index (BMI)and eosinophil blood count. By measuring that, we expected to study whether asthma/COPD patients have a need of stepping-up their treatment steps earlier.

## 2. Materials and Methods

The study was a retrospective analysis of clinical data of patients attending an outpatient pulmonology clinic in Łódź (Poland). Data was collected from clinic visits between 2013 and 2019.The study population included 119 patients (Table 1.) - 76 females and 43 males. Their mean age was 64.18±13.68 years.

| GINA/GOLD | GINA | | | | | GOLD | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | A | B | C | D |
| Patient count | 13 | 14 | 16 | 17 | 16 | 14 | 14 | 1 | 14 |

Table 1. Study group characteristics - patients with asthma and COPD assigned to GINA and GOLD recommendations.

The patients records were included in the study if they were diagnosed and treated for asthma or COPD during stable periods of their diseases during routine outpatient check-ups. History of recent (30-day) exacerbation of asthma/COPD excluded the patient from the study. Duration of the disease in both groups was minimum one year.

This was a retrospective analysis, in which for each study subject, past medical history laboratory test values and spirometry values were collected. The analysed laboratory test

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values were: red blood cell count, white blood cell count, haematocrit, haemoglobin concentration, neutrophil, eosinophil and basophil counts and percentage, platelet count.

Spirometry values collected were: forced expiratory volume in 1. second ($FEV_1$), $FEV_1$% predicted, forced vital capacity (FVC), FVC% predicted, maximal expiratory flow at 50% of vital capacity ($MEF_{50}$), $MEF_{50}$% predicted, maximal expiratory flow at 25% of vital capacity ($MEF_{25}$), $MEF_{25}$% predicted, maximal expiratory flow at 75% of vital capacity ($MEF_{75}$), $MEF_{75}$% predicted, peak expiratory flow (PEF), PEF% predicted, forced expiratory flow at 25-75% of forced vital capacity ($FEF_{25-75}$) and $FEF_{25-75}$% predicted.

Statistica 13.1 (TIBCO Software Inc., USA) was used to perform the statistical analysis. Shapiro – Wilk test was used to verify the normal distribution of data. Pearson correlation was calculated to estimate the correlations between various variables. A value of $p<0.05$ was considered statistically significant.

**3. Results**

The analysis of correlations in asthma patients showed a positive correlation in white blood cell count (WBC) (r=0,236, p=0.038) and ascending GINA treatment steps (the results are shown in Table 2.). Other whole blood count parameters did not correlate with ascending GINA treatment steps.

| Variable | WBC [g/L] | RBC [g/L] | Hb [g/dL] | HCT [%] | Neutrophils [%] | Eosinophils [%] | Basophils [%] | Neutrophils [g/L] | Eosinophils [g/L] | Basophils [g/L] | PLT [g/L] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GINA 1-5 | 0,2358 | 0,0706 | 0,0747 | 0,0606 | -0,0046 | 0,1797 | 0,0941 | 0,1610 | 0,1947 | 0,1817 | 0,1266 |
| p | p=0,038 | p=0,539 | p=0,516 | p=0,598 | p=0,968 | p=0,115 | p=0,412 | p=0,159 | p=0,088 | p=0,111 | p=0,269 |

Table 2. Correlations in asthma patients between WBC, RBC, Hb, HCT, Neutrophils, Eosinophils, Basophils, PLT and ascending GINA treatment steps.

Significant negative correlations were shown between ascending GINA treatment steps 1-5 and FEV1 volume, FEV1% of predicted value, FVC% of predicted value, MEF50 flow rate, MEF50% of predicted value, PEF% of predicted value( the results are shown in Table 3).

| Variable | $FEV_1$ [L] | $FEV_1$[%] | FVC [L] | FVC [%] | $MEF_{50}$[L/s] | $MEF_{50}$[%] | PEF[L/s] | PEF [%] |
|---|---|---|---|---|---|---|---|---|
| GINA 1-5 | -0,2566 | -0,5314 | -0,1329 | -0,4824 | -0,2410 | -0,2888 | -0,2142 | -0,3964 |
| P | p=0,023 | p=0,000 | p=0,246 | p=0,000 | p=0,034 | p=0,010 | p=0,060 | p=0,000 |

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Table 3. Correlations in asthma patients between $FEV_1$, FVC, $MEF_{50}$, PEF and ascending GINA treatment steps.

The analysis of correlations in COPD patients showed a positive correlation between ascending GOLD treatment groups and white blood cell count, neutrophil count, basophil percentage, platelet count (r=0.346; 0.309; 0.321; 0.401 respectively) (Table 4.).

| Variable | WBC [g/L] | RBC [g/L] | Hb [g/L] | HCT [%] | Neutrophils [%] | Eosinophils [%] | Basophils [%] | Neutrophils [g/L] | Eosinophils [g/L] | Basophils [g/L] | PLT [g/L] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GOLD A-D | 0,3458 | 0,1996 | 0,1220 | 0,1720 | 0,0362 | 0,1447 | 0,3208 | 0,3087 | 0,2549 | 0,0601 | 0,4012 |
| p | p=0,017 | p=0,179 | p=0,414 | p=0,248 | p=0,809 | p=0,332 | p=0,028 | p=0,035 | p=0,084 | p=0,688 | p=0,005 |

Table 4. Correlations in COPD patients between WBC, RBC, Hb, HCT, Neutrophils, Eosinophils, Basophils, PLT and GOLD treatment steps. Statistically significant with p<0,05.

Negative correlations were shown between ascending GOLD treatment groups (A-D) and FEV1 volume, FEV1% of predicted value, FVC volume, FVC% of predicted value, MEF50 flow rate, MEF50% of predicted value, PEF flow rate, PEF% of predicted value (r=-0.732; -0.575; -0.705; -0.498; -0.632; -0.558; -0.688; -0.597 respectively) (Table 5).

| Variable | $FEV_1$ [L] | $FEV_1$[%] | FVC [L] | FVC [%] | $MEF_{50}$[L/s] | $MEF_{50}$[%] | PEF[L/s] | PEF [%] |
|---|---|---|---|---|---|---|---|---|
| GOLD A-D | -0,7316 | -0,5748 | -0,7051 | -0,4982 | -0,6318 | -0,5579 | -0,6876 | -0,5971 |
| P | p=0,000 | p=0,000 | p=0,000 | p=0,000 | p=0,000 | p=0,000 | p=0,000 | p=0,000 |

Table 5. Correlations in COPD patients between $FEV_1$, FVC, $MEF_{50}$, PEF and ascending GOLD treatment steps

In both asthma and COPD patients there was no significant correlations between BMI and eosinophil count (Table 6).

| | Asthma | | COPD | |
|---|---|---|---|---|
| Variable | Eosinophil [G/L] | BMI | Eosinophil [G/L] | BMI |
| Eosinophil [G/L] | 1,0000 | -,2571 | 1,0000 | ,0422 |
| | p= --- | p=,023 | p= --- | p=,778 |
| BMI | -,2571 | 1,0000 | ,0422 | 1,0000 |
| | p=,023 | p= --- | p=,778 | p= --- |

Table 6. Correlations between asthma and COPD patients BMI and eosinophil count.

**4. Discussion**

In this study we used widely available tests suitable for both asthma and COPD, such whole blood count, spirometry, weight and height measurements. The limitations of the study include:1. Relatively small groups of patients and 2. only one patient in GOLD group C. However, the 2nd issue clearly refers to the small number of patients in this group worldwide (only 3,7% of COPD patients in 2017 [18]).

In this study we present that despite the more intensified treatment, patients on higher GINA treatment steps still present with worse spirometry values, such as FEV1 volume, FEV1% of predicted value, FVC volume, FVC% of predicted value, MEF50 flow rate, MEF50% of predicted value, PEF flow rate, PEF% of predicted value, than patients on lower GINA treatment steps.The similar observation is noticed in COPD patients, but patients on higher GOLD treatment steps present worse all measured in this study spirometry values, that is FEV1 volume, FEV1% of predicted value, FVC volume, FVC% of predicted value, MEF50 flow rate, MEF50% of predicted value, PEF flow rate, PEF% of predicted value, than patients on lower GOLD treatment steps. This may lead to a conclusion that patients can achieve good symptoms control despite low spirometry values and may somehow suggest that we should consider intensifying treatment earlier in some patients.

This study has shown a positive correlation between white blood cell count and severity of asthma and COPD. This is a confirmation of inflammatory process that takes place in airways in both asthma and COPD [1]. However, studies show that in COPD commonly we can observe neutrophilic inflammation and in asthma we can commonly observe eosinophilic inflammation. This study showed no significant correlation between peripheral eosinophil count and severity of asthma. There are conflicting studies on this subject, some showing an existing association between asthma severity and peripheral eosinophils [19], while some studies deny it [20].

In COPD patients higher GOLD group was associated with higher platelet count. This is important once acknowledging that thrombocytosis in COPD is associated with greater mortality after exacerbation [21]. Studies showed that antiplatelet therapy reduces all-cause mortality in COPD patients [22]. Further research is needed in order to acknowledge the role of thrombocytes in COPD. In COPD we also found a correlation between ascending GOLD treatment groups and percentage of basophils. Studies show that basophils can trigger emphysema in COPD patients [23]. This issue is a good starting point of further research of prevention on loss of pulmonary function.

This study showed no significant correlations between patients' BMI and eosinophil count in both asthma and COPD. This result is contrary to the studies that show the "obesity paradox" in COPD patients [16] and studies that show higher risk of exacerbations in obese asthma patients [15].

## Conclusions

Asthma and COPD, despite different pathomechanisms, are both characterized by chronic airways inflammation. In both cases spirometry test is essential for disease control. Negative correlations between ascending GINA and GOLD treatment steps and spirometry values may suggest that asthma and COPD patients may benefit from stepping-up the treatment steps earlier. Potential advantage of that more intensive treatment needs to be examined in the future. In relation to patients' BMI we cannot take significant conclusions, however the problem of obesity should be precisely examined in the future, because of opposing studies.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Conflicts of Interest:** The authors declare no conflict of interest in reference to this study

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