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Profiling blood hypereosinophilia in patients on dupilumab treatment for respiratory conditions: a real-life snapshot

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In relation to this work, authors declare no conflicts of interest.

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ABSTRACT

BACKGROUND

Transient and usually asymptomatic increase in blood eosinophil count (BEC) associated with dupilumab treatment has been described. Predicting factors related to BEC increase and symptoms occurrence are still poorly investigated.

OBJECTIVE

To investigate frequency, timing, duration, clinical relevance and potential predictors of BEC increase in a real-life multicentre cohort of patients affected by asthma and/or chronic rhinosinusitis with nasal polyps (CRSwNP) treated with dupilumab.

METHODS

BEC and clinical conditions at baseline and every 3 months after dupilumab treatment start were assessed. Any adverse drug reaction was also recorded. Remission of dupilumab-associated eosinophilia was defined by follow-up BEC values < 0.5 x10^9 cells/L.

RESULTS

Overall, 108 out of 195 (55%) patients experienced an increased BEC after dupilumab initiation but only 29 out of 195 (14.9%) showed hypereosinophilia. BEC peak occurred 6 months after the treatment start and resolved after 9 months (median time). Probability of developing hypereosinophilia was 3.3 times higher in patients with baseline BEC between 0.5 and 1.5 cells x 10^9L. Symptoms occurrence during BEC peak was higher in patients with comorbidities and in patients showing any increase of BEC.

CONCLUSIONS

In a real-life setting dupilumab treatment in asthma and/or CRSwNP patients was often associated with transient BEC increase but hypereosinophilia rarely occurred. Onset of symptoms co-occurring with BEC peak was observed in a minority of subjects. BEC should not preclude itself dupilumab initiation or continuation but deserves to be monitored for at least 8 months after the treatment start, particularly in the case of baseline eosinophilia/hypereosinophilia and/or comorbidities.

HIGHLIGHT BOX

What is already known about this topic?

Trials and real-life studies reported transient and usually asymptomatic increase of blood eosinophils count (BEC) in patients on dupilumab treatment.

What does this article add to our knowledge?

In patients with asthma and/or nasal polyps, increased baseline blood eosinophils count could predict hypereosinophilia occurrence. Multiple comorbidities and increase in eosinophils count after treatment initiation are associated with eosinophilia-associated symptoms.

How does this study impact current management guidelines

Our study highlights baseline patients' characteristics potentially associated with a higher risk of developing dupilumab-associated hypereosinophilia and related symptoms, and provides practical insights about BEC follow-up and timing of eosinophilia resolution (8 months).

Key words. asthma; CRSwNP; dupilumab; eosinophils; hypereosinophilia; real-life

Abbreviations

BEC	Blood eosinophils count
CRSwNP	Chronic rhinosinusitis with nasal polyps
RCT	Randomized Controlled Trial
EGPA	Eosinophilic granulomatosis with polyangiitis
HES	Hypereosinophilic syndrome
GERD	Gastro-oesophageal reflux disease
CRP	C Reactive protein
T2	Type 2
IQR	Interquartile range
OCS	Oral corticosteroids

INTRODUCTION

Dupilumab is a fully human monoclonal antibody selectively binding the alpha subunit of IL-4 / IL-13 receptor. As a result, the signalling of both cytokines is downregulated.[1] It's currently approved for several indications including atopic dermatitis, severe asthma, chronic rhinosinusitis with nasal polyps (CRSwNPs)[2], eosinophilic esophagitis, prurigo nodularis and chronic obstructive pulmonary disease .[2] In addition to the results of the randomized clinical trials (RCTs), [3–5] an increasing amount of real-word evidence supports its optimal efficacy profile in both asthma and CRSwNPs patients. [6–8]

In terms of safety, no major concerns have been raised so far, when considering both RCTs and real-life data, regardless the drug indication. [9,10] he most frequently reported adverse events are represented by injection site reactions, conjunctivitis and arthralgias; a not negligible proportion of patients experienced an increase in blood eosinophil count (BEC) over the treatment course. Although the issue is still controversial, the fluctuation of eosinophils in the blood stream might be related to dupilumab mechanism of action. In fact, as the drug prevents the eosinophilic tissue infiltration, a transitory peak of circulating cells could represent an expected consequence until the inflammatory processes are downregulated. The reason why only some individuals experience that effect has no univocal explanation so far. [11] According to published data, BEC increase is rare, transient, and usually not clinically relevant. [9,12] In addition, the occurrence of hypereosinophilic immunological disorders, including EGPA, in treated patients seems not to be more frequent when compared to other biologic treatments targeting T2 inflammation. [10,13]

So far, few real-life studies have addressed the issue as a primary focus, and the identification of potential predictors of eosinophils increase over dupilumab treatment is controversial. However, in the light of eosinophils relevance in the pathobiology of the conditions which represent the target for dupilumab use, the topic deserves to be widely explored.

The aim of our study was to evaluate the proportion of patients experiencing increase in BEC, with or without reaching the hypereosinophilia cut-off, over dupilumab treatment course and to investigate its frequency, timing, duration, clinical relevance and potential predictors in a real-life population affected by inflammatory conditions of the respiratory tract.

MATERIALS AND METHODS

We conducted a real-life multicentre prospective observational study involving thirteen Italian referral centres for severe asthma located across all the Country (Bari, Brescia, Cagliari, Catania, Genoa, Milan, Modena, Naples, Padua, Siena, Turin, Varese, Verona). Site related heterogeneity is minimized by the standardized approach in asthma diagnosis and management required by the national network all the participating centres are part of [14]. Biologic naïve adult patients prescribed with dupilumab for severe asthma with/without CRSwNP or CRSwNP and concomitant mild asthma consecutively referring to the participating centres between March and December 2022 were recruited. Dupilumab eligibility was established according to the current national regulatory indications. For severe asthma diagnosis ERS/ATS guidelines[15] and GINA recommendations[16] were considered; diagnosis of severe CRSwNP was confirmed according to EUFOREA/EPOS definition. [17] Patients receiving systemic corticosteroids in the 4 weeks preceding biologic initiation were not enrolled in order to exclude a potential determinant of biased BEC detection. Dupilumab schedule was established according to the regulatory indications (300 mg subcutaneously every two weeks).[2] For the purposes of the analysis and to prevent a low numerosity across categories, the diagnoses were divided into two macro-categories: asthma only and asthma plus polyposis/Samter's triad . Patients' characteristics, complete blood count including blood eosinophil count (BEC) and C reactive protein (CRP) were reported before treatment initiation, and every three months up to 30

months follow-up. In line with the currently accepted definitions, we classified as eosinophilia a BEC ranging from 0.5 to 1.5 cells x 10^9/L, as moderate hypereosinophilia a BEC ranging from 1.5 to 5.0 cells x 10^9/L, and as severe hypereosinophilia a BEC > 5.0 x 10^9/L.[18] Patient experiencing hypereosinophilia occurrence underwent more extensive blood sample assessment including inflammatory indexes, liver and renal function, troponin, coagulation profile, D-dimer, urine analysis at the time of onset, echocardiography and high resolution CT scan. In addition, the follow-up schedule was reduced to 4 weeks according to the clinical practice.

During the follow-up, timing and detailed value of eosinophilia first appearance as well as its maximum peak were registered, alongside with any change in clinical manifestations. Patients showing eosinophilia at baseline without further increase over the biologic treatment course, were not considered as cases. In terms of duration, eosinophilia / hypereosinophilia occurrence was considered resolved once BEC dropped to values < 0.5 x10^9 cells/L.

All the patients provided written informed consent and the study was approved by the Ethical Committee (4085CESC).

Statistical analysis

First a descriptive statistic was performed to explore overall patients' characteristics. Percentages and frequency rates were used for categorical variables and medians with interquartile range for continuous ones. Differences in sample distribution at baseline were assessed through Chi-squared and Fisher's exact test or Mann-Whitney-U non-parametric test, as appropriate. Secondly, the same analysis was applied only to the group of patients who developed eosinophilia to assess the differences between those with mild and those with moderate/severe eosinophilia.

The two selected outcome for survival analysis were eosinophilia development and eosinophilia resolution. The median time to the two outcome was examined by Kaplan–Meier estimates. The

association between clinical and demographic characteristics was investigated via Cox proportional hazard regression. The results were presented as hazard ratio with 95% confidential interval (CI). Survival analysis was applied considering right-censored data. To check for collinearity between independent variables correlation matrix and the Variance Inflation Factor (VIF) were used (supplemental material, Figure E1 and E2). The proportional-hazards (PH) assumption not being violated was assessed via Schoenfeld test (supplemental material, Table E1 and Figure E3). Post-hoc power analysis was conducted with the Hseih and Lavori's formula.[19]

Since the time of onset was not recorded for symptoms, it was modelled as an outcome via binomial logistic regression. The results were presented as odds ratio (OR) with 95% confidential interval.

Statistical analyses were performed in R v4.3. A p-value <0.05 was considered significant.

RESULTS

Patients' characteristics

Overall, 195 dupilumab-treated patients were included in the analysis (Table 1): 52.3% were females, median age was 55 years (IQR 45-64) with no difference based on sex (p=0.091). 187 patients were Caucasians, 8 of Indian ethnicity. Most of patients were diagnosed with severe asthma and CRSwNP (n=90, 46.1%), 39 (20.0%) had severe asthma only, 37 (19.0%) and 29 (14.9%) had mild asthma and CRSwNP and Samter's triad , respectively (Table E2). Median age was 47 years (IQR 42-61) in mild asthma + CRSwNP patients and 58 years (IQR: 49-64) in severe asthma + CRSwNPs subgroup. No differences in gender distribution were detected (p=0.118).

At least one chronic comorbidity was observed in 85 (43.6%) patients, with a median number of 2 (IQR 1-3) per patient in those with other comorbidities and 0 (IQR 0-1) in the overall sample (Table 1). The subgroup affected by comorbidities was significantly older (p<0.001), without differences in

terms of gender (p=0.392). Most frequent comorbidities included gastroesophageal reflux (GERD) (n=29, 34.1%), hypertension (n=25, 29.4%) and osteoporosis (n=18, 21.2%) Metabolic/endocrine conditions (n=16, 18.8%), anxiety-depressive syndrome (n=10, 11.7%), osteoporosis (n=10, 11.7%), bronchiectasis (n=9, 10.6%), sleep obstructive apnoea (n=8, 9.4%), history of malignancy (n=6, 7%), ophthalmic conditions (cataract, glaucoma) (n=6, 7%), cardiac diseased (n=5, 5.8%) and rheumatologic diseases (n=5, 5.8%) were less frequently observed No differences in the number of comorbidities could be described when comparing different asthma phenotypes (p=0.103). Atopy, defined as skin prick tests positivity or presence of positive specific serum IgE, was detected in 105 (53.8%) patients.

Eosinophilia

Overall, 108 out of 195 patients (55.4%) showed the occurrence of an increased BEC compared with baseline, following treatment administration (Table 1), but only 29 (14.9%) showed hypereosinophilia.

When considering the whole study population, 77/195 (39.5%) demonstrated blood eosinophilia at baseline (BEC between 0.5 and 1.5 cells $x10^9$L) and 37.6% of them (29/77) developed hypereosinophilia (BEC between 1.5 and 5 $x10^9$L) following dupilumab initiation.

With regard to the 108 patients experiencing any increase of eosinophil count during dupilumab treatment, at the first occurrence 77 patients (71.3%) demonstrated a BEC between 0.5 and 1.5 and 31 patients (28.7%) between 1.5 and 5.0 cells $x10^9$L (Table 1).

In terms of BEC peak, eosinophils ranged between 0.5 - 1.5 in 56.5% of patients (61/108), between 1.5 and 5 cells $x10^9$L in 41.7% of them (45/108), and only in 2 out of 108 (1.9%) the BEC raised above 5.0 cells $x10^9$L. For most of the patients developing BEC > 0.5, the peak of eosinophils ranged within the same class of eosinophilia characterising the first occurrence (88/108, 81.4%).

When moving to analyse patients experiencing hypereosinophilia, median time to its occurrence was 6.0 months (IQR 3.0-7.5). Blood and imaging investigations described in Methods detected no abnormalities suggesting a pathobiological relevance of eosinophils increase. Probability of developing hypereosinophilia was 3.3 (0.95 CI 3.2-11.7, p<0.001) times higher in patients with baseline BEC of 0.5 - 1.5 x10^9L compared to those with BEC < 0.5 (Figure 1). No other associations were found when considering specific diagnosis, atopy, gender, comorbidities (Table 2). With (a) 0.24 variance of baseline BEC being in 0.5-5 and (b) 0.17 R-squared, the 47 patients with hypereosinophilia provided 0.951 statistical power to detect a hazard ratio 3.27 at 0.05 significance level.

Overall, symptoms co-occurring with any eosinophils increase were observed in 22 (20.4%) patients, being the most frequent asthma exacerbation (50%) and myalgia (22.7%). Table 3 describes more in detail the patients experiencing clinical manifestation in concomitance with BEC variation. Probability of developing symptoms was 4.34 times (0.95CI 1.48-14.25, p=0.011) greater in patients with comorbidities. Subject showing any degree of eosinophilia after treatment initiation also had a higher probability of symptoms occurrence (OR: 13.1, 95%CI 3.3-88.5, p<0.001). No significant differences were found based on the maximum BEC peak.

Independently by symptoms onset, a medical action followed eosinophilia occurrence in 16 (14.8%) patients: 14 (87.5%) were prescribed with OCS and in 2 patients dupilumab administration schedule was prolonged at 28 days intervals. In 14 (12.9%) patients dupilumab was suspended, and 6 of them also received medical treatment for hypereosinophilia.

In terms of duration, 70.4% (76/108) of patients experiencing eosinophilia onset over dupilumab treatment showed a resolution during the study frame. The Kaplan-Meier median estimate time to resolution was 9.0 months 95%CI 6.0-10.0) (Figure 2, Panel A). The probability of BEC > 0.5 x10^9L resolution was higher in those with dupilumab discontinued (HR: 2.73 95%CI: 1.19-6.28, p=0.018)

(Figure 2, Panel B), and with maximum BEC of 0.5 – 1.5 compared to those with > 1.5 (HR: 1.49 95%CI: 1.08-1.72, p=0.025) (Figure 2, Panel C).

DISCUSSION

Our study investigated the frequency and features of blood eosinophils increase over dupilumab treatment, by exploring timing of occurrence, duration, clinical relevance and potential predictors in a real-life population of patient affected by inflammatory conditions of the respiratory tract.

According to the published literature hypereosinophilia onset during dupilumab treatment whether clinically relevant or not is a rare event, involving up to 14% of patients in randomized trials and up to 25% of patients in real-life reports related to subjects affected by upper and/or lower respiratory conditions.[12] Generally speaking, the phenomenon is usually transient and asymptomatic or mildly symptomatic.[9] More severe conditions like eosinophilic pneumonia, hypereosinophilic syndrome and EGPA have been occasionally reported,[11,20] but direct cause-effect association with dupilumab is still under debate, as the substantial steroid sparing effect of the drug might play a role in unmasking an already underlying disease.

However, few studies have been specifically designed to characterize the issue, in fact few data are available on hypereosinophilia characteristics, potential predictors of its occurrence and of its clinical relevance.

In our study we firstly distinguished between any BEC variation from baseline and the onset of hypereosinophilia. If the first case is relatively common, involving 55% of our study population, only 22% of patients were interested by the occurrence of hypereosinophilia, demonstrating a BEC > 1.5 $x10^9$ cells/L, and 20% of the study population (N.22 – Table 3) experienced symptoms co-occurring

with any BEC fluctuation. This observation is quite in line with the rate reported by other studies and suggests to consider BEC variation as an expected event, not raising itself particular concerns.[9] In fact, in our study asthma exacerbations were the most frequently reported events. In that regard, it is well known that blood eosinophils represent a biomarker of asthma-related inflammation and are expected to increase during T2-asthma exacerbations.[21] Under that perspective the co-occurrence of asthma poor control and BEC increase during the course of dupilumab might also be considered a hallmark of non-optimal drug efficacy more than a treatment-related adverse event.

Myalgia was the second most frequently reported adverse event. Recently a case report has been published describing the onset of myalgia in a patient prescribed with dupilumab for atopic dermatitis, still in the absence of concomitant blood count increase.[22] When reviewing dupilumab controlled trials on asthma,[9] myalgia co-occurring with fever, arthralgia and asthma exacerbation was reported in one patient, also showing 2.7 $x10^9$ eosinophils/L (Liberty Asthma Quest Study). Myalgia has been described as part of eosinophilic myositis, a group of extremely rare myopathies.[23] Although it might provide a pathobiological explanation, myositis was not diagnosed in dupilumab treated patients reporting myalgia mentioned above, nor in our population. Of note, in our cohort myalgia co-occurred with eosinophilia in all the patients experiencing the manifestation, but only one of them showed concomitant hypereosinophilia (Table 3). However, in all our cases myalgia was the only reported symptom, not leading to dupilumab interruption, thus considered by the clinicians as a non-specific and non-treatment related symptom.

Overall, in 10 out of 22 symptomatic patients BEC peak remained below the hypereosinophilia cut-off, suggesting that any variation of eosinophil count, even more when co-occurring with symptoms onset, deserves a careful follow-up.

Severe adverse events occurred in 2 females (1,8%). One patient developed a large vessel vasculitis (in concomitance with a BEC peak of 4.2 $x10^9$ cells/L) and the other one experienced

hypereosinophilic syndrome (HES) with pulmonary infiltrates, fever and asthenia (in concomitance with a BEC peak of 7.8x10^9 cells/L). Both patients improved after dupilumab discontinuation and OCS therapy. The cause-effect relationship between the onset of the new condition and the drug mechanism of action remains unclear. On one side a potential evolution from severe asthma to hypereosinophilic conditions including HES and EGPA has been described as part of the disease history.[24] On the other side the steroid sparing effect of biologic drugs, including dupilumab, alongside with their inability to prevent the above-mentioned trajectory might be hypothesized as an explanation. [13,25]

However, the probability of developing symptoms was significantly higher in patients with dupilumab associated eosinophilia when compared to patients whose eosinophils remained below 0.5x10^9 cells/L or decreased during treatment course, and associated with the presence of at least one comorbidity. On the other hand, no differences were found based on sex, age, type of diagnosis or eosinophils peak values.

Similarly, BEC > 0.5 x10^9 cells/L at baseline was the only parameter associated with a three-fold higher probability of experiencing hypereosinophilia, whether symptomatic or not. This observation is quite in line with the findings of both clinical trials and real-life experiences.[12,26,27] However, 84% (43/51) of the patients with eosinophilia at baseline (BEC > 0.5 x10^9 cells/L) which increased after dupilumab initiation showed a subsequent progressive reduction in blood count, that dropped to values < 0.5 x10^9 cells/L during follow-up. Kemp and colleagues also described a higher BEC peak in subject with a greater baseline BEC but in a population of CRSwNP patients.[26] Despite the distinct composition of the study population, in our case including asthma patients too, and the lower basal BEC values in our cohort (no patient with BEC > 1.5 x10^9 cells/L at baseline), our findings are comparable. However, on a practical ground our observations suggest that baseline eosinophilia should not preclude itself dupilumab prescription once performed an extensive differential

diagnosis, but in the case of baseline BEC > 0.5 x10^9 cells/L a careful follow-up in terms of blood samples and clinical assessment is required. When evaluating blood eosinophils, the potential biasing effect of systemic steroid should be always considered and if possible excluded. In our study only patients not receiving corticosteroids in the 4 weeks prior to dupilumab initiation were enrolled. However, in the case wash out was not feasible in relation to the disease activity, an historical unbiased BEC might provide some information. Eosinophils assessment should not be limited to the initial stage of dupilumab treatment. In fact, in our population the median time to eosinophil peak was 6 months, most of patients showing the maximum peak at the first detection of increased eosinophilia. When focusing on patients experiencing hypereosinophilia, in most of them eosinophils increase occurred within 8 months from dupilumab initiation, suggesting that besides that timeframe BEC assessment might be less stringent.

Although without distinguishing between eosinophilia and hypereosinophilia, the post-hoc analysis of dupilumab trials by Wechsler and colleagues similarly described the highest BEC between 4 and 24 weeks from the treatment start. [12]

In our cohort 70% of treated patients showed eosinophilia resolution (BEC < 0.5 x10^9 cells/L) within a median time of 9 months. This trend is quite aligned with the information coming from the clinical trials.[12] Of note, patients demonstrating higher eosinophils peak appeared to be less prone to eosinophilia resolution within the observation timeframe (Figure 2), which is quite expected as a greater BEC could take longer time to decrease, maybe not reaching the eosinophilia threshold (0.5 x10^9 cells/L). However, in the absence of associated clinical manifestations a mild eosinophilia, still persistent, can be acceptable especially in the case of successful efficacy outcomes during dupilumab treatment course.

Dupilumab discontinuation, which happened in 13 patients independently of the co-occurrence of eosinophils variation and symptoms, was associated with a more rapid resolution of blood

eosinophilia. If on one hand it is quite expected, on the other the observation further supports the idea that the eosinophils variation observed over dupilumab treatment is strictly associated to the drug mechanism of action rather than to the onset of an eosinophilic dysimmune condition. [11,23] In fact, eosinophilia resolution was maintained even after OCS therapy tapering in the case BEC increase was managed by both dupilumab discontinuation and steroid treatment.

A main limitation of our study, besides the observational design and the real-life setting, might be represented by the different approach and sensitivity of participating clinician in assessing and rating the clinical manifestation co-occurring with BEC variations. An additional limitation can be related to the lack of comparative investigations in terms of experimental lab investigations and innovative biomarkers in subjects developing or not BEC increase during dupilumab treatment. Under that perspective the contribution of our study to the clarification of mechanisms of eosinophilia variation in dupilumab treated patients is limited.

However, although larger studies are needed to confirm our observations, our report contributes to the evidence and practical management of a challenging issue by extensively investigating the features of eosinophils variation over dupilumab treatment course in a quite large population across our country.

In conclusion, taken together our findings suggest that dupilumab safety is confirmed. An extensive diagnostic work-up, particularly focusing on eosinophilic dysimmune conditions is required before dupilumab initiation, as well as before any other biologic drug prescription. The first 8 months follow-up represents the critical window for the onset of eosinophilia/hypereosinophilia, which are likely to resolve by 9 months, thus BEC assessment together with a careful clinical evaluation are required at least within that timeframe. Patients presenting baseline BEC > 0.5 $x10^9$ cells/L and suffering from comorbidities should be considered particularly at risk of eosinophil count fluctuation and symptoms occurrence.

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FIGURES LEGENDS

Figure 1 - Kaplan–Meier curves for hyper eosinophilia onset probability after treatment with Dupilumab based on baseline BEC. BEC=blood eosinophil count

Figure 2 - Kaplan–Meier curves for BEC > 0.5 maintenance probability after treatment with dupilumab (panel A) and distinguished by dupilumab discontinued (panel B) and maximum BEC of 0.5 – 1.5 vs > 1.5 (panel C). BEC=blood eosinophil count

TABLES LEGENDS

Table 1 - Characteristics of the patients treated with dupilumab distinguished by maximum BEC group after treatment. The 'no hypereosinophilia' group includes both those who have not developed it and those who have not shown any worsening.

Table 2 - Hazard ratios (HR) of hyper eosinophilia onset after dupilumab estimated in the multivariate Cox proportional hazard model with diagnosis type, atopy (yes/no), sex, age, comorbidities (yes/no and baseline BEC (0-500/500-1500).

Table 3 - Characteristic of patients experiencing symptoms in concomitance with the occurrence of blood eosinophils increase over dupilumab treatment.

Table 1 – Characteristics of the patients treated with dupilumab distinguished by maximum BEC group after treatment. The 'no hypereosinophilia' group includes both those who have not developed it and those who have not shown any worsening

	No BEC increase (n=87)	BEC 0.5 – 1.5 (n=61)	BEC 1.5 - 5 (n=45)	BEC >5 (n=2)	p-Value*	BEC Overall (n=195)
Sex					0.166	
Female	47 (54.0%)	27 (44.3%)	26 (57.8%)	2 (100.0%)		102 (52.3%)
Male	40 (46.0%)	34 (55.7%)	19 (42.2%)	0 (0.0%)		93 (47.7%)
Age (years)					0.562	
Median (IQR)	54.0 (43.0-62.2)	56.0 (47.0-65.8)	56.0 (51.0-57.1)	57.1 (48.6-65.7)		55.0 (45.0-64.0)
Atopy					0.998	
No	31 (35.6%)	33 (54.1%)	25 (55.6%)	1 (50.0%)		90 (46.2%)
Yes	56 (64.4%)	28 (45.9%)	20 (44.4%)	1 (50.0%)		105 (53.8%)
Diagnosis					0.272	
Asthma	28 (32.2%)	4 (6.6%)	6 (13.3%)	1 (50.0%)		39 (20.0%)
Asthma+polyposis	59 (67.8%)	57 (93.4%)	39 (86.7%)	1 (50.0%)		156 (80.0%)
Baseline BEC					0.035	
<0.5	63 (72.4%)	37 (60.7%)	16 (35.6%)	2 (100.0%)		118 (60.5%)
0.5 – 1.5	24 (27.6%)	24 (39.3%)	29 (64.4%)	0 (0.0%)		77 (39.5%)
Comorbidities number					0.538	
Median (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	3 (2-4)		0 (0-1)
Time BEC > 1.5 (months)					NA	
Median (IQR)	NA	NA	6.0 (3.0-7.0)	5.5 (4.3-6.8)		6.0 (3.0-7.5)
Resolution time (months)					0.354	
Median (IQR)	NA	9.0 (4.8-12.0)	9.0 (5.5-16.0)	NA		9.0 (4.8-14.3)
Dupilumab discontinued					0.003	
No	87 (100.0%)	59 (96.7%)	36 (80.0%)	0 (0.0%)		182 (93.3%)
Yes	0 (0.0%)	2 (3.3%)	9 (20.0%)	2 (100.0%)		13 (6.7%)
Symptoms					0.345	
No	85 (97.7%)	51 (83.6%)	34 (75.6%)	1 (50.0%)		171 (87.7%)
Yes	2 (2.3%)	10 (16.4%)	11 (24.4%)	1 (50.0%)		24 (12.3%)

** Chi-squared and Fisher's exact test. Mann-Whitney-U non-parametric test. Comparison was carried out between the group with BEC of 0.5 - 1.5 and that with BEC >1.5. BEC: blood eosinophils count*

Table 2 – Hazard ratios (HR) of hyper eosinophilia onset after dupilumab estimated in the multivariate Cox proportional hazard model with diagnosis type, atopy (yes/no), sex, age, comorbidities (yes/no and baseline BEC (0-500/500-1500).

	HR	0.95CI	p-Value
Diagnosis (polyps+asthma)	1.30	0.53-3.02	0.590
Atopy (yes)	0.67	0.36-1.24	0.202
Sex (male)	0.71	0.39-1.28	0.257
Age	1.02	0.99-1.04	0.126
Comorbidities (yes)	1.33	0.72-2.45	0.363
Baseline BEC (0.5-1.5)	3.27	1.80-5.94	<0.001

BEC=blood eosinophil count

Table 3 – Characteristic of patients experiencing symptoms in concomitance with the occurrence of blood eosinophils increase over dupilumab treatment.

Age	Gender	DIAGNOSIS	Blood eosinophils peak (cells x10^9L)	ASSOCIATED SYMPTOMS	TREATMENT	DUPILUMAB DISCONTINUED
74	F	CRSwNP + MILD ASTHMA	7.84	asthma exacerbation	2 weeks OCS trial	Y
78	F	SEVERE ASTHMA + CRSwNP	4.34	giant cell vasculitis	2 weeks OCS trial	Y
59	F	SEVERE ASTHMA + CRSwNP	4.28	injection site reaction	none	N
42	F	SEVERE ASTHMA + CRSwNP	3.8	asthma exacerbation	none	N
55	M	SEVERE ASTHMA + CRSwNP	3.3	asthma exacerbation	2 weeks OCS trial	Y
71	F	SEVERE ASTHMA + CRSwNP	3.22	asthma exacerbation	4 weeks OCS trial	Y
54	M	SEVERE ASTHMA + CRSwNP	2.91	paresthesia	none	Y
57	F	CRSwNP + MILD ASTHMA	2.29	headache	none	N
64	F	SEVERE ASTHMA + CRSwNP	1.94	Fever, asthma exacerbation	4 weeks OCS trial	Y
55	F	SAMTER'S TRIAD	1.65	asthma exacerbation	none	N
34	F	CRSwNP + MILD ASTHMA	1.6	myalgia	none	N
59	M	SEVERE ASTHMA + CRSwNP	1.5	conjunctivitis	none	N
54	F	SEVERE ASTHMA + CRSwNP	1.33	myalgia	2 weeks OCS trial	N
57	F	SAMTER'S TRIAD	1.3	myalgia	none	N
62	M	SEVERE ASTHMA	1.11	asthma exacerbation	2 weeks OCS trial	Y
62	M	SEVERE ASTHMA + CRSwNP	1.02	rhinitis	none	N
60	F	SEVERE ASTHMA + CRSwNP	0.98	asthma exacerbation	2 weeks OCS trial	N
47	F	CRSwNP + MILD ASTHMA	0.9	myalgia	nine	N
70	M	SEVERE ASTHMA + CRSwNP	0.9	asthma exacerbation, gynecomastia	2 weeks OCS trial	N
19	F	SEVERE ASTHMA	0.7	myalgia	none	N
44	F	SAMTER'S TRIAD	0.63	asthma exacerbation	none	N
30	F	SEVERE ASTHMA	0.52	asthma exacerbation	none	N

CRSwNP: Chronic rhinosinusitis with nasal polyps; OCS: oral corticosteroids