ORIGINAL ARTICLE

Mepolizumab Reduces Inflammatory Eosinophils in Nasal Polyp Tissue and Restores Histological Alterations

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Abstract

Background: Eosinophilic inflammation and IL-5 are key therapeutic targets in type 2 chronic rhinosinusitis with nasal polyposis (CRSwNP). Based on surface expression of CD62L, 2 eosinophil subpopulations, homeostatic and inflammatory, were recently described.
Objectives: First, we aimed to investigate the association between these subpopulations and the severity of CRSwNP, and second, to analyze the impact of mepolizumab on tissue eosinophil subpopulations.
Methods: Flow cytometry of nasal polyp (NP) tissue eosinophil subpopulations ($CD62L^{low}$, iEos; $CD62L^{bright}$, rEos) was performed in 15 CRSwNP patients (with and without severe asthma) treated with mepolizumab for a median of 7.5 months. NP tissue was analyzed at baseline and after the start of therapy. The correlation between the proportion of tissue iEos and the severity of CRSwNP and treatment response was assessed.
Results: The proportion of tissue iEos correlated with higher CRSwNP severity scores and quality of life questionnaire scores (SNOT-22, L-K score) at baseline. A higher and significant improvement in NPS was observed in patients who had been taking mepolizumab for longer than 6 months. Mepolizumab significantly reduced the proportion of iEos in nasal polyp tissue, although a significantly higher number of rEos remained. A greater reduction in tissue iEos was associated with a more significant improvement in quality of life (SNOT-22) and severity (L-K) scores. Finally, in addition to the reduction in tissue eosinophils, mepolizumab induced histological changes characterized by the decrease in edema and goblet cell hyperplasia.
Conclusions: Tissue iEos values correlate with poor sinonasal control in CRSwNP and are reduced by treatment with mepolizumab. A greater decrease in iEos is associated with a significantly greater therapeutic effect of mepolizumab.

Key words: Asthma. CRSwNP. Eosinophil subpopulations. Mepolizumab. T2 inflammation.

Resumen

Antecedentes: La inflamación eosinofílica y la IL-5 son objetivos terapéuticos clave en la rinosinusitis crónica con pólipos nasales (CRSwNP) Tipo 2; recientemente se han descrito dos subpoblaciones de eosinófilos (homeostática e inflamatoria), según la expresión de CD62L en su superficie.
Objetivos: En primer lugar, investigamos la asociación de dichas subpoblaciones con la gravedad de la CRSwNP y, en segundo lugar, analizamos el impacto de mepolizumab en las subpoblaciones de eosinófilos tisulares.
Métodos: Mediante citometría de flujo, se evaluaron las subpoblaciones de eosinófilos en los pólipos nasales (PN) (CD62L^bajo, iEos; CD62L^alto, rEos) de 15 pacientes con CRSwNP (con o sin asma grave), tratados con mepolizumab durante una mediana de 7,5 meses. Los PN fueron analizado basalmente y después del tratamiento. Se evaluó la correlación entre la proporción de iEos tisulares y la gravedad de la CRSwNP, así como la respuesta al tratamiento.
Resultados: La proporción de iEos en el tejido se correlacionó con puntuaciones más altas de gravedad de la CRSwNP y de los cuestionarios de calidad de vida (SNOT-22, puntuación L-K) antes del tratamiento (Basal). Se observó una mejoría significativa en la puntuación tras el tratamiento con mepolizumab, en aquellos pacientes que fueron tratados por más de seis meses. El mepolizumab redujo significativamente la proporción de iEos en el tejido de PN, mientras que se mantuvo un número significativamente mayor de rEos. Una mayor reducción de los iEos tisulares se asoció con una mejora más significativa en la calidad de vida (SNOT-22) y en las puntuaciones de gravedad (L-K). Finalmente, además de la reducción de eosinófilos tisulares, el mepolizumab induce cambios histológicos caracterizados por la disminución del edema y de la hiperplasia de células caliciformes.
Conclusiones: Los iEos tisulares se correlacionan con un mal control sinonasal en la CRSwNP y son reducidos por el tratamiento con mepolizumab. Una disminución mayor de iEos se asocia con un efecto terapéutico significativamente superior del mepolizumab.

Palabras clave: Asma. CRSwNP. Subpoblaciones de eosinófilos. Mepolizumab. Inflamación tipo 2.

Summary box

- **What do we know about this topic?**
  Eosinophil subpopulations, resident eosinophils (rEos) and inflammatory eosinophils (iEos), have been described. In patients with CRSwNP, iEos tend to accumulate significantly in nasal polyp tissue.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  The study supports the clinical relevance of iEos, considering the correlation between their tissue proportion and severity of nasal symptoms. We demonstrate the capacity of mepolizumab to decrease tissue infiltration by the iEos subpopulation. Our findings suggest a pathogenic role for iEos, which may represent a pivotal target of treatment of eosinophilic inflammation in CRSwNP.

Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal sinuses that is associated with a high symptom burden. Symptoms, such as loss of smell, anterior and posterior rhinorrhea, nasal congestion, and facial pressure, may be severe and often disabling. Phenotypically, CRS is classified as with nasal polyps (CRSwNP) or without nasal polyps. CRSwNP accounts for approximately 80% of cases [1,2]. Data from the literature underline that the presence of CRS in asthma patients is associated with worsening of asthma symptoms, increased risk of exacerbations, and greater use of oral corticosteroids (OCS) [3].

Most patients with CRSwNP exhibit type 2 inflammation with typical mucosal infiltration by eosinophils (>10/high power field [HPF]), lymphocytes, and mast cells and the involvement of key type 2 cytokines, such as interleukin (IL) 4, IL-5, and IL-13 [4]. In addition, other histological findings in CRSwNP include epithelial cell disruption, goblet cell hyperplasia with mucin hypersecretion, and basement membrane thickening [5].

The high levels of IL-5 demonstrated in polyp tissue support the role of eosinophils in the pathogenesis of CRSwNP, given the importance of this cytokine in eosinophil biology, particularly its proliferation and its differentiation-inducing and antiapoptotic effects on these cells [6]. Delayed eosinophil apoptosis may facilitate tissue eosinophilia in CRSwNP, thus enhancing pathophysiological events such as epithelial damage, epithelial-mesenchymal transition, and basement membrane thickening [7]. In fact, some studies have demonstrated correlations between upper airway remodeling and elevated levels of tissue eosinophils in CRSwNP [8].

Type 2 inflammation in CRSwNP is associated with higher rates of polyp recurrence after sinus surgery, and a demonstrated positive correlation with eosinophilic infiltration has been reported [9].

Recent studies have identified eosinophil subpopulations, with potential roles in tissue homeostasis (resident eosinophils [rEos]) and inflammatory response (inflammatory eosinophils [iEos]). Moreover, in patients with CRSwNP, iEos are significantly prone to accumulate in nasal polyp tissue [10,11].

As IL-5 is a well-known key driver of T2 inflammation, playing a pivotal role in differentiation, activation, chemotaxis, and survival of eosinophils, the anti–IL-5 monoclonal antibody mepolizumab is widely used for the treatment of asthma and other T2-mediated diseases [6,12,13]. More recently, in the phase 3 SYNAPSE trial, mepolizumab proved safe and effective in reducing nasal polyp size, nasal congestion scores, and the need for rescue OCS and endoscopic sinus surgery (ESS), leading to its approval for the treatment of recurrent, refractory, and severe isolated CRSwNP [14]. Mepolizumab was recently shown to restore the balance between peripheral blood iEos and rEos in patients with severe eosinophilic asthma with or without CRSwNP [15]. No data are available about the effect of mepolizumab on eosinophil subpopulations at tissue level.

The aim of this study was to evaluate the effects of mepolizumab on eosinophil subpopulations at the level of nasal polyp tissue and to investigate the impact on tissue iEos and clinical outcomes of mepolizumab in CRSwNP.

Methods

Study Population and Design

We performed a single-center noninterventional study in a cohort of 35 patients consecutively admitted to the Immunoallergology Unit at Careggi University Hospital in Florence, Italy with eosinophilic asthma (defined as ≥1 determination of >300/μL in blood in the previous 12 months) and CRSwNP and treated with at least 3 doses of mepolizumab 100 mg every 4 weeks owing to the severity of asthma and/or CRSwNP, according to the GINA and EPOS/EUFOREA guidelines [16,17]. None of the patients had received biological treatment before mepolizumab. Twenty patients were excluded because no nasal biopsy was available at baseline, ie, before initiation of mepolizumab. The remaining 15 mepolizumab-treated patients were enrolled in a longitudinal analysis because of the availability of NP tissue biopsy and ENT follow-up. All pretreatment biopsies were collected within 2 months before the first dose of mepolizumab. All patients were receiving the maximum dose of topical corticosteroids (mometasone, twice daily) according to the EPOS/EUFOREA guidelines. Adherence to topical treatment was confirmed in all patients. Nine patients had severe eosinophilic asthma, defined as uncontrolled disease despite optimized treatment with maximal doses of inhaled corticosteroids (ICS) and long-acting ß2-agonists (LABAs) in accordance with the criteria for GINA step 5 treatment (Figure 1). The Table shows the demographic and clinical

characteristics of the study population. The procedures followed in the study were approved by the Area Vasta Centro Regional Ethical Committee (19295_bio). Written informed consent was obtained from recruited patients.

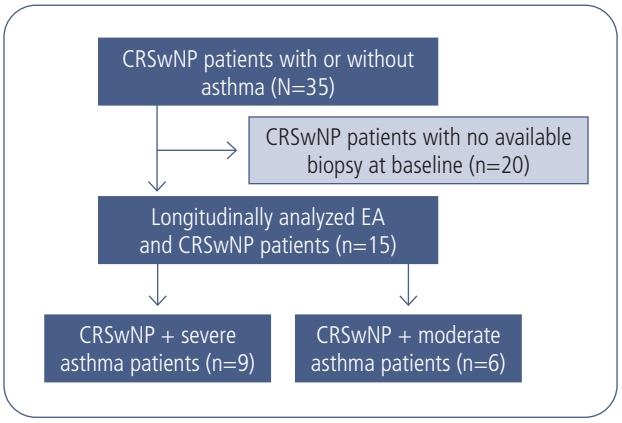


Figure 1. Flow chart showing selection of study participants. CRSwNP indicates chronic rhinosinusitis with nasal polyps; EA, eosinophilic asthma.

Table. Demographic and Clinical Characteristics of Longitudinally Analyzed Patients.[a]

	All patients (N=15)
Age, y	58.1 (2.9)
Male/Female	7/8
Asthma, No. (%)	15 (100)
CRSwNP, No. (%)	15 (100)
Atopy, No. (%)	8 (53.3)
ACT	19.5 (0.9)
ACQ-5	1.7 (0.3)
SNOT-22	53 (4.5)
NPS	5.3 (0.5)
L-K score	6.8 (0.5)
SST-16	4.9 (0.6)
Eosinophils/µL	1164 (220)
Eosinophils, %	13.6 (2.2)
Total IgE, kU/L	241.5 (66.5)
mOCS therapy, No. (%)	4 (26.7)
OCS dose, mg/d	3.1 (0.3)
Patients who underwent ESS, No. (%)	8 (53.3)
ESS number/patient	1.1 (1-3)

Abbreviations: ACQ-5, Asthma Control Questionnaire-5; ACT, Asthma Control Test; CRSwNP, chronic rhinosinusitis with nasal polyps; ESS, endoscopic sinus surgery; L-K, Lund-Kennedy; mOCS, maintenance oral corticosteroids; NPS, Nasal Polyp Score; SNOT-22, Sino-Nasal Outcome Test-22; SST-16, Sniffin' Sticks Test-16

[a]Data are expressed as mean (SE) unless otherwise indicated.

Clinical Scores

The clinical features of CRSwNP were analyzed at the baseline and the follow-up clinical examinations by applying the Sino-Nasal Outcome Test (SNOT-22), a self-assessment of disease burden using a 22-item questionnaire resulting in a total score of 0 to 110. The Nasal Polyp Score (NPS) was determined by endoscopy and ranged from 0 to 4 for each nostril, reflecting polyp severity. We also determined the Lund-Kennedy (L-K) score, which grades visual pathologic states within the nose and paranasal sinuses, including polyps, discharge, edema, scarring, and crusting and ranges from 0 to 5 for each nostril. The Sniffin' Sticks Test (SST-16) assessed the recognition of 16 different odors, yielding a score ranging from 0 to 16. Asthma symptoms were assessed using the Asthma Control Test (ACT) and the Asthma Control Questionnaire-5 (ACQ-5).

Reagents

Dulbecco phosphate-buffered saline (PBS) was purchased from Euroclone. Low-endotoxin RPMI 1640 medium (VLE-RPMI 1640, Biochrom AG) was supplemented with 2 mM L-glutamine, 2 mM 2-mercaptoethanol, 100 U/mL penicillin, 100 µg/mL streptomycin, 1% nonessential amino acids, and 1% sodium pyruvate (all from Sigma Chemical Co.). Fetal bovine serum was purchased from Euroclone. Antibodies for flow cytometry were purchased from Miltenyi Biotec.

Isolation of Human Eosinophils From Peripheral Blood and Flow Cytometry

Peripheral blood (15 mL) was collected immediately before administration of mepolizumab (trough level) in sterile EDTA-containing vacutainer tubes (BD Biosciences). Density-gradient centrifugation was performed using Lymphoprep™ (STEMCELL Technologies). Fifteen milliliters of whole blood was carefully layered over 20 mL of reagent in a 50-mL conical tube and centrifuged at 600*g* for 25 minutes at room temperature. The supernatant was discarded, and the bottom layer containing granulocytes and erythrocytes was collected. To remove erythrocytes, the cell suspension was mixed with 10 volumes of 1× Red Blood Cell Lysis Solution (Miltenyi Biotec) according to the manufacturer's instructions. Isolated granulocytes were counted, and their viability and quantity in peripheral blood were assessed. A total of 10^6 cells was stained with a panel of monoclonal antibodies (mAbs) targeting surface markers (recommended antibody dilution of 1:50 in a final volume of 100 µL). The cells were then analyzed using a BD FACSCanto II flow cytometer (Becton-Dickinson) and BD FACSDiva software.

Nasal Polyp Biopsy Collection

Samples from patients with CRSwNP were obtained during ESS. Two polyp tissue samples were taken from each patient: one was embedded in paraffin and processed for histology; the other was transferred to a GentleMACS Tube (Miltenyi Biotec) with 10 mL of complete medium and 2% fetal bovine serum for isolation of cells.

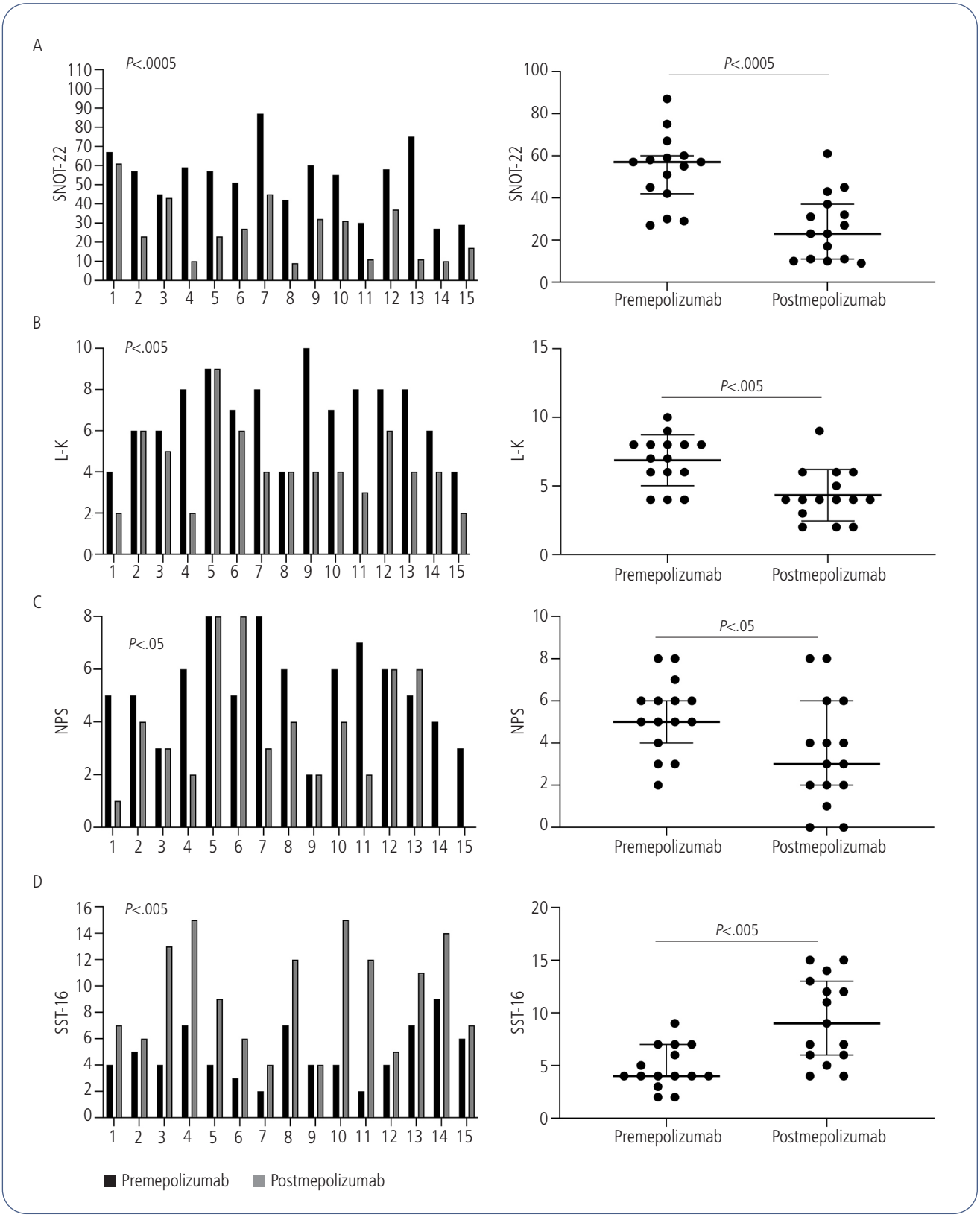


Figure 2. Mepolizumab improves clinical and endoscopic scores in CRSwNP patients. Mepolizumab treatment reduces the SNOT-22, NPS, and L-K scores and increases the SST-16 score in CRSwNP patients (n=15). Left, individual patient scores before and after mepolizumab treatment. Right, median (IQR). CRSwNP indicates chronic rhinosinusitis with nasal polyps; L-K, Lund Kennedy; NPS, Nasal Polyp Score; SNOT-22, Sino-Nasal Outcome Test-22; SST-16, Sniffin' Sticks Test-16. Comparisons were performed using the Mann-Whitney test.

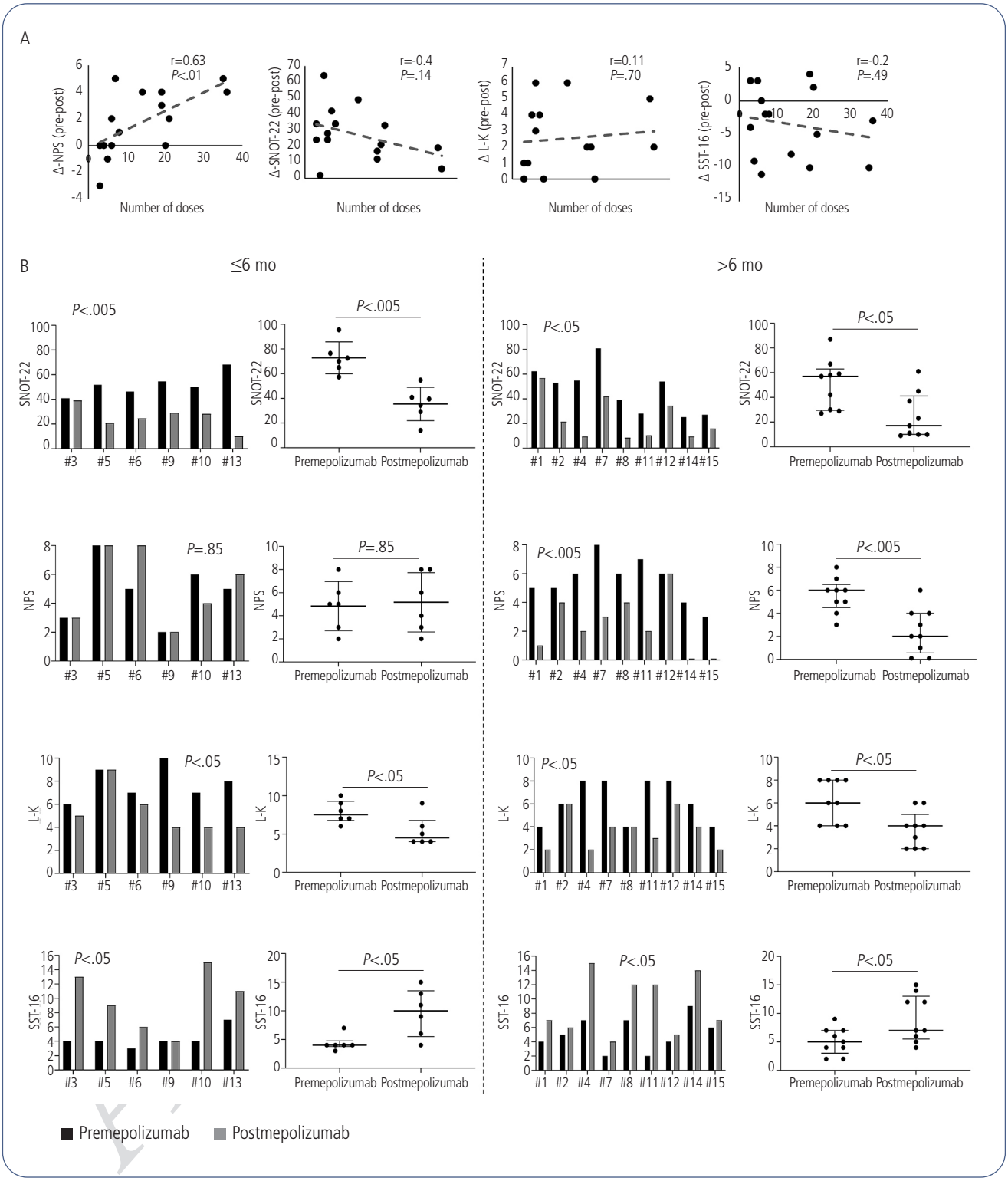


Figure 3. Mepolizumab improves CRSwNP scores in a time-dependent manner. A, The improvement in NPS, shown as Δ-NPS (NPS pretreatment minus NPS posttreatment) is correlated with the number of mepolizumab doses. No correlations were found with SNOT-22, L-K, or SST-16 scores. The analysis was performed based on the Spearman correlation test. B, Left panel: in mepolizumab-treated patients (≤6 months, n=6), the SNOT-22, L-K, and SST-16 results improved significantly. Left, scores for individual patients before and after mepolizumab treatment. The median (IQR) of the scores is shown on the right. Right panel: all clinical and endoscopic scores improved significantly in mepolizumab-treated patients (>6 months, n=9). Individual patient scores before and after mepolizumab treatment are shown on the left; the median (IQR) score is shown on the right. CRSwNP indicates chronic rhinosinusitis with nasal polyps; L-K, Lund-Kennedy score; NPS, Nasal Polyp Score; SNOT-22: Sino-Nasal Outcome Test-22; SST-16, Sniffin' Sticks Test-16. Comparisons were performed using the Mann-Whitney test.

Nasal Polyp Histology

Nasal polyp tissue was fixed in 4% formalin, paraffin-embedded, and sectioned at a thickness of 5 µm. Hematoxylin-eosin–stained polyp sections were examined histopathologically. Eosinophils, neutrophils, lymphocytes, and plasma cells were counted in 10 random fields by an observer blinded to the experimental conditions. Each polyp tissue sample was scored based on the absolute count of infiltrating cells/HPF. Regarding eosinophils, the absolute number per HPF was calculated as follows: N = total counted eosinophils/10. Regarding neutrophils, lymphocytes, and plasma cells, a grade based on cells/HPF was assigned, as follows: neutrophils, 1=absent, 2=<10/HPF, 3=>10/HPF; lymphocytes and plasma cells, 1=<10/HPF, 2=10-20/HPF, 3=>20/HPF). Mucosal ulceration and squamous metaplasia were scored as follows: 1=absent, 2=present. Fibrosis level and subepithelial edema were scored as follows: 1=absent, 2=partial, 3=extensive. Goblet cell hyperplasia was analyzed as the proportion of goblet cells over all epithelial cells counted in 10 random fields and scored as follows: 1=<5%, 2=5%-25%, 3=25%-50%, 4=50%-75%, 5=>75%.

Isolation of Human Eosinophils From Nasal Polyps and Flow Cytometry

Nasal polyp specimens were processed using the GentleMACS Dissociator (Miltenyi Biotec). The tissue in the GentleMACS Tube was centrifuged for 5 minutes at 300*g*; the supernatant was discarded and replaced with complete medium containing 2 mg/mL collagenase (Worthington) and 0.04 mg/mL DNase I (Roche Diagnostics). After incubation for 45 minutes at 37°C and 5% CO_2, a second mixing step was performed using the GentleMACS. The cell suspension was passed through a 70-µm cell strainer (Miltenyi Biotec). Cells were counted and stained with a panel of mAbs targeting surface markers. The cells were then analyzed using a BD FACSCanto II flow cytometer (Becton-Dickinson) and BD FACSDiva software.

Gating Strategy

Leukocytes were identified based on CD45 expression. Eosinophils were identified as Siglec8$^+$ CD16$^-$ cells among CD45$^+$ cells. All eosinophils from peripheral blood and nasal polyp tissue (CD45$^+$Siglec8$^+$CD16$^-$) were analyzed for CD62L expression. Fluorescence minus one analysis was performed on CD62L (Figure S1).

Statistical Analysis

The statistical analysis was performed using GraphPad Prism 10 software. Comparisons were performed using the Mann-Whitney test. Linear correlations were analyzed using the Spearman correlation test. Statistical significance was set at $P<.05$.

Results

Mepolizumab Improves CRSwNP in a Time-Dependent Manner: Early and Late Effects

All patients attended an ENT follow-up visit at a median of 7.5 (3-36) months after the start of treatment. Concerning the clinical and endoscopic parameters of CRSwNP, statistically significant decreases were observed in the SNOT-22 score (from 53.3 [4.5] to 26 [4.2]; $P<.001$), NPS (from 5.3 [0.5] to 3.5 [0.7]; $P<.05$), and L-K score (from 6.9 [0.5] to 4.3 [0.5]; $P<.005$). A statistically significant increase was recorded in the SST-16 between the first and last ENT visit (from 4.9 [0.6] to 9 [1.2]; $P<.05$) (Figure 2 and Table S1).

To investigate whether the duration of treatment would have impacted on the clinical effects of mepolizumab, we analyzed the correlation between the number of drug doses and the improvement in clinical scores, expressed as the difference (Δ) between parameters before and after treatment. As illustrated in Figure 3A, a direct positive correlation was found between the modification in NPS and the number of mepolizumab doses. No correlations were found for the SNOT-22 score, L-K score, or SST-16 score. Taking into account the heterogenous duration of therapy in our cohort, patients were placed into 2 groups according to the number of months of therapy (less than 6 months [n=6] or more than 6 months [n=9]). As illustrated in Figure 3B and Table S2 A-B, a more evident improvement in NPS was observed in patients who received treatment for longer. The other scores remained stable.

Given that all the patients had asthma, we also evaluated the clinical effect of mepolizumab on asthma control. As expected, mepolizumab led to a significant improvement in the ACT score (from 19.9 [1] to 24.1 [0.3]; $P<.005$) and ACQ-5 score (from 1.6 [0.3] to 0.2 [0.1]; $P<.0001$) (Figure S2).

Mepolizumab-Related Histological Changes in Nasal Polyp Tissue

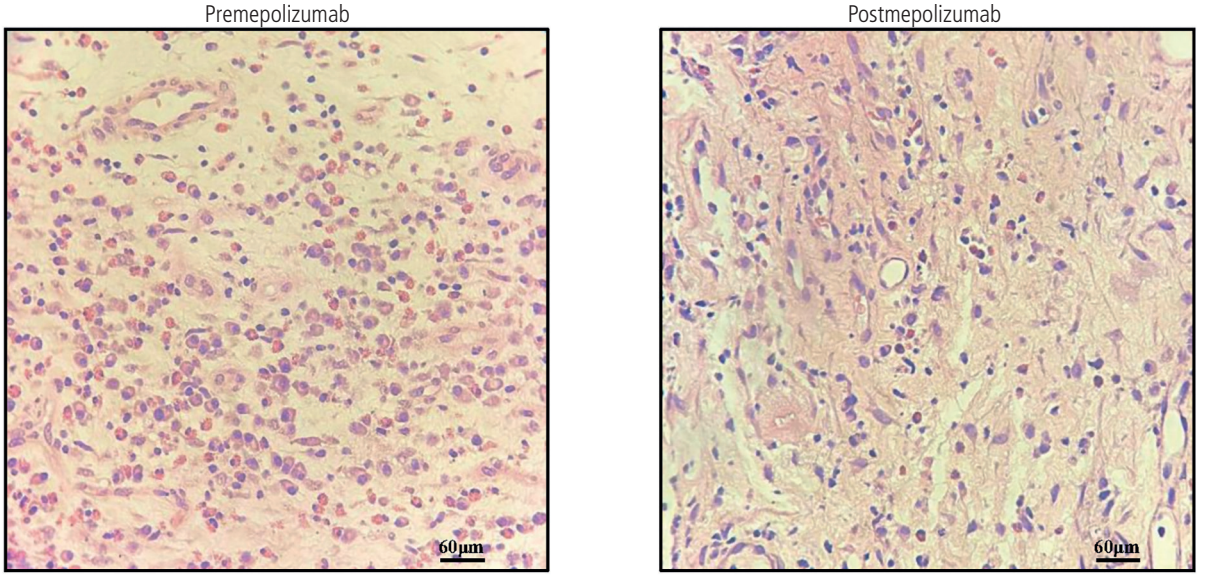
Histological analysis was carried out before and after treatment to better define the effects of mepolizumab on the various aspects of NP tissue inflammation. Two out of 15 patients (13.3%) did not display any residual NP tissue at the ENT follow-up visit after the start of therapy and were excluded from further analysis. A significant reduction in tissue eosinophilia was observed in the remaining patients with residual NP, with the eosinophil count decreasing from 26.9 (6.2) to 7.8 (1.9) cells/HPF ($P<.005$). Notably, a significant reduction was observed in both the goblet cell hyperplasia and the subepithelial edema scores. In contrast, there was no significant change in tissue neutrophils, lymphocytes, or plasma cells (Figure 4A, B, C).

Mepolizumab Decreases CD62L^{low} Inflammatory Eosinophils in Nasal Polyp Tissue

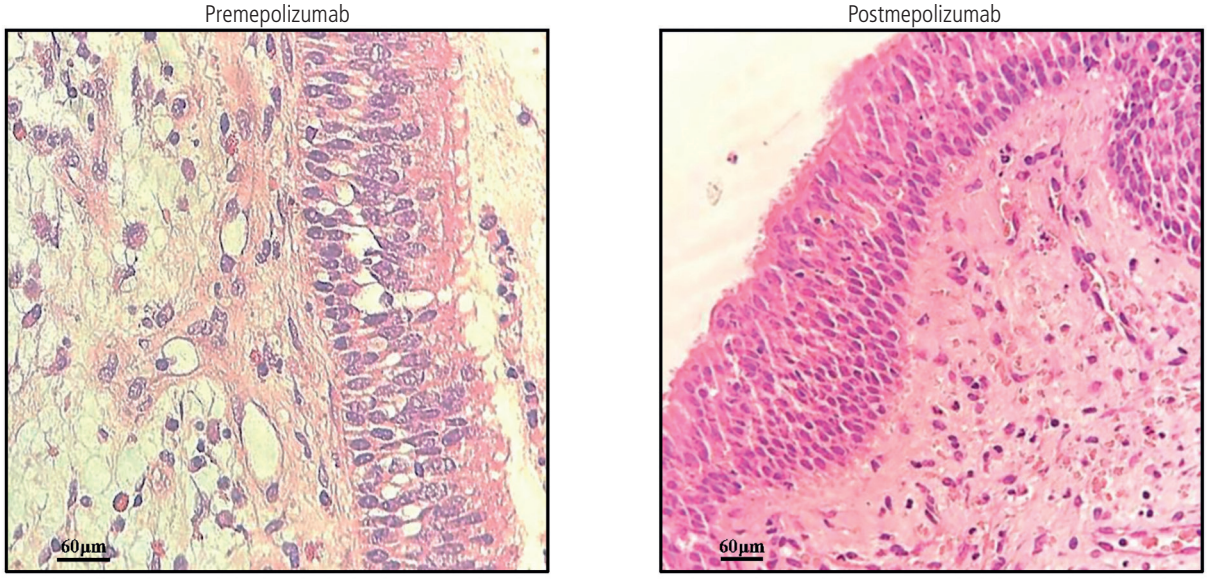
We first analyzed the proportion of blood and nasal polyp tissue CD62L^{low} eosinophil subpopulations in the 15 enrolled patients before initiation of mepolizumab. We observed a significantly higher percentage of CD62L^{low} cells (iEos) in tissue than in blood (42.9 [2.4] vs 14 [2.8]; $P<.005$), although no correlation was found between the circulating and tissue iEos ($r=0.28$, $P=.31$).

We then investigated the effect of mepolizumab on tissue eosinophil subpopulations in nasal polyp samples. Notably, beyond a decrease in circulating inflammatory CD62L^{low} eosinophils (13.3% [3.1%] vs 5.9% [1.2%], $P<.05$; 151 [69] vs 5 [1] cells/HPF, $P<.0001$), a significant reduction in these cells was observed at tissue level in patients treated with

A



B



C

	Premepolizumab	Postmepolizumab	*P* value
Eosinophils, cells/HPF	26.9 (6.2)	7.8 (1.9)	.004
Neutrophils (grading)	1.7 (0.3)	1.5 (0.2)	.78
Lymphocytes (grading)	2.5 (0.2)	2.8 (0.1)	.18
Plasma cells (grading)	2.5 (0.2)	2.6 (0.2)	.95
Mucosal ulceration	1.1 (0.1)	1.1 (0.1)	>.99
Squamous metaplasia	1.5 (0.2)	1.3 (0.1)	.66
Fibrosis	1.0 (0.0)	1.2 (0.1)	.48
Subepithelial edema	2.6 (0.2)	1.9 (0.2)	.02
Goblet cell hyperplasia	1.8 (0.3)	1.0 (0.0)	.04

Figure 4. Mepolizumab reduced eosinophilic infiltration, subepithelial edema, and goblet cell hyperplasia in nasal polyp tissue. A, Representative nasal polyp tissue microscopic images (hematoxylin-eosin: magnification, ×40) of an individual patient before and after treatment with mepolizumab. B, Effect of mepolizumab treatment on goblet cell hyperplasia. C, Effects of mepolizumab on the histological parameters of nasal polyp tissue.

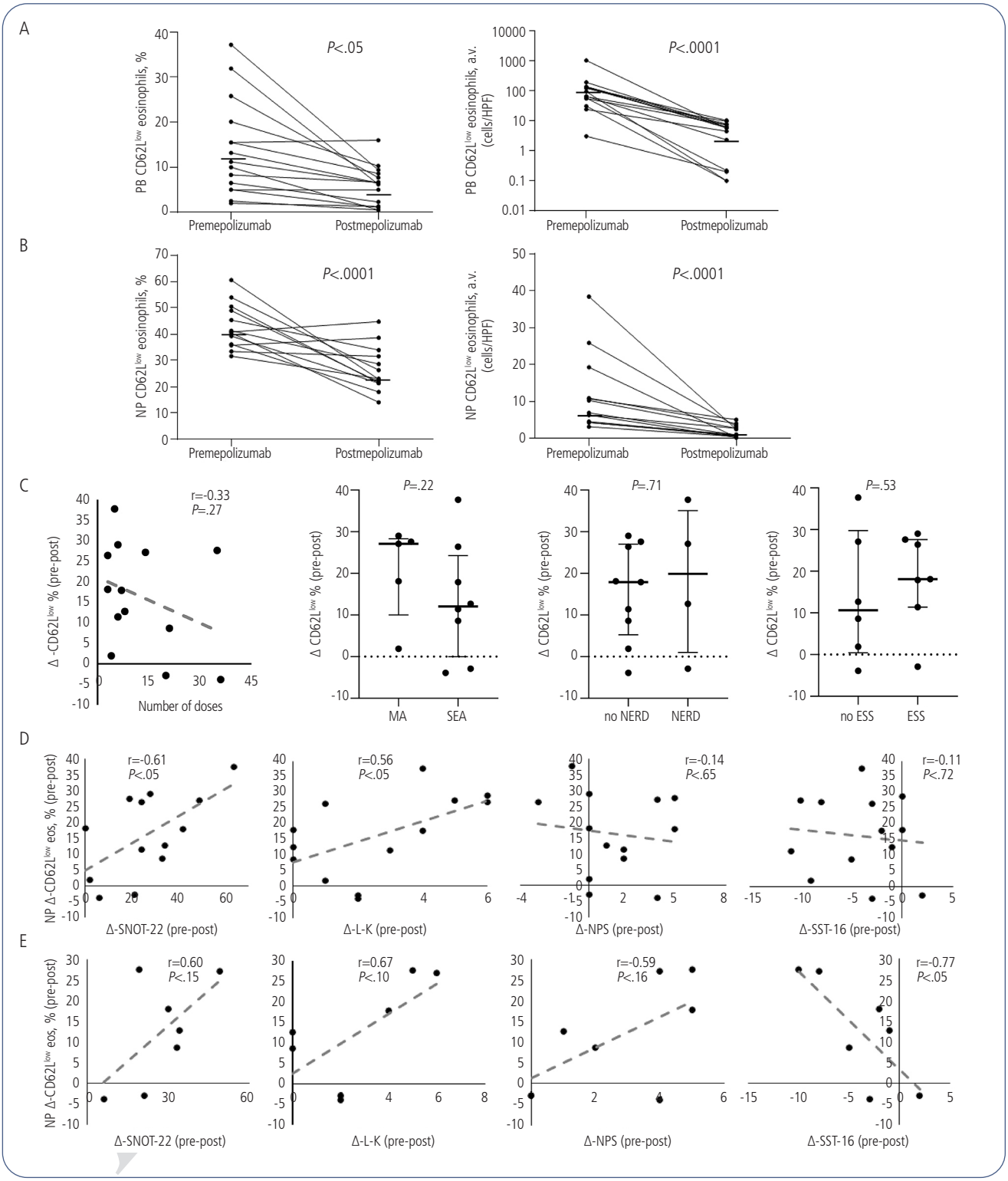


Figure 5. Effect of mepolizumab on eosinophil subpopulations in nasal polyp tissue. A, Mepolizumab decreased the peripheral blood CD62L^{low} percentage and absolute value. B, Mepolizumab decreased the NP CD62L^{low} percentage and absolute value. C, The Δ of NP CD62L^{low} did not depend on the number of mepolizumab doses, asthma severity, presence of NERD, or patient history of ESS. The analysis was performed using the Mann-Whitney test. D, The Δ of NP CD62L^{low} after treatment with mepolizumab correlated with the Δ of SNOT-22 and L-K in a statistically significant manner. E, The Δ of NP CD62L^{low} after treatment with mepolizumab correlated with the Δ of SST-16 in a statistically significant manner when only patients treated for >6 months are considered. ESS indicates endoscopic sinus surgery; HPF, high-power field; L-K: Lund-Kennedy; MA, moderate asthma; NERD, nonsteroidal anti-inflammatory drug–exacerbated respiratory disease NP, nasal Polyp; NPS, Nasal Polyp Score; PB, peripheral blood; SNOT-22, Sino-Nasal Outcome Test-22; SEA, severe eosinophilic asthma; SST-16, Sniffin' Sticks Test-16. The analysis was performed using the Spearman correlation test.

mepolizumab (42.6% [2.3%] vs 26.6% [2.4%], *P*<.0001; 12 [3] vs 2 [0.5] cells/HPF, *P*<.0001) (Figure 5A, B). The effect of mepolizumab on iEos was independent of the duration of therapy and the severity of asthma. In the analysis of patients with and without NSAID-exacerbated respiratory disease, no differences were recorded for the impact of mepolizumab on the reduction in tissue $CD62L^{low}$ cells. This was also true for patients who did and did not undergo ESS, although the number of patients in our study was limited (Figure 5C).

A Higher Degree of Tissue iEos Reduction Is Associated With a More Marked Improvement in CRSwNP

To verify whether the reduction in tissue $CD62L^{low}$ iEos was associated with a greater improvement in CRSwNP scores, we investigated the correlation between the delta change (Δ) in iEos percentage and the variation in clinical and endoscopic parameters. A statistically significant positive correlation was found between Δ-$CD62L^{low}$ in tissue and both Δ-SNOT-22 and Δ-LK (r=0.61 [*P*<.05] and r=0.56 [*P*=.05], respectively). No correlation was demonstrated between Δ-$CD62L^{low}$ and both Δ-NPS and Δ-SST-16 (r=–0.14 [*P*=.65] and r=–0.11 [*P*=.72]) (Figure 5D). As far as the reduction in blood iEos is concerned, the only correlation we observed was the improvement in the SNOT-22 score (r=–0.52 [*P*<.05]). When we considered patients with >6 months of treatment, a similar trend was observed for the SNOT22 and L-K scores, although this was not statistically significant, probably owing to the low number of patients. Conversely, the coefficient of correlation for the Δ-SST-16 showed a statistically significant increase (r=0.77 [*P*<.05]) (Figure 5E).

Discussion

The present study, which was performed in a real-world setting, confirms that in addition to improving asthma control, mepolizumab is significantly effective in CRSwNP, reducing nasal symptoms and endoscopic features. Additionally, our findings support the clinical relevance of $CD62L^{low}$ iEos, considering the correlation between severity of nasal symptoms and percentage of iEos in tissue. More importantly, to our knowledge, this is the first study to demonstrate the capacity of mepolizumab to decrease infiltration of tissue by the iEos subpopulation.

A mepolizumab-induced decrease in nasal polyp tissue eosinophilia has been reported [18]. Our study confirms a strong reduction in tissue eosinophils in nasal polyps with mepolizumab, reaching over 70%; however, more importantly, we observed that this effect was preferentially related to iEos. We had previously reported a decrease in blood iEos in mepolizumab-treated asthma patients and that this biological effect correlated with an improvement in clinical outcomes. IL-5, as well as other cytokines (IL-3, GM-CSF, TSLP), are involved in Eos activation and in modulation of surface CD62L expression, both in vitro and in vivo, as shown in our previous study [15]. We also reported that iEOS accumulate preferentially in tissue [11]. Concerning the clinical implications of this study, we highlight iEos as target cells of mepolizumab and stress that the clinical effectiveness of this monoclonal antibody is related to its capacity to rebalance the iEos/rEos ratio, although additional IL-5–dependent mechanisms are involved. Specifically, our results show how important it is to address tissue iEos to obtain a clinical response, as the degree of improvement correlates with the degree of reduction in iEos in tissue. Finally, to our knowledge, data showing the tissue effect of antieosinophilic therapy on eosinophil subphenotypes have not been published to date. A key finding in the current study is the positive correlation between the impact of mepolizumab on iEos and its therapeutic effects in CRSwNP. Given that iEos are more abundant in polyp tissue than in blood [11], this finding suggests a pathogenic role for iEos, indicating that they may represent a pivotal target of treatment in eosinophilic inflammation. Otherwise, iEOS can release a range of granule mediators, including major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin, as well as cytokines (eg, IL-4, IL-3, IL-5, TNF-α, TGF-ß) and cytosolic Charcot-Leyden crystal protein/galectin-10 (CLC/Gal-10) [19,20].

The remaining eosinophils found in the nasal tissue of mepolizumab-treated patients largely (about 75%) belong to the resident subpopulation (rEos). This observation resembles the effects of mepolizumab in the blood of severe eosinophilic asthma patients, where the reduction in iEos cells was paralleled by the increase in rEos cells, thus restoring a healthy balance among eosinophil subpopulations [15]. The residual resident/homeostatic eosinophils observed during treatment with mepolizumab could represent a further mechanism supporting the clinical efficacy of the drug, taking into account that rEos are presumed to exert immunomodulatory functions, thanks to their anti-inflammatory signature [21,22]. As regards the effect of mepolizumab on the percentage of iEos at tissue level, which in our case series was found to be independent of the duration of treatment and the severity of asthma, we can hypothesize that it is related to the rapid effect exerted by the drug.

We recorded a more significant improvement in NPS in patients who had received more doses of mepolizumab and for a period longer than 6 months. Moreover, a faster improvement in SNOT-22 was recorded. These data suggest an early and long-term effect of mepolizumab on endoscopic severity scores. This observation is highly consistent with the indication from EPOS/EUFOREA 2023 to assess the response to biological therapy in CRSwNP at least 6 months after initiation of treatment [17]. Our data suggest that the impact of mepolizumab on eosinophils, and specifically on iEos, seems to be more related to the early effect on SNOT-22 than to the late-onset effect on NPS. The improved NPS in affected patients after long-term treatment could be dependent on the blockage of IL-5 functions on a variety of cells expressing IL-5R (eg, plasma cells, mast cells), which are also involved beyond eosinophils in the inflammatory process at nasal tissue level [23,24].

The histological analysis conducted in our mepolizumab-treated patients revealed a significant reduction in mucous cell hyperplasia and subepithelial edema. This change could be the consequence of reduced eosinophil infiltration, with decreased values for the proinflammatory mediators/cytokines

released by these cells. However, it could be also related to the direct blockage of IL-5 and of its activity on mucous cells. In fact, it has been clearly shown that even if IL-13 is a key component in stimulating mucus hypersecretion [25], IL-5 production enhances airway mucous metaplasia in mouse models of asthma [26]. The changes in goblet cells during type 2 inflammation are included in the wider concept of airway remodeling. Therefore, the effects of mepolizumab on goblet cells we observed are consistent with the previous reduction in remodeling-related markers such as tenascin, lumican, and procollagen III reported in mepolizumab-treated patients [27,28]. The data reported above may support the potential effect of mepolizumab on the complex phenomenon of upper and lower airway remodeling.

Although small in size, our case series is notable in that the nasal polyps had disappeared completely (endoscopic remission) in approximately 15% of longitudinally followed patients, pointing to full inhibition of the inflammatory process in at least a proportion of patients. Nevertheless, we were unable to confirm the resolution at mucosal level. Future research should attempt to explain the decrease in the nasal polyp size without complete resolution in the remaining patients. After all, other factors, such as IL-4/IL-13 signals, are strongly involved in the inflammatory process in CRSwNP associated with type 2 cell responses and epithelial barrier, mucociliary, and olfactory dysfunction [29].

Our study is subject to a series of limitations. First, the number of enrolled patients with a biopsy finding before and after treatment with mepolizumab was relatively low. In addition, the analysis may have been affected by the fact that we considered only 43% of the patients owing to the availability of an ENT evaluation before and after treatment. Second, our study was performed at a single center, although this guarantees standardization of enrollment, ENT procedures, and flow cytometry in a high-experience laboratory. Finally, since our study was performed under real-world conditions, it did not include a placebo or a control group.

In conclusion, this is the first study to describe the relationship between tissue iEos and clinical/endoscopic severity in CRSwNP patients and to highlight the capacity of mepolizumab in reducing iEos, goblet cell hyperplasia, and edema at tissue level. The effect observed on iEos was positively associated with the improvement in nasal symptoms, thus reinforcing the relationship between the biological effects and clinical outcomes obtained with mepolizumab.

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Conflicts of Interest

AM has received fees for advisory boards and lectures from AstraZeneca, Sanofi, GSK, Takeda, and Chiesi. AV has received fees for advisory boards and lectures from AstraZeneca, Sanofi, GSK, Takeda, and Firma. LC has received fees for advisory boards and lectures from GSK, Novartis, Alk-Abello, Sanofi, and Menarini. PP has received fees for advisory boards and lectures from GSK, Novartis, and Leofarma. OR has received fees from Alk-Abello, Novartis, Generic, Firma, Sanofi, GSK, and Recordati. EV has received fees for lectures from GSK. GM has received fees for lectures from Sanofi, GSK, and Novartis. The remaining authors declare that they have no conflicts of interest.

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