Received: 6 April 2023 | Revised: 28 June 2023 | Accepted: 10 July 2023

DOI: 10.1111/all.15844

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

ORIGINAL ARTICLE

Dupilumab-induced eosinophilia in patients with diffuse type 2 chronic rhinosinusitis

Fabio S. Ryser[1,2] | Ayla Yalamanoglu[3] | Alan Valaperti[3] | Catrin Brühlmann[4] | Tina Mauthe[4] | Stephan Traidl[5,6,7] | Michael B. Soyka[4] | Urs C. Steiner[1,8]

[1]Department of Rheumatology and Immunology, University Hospital Bern, University of Bern, Bern, Switzerland

[2]Faculty of Medicine, University of Bern, Bern, Switzerland

[3]Department of Immunology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

[4]Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Zurich, University of Zurich, Zurich, Switzerland

[5]Department of Dermatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

[6]Department of Dermatology and Allergy, Hannover Medical School, Hannover, Germany

[7]Swiss Institute for Asthma and Allergy Research, Davos, Switzerland

[8]Institute of Clinical Chemistry, Inselspital Bern University Hospital, University of Bern, Bern, Switzerland

Correspondence
Urs C. Steiner, Universitätsklinik für Rheumatologie und Immunologie, Inselspital, Universitätsspital Bern, Haus 5, Freiburgstrasse 16p, 3010 Bern, Switzerland.
Email: urs.steiner@insel.ch

Abstract

Background: Dupilumab, a monoclonal anti-IL-4Rα antibody, is approved for several type 2 mediated inflammatory diseases like asthma, atopic dermatitis, and diffuse type 2 chronic rhinosinusitis (CRS). Clinical studies had reported a transient increase in blood eosinophils during dupilumab therapy.

This study aimed to assess the impact of elevated blood eosinophils on clinical outcome and to investigate the cause of high blood eosinophil levels under dupilumab therapy.

Methods: Patients suffering from diffuse type 2 CRS treated with dupilumab were examined on days 0, 28, 90, and 180 after therapy start. Sino-Nasal-Outcome-Test Score (SNOT-22), Total Nasal Polyp Score (TNPS), and blood samples were collected. Cytokine measurements and proteomics analysis were conducted. Flow cytometry analysis measured receptor expression on eosinophils.

Results: Sixty-eighty patients were included. Baseline eosinophilia ≥0.3G/L was observed in 63.2% of patients, and in 30.9% of patients, eosinophils increased by ≥0.5G/L under dupilumab. Subjects with eosinophilia ≥0.3G/L at baseline had the best SNOT-22 mean change compared to no eosinophilia. Eosinophil elevation during dupilumab therapy had no impact on clinical scores. The eosinophil adhesion molecule VCAM-1 decreased significantly during therapy in all patients. The chemokine receptor CXCR4 was significantly down- and IL-4 upregulated in subjects with eosinophil increase.

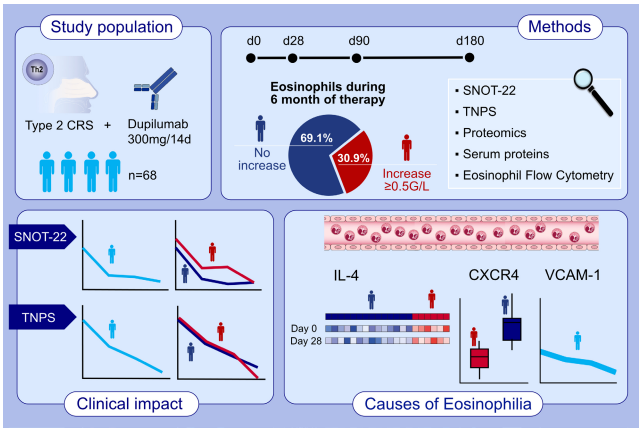
Conclusion: Our findings suggest that increased eosinophils in type 2 CRS are associated with a good clinical response to dupilumab. Patients with elevated IL-4 at baseline developed dupilumab-induced transient eosinophilia. We identified the

Abbreviations: CCR3, C-C chemokine receptor 3; CRS, Chronic rhinosinusitis; CRSwNP, Chronic rhinosinusitis with nasal polyps; CXCR4, CXC chemokine receptor 4 (stromal cell-derived factor 1 receptor); ECP, Eosinophilic cationic protein; EGPA, Eosinophilic Granulomatosis with Polyangiitis; ELISA, Enzyme-linked Immunosorbent Assay; EPOS20, European Position Paper on Rhinosinusitis and Nasal Polyps 2020; EUFOREA, European Forum for Research and Education in Allergy and Airway Diseases; FACS, Fluorescence-activated cell sorting; FEIA, Fluorescence-Enzyme-Immunoassay; GCPR, G-protein coupled receptor; ICAM-1, Intercellular adhesion molecule 1; IgE, Immunoglobulin E; IL-13, Interleukin 13; IL-4, Interleukin 4; IL-4Rα, Interleukin 4 receptor alpha; IL-5, Interleukin 5; NERD, Non-steroidal anti-inflammatory drug-exacerbated respiratory disease; RCT, Randomized Controlled Study; SNOT-22, Sino-nasal outcome test 22 questionnaire; Th2, T-helper cell 2; TNPS, Total nasal polyp score; VCAM-1, vascular adhesion molecule 1; VLA-4, very late antigen 4 (CD49d, Integrin α-4).

Michael B. Soyka and Urs C. Steiner contributed equally.

downregulation of VCAM-1 and surface markers CD49d and CXCR4 on eosinophils as possible explanations of dupilumab-induced eosinophilia.

KEYWORDS
diffuse type 2 chronic rhinosinusitis, dupilumab, eosinophilia, Interleukin-4, VCAM-1



GRAPHICAL ABSTRACT
Transient blood eosinophilia seems to have no clinical impact in patients with type 2 CRS treated with dupilumab. High IL-4 levels at baseline induce dysregulation of migration, chemotaxis, and homing of eosinophils by decreased CXCR4 on eosinophils and VCAM-1. CXCR4 might be a useful marker to predict blood eosinophilia in patients with type 2 CRS under dupilumab.

1 | INTRODUCTION

Chronic Rhinosinusitis (CRS) affects about 10.9% of the global population and significantly impacts patients' health-related quality of life.[1–3] Type 2 inflammation is the predominant inflammatory endotype of CRS with Nasal Polyps (CRSwNP) in Europe, affecting 84%–91% of patients suffering from CRS.[4–7] Type 2 inflammation is characterized by Th2 T-cells, innate lymphoid cells 2 (ILC2), eosinophils, and mast cells (MC). The corresponding cytokine milieu includes IL-4, IL-5, and IL-13.[8] IL-4 induces cell activation, trafficking to the site of inflammation, and B-cell IgE isotype switching. IL-5 promotes eosinophilic growth, survival, and differentiation, and IL-13 supports maturation and mucus production of epithelial cells and induction of tuft cells and PGE2-mediated inflammation.[9–13]

In recent years, novel monoclonal antibody therapies have been developed targeting receptors and mediators of Th2 inflammation. Dupilumab is a human monoclonal antibody that binds to the IL-4-receptor alpha subunit (IL-4Rα) and inhibits the IL-4 and IL-13 signaling. Several randomized placebo-controlled trials have demonstrated the efficacy of dupilumab in patients suffering from type 2 mediated inflammatory diseases such as CRSwNP, asthma, prurigo nodularis, atopic dermatitis, and eosinophilic esophagitis.[14–20]

As a common adverse event, patients treated with dupilumab may develop transient eosinophilia. Eosinophils can increase up to ≥3.0G/L and are highest in patients with asthma, followed by CRS and atopic dermatitis.[21] Most often transient eosinophilia had no clinical impact.[22–24] However, Eosinophilic Granulomatosis with Polyangiitis (EGPA) has been described.[25]

The exact pathomechanism of dupilumab-associated eosinophilia has not yet been fully elucidated. It is hypothesized that blocking IL-4Rα induces downregulation of the adhesion molecule VCAM-1 and the chemokines eotaxin-1, 2, and 3.[26–29]

The aim of this study was to characterize patients who developed transient eosinophilia under dupilumab treatment and to evaluate the clinical impact of blood eosinophilia.

2 | METHODS

2.1 | Study design and population

This retrospective and prospective case–control study includes patients suffering from uncontrolled diffuse type 2 CRS treated with dupilumab (anti-IL4Rα-monoclonal antibody) between June 2020 and October 2022. The study was approved by the ethics committee of the canton of Zurich (KEK-2020-02955, 2021–01213) and was conducted in accordance with the Declaration of Helsinki.[30]

All participants were recruited at the Department of Otorhinolaryngology, Head and Neck Surgery, at the University Hospital Zurich, Zurich, Switzerland.

Sixty-eight patients diagnosed with severe uncontrolled diffuse type 2 CRS, according to the EPOS20 guidelines, were included in this study.[6] The indication of dupilumab therapy was set by senior physicians in line with the EUFOREA criteria for biologics treatment.[31] All patients had undergone at least one sinus surgery or had a contraindication for surgical procedures. Clinical data and

biosamples from 41 subjects of the retrospective study and from 27 subjects of the prospective project, were evaluated for analysis.

Dupilumab (Dupixent® by Sanofi and Regeneron Pharmaceuticals, Inc.) 300 mg was injected subcutaneously by the patients themselves, every 2 weeks. Instructions on medication use and application were provided by the Department of Immunology at the University Hospital Zurich, Zurich, Switzerland. All subjects continued topical glucocorticoid treatments, including mometasone/fluticasone sprays, fluticasone drops, or budesonide rinses, as prescribed by the treating physician. No patient needed rescue medication of systemic glucocorticoid. Control samples were provided by 11 healthy subjects who reported no inflammatory or allergic disorders. Fifteen individuals suffering from diffuse type 2 CRS and treated with dupilumab for more than 6 months were recruited additionally as a control group with long-term treatment.

2.2 | Follow-ups

All participants of the study were seen at the start of treatment (day 0) and on days 28, 90, and 180 (±7 days) after therapy start in the interdisciplinary consultation of the Department of Otorhinolaryngology, Head and Neck Surgery, and the Department of Immunology at the University Hospital Zurich. Blood samples (EDTA tubes, heparin tubes, and serum tubes) and the Total Nasal Polyp Score (TNPS) evaluated by rhinoscopy were assessed on each visit. The Sino-Nasal-Outcome Test 22 (SNOT 22) questionnaire, which includes standardized questions about nasal patency, rhinorrhea, sense of smell, sleep, emotions, and pain, was electronically filled out by the participants on their mobile device each week until 6 months after the first injection.

2.3 | Definition of eosinophilia during dupilumab therapy

Blood eosinophil levels were measured at each visit. We identified patients with baseline eosinophilia by elevated blood eosinophil counts at a threshold of ≥0.3 G/L on day 0. Patients with an increase of eosinophils during ongoing dupilumab therapy were characterized by an eosinophil level change of at least 0.5 G/L from day 0 to either day 28, 90, or 180.

2.4 | Assay of VCAM-1, Eotaxin, IL-4, ICAM-1, IL-5, and ECP

IL-4, ICAM-1, VCAM-1, and eotaxin-1 (CCL11) in the serum were measured with the flow Cytometric Bead Array (CBA) of BioLegend® by using LEGENDplex™ technique following the manufacturer's protocol. Blood serum samples of the whole cohort on day 0 (30 samples), day 28 (22 samples), and day 90 (11 samples) were used. Missing samples occurred randomly. IL-5 in the serum was measured with CBA of BD Biosciences following the manufacturer's protocol. Serum levels of ECP were analyzed using the Fluorescence Enzyme Immunoassay (FEIA) technology of ThermoFisher and preclinical precautions were followed as instructed by the local laboratory technicians.

2.5 | Flow cytometry staining of eosinophilic granulocytes

Leukocytes were isolated using HetaSep (Stemcell) as described by the manufacturer. Briefly, heparinized blood was mixed with HetaSep and centrifuged with the brake off at 100*g* for 5 min. The leukocyte-enriched upper layer was pipetted off and washed with flow cytometry buffer (DPBS +1% fetal bovine serum +2 mM EDTA). Cells were then stained with fluorochrome-conjugated monoclonal antibodies on ice for 20 min. The following receptor expression levels on eosinophils were evaluated: CCR3, CCR4, CD11b, CD162, CD49d, CD62L, CD66b, CD88, CRTH2, CXCR3, CXCR4 (Table S1). Afterwards, the cells were washed and fixed at room temperature with 4% paraformaldehyde for 10 min. First granulocytes were gated based on unique forward scatter (FSC) and side scatter characteristics (SSC), then cell doublets were excluded, and finally eosinophils were identified as CD16 negative cell population.[32] The samples were acquired with a BD LSRFortessa and data was analyzed using FlowJo software.

2.6 | Olink® analysis

Twenty available sera from days 0 and 28 of the prospective cohort were collected and analyzed using the Olink® technique. In accordance with the manufacturer's instructions, Olink proteomics analysis was performed including the "inflammation" panel. This panel includes 92 proteins. The results were statistically evaluated as NPX (normalized protein expression) values by a shiny application using limma, ggplot2, and pheatmap packages.

2.7 | Statistical analysis

Descriptive statistics are reported depending on the distribution in means and standard deviation respectively in medians and IQR (interquartile range). For parametric variables, the paired Student's *t*-test was applied, or its nonparametric equivalent, the Wilcoxon signed-rank test. Normal distribution was tested using the Shapiro–Wilk test. Correlations between variables were analyzed using Spearman's Correlation. A linear regression model tested the correlation findings. An R-based shiny app using the limma package was used for Olink® analysis. Sensitivity and specificity were assessed using the receiver operating curve method, Youden's index, and the area under the curve. Multiple testing was corrected using the Bonferroni correction method. All statistical analyses were conducted using R and R-studio by IBM. A p-value of $<.05$ was assumed significant.

3 | RESULTS

The analysis was based on 68 consenting patients with a mean age of 50.7 years (SD 13.4). Two patients reported side effects (tinnitus, anxiety disorder) and one patient showed a lack of adherence leading to discontinuation of dupilumab after three injections (after 6 weeks). Sixty-five patients completed the follow-up after 6 months of dupilumab therapy. Forty-three subjects showed eosinophilia of ≥0.3G/L at therapy start and 21 patients developed an increase of eosinophils of ≥0.5G/L during 6 months of dupilumab treatment (Table 1).

3.1 | Clinical response to dupilumab treatment in CRS

All groups had a significant clinical response to dupilumab treatment, measured by SNOT-22 score and TNPS. Patients with eosinophilia ≥0.3G/L at baseline showed the most benefit from the dupilumab treatment after 1, 3, and 6 months, with a mean change of 51.2% of the SNOT-22 score (mean differences: 22.6, 26.4, 26) after 1 month, compared to a mean change of 32.5% in patients without eosinophilia at baseline. However, the TNPS (mean differences: 3, 3, 5) changed equally by 50% in the two groups (Figure 1, Table S2).

TABLE 1 Demographics and characteristics.

	Total ($N = 68$)
Age	
Mean age	50.7 (SD 13.4; range 27–76)
Sex	
Men	45 (66.2%)
Women	23 (33.8%)
NERD	
No NERD	44 (64.7%)
NERD	24 (35.3%)
Asthma	
No asthma	16 (23.5%)
Asthma	52 (76.5%)
No of Sinus Surgeries	
0	15 (22.1%)
1	21 (30.9%)
2	15 (22.1%)
3	9 (13.2%)
4	6 (8.8%)
5	2 (2.9%)
Elevated eosinophils (≥0.3G/l) at baseline (day 0)	
No elevated eosinophils d0	25 (36.8%)
Elevated eosinophils d0	43 (63.2%)
Eosinophil increase (≥0.5G/l) during 6 months of treatment	
No eosinophil increase	47 (69.1%)
Eosinophil increase	21 (30.9%)

Abbreviation: NERD, nsaid exacerbated respiratory disease.

3.2 | Eosinophil levels under dupilumab treatment

Overall, the eosinophilic granulocyte count did not increase over the 6 months. Means were 0.46G/L, 0.57G/L, 0.55G/L, and 0.46G/L on days 0, 28, 90, and 180, respectively. The highest individual eosinophil count was 2.57G/L on day 28 of dupilumab treatment; this one had started at 1.01G/L on day 0 (Figure 2A). Patients with eosinophils of ≥0.3G/L at baseline, had no significant further increase in blood eosinophil levels under dupilumab treatment (Figure 2C).

Subjects with an increase of eosinophils ≥0.5G/L during dupilumab treatment showed higher eosinophil levels on days 28 and 90 (Figure 2B).

3.3 | Serum biomarker expression under dupilumab treatment

Overall IL-5 showed a nonsignificant increasing trend on days 90 and 180 compared to day 0 (p-value .23, .76). In the patient group with an eosinophil increase of ≥0.5G/L under dupilumab treatment, IL-5 had the same trend of increase on day 90 compared to day 0 of dupilumab treatment (p-value .12). Overall ECP showed no significant changes over time. In the group with an eosinophil increase, we found the same trend on day 28 (p-value .31). In the group without increased eosinophils, we observed a nonsignificant reduction of ECP at day 28 (p-value: .16, Figure 2G).

Eotaxin-1 (CCL11) and ICAM-1 showed unchanged overall expression over time and no changes during dupilumab therapy (data not shown). VCAM-1 was overall irregularly expressed with a high standard deviation at all timepoints (means day 0: Mean 4415.6 ± 6894.3, day 28: Mean 6781.4 ± 9617.7, day 90: Mean 3991.2 ± 6492.0, day 180: Mean 1456.6 ± 713.1) and significantly decreased on day 180 compared to day 0 (p-value .048, CI 169.7–12,299.71, V = 54) (Figure 2H). IL-4 showed overall a nonsignificant higher expression on days 90 and 180 compared to day 0. In the group with eosinophil increase, VCAM-1, ICAM, eotaxin-1, and IL-4 showed no significant different expressions on days 28, 90, or 180 compared to baseline. Furthermore, there were no significant differences between the groups with and without eosinophil increase (data not shown).

Comparing the mean changes of VCAM-1, ICAM-1, IL-4, and Eotaxin-1 under dupilumab treatment with the mean changes of eosinophil counts between days 0 and days 28 or 90 overall, we found no significant correlation.

3.4 | Flow cytometric analysis of eosinophils surface markers

CD49d (Integrin α) was significantly higher expressed in healthy controls compared to the patients in our cohort on days 0, 28, and 90 (Figure 3A). In the group with an eosinophil increase during dupilumab treatment, CXCR4 was significantly downregulated compared to the

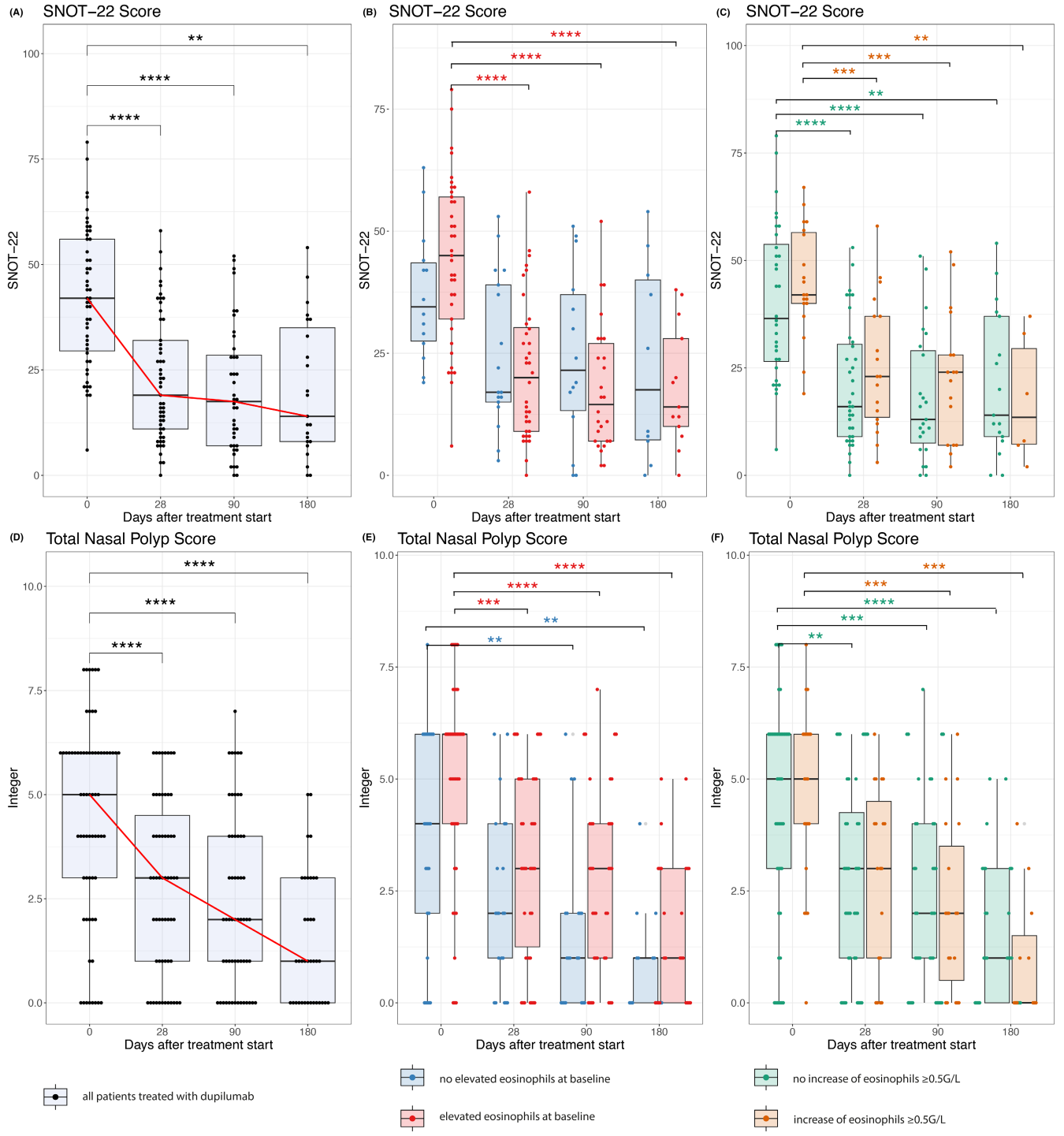


FIGURE 1 SNOT-22 Score and Total Nasal Polyp Score in type 2 CRS patients treated with dupilumab on days 0, 28, 90, and 180 after treatment start. Subgroups are defined by elevated eosinophils ≥0.3G/l at baseline (red) and by an increase of eosinophils of at least ≥0.5G/L during 6 months of therapy (orange). (A) and (D) SNOT-22 Score and TNPS overall, (B) and (E) SNOT-22 Score and TNPS grouped by eosinophils ≥0.3G/L (red) and normal eosinophils ≤0.3G/l (blue) at baseline, (C) and (F) SNOT-22 Score and TNPS grouped by an increase of eosinophils ≥0.5G/L during 6 months of therapy (orange) and no increase of eosinophils ≥0.5G/L during 6 months (green). SNOT-22: Sino-Nasal-Outcome Test 22, TNPS: Total Nasal Polyp Score. *p*-values are reported above the brackets: 0.05*, 0.01**, 0.001***, 0.0001****.

group without eosinophil increase on days 0 and 28 (Figure 3D). Expression levels of other receptors (CCR3, CCR4, CD11b, CD162, CD62L, CD66b, CD88, CRTH2, CXCR3) showed no significant changes in the analysis overall and of the subgroups under dupilumab therapy.

3.5 | Correlation of serum biomarkers, surface receptor expression, and eosinophils

Spearman's correlation of blood eosinophil levels and the different measured biomarkers (ECP, IL-5, VCAM-1, ICAM-1, IL-4,

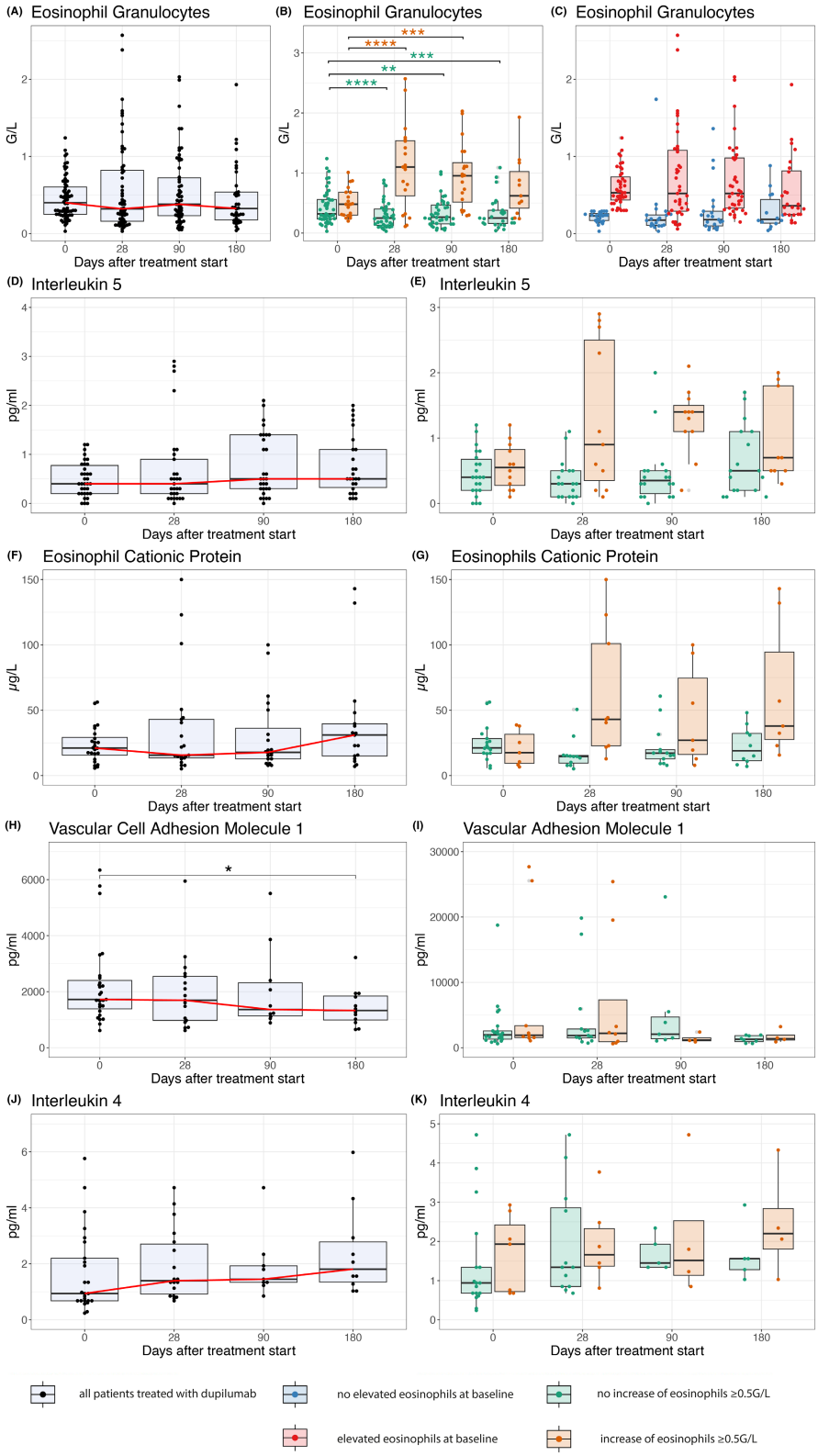


FIGURE 2 Blood eosinophil levels and serum expression levels of ECP, IL-5, VCAM-1, and IL-4 on days 0, 28, 90, and 180 overall and eosinophil increase under dupilumab therapy. Overall expression of (A) level of blood eosinophils, (D) IL-5-, (F) ECP-, (H): VCAM-1-, and (J) IL-4-concentration. Grouped by an eosinophil increase of ≥0.5G/l (orange) or no eosinophil increase (green): (B) level of blood eosinophils, (E) IL-5-, (G): ECP-, (I): VCAM-1- and (K) IL-4 concentration. (C) blood eosinophils grouped by elevated eosinophils at baseline (red), non-elevated eosinophils at baseline (blue). *p*-values are reported above the brackets: 0.05*, 0.01**, 0.001***, 0.0001****.

eotaxin-1) on all timepoints showed only positive correlations between IL5, ECP, and eosinophils (Figure S1). There were no significant correlations between VCAM-1, IL-4, ICAM-1, Eotaxin-1, and eosinophils.

The development of high eosinophil levels under dupilumab is associated with a low expression of CXCR4. CXCR4 expression also shows a significant impact on eosinophils in a linear regression model ($r2 = -0.0083$, SE 0.0023, $t = -3.6$, p-value $< .001$, $y = -0.008324 * x + 0.784597$). CD49d as well as CCR3 correlate positively with CXCR4 (Figure 4). VCAM-1, ICAM-1, and eotaxin-1 show no association with the receptor expression levels (data not shown).

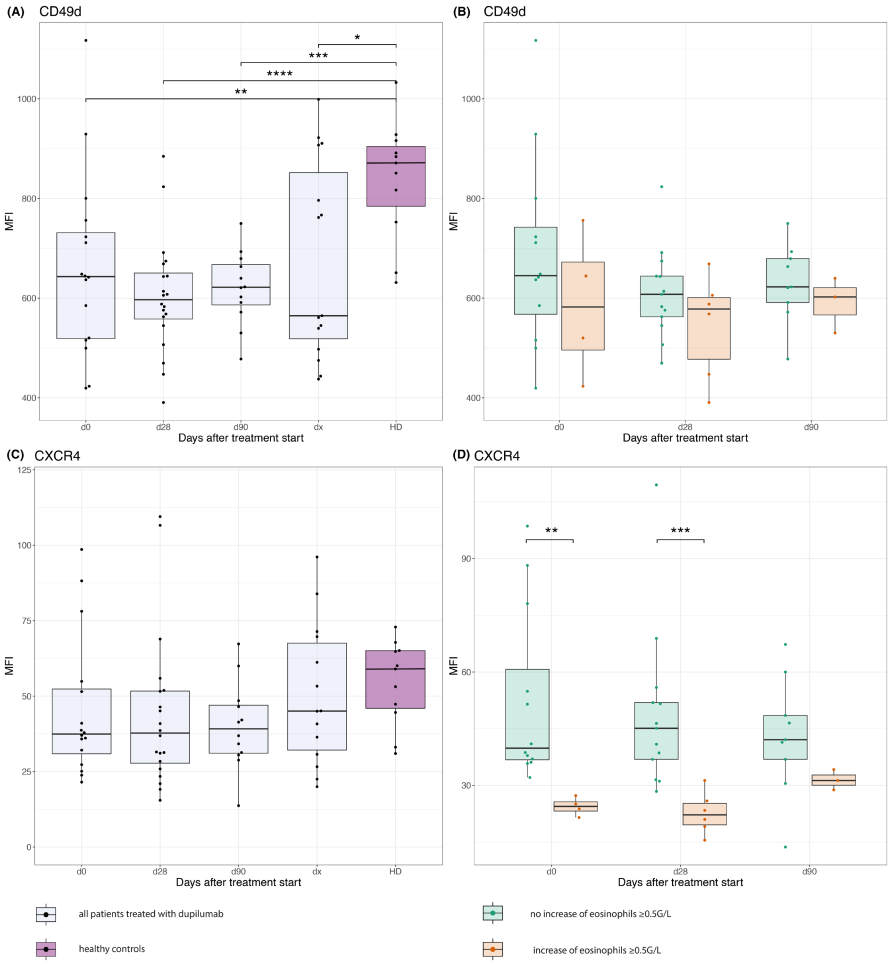


FIGURE 3 Eosinophil receptor CD49d and CXCR4 expressions under dupilumab therapy. Boxplots of eosinophil surface markers measured by flow cytometry on days 0, 28, 90, and 180, compared with healthy controls (HD). Dx indicates patients with long-term treatment of dupilumab (>6 months), (A) and (C) CD49d (Integrin-α-4) and CXCR4 expression overall, mean differences between HD and the different timepoints days 0, 28, 90 and with long-term treatment of dupilumab (dx). (B) and (D) CD49d and CXCR4 expression grouped by an increase of eosinophils ≥0.5G/L and no increase of eosinophils. Adjusted p-values are reported above the brackets: 0.05*, 0.01**, 0.001***, 0.0001****.

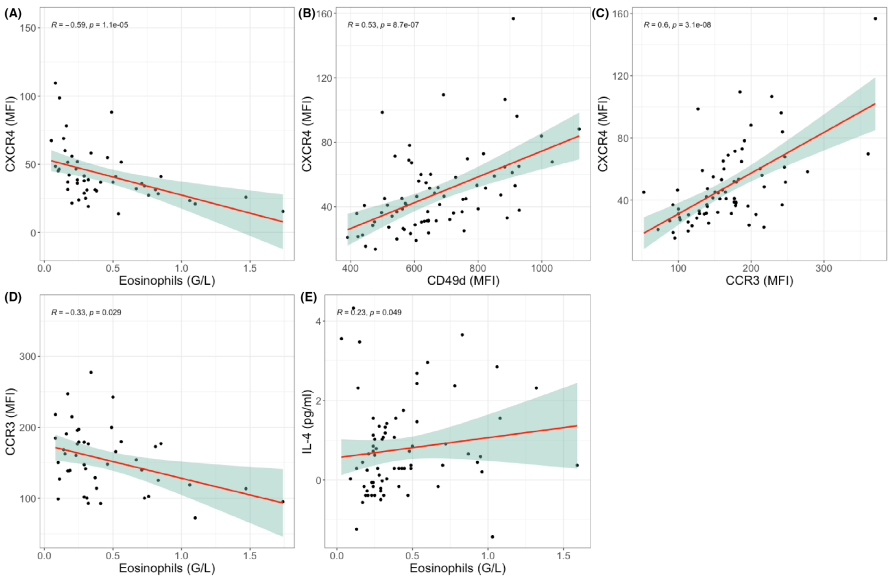


FIGURE 4 (A) Spearman's correlation of CXCR4 and eosinophils of all patients, the linear trend is shown by a red line and confidence interval by a green shadow, (B) correlation of CXCR4 and CD49d, (C) correlation of CXCR4 and CCR3, (D) correlation of CCR3 and eosinophils, (E) correlation of IL-4 and eosinophils.

3.6 | Proteomics analysis

For further evaluation of dysregulated proteins between patients with eosinophil increase and without, Olink® analysis was performed. On day 0 of treatment, five proteins were already expressed differently, including IL4, CCL25, CCL19, and IFN-γ. The same proteins were either upregulated or downregulated on day 28 of treatment (Figure 5).

To examine IL-4 and CXCR4 as possible predictors for dupilumab-induced eosinophilia, we conducted a ROC analysis. The area under the curve for IL-4 was 0.738 (73.8%) and for CXCR4 94%. For IL-4 the sensitivity was 83% and specificity 50% at a threshold of

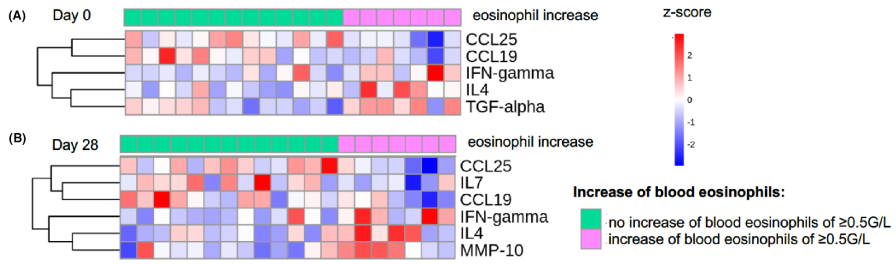


FIGURE 5 Heatmaps of dysregulated proteins analyzed by Olink® method. Values are reported standardized. The z-score legend is presented on the right side of the heatmap. Groups are indicated above the heatmaps. Green: subjects without an increase of eosinophils, pink: subjects with an increase of eosinophils of at least 0.5G/L during 6 months of therapy. (A) Samples on day 0 of dupilumab therapy, (B) day 28.

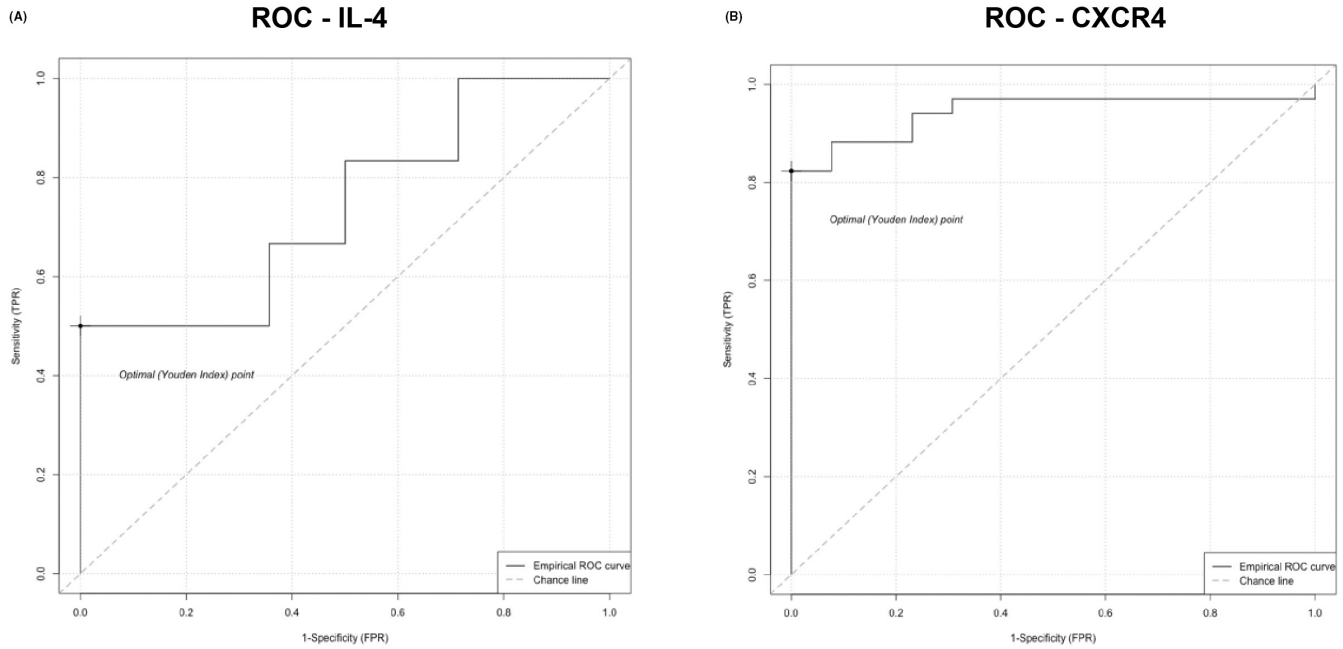


FIGURE 6 ROC curve for development of dupilumab-induced eosinophilia of (A) IL-4 measured by Olink® and (B) CXCR4. The optimal point is indicated by the Youden index.

1.35 pg/mL. The sensitivity for CXCR4 was 88% and specificity 92% at a threshold of 31.5 Mean Fluorescence Intensity (MFI) (Figure 6).

4 | DISCUSSION

In this study, we investigated patients with diffuse type 2 CRS under therapy with dupilumab for the outcome and development of eosinophilia. A rapid clinical response was achieved in all patients included regardless of eosinophil counts. Patients with eosinophilia ≥0.3G/L at baseline had an even better clinical response. An increase of eosinophils while on dupilumab was found in 30.9%. They showed elevated IL-4 values and decreased CXCR4 expression on eosinophils before treatment start with dupilumab.

In our cohort, all groups reached the minimal clinically important difference of 12 points (MCID) of the SNOT-22 score after 1 month under dupilumab therapy.[33] In line with the findings of Bertlich M. et al., and Fujieda et al., who reported a better clinical response to dupilumab in patients with severe eosinophilic CRS, we observed the best SNOT-22 mean changes in patients with eosinophilia at baseline.[34,35] In these patients, we also observed no further increase in blood eosinophils during dupilumab treatment.

The 21 patients with an eosinophil increase of ≥0.5G/l during dupilumab treatment reported the same clinical response compared to patients without an eosinophil increase. None of the patients with eosinophil increase developed eosinophil-induced symptoms, and therapy with dupilumab could be continued in all of them. Unlike Deimling et al., we also observed no cases of EGPA.[25] Therefore, the increase of eosinophils does generally not seem to be clinically relevant. This is in line with a recently published systematic review by Wechsler ME. and Wollenberg et al.[24,26] Furthermore, transient eosinophilia seems to recover after about 6 months as described by Bachert et al.[16]

The overall IL-5 concentration in our cohort showed a slight but not significant increase. This is in contrast to the findings described

in other studies.[16,36] In the subgroup analysis, the increase of IL-5 could be clearly assigned to the cohort with an eosinophil increase during dupilumab therapy. Also, for ECP, we observed an increase in the subgroup with dupilumab-induced eosinophilia, whereas the overall ECP was not increasing. The slight increase of IL-5 positively correlates with blood eosinophils (Figure S1). Previous studies indicate that Th2 and ILC2 cells are downregulated in patients treated with dupilumab. This might suggest that the elevated number of secreting eosinophils in our study could be responsible for the increase of IL-5 and ECP.[37–42] However, in this study we did not investigate lymphocytes and MC as possible sources of IL-5 secretion, therefore the source of elevated IL-5 cannot be definitively attributed and should be further investigated.

We could demonstrate that VCAM-1 is reduced in the serum of dupilumab-treated patients as hypothesized by Castro et al. and Hamilton et al.[17,28]

VCAM-1 is an adhesion molecule, which binds to the surface receptor CD49d (Integrin alpha4/beta1) of eosinophils and enables them to roll along the endothelium for tethering and migration into inflamed tissue.[43,44] VCAM-1 is significantly upregulated in IL-4-mediated inflammation and is induced by IL-4 through the STAT6 pathway.[45–47] In our cohort, we observed a reduction of VCAM-1 overall after 6 months of dupilumab therapy. This finding suggests that the reduction of VCAM-1 resulting from blocking the IL-4 receptor leads to a decrease in rolling, adhesion, and migration of eosinophils, which ultimately results in an increase of circulating blood eosinophils.

CD49d (VLA-4) is the corresponding receptor for VCAM-1 on eosinophils; it showed a decreased expression at almost all time points compared to healthy controls. CD49d is downregulated by IL-4 through the STAT6 pathway.[48,49] Accordingly, CD49d on eosinophils is reduced in patients with type 2 inflammation.[50] Decreased CD49d on eosinophils reduces adhesion and migration and leads to an increase in peripheral blood eosinophil levels. CD49d expression on eosinophils did not change in patients treated with dupilumab, while VCAM-1 was downregulated.

CXCR4 on eosinophils was significantly downregulated on days 0 and 28 in patients with an increase of eosinophils of at least 0.5G/L during 6 months of dupilumab treatment and compared to patients without an increase of eosinophils, it correlated negatively with the blood eosinophil counts. CXCR4/CXCL12 plays an important role in chemotaxis, cell growth, and cell migration of eosinophils.[51–53] Nagase et al. further reported that CXCR4 surface expression was downregulated by IL-4.[54,55] CXCR4 is also crucial for homing of eosinophils into the CXCL12-rich tissue including bone marrow.[56] Therefore, eosinophils with low CXCR4 have a reduced capacity for migration in tissues and for homing in CXCL12-rich tissues, leading to an increase in circulating blood eosinophils. The negative correlation of CXCR4 and eosinophil levels shown in our study supports this hypothesis (Figure 4A). The difference in CXCR4 expression among the groups with and without eosinophilia was not anymore significant on day 90 (Figure 3D) suggesting that the CXCR4 receptor expression levels on eosinophils seemed to improve when the IL-4 receptor was blocked with dupilumab. CXCR4 expression positively correlated with CD49d and CCR3, an eotaxin receptor (Figure 4B,C). Eosinophils with low expression of CXCR4 had also low numbers of CCR3 and CD49d receptors on the surface. Low CCR3 expression leads to worsened chemotaxis and migration.[57] The lack of CD49d prevents eosinophil tethering, rolling, and migration.[48,49] The combination of these different factors seems to impede the migration of eosinophils in the described patient subsets and induce an increase of blood eosinophils.

In patients with an increase in eosinophils during dupilumab therapy, IL-4 levels were initially significantly higher (Figure 5A). IL-4, secreted by MC, ILC2, and Th2 cells, mediates type 2 inflammation including B-cell isotype switch, remodeling, and macrophage activation.[58] It is usually upregulated in type 2 inflammation including asthma, atopic dermatitis, or type 2 CRS. Recently, IL-4 was described as a main driver of type 2 airway inflammation.[59] As Nagase et al. described, the high levels of IL-4 may cause the low expression of CXCR4 in patients with an increase in eosinophils, which is supported by the finding of an inverse correlation of IL-4 and CXCR4 (Figure S2).[54,55]

The ROC curve revealed a weak area under the curve for the prediction of dupilumab-induced eosinophilia using the baseline IL-4 concentration. However, the ROC curve of CXCR4 was excellent, with an area of 94% under the curve, and a sensitivity of 88%. Although dupilumab-induced transient eosinophilia seems not clinically relevant in the majority of cases,[22–25] there are case reports with dupilumab-associated hypereosinophilic syndromes.[60–62] Therefore, CXCR4 on eosinophils might be a valuable predictive marker for stratifying patients who might develop eosinophilia and should be monitored more carefully under the treatment with dupilumab.

In this study, we were able to describe that dysregulation of migration and chemotaxis of eosinophils may induce blood eosinophilia in some patients with diffuse type 2 CRS treated with dupilumab. However, other effects of dupilumab on lymphocytes, MC, or on the lifespan of eosinophils as well as on the production of eosinophils have not been investigated.

In conclusion, the dupilumab-induced transient eosinophil increase in a selected patient group may be affected by an initially elevated IL-4 concentration with a consecutive downregulated CXCR4 receptor on eosinophils and the concomitant decrease of VCAM-1 caused by dupilumab. The blocking of IL-4 by dupilumab results in a recovery of CXCR4 expression, enabling eosinophils to home into tissue. Although transient eosinophilia under dupilumab treatment is usually not clinically relevant, IL-4 and CXCR4 might serve as predictive markers.

AUTHOR CONTRIBUTIONS

FSR: conceptualization, methodology, formal analysis, writing-original draft, visualization; AY, AV, CB, TM, ST: investigation, writing-reviewing and editing; MBS: conceptualization, writing-reviewing and editing; UCS: conceptualization, supervision, project administration, writing-original draft.

ACKNOWLEDGEMENTS

We thank Lukas Heeb for the excellent flow cytometry analysis of the eosinophils, Rebecca Masek for the vigilant sample processing, and the Boymanlab for the safe storage of our biosamples. This is an unsponsored study and the costs have been covered by the authors' institutions. Open access funding provided by Inselspital Universitatsspital Bern.

CONFLICT OF INTEREST STATEMENT

UCS and MBS are consultants for different companies including: Sanofi, GSK, Astra Zeneca, TAKEDA, Novartis. All other authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Fabio S. Ryser https://orcid.org/0000-0002-9388-2289
Urs C. Steiner https://orcid.org/0000-0002-3905-2508

REFERENCES

1. Hastan D, Fokkens WJ, Bachert C, et al. Chronic rhinosinusitis in Europe—an underestimated disease. A GA(2)LEN study. *Allergy*. 2011;66(9):1216-1223. doi:10.1111/j.1398-9995.2011.02646.x
2. Remenschneider AK, Scangas G, Meier JC, et al. EQ-5D-derived health utility values in patients undergoing surgery for chronic rhinosinusitis. *Laryngoscope*. 2015;125(5):1056-1061. doi:10.1002/lary.25054
3. Fu CH, Huang CC, Chen YW, Chang PH, Lee TJ. Nasal nitric oxide in relation to quality-of-life improvements after endoscopic sinus surgery. *Am J Rhinol Allergy*. 2015;29(6):e187-e191. doi:10.2500/ajra.2015.29.4249
4. Kato A, Peters AT, Stevens WW, Schleimer RP, Tan BK, Kern RC. Endotypes of chronic rhinosinusitis: relationships to disease phenotypes, pathogenesis, clinical findings, and treatment approaches. *Allergy*. 2022;77(3):812-826. doi:10.1111/all.15074
5. Chen S, Zhou A, Emmanuel B, Thomas K, Guiang H. Systematic literature review of the epidemiology and clinical burden of chronic rhinosinusitis with nasal polyposis. *Curr Med Res Opin*. 2020;36(11):1897-1911. doi:10.1080/03007995.2020.1815682
6. Fokkens WJ, Lund VJ, Hopkins C, et al. European position paper on Rhinosinusitis and nasal polyps 2020. *Rhinology*. 2020;58(Suppl S29):1-464. doi:10.4193/Rhin20.600
7. Grayson JW, Hopkins C, Mori E, Senior B, Harvey RJ. Contemporary classification of chronic Rhinosinusitis beyond polyps vs No polyps: a review. *JAMA Otolaryngol Head Neck Surg*. 2020;146(9):831-838. doi:10.1001/jamaoto.2020.1453
8. Kato A, Schleimer RP, Bleier BS. Mechanisms and pathogenesis of chronic rhinosinusitis. *J Allergy Clin Immunol*. 2022;149(5):1491-1503. doi:10.1016/j.jaci.2022.02.016
9. Gandhi NA, Bennett BL, Graham NM, Pirozzi G, Stahl N, Yancopoulos GD. Targeting key proximal drivers of type 2 inflammation in disease. *Nat Rev Drug Discov*. 2016;15(1):35-50. doi:10.1038/nrd4624
10. Feldman S, Kasjanski R, Poposki J, et al. Chronic airway inflammation provides a unique environment for B cell activation and antibody production. *Clin Exp Allergy*. 2017;47(4):457-466. doi:10.1111/cea.12878
11. Stevens WW, Ocampo CJ, Berdnikovs S, et al. Cytokines in chronic Rhinosinusitis. Role in eosinophilia and aspirin-exacerbated respiratory disease. *Am J Respir Crit Care Med*. 2015;192(6):682-694. doi:10.1164/rccm.201412-2278OC
12. Gevaert P, Han JK, Smith SG, et al. The roles of eosinophils and interleukin-5 in the pathophysiology of chronic rhinosinusitis with nasal polyps. *Int Forum Allergy Rhinol*. 2022;12(11):1413-1423. doi:10.1002/alr.22994
13. Kotas ME, Patel NN, Cope EK, et al. IL-13-associated epithelial remodeling correlates with clinical severity in nasal polyposis. *J Allergy Clin Immunol*. 2023;151(5):1277-1285. doi:10.1016/j.jaci.2022.12.826
14. Simpson EL, Bieber T, Guttman-Yassky E, et al. Two phase 3 trials of Dupilumab versus placebo in atopic dermatitis. *N Engl J Med*. 2016;375(24):2335-2348. doi:10.1056/NEJMoa1610020
15. Simpson EL, Akinlade B, Ardeleanu M. Two phase 3 trials of Dupilumab versus placebo in atopic dermatitis. *N Engl J Med*. 2017;376(11):1090-1091. doi:10.1056/NEJMc1700366
16. Bachert C, Han JK, Desrosiers M, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. *Lancet*. 2019;394(10209):1638-1650. doi:10.1016/S0140-6736(19)31881-1
17. Castro M, Corren J, Pavord ID, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N Engl J Med*. 2018;378(26):2486-2496. doi:10.1056/NEJMoa1804092
18. Hirano I, Dellon ES, Hamilton JD, et al. Efficacy of Dupilumab in a phase 2 randomized trial of adults with active eosinophilic esophagitis. *Gastroenterology*. 2020;158(1):111-122. doi:10.1053/j.gastro.2019.09.042
19. Calugareanu A, Jachiet M, Tauber M, et al. Effectiveness and safety of dupilumab for the treatment of prurigo nodularis in a French multicenter adult cohort of 16 patients. *J Eur Acad Dermatol Venereol*. 2020;34(2):e74-e76. doi:10.1111/jdv.15957
20. Chong LY, Piromchai P, Sharp S, et al. Biologics for chronic rhinosinusitis. *Cochrane Database Syst Rev*. 2021;3(3):CD013513. doi:10.1002/14651858.CD013513.pub3
21. Rabe KF, Nair P, Brusselle G, et al. Efficacy and safety of Dupilumab in glucocorticoid-dependent severe asthma. *N Engl J Med*. 2018;378(26):2475-2485. doi:10.1056/NEJMoa1804093
22. Caminati M, Olivieri B, Dama A, et al. Dupilumab-induced hypereosinophilia: review of the literature and algorithm proposal for clinical management. *Expert Rev Respir Med*. 2022;16(7):713-721. doi:10.1080/17476348.2022.2090342
23. Vinciguerra A, Rampi A, Yacoub MR, et al. Hypereosinophilia management in patients with type 2 chronic rhinosinusitis treated with dupilumab: preliminary results. *Eur Arch Otorhinolaryngol*. 2022;279(11):5231-5238. doi:10.1007/s00405-022-07389-5
24. Wechsler ME, Klion AD, Paggiaro P, et al. Effect of Dupilumab on blood eosinophil counts in patients with asthma, chronic Rhinosinusitis with nasal polyps, atopic dermatitis, or eosinophilic esophagitis. *J Allergy Clin Immunol Pract*. 2022;10(10):2695-2709. doi:10.1016/j.jaip.2022.05.019
25. von Deimling M, Koehler TC, Frye BC, Maerker-Hermann E, Venhoff N. Two cases with new onset of ANCA-positive eosinophilic granulomatosis with polyangiitis under treatment with dupilumab: coincidence or causality? *Ann Rheum Dis*. 2023;82:580-582. doi:10.1136/ard-2022-223596
26. Wollenberg A, Beck LA, Blauvelt A, et al. Laboratory safety of dupilumab in moderate-to-severe atopic dermatitis: results from three phase III trials (LIBERTY AD SOLO 1, LIBERTY AD SOLO 2,

LIBERTY AD CHRONOS). *Br J Dermatol*. 2020;182(5):1120-1135. doi:10.1111/bjd.18434

27. Hoeck J, Woisetschlager M. STAT6 mediates eotaxin-1 expression in IL-4 or TNF-alpha-induced fibroblasts. *J Immunol*. 2001;166(7):4507-4515. doi:10.4049/jimmunol.166.7.4507
28. Hamilton JD, Harel S, Swanson BN, et al. Dupilumab suppresses type 2 inflammatory biomarkers across multiple atopic, allergic diseases. *Clin Exp Allergy*. 2021;51(7):915-931. doi:10.1111/cea.13954
29. Jonstam K, Swanson BN, Mannent LP, et al. Dupilumab reduces local type 2 pro-inflammatory biomarkers in chronic rhinosinusitis with nasal polyposis. *Allergy*. 2019;74(4):743-752. doi:10.1111/all.13685
30. General Assembly of the World Medical A. World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. *J Am Coll Dent*. 2014;81(3):14-18. PubMed PMID: 25951678.
31. Fokkens WJ, Lund V, Bachert C, et al. EUFOREA consensus on biologics for CRSwNP with or without asthma. *Allergy*. 2019;74(12):2312-2319. doi:10.1111/all.13875
32. Wacht G, Poirot A, Charles AL, et al. FACS—based isolation of human eosinophils allows purification of high quality RNA. *J Immunol Methods*. 2018;463:47-53. doi:10.1016/j.jim.2018.09.003
33. Phillips KM, Hoehle LP, Caradonna DS, Gray ST, Sedaghat AR. Minimal clinically important difference for the 22-item Sinonasal outcome test in medically managed patients with chronic rhinosinusitis. *Clin Otolaryngol*. 2018;43(5):1328-1334. doi:10.1111/coa.13177
34. Bertlich M, Freytag S, Dombrowski T, et al. Subgroups in the treatment of nasal polyposis with dupilumab: a retrospective study. *Medicine (Baltimore)*. 2022;101(45):e31031. doi:10.1097/MD.0000000000031031
35. Fujieda S, Matsune S, Takeno S, et al. Dupilumab efficacy in chronic rhinosinusitis with nasal polyps from SINUS-52 is unaffected by eosinophilic status. *Allergy*. 2022;77(1):186-196. doi:10.1111/all.14906
36. Le Floc'h A, Allinne J, Nagashima K, et al. Dual blockade of IL-4 and IL-13 with dupilumab, an IL-4Ralpha antibody, is required to broadly inhibit type 2 inflammation. *Allergy*. 2020;75(5):1188-1204. doi:10.1111/all.14151
37. Imai Y, Kusakabe M, Nagai M, Yasuda K, Yamanishi K. Dupilumab effects on innate lymphoid cell and helper T cell populations in patients with atopic dermatitis. *JID Innov*. 2021;1(1):100003. doi:10.1016/j.xjidi.2021.100003
38. Trichot C, Faucheux L, Karpf L, et al. T(H) cell diversity and response to dupilumab in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2021;147(2):756-759. doi:10.1016/j.jaci.2020.05.037
39. Bakker DS, van der Wal MM, Heeb LEM, et al. Early and long-term effects of Dupilumab treatment on circulating T-cell functions in patients with moderate-to-severe atopic dermatitis. *J Invest Dermatol*. 2021;141(8):1943-1953. doi:10.1016/j.jid.2021.01.022
40. Matsuyama T, Takahashi H, Tada H, Chikamatsu K. Circulating T cell subsets and ILC2s are altered in patients with chronic Rhinosinusitis with nasal polyps after Dupilumab treatment. *Am J Rhinol Allergy*. 2023;37(1):58-64. doi:10.1177/19458924221132065
41. Tomassen P, Vandeplas G, Van Zele T, et al. Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. *J Allergy Clin Immunol*. 2016;137(5):1449-1456. doi:10.1016/j.jaci.2015.12.1324
42. Venge P, Bystrom J, Carlson M, et al. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. *Clin Exp Allergy*. 1999;29(9):1172-1186. doi:10.1046/j.1365-2222.1999.00542.x
43. Fukuda T, Fukushima Y, Numao T, et al. Role of interleukin-4 and vascular cell adhesion molecule-1 in selective eosinophil migration into the airways in allergic asthma. *Am J Respir Cell Mol Biol*. 1996;14(1):84-94. doi:10.1165/ajrcmb.14.1.8534490 PubMed PMID: 8534490.
44. Kong DH, Kim YK, Kim MR, Jang JH, Lee S. Emerging roles of vascular cell adhesion Molecule-1 (VCAM-1) in immunological disorders and cancer. *Int J Mol Sci*. 2018;19(4):1-16. doi:10.3390/ijms19041057
45. Fritz DK, Kerr C, Tong L, Smyth D, Richards CD. Oncostatin-M up-regulates VCAM-1 and synergizes with IL-4 in eotaxin expression: involvement of STAT6. *J Immunol*. 2006;176(7):4352-4360. doi:10.4049/jimmunol.176.7.4352 PubMed PMID: 16547273.
46. Schleimer RP, Sterbinsky SA, Kaiser J, et al. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. *J Immunol*. 1992;148(4):1086-1092. PubMed PMID: 1371130.
47. Fukushi J, Ono M, Morikawa W, Iwamoto Y, Kuwano M. The activity of soluble VCAM-1 in angiogenesis stimulated by IL-4 and IL-13. *J Immunol*. 2000;165(5):2818-2823. doi:10.4049/jimmunol.165.5.2818 PubMed PMID: 10946314.
48. Sasaki K, Pardee AD, Okada H, Storkus WJ. IL-4 inhibits VLA-4 expression on Tc1 cells resulting in poor tumor infiltration and reduced therapy benefit. *Eur J Immunol*. 2008;38(10):2865-2873. doi:10.1002/eji.200838334
49. Sasaki K, Zhao X, Pardee AD, et al. Stat6 signaling suppresses VLA-4 expression by CD8+ T cells and limits their ability to infiltrate tumor lesions in vivo. *J Immunol*. 2008;181(1):104-108. doi:10.4049/jimmunol.181.1.104
50. Kayaba H, Yamada Y, Cui CH, et al. Expression of VLA-4 on eosinophils decreases in patients with eosinophilia. *Int Arch Allergy Immunol*. 2001;125(Suppl 1):33-37. doi:10.1159/000053850 PubMed PMID: 11408770.
51. McBrien CN, Menzies-Gow A. The biology of eosinophils and their role in asthma. *Front Med (Lausanne)*. 2017;4:93. doi:10.3389/fmed.2017.00093
52. Diem L, Hoepner R, Bagnoud M, Salmen A, Chan A, Friedli C. Natalizumab induced blood eosinophilia: a retrospective pharmacovigilance cohort study and review of the literature. *J Neuroimmunol*. 2021;353:577505. doi:10.1016/j.jneuroim.2021.577505
53. Britton C, Poznansky MC, Reeves P. Polyfunctionality of the CXCR4/CXCL12 axis in health and disease: implications for therapeutic interventions in cancer and immune-mediated diseases. *FASEB J*. 2021;35(4):e21260. doi:10.1096/fj.202001273R PubMed PMID: 33715207.
54. Nagase H, Miyamasu M, Yamaguchi M, et al. Expression of CXCR4 in eosinophils: functional analyses and cytokine-mediated regulation. *J Immunol*. 2000;164(11):5935-5943. doi:10.4049/jimmunol.164.11.5935 PubMed PMID: 10820276.
55. Dulkys Y, Buschermohle T, Escher SE, Kapp A, Elsner J. T-helper 2 cytokines attenuate senescent eosinophil activation by the CXCR4 ligand stromal-derived factor-1alpha (CXCL12). *Clin Exp Allergy*. 2004;34(10):1610-1620. doi:10.1111/j.1365-2222.2004.02063.x PubMed PMID: 15479278.
56. Hong SG, Sato N, Legrand F, et al. Glucocorticoid-induced eosinopenia results from CXCR4-dependent bone marrow migration. *Blood*. 2020;136(23):2667-2678. doi:10.1182/blood.2020005161
57. Jiang Y, Pan Q, Zhu X, et al. Knockdown of CCR3 gene inhibits proliferation, migration and degranulation of eosinophils in mice by downregulating the PI3K/Akt pathway. *Int Immunopharmacol*. 2022;113(Pt B):109439. doi:10.1016/j.intimp.2022.109439
58. Kato A. Immunopathology of chronic rhinosinusitis. *Allergol Int*. 2015;64(2):121-130. doi:10.1016/j.alit.2014.12.006
59. Scott G, Asrat S, Allinne J, et al. IL-4 and IL-13, not eosinophils, drive type 2 airway inflammation, remodeling and lung function decline. *Cytokine*. 2023;162:156091. doi:10.1016/j.cyto.2022.156091
60. Nishiyama Y, Koya T, Nagano K, et al. Two cases of dupilumab-associated eosinophilic pneumonia in asthma with eosinophilic chronic rhinosinusitis: IL-5-driven pathology? *Allergol Int*. 2022;71(4):548-551. doi:10.1016/j.alit.2022.03.005
61. Descamps V, Deschamps L, El Khalifa J, et al. Eosinophilic vasculitis associated with persistent dupilumab-induced hypereosinophilia

in severe asthma. *Respir Med Res*. 2021;79:100821. doi:10.1016/j.resmer.2021.100821

62. Lommatzsch M, Stoll P, Winkler J, et al. Eosinophilic pleural effusion and stroke with cutaneous vasculitis: two cases of dupilumab-induced hypereosinophilia. *Allergy*. 2021;76(9):2920-2923. doi:10.1111/all.14964

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ryser FS, Yalamanoglu A, Valaperti A, et al. Dupilumab-induced eosinophilia in patients with diffuse type 2 chronic rhinosinusitis. *Allergy*. 2023;00:1-12. doi:10.1111/all.15844