



Early View

Original research article

Dupilumab increases aspirin tolerance in NSAID-exacerbated respiratory disease (N-ERD)

Sven Schneider, Katharina Poglitsch, Christina Morgenstern, Tamara Quint, Katharina Gangl, Christoph Sinz, Tina Bartosik, Nicholas James Campion, David Tianxiang Liu, Lukas David Landegger, Aldine Tu, Victoria Stanek, Marianne Rocha-Hasler, Christine Bangert, Julia Eckl-Dorna

Please cite this article as: Schneider S, Poglitsch K, Morgenstern C, *et al.* Dupilumab increases aspirin tolerance in NSAID-exacerbated respiratory disease (N-ERD). *Eur Respir J* 2022; in press (<https://doi.org/10.1183/13993003.01335-2022>).

This manuscript has recently been accepted for publication in the *European Respiratory Journal*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJ online.

Copyright ©The authors 2022. This version is distributed under the terms of the Creative Commons Attribution Non-Commercial Licence 4.0. For commercial reproduction rights and permissions contact permissions@ersnet.org

Dupilumab increases aspirin tolerance in NSAID-exacerbated respiratory disease (N-ERD)

Sven Schneider¹, Katharina Poglitsch², Christina Morgenstern³, Tamara Quint², Katharina Gangl¹, Christoph Sinz², Tina Bartosik¹, Nicholas James Campion, David Tianxiang Liu¹, Lukas David Landegger¹, Aldine Tu¹, Victoria Stanek¹, Marianne Rocha-Hasler¹, Christine Bangert^{2*}, Julia Eckl-Dorna¹

Affiliations:

¹ Department of Otorhinolaryngology, General Hospital and Medical University of Vienna, Vienna, Austria

² Department of Dermatology, General Hospital and Medical University of Vienna, Vienna, Austria

³ Science Impuls, Seeboden, Austria

***CORRESPONDENCE**

Christine Bangert, MD,
Department of Dermatology
Medical University of Vienna, General Hospital of Vienna
Waehringer Guertel 18-20
A-1090 Vienna, Austria
Tel: +43-1-40400-77020
Email: christine.bangert@meduniwien.ac.at

RUNNING TITLE

Dupilumab increases aspirin tolerance in N-ERD

TAKE HOME MESSAGE

This study for the first time prospectively assessed the impact of dupilumab on aspirin hypersensitivity in patients suffering from NSAID-exacerbated respiratory disease and provides insights into association of nasal biomarkers with clinical parameters.

AUTHOR CONTRIBUTION STATEMENT

SS, CB, TQ and JED designed the study, wrote the study protocol, and gained ethical approval for the study. SS, KP, TQ, KG, CS, TB, NJC, DTL and LDL recruited all the patients and carried out the study. AT and VS performed the experiments. SS, CM, MRH, CB and JED performed the analysis. SS, CM, CB, and JED wrote the manuscript. All authors critically revised the manuscript together.

FUNDING

Marianne Rocha-Hasler was supported by the externally funded research grant NCR-20-20749 of AstraZeneca.

ACKNOWLEDGEMENT

We thank Guenther Regelsberger from the Department of Neurology, Medical University of Vienna for assistance with MSD measurements.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

SS served as a speaker and/or consultant and/or advisory board member for Sanofi and Novartis. SS is an investigator for Novartis and AstraZeneca (grants paid to his institution). TB received personal fees from Sanofi. LDL served as an independent consultant for Conclave Capital and Gerson Lehrman Group. LDL is an investigator for Decibel Therapeutics and Amgen (grants paid to his institution). CB has received personal fees from Mylan, LEO Pharma, Pfizer, Sanofi Genzyme, Eli Lilly, Novartis, and AbbVie. CB is an investigator for Novartis, Sanofi, AbbVie, Eli Lilly, LEO Pharma and Galderma (grants paid to her institution). JED served as a speaker and/or consultant and/or advisory board member for Sanofi, Allergopharma, AstraZeneca, GSK and Novartis. JED is an investigator for Novartis and AstraZeneca (grants paid to her institution). All other authors declare no conflict of interest.

TOTAL WORD COUNT: 3000 (excluding references)

ABSTRACT

Non-steroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (N-ERD) comprises the triad of chronic rhinosinusitis with nasal polyps, asthma, and intolerance to NSAIDs. Dupilumab treatment, targeting the IL-4 receptor alpha, significantly reduces polyp burden as well as asthma symptoms. Here we aimed to investigate the effect of dupilumab on aspirin intolerance, burden of disease, as well as on nasal cytokine profiles in patients suffering from N-ERD.

In this open-label trial, adult patients with confirmed N-ERD were treated with dupilumab for six months. Clinical parameters (e.g., total polyp scores, quality of life questionnaires, smell test, spirometry), oral aspirin provocation testing, blood, nasal and urine sampling were monitored up to six months after starting dupilumab therapy at regular intervals.

Of the thirty-one patients included in the study, thirty completed both aspirin provocation testing. After six months of treatment with dupilumab, 23.3% (n=7/30) of patients developed complete aspirin tolerance and an additional 33.3% of patients (n=10/30) tolerated higher doses. Polyp burden was significantly reduced (total polyp score: -2.68 ± 1.84 , $p < 0.001$) and pulmonary symptoms (asthma control test: $+2.34 \pm 3.67$, $p < 0.001$), as well as olfactory performance improved (UPSIT: $+11.16 \pm 9.54$, $p < 0.001$) in all patients after therapy. Patients with increased aspirin tolerance showed a significant decrease in urine leukotriene E4 levels and their improvement in clinical parameters was associated with the reduction of eotaxin-1, CCL17, IL-5, IL-17A and IL-6.

57% of N-ERD patients tolerated higher doses of aspirin under dupilumab therapy.

Abstract Word Count: 241

Keywords: NSAID exacerbated respiratory disease, aspirin hypersensitivity, asthma, CRSwNP, nasal polyposis, dupilumab

Introduction

Non-steroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (N-ERD) is characterized by the presence of chronic rhinosinusitis with nasal polyps (CRSwNP), asthma and an intolerance towards aspirin or other cyclooxygenase-1 (COX-1) inhibiting NSAIDs (1). The prevalence is between 0.3% and 0.9% in the general population, yet 7% of asthmatic patients and 10-21% of patients diagnosed with CRSwNP suffer from N-ERD, respectively (2-4). Affected patients have a severely reduced quality of life with chronic nasal obstruction, secretion and impaired sense of smell being the most burdensome symptoms (5, 6). Upon ingestion of NSAIDs inhibiting the cyclooxygenase-1 enzyme, N-ERD patients experience severe upper and lower respiratory tract symptoms. These are caused by dysregulation of the arachidonic acid metabolism with an overproduction of cysteinyl leukotrienes and prostaglandin D₂ accompanied by decreased levels of the anti-inflammatory prostaglandin PGE₂ (7).

In recent years, biologicals targeting cytokines involved in type 2 inflammation have become available as an important therapeutic option for refractory nasal polyps (8, 9). In this line, omalizumab - binding to the Cε3 region of IgE and thus blocking binding of IgE to its receptor present on e.g. mast cells - was the first biological shown to reduce not only nasal polyp burden, but also to induce aspirin tolerance in N-ERD patients (10-12). Dupilumab, targeting the IL-4 receptor α chain, has become a mainstay therapy for patients with difficult-to-treat nasal polyposis. Treatment significantly reduces polyp size and leads to improved quality of life, including improved olfactory function (13, 14). This is accompanied by a reduction of local ECP, eotaxin-3, IL-5 and total IgE levels in nasal secretions (13). In phase 3 dupilumab studies, self-reported N-ERD patients were included as a subgroup and showed markedly improvement in total symptom score and Sino-Nasal Outcome Test-22 (SNOT-22) as compared to aspirin-tolerant subjects (15). However, so far, the effect of dupilumab on aspirin tolerance has not been thoroughly addressed in a prospective study design.

Thus, we conducted a prospective open-label trial in 31 N-ERD patients to investigate the effect of dupilumab on aspirin tolerability before and six months after

treatment by performing an aspirin-challenge testing to optimize recommendation on patients' NSAID intake. We further sought to verify the impact of dupilumab on polyp size, asthma, and quality of life in these patients. Additionally, we analyzed the effect of dupilumab on 33 different inflammatory biomarkers in nasal secretions of affected patients, on urine levels of metabolites of the arachidonic acid pathway and sought to identify differences in patients with dupilumab-induced aspirin tolerance versus those who remained aspirin sensitive.

METHODS

Study design and patients

This prospective open-label single-center study was conducted at the Department of Otorhinolaryngology and the Department of Dermatology at the Medical University of Vienna, Austria. It was approved by the local ethics committee (EK 1044/2020) and registered at EudraCT (2019-004889-18) and ClinGov (NCT04442256).

All patients signed an informed consent prior to study participation. Inclusion criteria included a minimum age of 18 years and a diagnosis of N-ERD (CRSwNP, asthma, aspirin intolerance).

CRSwNP was diagnosed according to European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020 criteria (16). Asthma was diagnosed based on the Global Initiative for Asthma guidelines (GINA). N-ERD was diagnosed by patients' medical history according to the European Academy of Allergology and Clinical Immunology (EAACI) position paper on diagnosis and management of N-ERD and was confirmed by hypersensitivity reactions to oral aspirin provocation challenge before initiating treatment with dupilumab (17). For provocation testing patients needed to have a stable asthma and/or FEV1 >70%. Patients were carefully monitored for 24 hours in the hospital after positive reaction to aspirin.

Dupilumab was administered subcutaneously for a total of six months, dosed 300 mg biweekly after a loading dose of 600 mg according to dosage for atopic dermatitis. Patients maintained their usual topical/systemic asthma medication and nasal treatment regimen throughout the study. Concomitant aspirin desensitization or treatment with other biologics for CRSwNP or asthma was not permitted.

Study measures

The primary study endpoint was the proportion of patients developing tolerance to aspirin as examined at baseline and after the 24-week treatment (Fig. E1). Key secondary endpoints were change of nasal polyp size (total polyp score=TPS) (18), disease-specific quality of life (QoL) for CRSwNP measured with SNOT-22 (19), Total Nasal Symptom Score (TNSS), disease-specific QoL for asthma measured with Asthma Control Test (ACT) score from baseline up to 24 weeks after start of dupilumab treatment (baseline, four weeks, 12 weeks, 24 weeks). Sense of smell was evaluated using the University of Pennsylvania Smell Identification Test (UPSIT)

at baseline and after 24 weeks (20). Respiratory parameters were measured by spirometry and included FEV1, maximal expiratory flow (MEF)-50 and MEF-75 at baseline and after 12 and 24 weeks of therapy. For further information, please refer to supplementary methods.

Aspirin provocation testing

Aspirin provocation testing was performed at baseline and after 24 weeks of dupilumab treatment by oral exposure to aspirin performed in accordance with previously established protocols starting with a dose of 125 mg that was increased to 250 mg after two hours, and when no reaction occurred, a maximum of 500 mg was given after another two hours observation (12). Vital signs were monitored, and spirometry was performed 30 minutes before and after each drug intake. Reactions were evaluated by spirometry and/or documentation of clinical nasal or extrapulmonary symptoms. Lower respiratory tract reactions were deemed positive if a decrease in forced expiratory volume in 1 second [FEV1] > 20% was observed. Upper respiratory tract reactions were categorized as positive when symptoms of nasal congestion, rhinorrhea, sneezing, nasal/eye itching, or eye redness/tearing were observed. Combined upper and lower respiratory reactions were categorized as positive when decrease in FEV1 by at least 20% with presence of nasal congestion, rhinorrhea, sneezing, nasal/eye itching, or eye redness/tearing occurred.

Subsequent to positive reactions, aspirin provocation testing was stopped, and patients received appropriate symptomatic treatment. Patients were observed for up to 8 hours after the last dose of aspirin in order to exclude late anaphylactic reactions.

ELISA, MSD measurements and statistics:

Please refer to online repository.

Results

Induction of aspirin tolerance in N-ERD patients after 24 weeks of dupilumab treatment

31 patients with a conclusive history of N-ERD and concomitant symptoms were enrolled, with 30 patients completing both aspirin provocations before and after dupilumab treatment (Fig. E1B, Table 1). At baseline aspirin provocation, the majority of patients (n=19/31, 61%) presented with a combination of both upper (e.g., nasal congestion, rhino conjunctivitis or rhinorrhoea) and lower respiratory tract symptoms (e.g., pulmonary obstruction, coughing or dyspnoea). 32% (n=10/31) of patients showed isolated upper and 6% (n=2/31) only lower respiratory tract reactions (Table 1 and Fig. 1). At baseline, 23/31 patients showed respiratory symptoms at a dosage of 125 mg, six patients tolerated a cumulative dose of 375 mg and two patients reacted at the highest cumulative doses of 875 mg aspirin (Table 2). All patients received dupilumab treatment for six months. While one patient, who reacted with a cumulative dose of 875 mg of aspirin during the first provocation, was unwilling to perform the second aspirin challenge, 23.3% (n=7/30) of the 30 patients attending the second provocation test tolerated the highest cumulative dosage of 875 mg without showing any signs of hypersensitivity reaction ($p<0.001$, Table 2) (=Complete aspirin tolerance). An additional 33.3% of patients (n=10/30) tolerated a higher dose at the second challenge (=Partial aspirin tolerance), thus, a total of 57% (n=17/30) had reduced NSAID hypersensitivity after six months of dupilumab therapy. 12/30 patients showed symptoms at the same dosage as in the baseline visit (40.0%) and one patient (3.3%) tolerated less aspirin in the second challenge. As opposed to baseline, patients suffered mainly from isolated upper respiratory tract reactions (n=16/30, Fig. 1) and fewer patients experienced both upper and lower respiratory tract reactions (n=6/30) at the second aspirin provocation.

Clinical effectiveness of dupilumab in N-ERD patients

After 24 weeks of dupilumab treatment we observed an overall significant decrease in polyp size (TPS: -2.68 ± 1.84 , $p<0.001$, Table E1, Fig. 2A). This was accompanied by improved TNSS (-3.46 ± 3.39 , $p<0.001$) and total SNOT-22 levels (-26.73 ± 23.49 , $p<0.001$) as compared to baseline (Fig. 2B and C). Dupilumab treatment also resulted in better smell perception (UPSIT score: $+11.16\pm9.54$, $p<0.001$, Fig. 2D) and improvement of asthma symptoms (ACT: $+2.49\pm3.66$, $p<0.001$, Fig. 2E).

Furthermore, the spirometry-derived parameters FEV1 ($+2.28 \pm 5.44$, $p=0.01$) and MEF50 ($+0.26 \pm 0.79$, $p=0.02$) showed a trend towards improvement (Fig. 2F and G). Patients performed better in QoL scores PHQ-2 and EQ-5D-3L (Fig. E2 A-C).

Biomarker changes in serum, urine, and nasal secretions upon dupilumab treatment

Dupilumab treatment in N-ERD patients was associated with a reduction in total serum IgE levels (-165.13 ± 184.06 , $p<0.001$; Table E1, Fig. 3A) and an increase in serum ECP levels ($+49.02 \pm 48.82$, $p<0.001$; Table E1, Fig. 3B) as well as absolute ($+0.21 \pm 0.35$, $p=0.003$; Fig. 3C) and percentage of ($+1.71 \pm 4.07$, $p=0.01$; Fig. 3D) blood eosinophil levels after 24 weeks. In addition, after six months of treatment, we observed a drop in total IgE levels in nasal secretions (-1195.77 ± 1777.02 , $p<0.001$, Fig. 3E). Urinary levels of 11β -Prostaglandin $F2\alpha$ (-488.56 ± 943.07 , $p=0.03$) as well as Leukotriene E4 (-295.19 ± 655.45 , $p=0.02$) were also significantly reduced (Fig. 3F, G, Table E1). Of the 33 biomarkers assessed in nasal secretions, we observed a significant decrease (Table E2) in type 2 associated cytokines such as TSLP (Fig. 4A), CCL17 (Fig. 4B), IL-5 (Fig. 4C), IL-13 (Fig. 4D) as well as eotaxin-1 (Fig. 4E), and eotaxin-3 (Fig. 4F). The inflammatory cytokines IL-12p40 (Fig. 4G), TNF- α (Fig. 4H) and IL-6 (Fig. 4I) as well as T-cell subset associated cytokines IL-17A (Fig. 4J) and IL-22 (Fig. 4K) also decreased over time. For all the other cytokines assessed, no significant differences between week 0 and week 24 of treatment were observed (Fig. E3 and Table E2).

Aspirin-tolerant but not -intolerant patients show an associated change in clinical parameters with biomarkers

Baseline characteristics of patients who tolerated higher levels of aspirin during the second provocation after six months of dupilumab treatment (referred to as aspirin-tolerant) did not differ from those who remained intolerant for aspirin (referred to as aspirin-intolerant) except for the number of previous surgeries at week 0 ($\text{mean}_{\text{aspirin-tolerant}}=2.65 \pm 1.54$, $\text{mean}_{\text{aspirin-intolerant}}=1.46 \pm 0.97$, $p=0.02$) (Table 1). Dupilumab treatment resulted in comparable reduction of clinical parameters in both groups after six months (Fig. 5A and B, Fig. E2D and data not shown). With regards to biomarkers, only the aspirin-tolerant, but not the aspirin-intolerant groups showed a significant reduction in urine Leukotriene E4 ($\text{mean}_{\text{aspirin-intolerant}}=-441.81 \pm 703.33$, $p=0.02$, Fig. 5C) and 11β -Prostaglandin $F2\alpha$ levels ($\text{mean}_{\text{aspirin-intolerant}}=-$

681.62±1003.91, $p=0.03$, Fig. 5D) at week 24. Surprisingly, the change in nasal secretion biomarker levels after six months of dupilumab administration between both groups was comparable, with IL-17A, IL-5 and IL-6 showing a trend towards stronger reduction in the aspirin-intolerant and CCL17 showing a trend towards a more pronounced decrease in aspirin-tolerant patients (Fig. 5E, Fig. E4 and Table E2). In an additional analysis differentiating between patients who developed complete aspirin tolerance and those with a partial tolerance towards aspirin as compared to those who remained aspirin-intolerant, we found no difference in TPS or smell performance between the groups (Fig. E5). Whilst cytokine levels confirmed previous observations (Fig. E6), the patients with partial aspirin tolerance had a trend towards higher baseline Leukotriene E4 and 11β -Prostaglandin $F2\alpha$ levels baseline levels and were the only group showing significant reduction in urine Leukotriene E4 (mean_{partial aspirin tolerance} = -644.77±773.33, $p=0.03$, Fig. E5C) and 11β -Prostaglandin $F2\alpha$ levels (mean_{partial aspirin tolerance} = -951.07±1053.47, $p=0.01$, Fig. E5D) at week 24. However, the statistical results need to be carefully evaluated with regards to the considerably small sample size in the three-group comparison.

The decrease in nasal but not urine-derived biomarkers is associated with clinical improvement in aspirin-tolerant patients

Last, we assessed the association of changes in nasal TPS and UPSIT score with changes in nasal and urine biomarkers in both the aspirin-tolerant and aspirin-intolerant group after dupilumab treatment. Interestingly, we noted that the decrease in percentage change between end of treatment and baseline for nasal eotaxin-1 (Fig. 6A, $p_{\text{aspirin-tolerant}}=0.02$, $p_{\text{aspirin-intolerant}}=0.62$) and IL-6 (Fig. 6B, $p_{\text{aspirin-tolerant}}=0.05$, $p_{\text{aspirin-intolerant}}=0.91$) showed an association with the change of TPS from baseline in the aspirin-tolerant group only. This was not the case for the other parameters assessed, namely nasal IL-5 (Fig. 6C), IL-17A (Fig. 6D) and CCL17 (Fig. 6E) or urine Leukotriene E4 (Fig. 6F) and 11β -Prostaglandin $F2\alpha$ (Fig. 6G) levels. Although no association was found for change in nasal derived eotaxin-1 (Fig. 6H) or IL-6 (Fig. 6I) with change in UPSIT score, a moderate negative correlation was observed for change in UPSIT with decrease in nasal derived IL-5 (Fig. 6J, $p_{\text{aspirin-tolerant}}=0.01$, $p_{\text{aspirin-intolerant}}=0.65$), IL-17A (Fig. 6K, $p_{\text{aspirin-tolerant}}=0.05$, $p_{\text{aspirin-intolerant}}=0.53$) and CCL17 (Fig. 6L, $p_{\text{aspirin-tolerant}}=0.02$, $p_{\text{aspirin-intolerant}}=0.25$) in the aspirin-tolerant but not in the aspirin-intolerant group. Again, urine biomarkers Leukotriene E4 (Fig. 6M) and

11 β -Prostaglandin F2 α (Fig. 6N) did not correlate with change in UPSIT levels in any of the two patient groups.

Discussion

In this single-center prospective open-label study, we demonstrated that treatment with dupilumab in N-ERD patients leads to a significant reduction in polyp size accompanied by an improvement in quality of life and smell perception. Importantly, 23.3% of 30 patients (7/30) developed a complete tolerance and 33.3% (10/30) tolerated higher doses of NSAID after six months of dupilumab therapy. Patients showed diminished levels of urine leukotriene E4 and prostaglandin F2 as well as nasal type 2 response-associated biomarkers after six months of treatment. In aspirin-tolerant patients, the improvement in clinical parameters was associated with the reduction of nasal-derived eotaxin-1, CCL17, IL-5, IL-17A and IL-6.

After six months of treatment with dupilumab, we observed a reduction in polyp score by -2.68 ± 1.84 points which is comparable to data from the large multicentre placebo-controlled trials SINUS-24 and SINUS-52 for treatment of CRSwNP with dupilumab (13). A subanalysis of the latter studies in N-ERD patients and trials in other N-ERD cohorts also proved the significant impact of dupilumab on polyp burden in this difficult-to-treat patient group (15, 21, 22). Consistent with previous data we also observed better control of lower airway disease with an improvement in MEF50 ($+0.26 \pm 0.79$) and the ACT score ($+2.49 \pm 3.66$) (23, 24).

Smell perception after six months of therapy as measured by UPSIT score ($+11.16 \pm 9.54$) improved significantly comparable to results obtained in previous studies with dupilumab in CRSwNP and in N-ERD patients (13, 25, 26). Although the underlying mechanism is not yet fully understood, it is thought that both the impaired nasal airflow and the direct effect of inflammatory processes on the mucosa and olfactory neurons play a role in the olfactory loss in CRS (27). In fact, cytokines associated with type 2 inflammation, which are known to be elevated in N-ERD patients (9, 28), as well as TNF- α have been shown to have a direct negative impact on olfactory neurogenesis and regeneration (29, 30). In accordance with previous observations in CRSwNP patients without aspirin sensitivity (13, 14), we observed a significant decrease of eotaxin-3 and IL-5 in nasal secretions of dupilumab-treated patients. This reduction was accompanied by an increase in eosinophil levels in the blood which may be explained by the effect of dupilumab in reducing eosinophil

attracting cytokines and thus eosinophil migration into tissue whilst not affecting production of these cells in the bone marrow (13). Other type 2 response-associated molecules such as TSLP, CCL17 and IL-13 as well as TNF- α were also decreased after treatment. Interestingly, the reduction of CCL17 and IL-5 showed a significant direct association of smell improvement in patients developing aspirin tolerance during therapy. Our results thus indicate that the observed ameliorated smell perception after dupilumab therapy may not only be explained by improved nasal patency due to reduced polyp size, but also by the reduction of relevant biomarker levels in nasal secretions.

This is, to the best of our knowledge, the first study reporting the effect of dupilumab on aspirin tolerance in a prospective study design within a representative group of 31 well-defined N-ERD patients as confirmed by aspirin provocation testing (17).

Overall, we found that 57% of patients tolerated more aspirin after dupilumab therapy with more than half of them (33.3% of all patients) bearing the highest dose. These results are similar to a recent small study in five patients observing increased (40% of patients) or complete (60% of patients) aspirin tolerance in N-ERD patients after at least 3 months of dupilumab treatment (31). Furthermore, patient becoming aspirin tolerant after 6 months showed a trend towards lower baseline TPS score before start of treatment. Though it did not reach significance, this observation warrants further studies to investigate the influence of the baseline TPS score on patients' outcome with regards to aspirin tolerance development. Patients who developed dupilumab-induced aspirin tolerance showed a significant reduction in urine mast cell-derived arachidonic acid metabolites and a trend towards a stronger decrease in nasal CCL17 levels from baseline as compared to subjects who remained aspirin sensitive. The observation that the leukotriene C4 synthetase in human mast cells can also be activated by IL-4 may explain the beneficial effect of dupilumab on aspirin sensitivity (32). However, why this beneficial effect is observed only in a subset of patients can only be speculated on: it is conceivable that the IL-4 receptor-mediated augmentation of leukotriene synthesis may be more prevalent in some of our patients showing dupilumab-induced aspirin tolerance whilst in others cysteinyl leukotriene synthesis may be more dependent on, e.g., IL-3 or IgE receptor-mediated pathway activation (32, 33). This may also explain the differential results regarding development of full aspirin tolerance with omalizumab, where 56% (12) and 62.5% (34) of patients successfully managed the highest aspirin dose during the challenge

after treatment. Omalizumab directly targets activation of mast cells, which are known to be in a hypersensitive state and to play an important role in acute aspirin reactivity in N-ERD patients (11, 12, 35, 36), finally resulting in a better therapeutic outcome with regard to aspirin tolerance using the strategy of IgE inhibition.

Interestingly, the observed reduction in nasal polyp scores with an average TPS of -2.68 ± 1.84 points is more pronounced than the one observed with omalizumab in N-ERD patients (TPS -1.9 ± 0.3 , (12)). These results are consistent with previous studies suggesting a stronger effect of dupilumab on reduction of polyp size as compared to omalizumab (10, 13, 21, 37, 38). Furthermore, in a recent comparison of different biologic therapies in N-ERD patients, dupilumab led to higher rates of both subjective and objective symptom improvements as compared to anti-IL5/IL-5R α or anti-IgE therapy (39). Targeting the IL-4R α , shared by IL-4 and IL-13, might result in a broad inhibition of multiple traits of type 2 responses important in CRSwNP pathogenesis such as activation of eosinophils, mucus secretion, and airway remodelling and, consequently, lead to more pronounced clinical improvement.

The major limitation of this study is the lack of a placebo group. However, as all patients included in this study had a long disease duration, were heavily suffering and have previously unsuccessfully tried other treatment strategies. We felt that it was ethically not justifiable to potentially deny them access to any treatment with biologicals for a period of six months. Furthermore, at the time of the study dupilumab was already licensed as treatment for CRSwNP and there is substantial evidence of the effectiveness of dupilumab over placebo in N-ERD (15).

In summary, we show that inhibition of IL-4R α signalling results in a strong reduction in polyp burden in CRSwNP patients with comorbid N-ERD. This was accompanied by increased aspirin tolerance in 57% of the patients. As dupilumab-induced aspirin tolerance was not achieved in all patients, aspirin challenge is recommended prior to NSAID prescription to determine status of sensitivity in N-ERD patients receiving dupilumab. Although the development of aspirin tolerance led to a significant reduction in urine-derived arachidonic acid metabolites involved in N-ERD, biomarkers clearly identifying patients who will respond with aspirin tolerance to different biologicals are still missing and warrant further investigation.

References:

1. Ledford DK, Wenzel SE, Lockey RF. Aspirin or other nonsteroidal inflammatory agent exacerbated asthma. *J Allergy Clin Immunol Pract*. 2014;2(6):653-7.
2. Li KL, Lee AY, Abuzeid WM. Aspirin Exacerbated Respiratory Disease: Epidemiology, Pathophysiology, and Management. *Med Sci (Basel)*. 2019;7(3).
3. Khan A, Vandeplas G, Huynh TMT, Joish VN, Mannent L, Tomassen P, et al. The Global Allergy and Asthma European Network (GALEN rhinosinusitis cohort: a large European cross-sectional study of chronic rhinosinusitis patients with and without nasal polyps. *Rhinology*. 2019;57(1):32-42.
4. Rajan JP, Wineinger NE, Stevenson DD, White AA. Prevalence of aspirin-exacerbated respiratory disease among asthmatic patients: A meta-analysis of the literature. *The Journal of allergy and clinical immunology*. 2015;135(3):676-81 e1.
5. Ta V, White AA. Survey-Defined Patient Experiences With Aspirin-Exacerbated Respiratory Disease. *J Allergy Clin Immunol Pract*. 2015;3(5):711-8.
6. Schneider S, Campion NJ, Villazala-Merino S, Liu DT, Bartosik T, Landegger LD, et al. Associations between the Quality of Life and Nasal Polyp Size in Patients Suffering from Chronic Rhinosinusitis without Nasal Polyps, with Nasal Polyps or Aspirin-Exacerbated Respiratory Disease. *J Clin Med*. 2020;9(4).
7. Lyly A, Laidlaw TM, Lundberg M. Pathomechanisms of AERD-Recent Advances. *Front Allergy*. 2021;2:734733.
8. Stevens WW, Peters AT, Tan BK, Klingler AI, Poposki JA, Hulse KE, et al. Associations Between Inflammatory Endotypes and Clinical Presentations in Chronic Rhinosinusitis. *J Allergy Clin Immunol Pract*. 2019;7(8):2812-20 e3.
9. Bangert C, Villazala-Merino S, Fahrenberger M, Krausgruber T, Bauer WM, Stanek V, et al. Comprehensive Analysis of Nasal Polyps Reveals a More Pronounced Type 2 Transcriptomic Profile of Epithelial Cells and Mast Cells in Aspirin-Exacerbated Respiratory Disease. *Front Immunol*. 2022;13:850494.
10. Gevaert P, Omachi TA, Corren J, Mullol J, Han J, Lee SE, et al. Efficacy and safety of omalizumab in nasal polyposis: 2 randomized phase 3 trials. *The Journal of allergy and clinical immunology*. 2020;146(3):595-605.
11. Hayashi H, Fukutomi Y, Mitsui C, Kajiura K, Watai K, Kamide Y, et al. Omalizumab for Aspirin Hypersensitivity and Leukotriene Overproduction in Aspirin-exacerbated Respiratory Disease. A Randomized Controlled Trial. *American journal of respiratory and critical care medicine*. 2020;201(12):1488-98.
12. Quint T, Dahm V, Ramazanov D, Arnoldner MA, Kurz H, Janik S, et al. Omalizumab-Induced Aspirin Tolerance in Nonsteroidal Anti-Inflammatory Drug-Exacerbated Respiratory Disease Patients Is Independent of Atopic Sensitization. *J Allergy Clin Immunol Pract*. 2022;10(2):506-16 e6.
13. Bachert C, Han JK, Desrosiers M, Hellings PW, Amin N, Lee SE, 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):1638-50.
14. Jonstam K, Swanson BN, Mannent LP, Cardell LO, Tian N, Wang Y, et al. Dupilumab reduces local type 2 pro-inflammatory biomarkers in chronic rhinosinusitis with nasal polyposis. *Allergy*. 2019;74(4):743-52.
15. Mullol J, Laidlaw TM, Bachert C, Mannent LP, Canonica GW, Han JK, et al. Efficacy and safety of dupilumab in patients with uncontrolled severe chronic rhinosinusitis with nasal polyps and a clinical diagnosis of NSAID-ERD: Results from two randomized placebo-controlled phase 3 trials. *Allergy*. 2022;77(4):1231-44.
16. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. *Rhinology*. 2020;58(Suppl S29):1-464.
17. Kowalski ML, Agache I, Bavbek S, Bakirtas A, Blanca M, Bochenek G, et al. Diagnosis and management of NSAID-Exacerbated Respiratory Disease (N-ERD)-a EAACI position paper. *Allergy*. 2019;74(1):28-39.

18. Meltzer EO, Hamilos DL, Hadley JA, Lanza DC, Marple BF, Nicklas RA, et al. Rhinosinusitis: developing guidance for clinical trials. *The Journal of allergy and clinical immunology*. 2006;118(5 Suppl):S17-61.
19. Baumann I, Plinkert PK, De Maddalena H. [Development of a grading scale for the Sino-Nasal Outcome Test-20 German Adapted Version (SNOT-20 GAV)]. *HNO*. 2008;56(8):784-8.
20. Doty RL, Shaman P, Kimmelman CP, Dann MS. University of Pennsylvania Smell Identification Test: a rapid quantitative olfactory function test for the clinic. *The Laryngoscope*. 1984;94(2 Pt 1):176-8.
21. Laidlaw TM, Mullol J, Fan C, Zhang D, Amin N, Khan A, et al. Dupilumab improves nasal polyp burden and asthma control in patients with CRSwNP and AERD. *J Allergy Clin Immunol Pract*. 2019;7(7):2462-5 e1.
22. Bertlich M, Ihler F, Bertlich I, Weiss BG, Groger M, Haubner F. Management of chronic rhinosinusitis with nasal polyps in Samter triad by low-dose ASA desensitization or dupilumab. *Medicine (Baltimore)*. 2021;100(40):e27471.
23. Lans R, Fokkens WJ, Adriaensen G, Hoven DR, Drubbel JJ, Reitsma S. Real-life observational cohort verifies high efficacy of dupilumab for chronic rhinosinusitis with nasal polyps. *Allergy*. 2022;77(2):670-4.
24. Laidlaw TM, Bachert C, Amin N, Desrosiers M, Hellings PW, Mullol J, et al. Dupilumab improves upper and lower airway disease control in chronic rhinosinusitis with nasal polyps and asthma. *Ann Allergy Asthma Immunol*. 2021;126(5):584-92 e1.
25. Buchheit KM, Sohail A, Hacker J, Maurer R, Gakpo D, Bensko JC, et al. Rapid and sustained effect of dupilumab on clinical and mechanistic outcomes in aspirin-exacerbated respiratory disease. *The Journal of allergy and clinical immunology*. 2022.
26. Mullol J, Bachert C, Amin N, Desrosiers M, Hellings PW, Han JK, et al. Olfactory Outcomes with Dupilumab in Chronic Rhinosinusitis with Nasal Polyps. *J Allergy Clin Immunol Pract*. 2021.
27. Yan X, Whitcroft KL, Hummel T. Olfaction: Sensitive indicator of inflammatory burden in chronic rhinosinusitis. *Laryngoscope Invest Otolaryngol*. 2020;5(6):992-1002.
28. Scott WC, Cahill KN, Milne GL, Li P, Sheng Q, Huang LC, et al. Inflammatory heterogeneity in aspirin-exacerbated respiratory disease. *The Journal of allergy and clinical immunology*. 2020.
29. Rouyar A, Classe M, Gorski R, Bock MD, Le-Guern J, Roche S, et al. Type 2/Th2-driven inflammation impairs olfactory sensory neurogenesis in mouse chronic rhinosinusitis model. *Allergy*. 2019;74(3):549-59.
30. Kim DK, Choi SA, Eun KM, Kim SK, Kim DW, Phi JH. Tumour necrosis factor alpha and interleukin-5 inhibit olfactory regeneration via apoptosis of olfactory sphere cells in mice models of allergic rhinitis. *Clin Exp Allergy*. 2019;49(8):1139-49.
31. Mustafa SS, Vadmalai K. Dupilumab increases aspirin tolerance in aspirin-exacerbated respiratory disease. *Ann Allergy Asthma Immunol*. 2021;126(6):738-9.
32. Hsieh FH, Lam BK, Penrose JF, Austen KF, Boyce JA. T helper cell type 2 cytokines coordinately regulate immunoglobulin E-dependent cysteinyl leukotriene production by human cord blood-derived mast cells: profound induction of leukotriene C(4) synthase expression by interleukin 4. *J Exp Med*. 2001;193(1):123-33.
33. Lang DM, Aronica MA, Maierson ES, Wang XF, Vasas DC, Hazen SL. Omalizumab can inhibit respiratory reaction during aspirin desensitization. *Ann Allergy Asthma Immunol*. 2018;121(1):98-104.
34. Hayashi N, Tsukamoto Y, Sallas WM, Lowe PJ. A mechanism-based binding model for the population pharmacokinetics and pharmacodynamics of omalizumab. *Br J Clin Pharmacol*. 2007;63(5):548-61.
35. Boyce JA. Aspirin sensitivity: Lessons in the regulation (and dysregulation) of mast cell function. *The Journal of allergy and clinical immunology*. 2019;144(4):875-81.
36. O'Sullivan S, Dahlen B, Dahlen SE, Kumlin M. Increased urinary excretion of the prostaglandin D2 metabolite 9 alpha, 11 beta-prostaglandin F2 after aspirin challenge supports mast cell activation in aspirin-induced airway obstruction. *The Journal of allergy and clinical immunology*. 1996;98(2):421-32.

37. Agache I, Song Y, Alonso-Coello P, Vogel Y, Rocha C, Sola I, et al. Efficacy and safety of treatment with biologicals for severe chronic rhinosinusitis with nasal polyps: A systematic review for the EAACI guidelines. *Allergy*. 2021;76(8):2337-53.
38. Armengot-Carceller M, Gomez-Gomez MJ, Garcia-Navalon C, Domenech-Campos E, Munoz-Fernandez N, Miguel AG, et al. Effects of Omalizumab Treatment in Patients With Recalcitrant Nasal Polyposis and Mild Asthma: A Multicenter Retrospective Study. *Am J Rhinol Allergy*. 2021;35(4):516-24.
39. Wangberg H, Spierling Bagsic SR, Osuna L, White AA. Appraisal of the Real-World Effectiveness of Biologic Therapies in Aspirin-Exacerbated Respiratory Disease. *J Allergy Clin Immunol Pract*. 2022;10(2):478-84 e3.

Figure legends:

Figure 1: Respiratory symptoms in response to aspirin provocation before and 24 weeks after dupilumab treatment. Number of patients reacting with different types of respiratory reactions (upper: black, lower: orange, both: red, none: salmon) to oral aspirin provocation before and after 24 weeks of treatment with dupilumab are displayed. Number of patients at each visit and significance of changes between baseline and week 24 are indicated in individual graphs: *** $p < .001$, **** $p < .0001$.

Figure 2: Changes in clinical responses from baseline over time in N-ERD patients during 24 weeks of dupilumab treatment. (A) Mean score of total polyp score (TPS) (B) mean difference from baseline in total nasal symptom score (TNSS), (C) mean difference from baseline in Sino-Nasal Outcome Test-22 (SNOT-22), (D) mean score of University of Pennsylvania Smell Identification Test (UPSIT), (E) mean difference from baseline in Asthma Control Test (ACT), (F) mean percentage of forced expiratory volume in 1 second (FEV1) and (G) mean score of maximal expiratory flow 50% (MEF50) are displayed at baseline as well as 4, 12 and 24 weeks after treatment where applicable. Number of patients at each visit and significance of changes between baseline and week 24 are indicated in individual graphs: * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$.

Figure 3: Analysis of biomarkers in blood and urine of N-ERD patients in response to 24 weeks of dupilumab treatment.

(A-D) Boxplots representing levels of (A) total IgE (kU/L), (B) eosinophilic cationic protein (ECP, $\mu\text{g/L}$), (C) absolute (count) and (D) percentage of eosinophils in blood are displayed at baseline and after 24 weeks of dupilumab treatment. (E) Boxplots showing levels of total IgE (ng/ μL) in nasal secretions are displayed at baseline and after 24 weeks of dupilumab treatment. (F-G) Boxplots representing levels of (F) 11β -Prostaglandin $\text{F}_{2\alpha}$ and (G) Leukotriene E4 levels (pg/mL) in urine are shown at week 0 and 24 of dupilumab treatment. Line within each box represents the median, bottom border represents the 25th percentile and top border the 75th percentile of the data. Whiskers extend 1.5 times the interquartile range and diamond shaped points are outliers.

Number of patients at each visit and significance of changes between baseline and week 24 are indicated in individual graphs: * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$

Figure 4: Analysis of selected biomarkers in nasal secretions of N-ERD patients in response to dupilumab treatment. Boxplots representing levels (pg/mL) of **(A)** Thymic stromal lymphopoietin (TSLP), **(B)** CCL17, **(C)** IL-5, **(D)** IL-13, **(E)** Eotaxin-1, **(F)** Eotaxin-3 **(G)** IL-12 p40, **(H)** TNF- α , **(I)** IL-6, **(J)** IL-17A and **(K)** IL-22 levels in N-ERD patients (n=31) are displayed at baseline and after 24 weeks of dupilumab treatment. Line within each box represents the median, bottom border represents the 25th percentile and top border the 75th percentile of the data. Whiskers extend 1.5 times the interquartile range and diamond shaped points are outliers- Significance of changes between baseline and week 24 are indicated in individual graphs: * $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$.

Figure 5: Analysis of clinical responses, urine, and nasal secretion biomarkers in aspirin-tolerant and aspirin-intolerant N-ERD-patients after 24 weeks of dupilumab treatment. Change over time of **(A)** TPS represented as mean score. **(B)** University of Pennsylvania Smell Identification Test (UPSIT) mean score before and after 24 weeks of treatment. **(C-D)** Boxplots representing **(C)** urine 11 β -Prostaglandin F2 α (pg/mL) and **(D)** urine Leukotriene E4 levels (pg/mL). Line within each box represents the median, bottom border represents the 25th percentile and top border the 75th percentile of the data. Whiskers extend 1.5 times the interquartile range and diamond shaped points are outliers. **(E)** Median percentage change from baseline of selected biomarkers in nasal secretions. All analyses performed in patients showing aspirin tolerance (orange) or no aspirin tolerance (blue) at the second provocation after 24 weeks of treatment. Significance of changes between baseline and week 24 are indicated in individual graphs: * $p < .05$, ** $p < .01$, ns=not significant.

Figure 6: Correlation between clinical parameters and nasal biomarkers in aspirin-tolerant and aspirin-intolerant N-ERD patients after 24 weeks of dupilumab treatment. Correlation of change from baseline (percent) of **(A-G)** total polyp score (TPS, x-axis) versus nasal **(A)** Eotaxin-1, **(B)** IL-6, **(C)** IL-5, **(D)** IL-17A, **(E)** CCL17 as well as urine **(F)** Leukotriene E4 and **(G)** 11 β -Prostaglandin F2 α . Correlation of change from baseline (%) of **(H-N)** University of Pennsylvania Smell Identification Test (UPSIT, x-axis) versus nasal **(H)** Eotaxin-1, **(I)** IL-6, **(J)** IL-5, **(K)** IL-17A, **(L)** CCL17 as well as **(M)** urine Leukotriene E4 and **(N)** urine 11 β -Prostaglandin

F2 α . Correlation as determined by Spearman correlation coefficient and significance levels are indicated in the figure for aspirin-tolerant (orange, n=17) and aspirin-intolerant (blue, n=13) patients. The respective lines within the scatter plots represent the linearly regressed trendline and the shaded regions the 95% confidence interval.

Figure E1: Study design. (A) Study design and **(B)** Study flow chart. TPS=total polyp score, TNSS=Total Nasal Symptom Score, SNOT-22=Sino-Nasal Outcome Test-22, UPSIT=University of Pennsylvania Smell Identification Test, QoL=Quality of life scores, ACT=Asthma Control Test.

Figure E2: Changes in quality of life from baseline over time in N-ERD patients during 24 weeks of dupilumab treatment. (A) Mean score in PHQ-2 score. **(B-C)** Percentage of respondents in the EQ-5D-3L at **(B)** Baseline and after **(C)** 24 weeks. Dark grey bars represent patients reporting “No problem” and light grey bars “any problem”. **(D)** Mean score in PHQ-2 score in aspirin-tolerant (orange, n=17) and aspirin-intolerant (blue, n=13) patients. Number of patients at each visit and significance of changes between baseline and week 24 are indicated in individual graphs: **p<.01.

Figure E3: Analysis of selected biomarkers in nasal secretions of N-ERD patients in response to dupilumab treatment. Boxplots showing levels (pg/mL) of **(A)** G-CSF, **(B)** GM-CSF, **(C)** IFN- γ , **(D)** IL-1RA, **(E)** IL-1 α , **(F)** IL-1 β , **(G)** IL-2, **(H)** IL-3, **(I)** IL-4, **(J)** IL-7, **(K)** IL-8, **(L)** IL-9, **(M)** IL-10, **(N)** IL-12p70, **(O)** IL-15, **(P)** IL-16, **(Q)** IL-17E/IL-25, **(R)** IL-21, **(S)** IL-27, **(T)** IL-33, **(U)** TNF- β , and **(V)** VEGF levels in N-ERD patients (n=31) are displayed at baseline and after 24 weeks of dupilumab treatment. Line within each box represents the median, bottom border represents the 25th percentile and top border the 75th percentile of the data. Whiskers extend 1.5 times the interquartile range and diamond shaped points are outliers. Significance of changes between baseline and week 24 are indicated in individual graphs: ns=not significant.

Figure E4: Analysis of nasal secretion biomarkers in aspirin-tolerant and aspirin-intolerant N-ERD-patients after 24 weeks of dupilumab treatment. Boxplots representing levels (pg/mL) of **(A)** Eotaxin-1, **(B)** Eotaxin-3, **(C)** CCL17, **(D)** TNF- α , **(E)** TSLP, **(F)** IL-5, **(G)** IL-6, **(H)** IL-12p40, **(I)** IL-15, **(J)** IL-17A, **(K)** IL-17E/IL-25, **(L)** IL-22, **(M)** G-CSF, **(N)** GM-CSF, **(O)** IFN- γ , **(P)** IL-1RA, **(Q)** IL-1 α , **(R)** IL-1 β , **(S)** IL-2, **(T)** IL-3, **(U)** IL-4, **(V)** IL-7, **(W)** IL-8, **(X)** IL-9, **(Y)** IL-10, **(Z)** IL-12p70, **(AA)** IL-

13, **(AB)** IL-16, **(AC)** IL-21, **(AD)** IL-27, **(AE)** IL-33, **(AF)** TNF- β and **(AG)** VEGF levels in N-ERD patients (n=31) are displayed at baseline and after 24 weeks of dupilumab treatment in aspirin-tolerant (orange) or aspirin-intolerant (blue) patients. Line within each box represents the median, bottom border represents the 25th percentile and top border the 75th percentile of the data. Whiskers extend 1.5 times the interquartile range and diamond shaped points are outliers. Significance of changes between baseline and week 24 are indicated in individual graphs: *p<.05, **p<.01, ***p<.001, ns=not significant.

Figure E5: Analysis of clinical responses, urine, and nasal secretion biomarkers in complete and partial aspirin-tolerant as well as aspirin-intolerant N-ERD-patients after 24 weeks of dupilumab treatment. Change over time of **(A)** TPS represented as mean score. **(B)** University of Pennsylvania Smell Identification Test (UPSIT) mean score before and after 24 weeks of treatment. **(C-D)** Boxplots representing **(C)** urine 11 β -Prostaglandin F2 α (pg/mL) and **(D)** urine Leukotriene E4 levels (pg/mL). Data are presented from complete (green) or partial (orange) aspirin tolerant as well as aspirin intolerant (blue) patients. Line within each box represents the median, bottom border represents the 25th percentile and top border the 75th percentile of the data. Whiskers extend 1.5 times the interquartile range and diamond shaped points are outliers. Significance of changes between baseline and week 24 are indicated in individual graphs: *p<.05, **p<.01, ns=not significant.

Figure E6: Analysis of nasal secretion biomarkers complete and partial aspirin-tolerant as well as aspirin-intolerant N-ERD-patients after 24 weeks of dupilumab treatment. Boxplots representing levels (pg/mL) of **(A)** Eotaxin-1, **(B)** Eotaxin-3, **(C)** CCL17, **(D)** TNF- α , **(E)** TSLP, **(F)** IL-5, **(G)** IL-6, **(H)** IL-12p40, **(I)** IL-15, **(J)** IL-17A, **(K)** IL-17E/IL-25, **(L)** IL-22, **(M)** G-CSF, **(N)** GM-CSF, **(O)** IFN- γ , **(P)** IL-1RA, **(Q)** IL-1 α , **(R)** IL-1 β , **(S)** IL-2, **(T)** IL-3, **(U)** IL-4, **(V)** IL-7, **(W)** IL-8, **(X)** IL-9, **(Y)** IL-10, **(Z)** IL-12p70, **(AA)** IL-13, **(AB)** IL-16, **(AC)** IL-21, **(AD)** IL-27, **(AE)** IL-33, **(AF)** TNF- β and **(AG)** VEGF levels are displayed at baseline and after 24 weeks of dupilumab treatment in N-ERD patients (n=31) with complete (green) or partial (orange) aspirin tolerance as well as aspirin intolerant patients (blue) after treatment. Line within each box represents the median, bottom border represents the 25th percentile and top border the 75th percentile of the data. Whiskers extend 1.5 times the interquartile range and diamond shaped points are outliers. Significance of

changes between baseline and week 24 are indicated in individual graphs: * $p < .05$, ** $p < .01$, *** $p < .001$, ns=not significant.

Characteristics	N	Total at baseline		N	Aspirin-intolerant after 6 months treatment with dupilumab		N	Aspirin-tolerant after 6 months treatment with dupilumab		p value*
Age, years (min-max); Mean	31	26–70; 46.13		13	33-63; 45.92		17	26-70; 46.65		NS (0.87)
Sex, n (%)										NS (0.25)
Men		17 (55%)			9 (69%)			7 (41%)		
Women		14 (45%)			4 (31%)			10 (59%)		
Reaction to aspirin provocation before treatment (yes, no), n (%)										
Upper respiratory tract reaction	31	Yes 10 (32%)	No 21 (68%)	13	Yes 3 (23%)	No 10 (77%)	17	Yes 6 (35%)	No 11 (65%)	NS (0.35)
Lower respiratory tract reaction	31	2 (6%)	29 (94%)	13	2 (15%)	11 (85%)	17	0 (0%)	17 (100%)	NS (0.75)
Both upper and lower respiratory tract reaction	31	19 (61%)	12 (39%)	13	8 (62%)	5 (38%)	17	11 (65%)	6 (35%)	NS (0.21)
Comorbidities (yes, no), n (%)										
Allergy	30	22 (73%)	8 (27%)	13	10 (77%)	3 (23%)	16	11 (69%)	5 (31%)	NS (0.94)
Asthma	31	30 (97%)	1 (3%)	13	12 (92%)	1 (8%)	17	17 (100%)	0 (0%)	NS (0.89)
Number of previous surgeries (min-max); Mean (±SD)	31	0-6; 2.06 (1.46)		13	0-3; 1.46 (0.97)		17	0-6; 2.65 (1.54)		0.02
Scores (scale), Mean (±SD)										
TPS combined (0-8)	31	3.58 (2.42)		13	4.46 (2.33)		17	2.76 (2.28)		NS (0.06)
UPSIT score (0-40)	30	14.70 (8.68)		12	13.33 (8.62)		17	16.12 (8.85)		NS (0.24)
SNOT-22 (0-110)	30	41.43 (23.27)		13	46.85 (22.34)		16	37.12 (24.54)		NS (0.28)
ACT (0-25)	29	21.07 (3.85)		13	20.23 (2.74)		15	21.53 (4.61)		NS (0.09)
FEV1 (%) Median (min-max); Mean (±SD)	29	71.09 (51.13-86.58); 70.51 (8.87)		13	71.09 (51.13-82.71); 71.30 (8.58)		15	71.77 (53.00-86.58); 69.88 (9.64)		NS (0.69)
MEF50 (L/s) Median (min-max); Mean (±SD)	29	2.29 (0.86–4.31); 2.40 (0.81)		13	2.29 (0.86-3.33); 2.29 (0.70)		15	2.23 (1.01-4.31); 2.46 (0.93)		NS (0.60)
MEF75 (L/s) Median (min-max); Mean (±SD)	23	4.85 (2.71–9.39); 4.69 (1.48)		9	4.75 (2.89-6.63); 4.60 (1.28)		13	4.85 (2.71-9.39); 4.68 (1.70)		NS (0.89)
Biomarkers, Median (min-max); Mean (±SD)										
Total IgE (kU/L)	30	194.5 (26.90-1051.00); 264.82 (264.71)		12	250.5 (62.3-1051.0); 362.02 (324.97)		17	146.0 (26.9-896.0); 206.79 (204.24)		NS (0.18)
Total IgE from nasal secretion (ng/μL)	31	528.87 (65.18 – 6813.47); 1389.91 (1845.84)		13	1652.23 (81.26 – 6813.47); 2275.51 (2343.61)		17	293.60 (65.18 – 3728.98); 786.31 (1070.86)		NS (0.06)
Eosinophils (%)	28	4.60 (0.70-12.00); 5.67 (3.00)		11	6.7 (1.9-9.7); 6.30 (3.15)		16	4.3 (0.7-12.0); 5.27 (3.02)		NS (0.49)
ECP ^e (μg/L)	23	39.9 (9.38 – 200.0); 57.23 (43.85)		10	47.7 (9.38-109.0); 54.24 (31.92)		12	38.5 (18.80-200.0); 61.36 (54.32)		NS (0.82)

Table 1. Patient demographics and clinical characteristics.

TPS: Total polyp score, UPSIT: University of Pennsylvania Smell Identification Test, SNOT: Sino-nasal Outcome Test, ACT: Asthma Control Test, SD: Standard deviation, FEV1: Forced expiratory volume in one second, MEF50: Maximal expiratory flow at 50%, MEF75: Maximal expiratory flow at 75%, Total IgE: total immunoglobulin E, ^oECP: Eosinophil cationic protein,
* *p* value from comparison of Aspirin-intolerant and Aspirin-tolerant patients at baseline.

Table 2: Cumulative dose for aspirin provocation at baseline and 24 weeks after dupilumab treatment.

Patient ID	Sex	Age (yr)	Cumulative provoking Aspirin dose [mg]	
			Baseline	Week 24
Complete Aspirin tolerance				
001	M	54	375	875
002	F	43	125	875
003	F	47	125	875
004	F	31	125	875
005	F	49	375	875
006	F	33	125	875
007	F	48	125	875
Partial Aspirin tolerance				
008	M	50	125	375
009	F	27	125	375
010	F	70	125	375
011	M	36	375	875
012	M	43	125	375
013	M	38	125	375
014	F	67	125	375
015	M	68	125	375
016	F	33	125	375
017	M	65	125	375
No Aspirin tolerance				
018	M	40	125	125
019	M	36	125	125
020	M	51	125	125
021	F	47	125	125
022	M	56	375	375
023	M	63	375	375
024	M	33	375	125
025	M	35	125	125
026	F	42	125	125
027	M	51	125	125
028	M	56	125	125
029	F	47	875	875
030	F	45	125	125
No second provocation				
031	M	40	875	NA

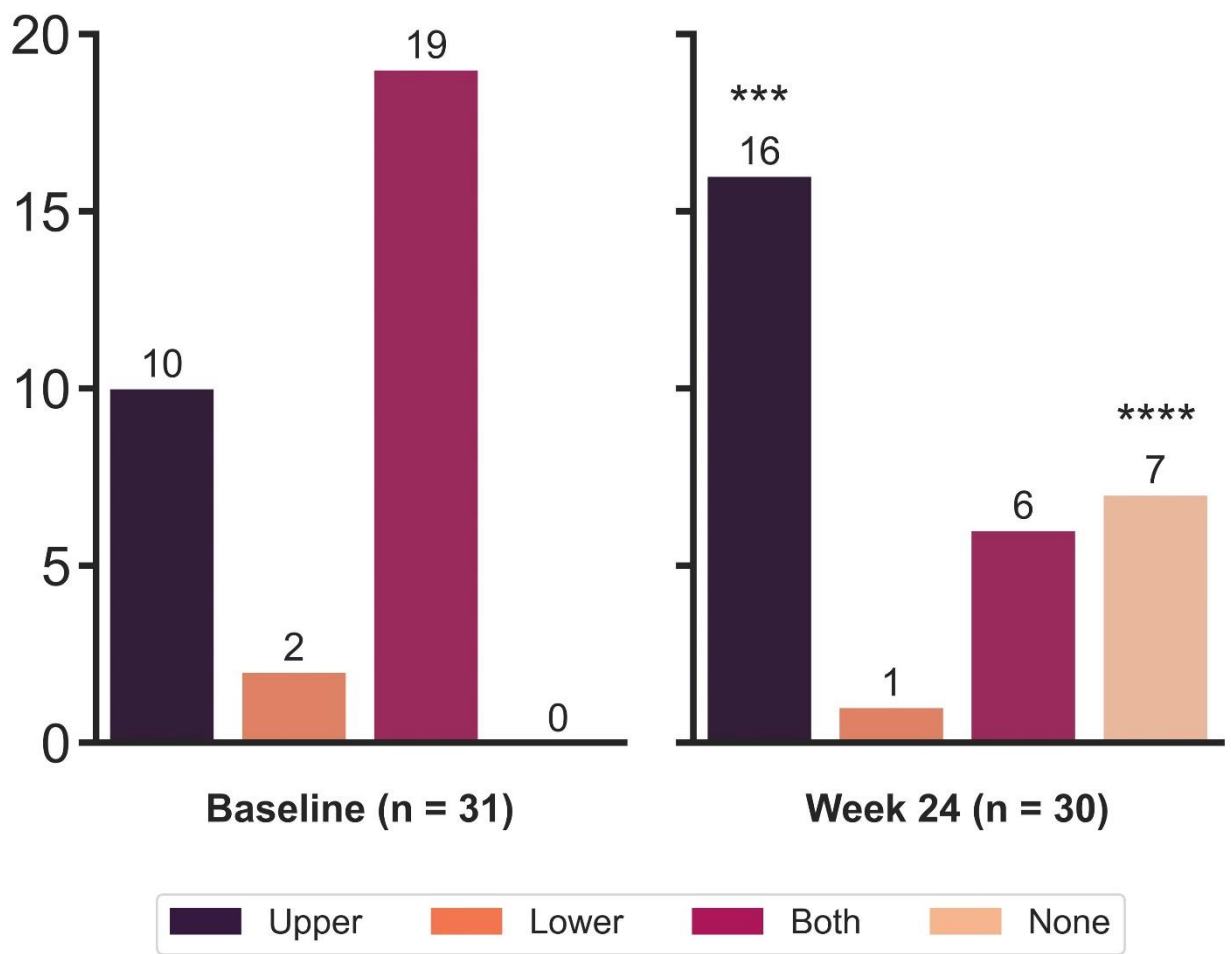


Figure 1

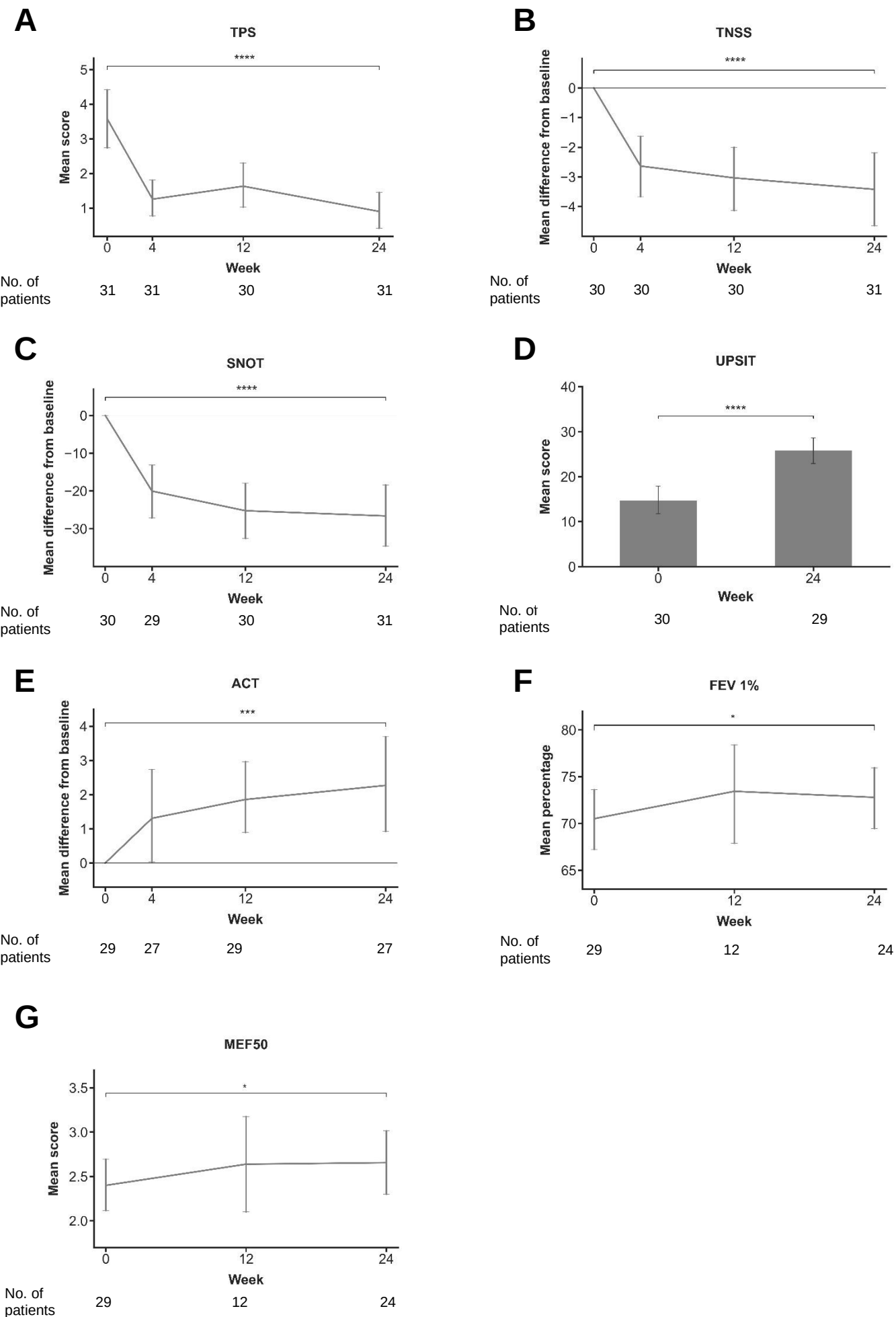
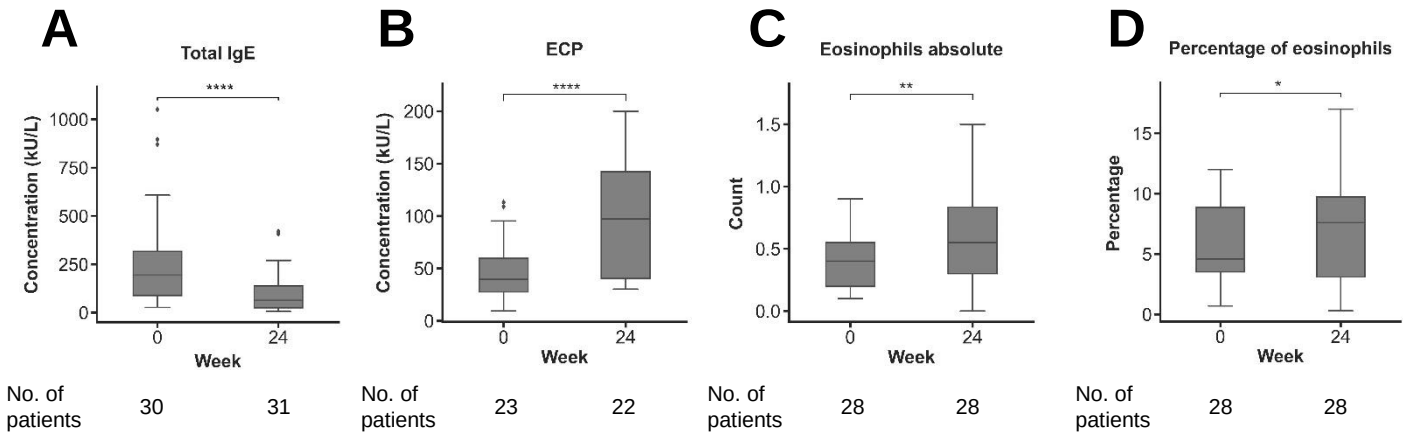
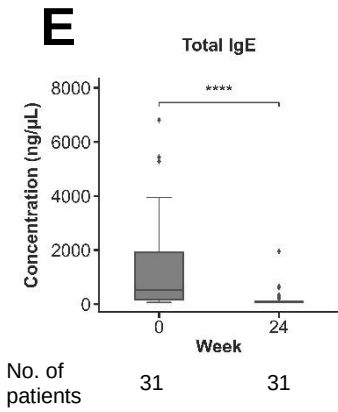


Figure 2

Blood



Nasal secretions



Urine

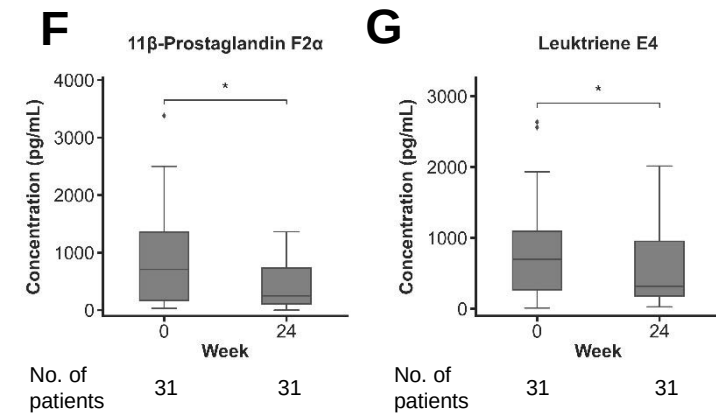


Figure 3

Nasal secretions

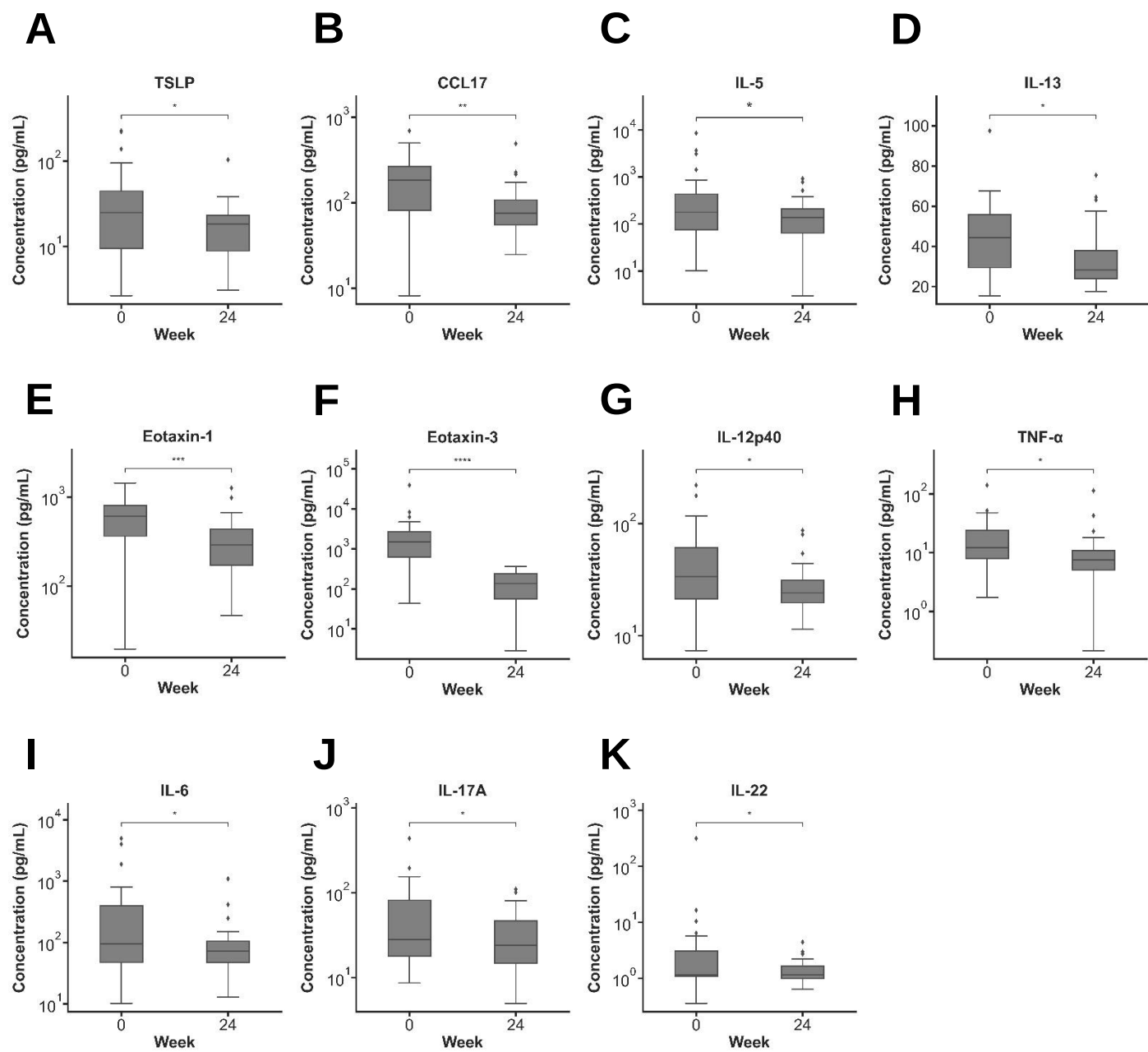


Figure 4

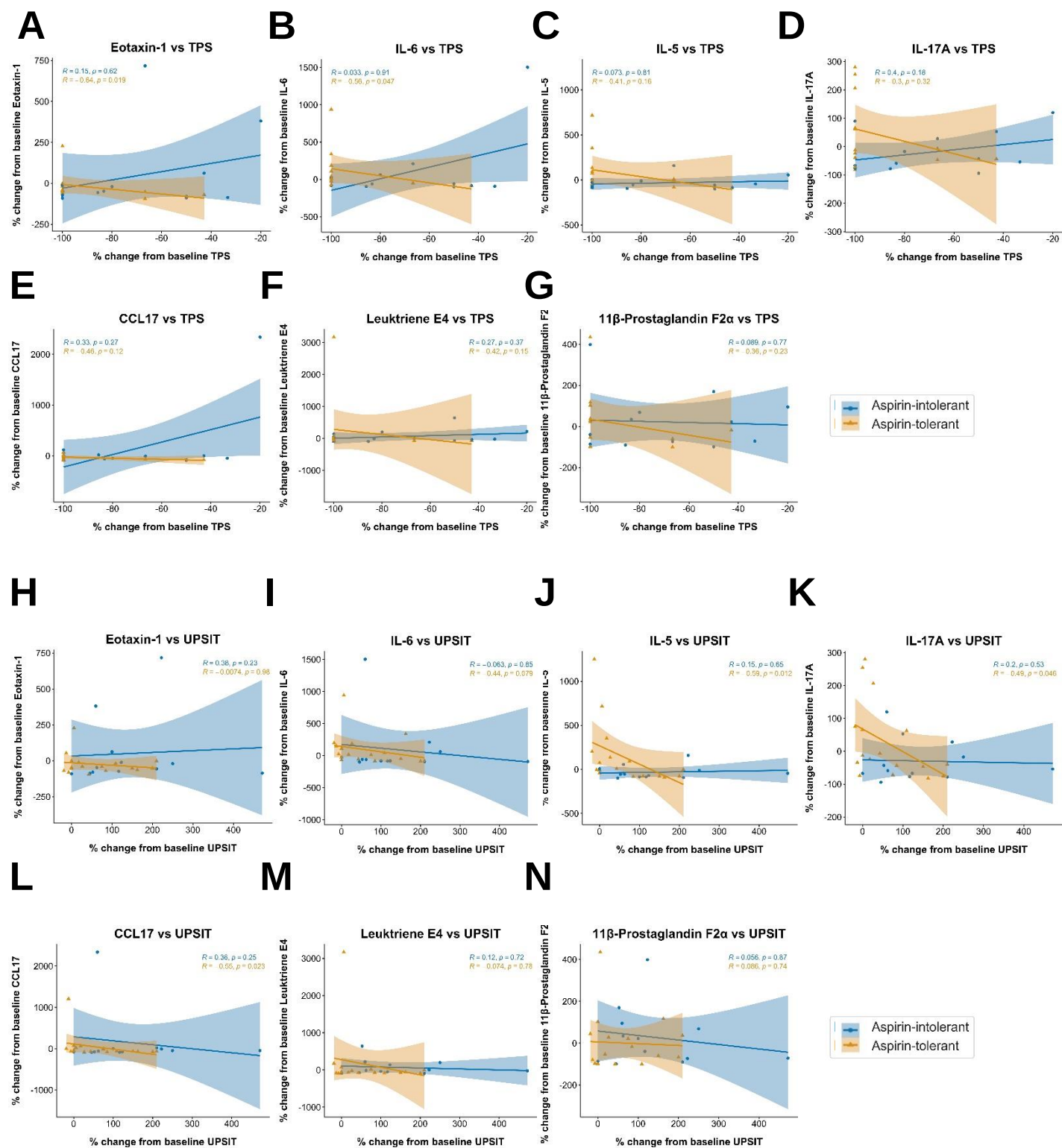


Figure 6

Supplementary methods and tables for Dupilumab increases aspirin tolerance in NSAID- exacerbated respiratory disease (N-ERD)

Schneider S.¹, Poglitsch K.², Morgenstern C.³, Quint T.², Gangl K.¹, Sinz C.², Bartosik T.¹, Campion N.J., Liu D.T.¹, Landegger L.D.¹, Tu A.¹, Stanek V.¹, Rocha-Hasler M.¹, Bangert C.^{2*}, Eckl-Dorna J.¹

Affiliations:

¹ Department of Otorhinolaryngology, General Hospital and Medical University of Vienna, Vienna, Austria

² Department of Dermatology, General Hospital and Medical University of Vienna, Vienna, Austria

³ Science Impuls, Seeboden, Austria

***CORRESPONDENCE**

Christine Bangert, MD,
Department of Dermatology
Medical University of Vienna, General Hospital of Vienna
Waehringer Guertel 18-20
A-1090 Vienna, Austria
Tel: +43-1-40400-77020
Email: christine.bangert@meduniwien.ac.at

SUPPLEMENTARY METHODS

Additional study measures

Extent of nasal polyps was evaluated using a rigid 0° endoscope. After application of local anesthetic and decongestant, TPS was graded for each nasal cavity from 0 to 4 (0= no visible polyps, 1= small polyps in the middle meatus, 2= nasal polyps reaching below the lower border of the middle turbinate, 3= large polyps reaching the lower border of the inferior turbinate or polyps medial to the middle turbinate, 4= large polyps causing complete obstruction of the inferior nasal cavity) (1). Nasal secretions were collected at baseline and at week 24 and processed as previously described using nasosorptions (Nasosorption FX-I, Hunt Developments (UK) Limited, Midhurst, West Sussex, United Kingdom) (2). Urine was collected at baseline and week 24 after treatment. Blood eosinophil counts, serum total IgE levels and ECP were assessed at baseline and after 24 weeks and potential drug-related side effects were monitored at every visit. The study design is displayed in Fig. E1.

Sample size considerations:

The sample size of the study was calculated based on the half-width of the 95% confidence interval for the proportion of patients with an improvement in the primary endpoint between visit 1 (baseline) and visit 4 (24 weeks after treatment with dupilumab). With 30 patients and observed proportions of 50%, 70% or 80%, the respective half-width of the 95% confidence interval will be 18, 16, or 14 percentage points. Given the explorative nature of the study this is considered as a sufficient level of precision.

MSD Measurement

The following cytokines were assayed in nasal secretions using the electrochemiluminescence technology MSD multiplex U-Plex platform: Interleukin (IL)-1 α , IL-1 β , IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17E/IL-25, IL-21, IL-22, IL-27, IL-33, Tumor Necrosis Factor-alpha (TNF- α), TNF- β , Interferon-gamma (IFN- γ), Thymic Stromal Lymphopoietin (TSLP), Eotaxin-1, Eotaxin-3, Thymus and Activation-Regulated Chemokine (TARC=CCL17), Vascular Endothelial Growth Factor-A (VEGF-A), Granulocyte Colony-Stimulating Factor (G-CSF) and Granulocyte-Macrophage

Colony-Stimulating Factor (GM-CSF). Nasal secretions of left and right nostrils were pooled prior to application to MSD plates. All measurements were performed according to the manufacturer's instructions (<https://www.mesoscale.com/en>). Briefly, linkers were complexed with 200µL target antibody, diluted to 1x and plates were coated with 50µL of this solution on a shaker for one hour at room temperature, combinations of linkers with antibodies were noted. Plates were washed thrice with PBS 0.05% Tween 20 (PBS-T), dried and stored at 4°C for a maximum of seven days prior to use. Standards were freshly reconstituted and serially diluted in 4-fold steps in diluent 43 provided with the kit. 50µL sample or standard were incubated on the plate with agitation overnight at 4°C under a seal. Plates were washed thrice, detection antibodies were diluted with diluent 3 provided and 50µL of this mixture were applied to plates for one hour at room temperature with agitation under a seal. Plates were washed thrice with PBS-T and immediately measured in 150µL of MSD GOLD read buffer (provided) on a MESO SECTOR S 600 plate reader. DISCOVERY WORKBENCH software (MSD, Rockville, Maryland, USA) was inputted with batch-specific standard concentrations and sample analyte concentrations were extrapolated by the software.

Missing concentration values that resulted from readings below or above the detection limits were imputed using the mean of the estimated lower and higher detection limit values for the specific cytokine across all batch readings.

ELISA measurement of total IgE levels in nasal secretion

IgE levels within nasal secretions were measured by using the Human IgE ELISA Antibody Pair Kit (Stemcell Technologies, Vancouver, Canada) according to the manufacturer's instructions. Briefly, ELISA plates (i.e., 384 well plates, Nunc Maxisorp, Roskilde, Denmark) were coated with 1 µg/ml Human IgE Capture Antibody in PBS overnight at 4°C and blocked with PBS, 0.05% v/v Tween-20, 0.1% v/v BSA (incubation buffer). As a standard, purified Bet v 1 specific IgE (12.5 µg/ml) was used (3). Samples were diluted either 1:5 or 1:10 in incubation buffer. Samples and standards were added to the plate and then incubated overnight at 4°C. After washing, 0.3µg/ml Human IgE Biotinylated Detection Antibody was added and left for 1 hour at room temperature. Subsequently, the plates were washed and incubated with Streptavidin Alkaline Phosphatase for 1 hour at room temperature. After thorough washing, the 4-Nitrophenyl phosphate disodium salt hexahydrate (pNPP) substrate

solution (pNPP in 10% v/v Diethanolamine, 2% Sodium azide in H₂O, pH 9.8) was added to the plate and plates were incubated for 1 hour at room temperature protected from light. OD levels were measure at 405 nm. All measurements were done in triplicates, background was subtracted.

ELISA measurement of 11 β -Prostaglandin F₂ α and Leukotriene E₄ levels in urine.

11 β -Prostaglandin F₂ α and Leukotriene E₄ levels in urine were measured using the Leukotriene E₄ ELISA Kit (Cayman Chemicals, Michigan, USA) and 11 β -Prostaglandin F₂ α (Cayman Chemicals, Michigan, USA) respectively, following the manufacturer's protocol similar to the ELISA described above. All measurements were done in triplicates, background was subtracted.

Statistics:

Data from clinical questionnaires were included in the analysis if they were greater than 80% complete and contained at least two patient visits. Missing values were imputed using the mean of the two closest visits (Number and percentage of imputed missing values: please refer to table E3). Mean and standard deviation as well as median and range were calculated for metric variables and absolute and relative frequencies were calculated for categorical variables. Tolerated aspirin dosages at baseline and after 24 weeks were summarized in a table.

McNemar's exact test was used for dichotomous data to assess significance between baseline and week 24 of matched samples. Patients with missing values in a particular outcome variable were excluded in the calculation of the respective summary statistics. Plots for secondary endpoints (TPS, TNSS, SNOT-22, UPSIT and ACT) are presented as mean difference from baseline (with 95% confidence intervals and *p* values). Statistical significance was assessed with non-parametric Wilcoxon signed-rank test apart from FEV₁ and MEF₅₀ where a two-sided t-test was used according to the assumption of normality. A significance level of 0.05 was applied.

Levels of cytokines and chemokines were not normally distributed as assessed by Shapiro-Wilks test. Statistical analysis on cytokines and chemokines was carried out for paired samples using the Wilcoxon signed-rank test and for non-paired samples (comparing aspirin-tolerant vs. aspirin-intolerant patients) using the Mann-Whitney *U* test.

Change in cytokine and chemokine levels from baseline to 24 weeks was presented as median percentage change from baseline with median absolute deviations. Differences in medians between aspirin-tolerant and aspirin-intolerant patients were assessed using the Mann-Whitney *U* test for independent samples.

Correlation plots of cytokines and chemokines with clinical parameters were drawn using percentage change from baseline and calculation of Spearman correlation coefficient.

Statistical analyses were conducted using Python 3.9.6 and R 4.1.0.

References:

1. Meltzer EO, Hamilos DL, Hadley JA, Lanza DC, Marple BF, Nicklas RA, et al. Rhinosinusitis: developing guidance for clinical trials. *The Journal of allergy and clinical immunology*. 2006;118(5 Suppl):S17-61.
2. Leaker BR, Malkov VA, Mogg R, Ruddy MK, Nicholson GC, Tan AJ, et al. The nasal mucosal late allergic reaction to grass pollen involves type 2 inflammation (IL-5 and IL-13), the inflammasome (IL-1beta), and complement. *Mucosal Immunol*. 2017;10(2):408-20.
3. Laffer S, Vangelista L, Steinberger P, Kraft D, Pastore A, Valenta R. Bip 1, a monoclonal antibody with specificity for the major birch pollen allergen Bet v 1, modulates IgE binding to the allergen. *International archives of allergy and immunology*. 1997;113(1-3):260-1.

Table E1. Change from baseline to week 24 in clinical endpoints.

Clinical Endpoint (range)	Total				Aspirin-intolerant				Aspirin-tolerant			
	Baseline, Mean ($\pm$ SD) ¹	Week 24, Mean ($\pm$ SD)	Mean differenc e from baseline	<i>p</i> value	Baseline, Mean ($\pm$ SD)	Week 24, Mean ($\pm$ SD)	Mean difference from baseline	<i>p</i> value	Baseline, Mean ($\pm$ SD)	Week 24, Mean ($\pm$ SD)	Mean difference from baseline	<i>p</i> value
	Median (min-max)	Median (min- max)	Mean ($\pm$ SD)		Median (min-max)	Median (min-max)	Mean ($\pm$ SD)		Median (min-max)	Median (min-max)	Mean ($\pm$ SD)	
TPS combined (0-8)	N=31 3.58 (2.42) 3 (0 – 8)	N=31 0.90 (1.45) 0 (0 – 4)	-2.68 (1.84)	<i>p</i> <0.001	N=13 4.46 (2.33) 5 (1 – 8)	N=13 1.62 (1.71) 1 (0 – 4)	-2.84 (1.61)	<i>p</i> <0.001	N=17 2.76 (2.28) 3 (0 – 7)	N=17 0.35 (1.00) 0 (0 – 4)	-2.41 (1.94)	0.001
UPSIT (0-40)	N=30 14.7 (8.68) 10.5 (6 – 37)	N=29 25.86 (8.07) 26 (10 – 40)	11.16 (9.54)	<i>p</i> <0.001	N=12 13.33 (8.62) 10 (6 – 37)	N=13 27.46 (9.04) 29 (13 – 40)	14.13 (9.19)	0.002	N=17 16.12 (8.85) 11 (8 – 33)	N=15 24 (7.09) 24 (10 – 35)	7.88 (8.17)	0.004
SNOT-22 (0-110)	N=30 41.43 (23.27) 40.25 (5 – 83)	N=30 14.7 (12.6) 12 (1– 66)	-26.73 (23.49)	<i>p</i> <0.001	N=13 46.85 (22.34) 42.5 (13 – 80)	N=13 14.35 (8.82) 13 (2 – 30)	-32.5 (19.76)	<i>p</i> <0.001	N=16 37.12 (25.54) 32 (5 – 83)	N=16 16.72 (16.01) 12 (1 – 68)	-20.4 (21.07)	0.001
TNSS (0-12)	N=30 5.27 (2.88) 5 (0 – 11)	N=31 1.81 (1.66) 2 (0 – 6)	-3.46 (3.39)	<i>p</i> <0.001	N=13 5.23 (3.06) 5 (1 – 11)	N=13 1.85 (1.68) 1 (0 – 6)	-3.38 (3.41)	0.008	N=16 5.12 (2.83) 5 (0 – 11)	N=17 1.65 (1.66) 2 (0 – 5)	-3.47 (3.47)	0.003
ACT (0-25)	N=29 21.07 (3.85) 22 (12 – 25)	N=27 23.56 (2.19) 25 (16 – 25)	2.49 (3.66)	<i>p</i> <0.001	N=13 20.23 (2.74) 21 (16 – 25)	N=10 22.90 (2.85) 24 (16 – 25)	2.67 (2.25)	0.004	N=15 21.53 (4.61) 23 (12 – 25)	N=16 23.88 (1.71) 25 (20 – 25)	2.35 (4.42)	0.03
FEV1^a (%)	N=29 70.51 (8.87) 71.09 (51.13 – 86.58)	N=24 72.79 (8.19) 74.63 (51.99 - 84.88)	2.28 (5.44)	0.013	N=13 71.3 (8.58) 71.09 (51.13 – 82.71)	N=12 75.1 (6.42) 78.02 (64.78 – 84.59)	3.8 (5.24)	0.02	N=15 69.88 (9.64) 71.77 (53 – 86.58)	N=12 70.48 (9.34) 71.47 (51.99 – 84.88)	0.6 (5.32)	NS (0.3)
MEF50^b (L/s)	N=29 2.40 (0.81) 2.29 (0.86 – 4.31)	N=24 2.66 (0.89) 2.50 (1.14 – 4.41)	0.26 (0.79)	0.016	N=13 2.29 (0.7) 2.29 (0.86 – 3.33)	N=12 2.88 (0.77) 2.64 (1.9 – 4.41)	0.59 (0.85)	0.03	N=15 2.46 (0.93) 2.23 (1.01 – 4.31)	N=12 2.43 (0.98) 2.25 (1.14 – 4.28)	-0.03 (0.66)	NS (0.29)
Mef75^c (L/s)	N=23 4.69 (1.48)	N=18 4.89 (1.54)	0.2	<i>p</i> <0.001	N=9 4.6 (1.28)	N=9 5.27 (1.65)	0.67	0.006	N=13 4.68 (1.7)	N=9 4.5 (1.41)	-0.18	0.02

	4.85 (2.71 – 9.39)	4.52 (2.74 – 7.97)	(1.21)		4.75 (2.89 – 6.63)	5.4 (3.07 – 7.97)	(1.08)		4.85 (2.71 – 9.39)	4.12 (2.74 – 7.47)	(1.25)	
Eosinophils, absolute (cells/ μL)	N=28 0.42 (0.24) 0.4 (0.1 – 0.9)	N=28 0.63 (0.45) 0.55 (0 – 1.5)	0.21 (0.35)	0.003	N=11 0.51 (0.3) 0.5 (0.1 – 0.9)	N=11 0.61 (0.43) 0.5 (0 – 1.5)	0.1 (0.24)	0.28	N=16 0.37 (0.17) 0.35 (0.1 – 0.7)	N=16 0.69 (0.46) 0.65 (0.1 – 1.48)	0.32 (0.4)	0.021
Percentage of Eosinophils	N=28 5.67 (3.00) 4.6 (0.7 – 12)	N=28 7.38 (4.86) 7.6 (0.3 – 17)	1.71 (4.07)	0.01	N=11 6.3 (3.15) 6.7 (1.9 – 9.7)	N=11 7.74 (5.02) 7.0 (0.8 – 17)	1.44 (3.17)	0.07	N=16 5.27 (3.02) 4.3 (0.7 – 12)	N=16 7.57 (4.71) 8.55 (0.9 – 15)	2.3 (4.32)	0.039
Blood total IgE^d (kU/L)	N=30 264.82 (264.71) 194.50 (26.9 – 1051)	N=31 99.69 (106.9) 64.2 (5.9 – 420)	-165.13 (184.06)	p<0.001	N=12 362.03 (324.97) 250.5 (62.3 – 1051)	N=13 116.46 (118.17) 87.5 (16.2 – 420)	245.57 (239.91)	0.02	N=17 206.79 (204.24) 146 (26.9 – 896)	N=17 88.96 (102.57) 40.1 (5.9 – 407)	117.83 (108.2)	p<0.001
Nasal secretion total IgE^d (ng/μL)	N=31 1389.91 (1845.84) 528.87 (65.18 – 6813.47)	N=31 194.14 (358.54) 80.53 (53.61 – 1954.98)	-1195.77 (1777.02)	p<0.001	N=13 2275.51 (2343.61) 1652.23 (81.26 – 6813.47)	N=13 320.89 (533.59) 80.53 (55.25 – 1954.98)	-1954.62 (2256.51)	p<0.001	N=17 786.31 (1070.86) 293.6 (65.18 – 3728.98)	N=17 101.97 (70.75) 78.7 (53.61 – 336.66)	-684.34 (1040.22)	p<0.001
ECP^e (μg/L)	N=23 57.23 (43.85) 39.9 (9.38 – 200)	N=22 106.25 (62.29) 109.5 (30.2 – 200)	49.02 (48.82)	p<0.001	N=10 54.24 (31.92) 47.7 (9.38 – 109)	N=10 114.24 (55.39) 127 (36.2 – 200)	60.0 (55.37)	p<0.001	N=12 61.36 (54.32) 38.5 (18.8 – 200)	N=12 99.59 (69.21) 72.55 (30.2 – 200)	38.23 (34.65)	p<0.001
Leukotriene E4	N=31 801.38 (673.48) 701.77 (10.64 – 2635.19)	N=31 506.19 (480.19) 319.87 (27.47 – 1024.72)	-295.19 (655.45)	0.02	N=13 825.37 (770.17) 792.48 (20.51 – 2635.19)	N=13 632.75 (577.27) 403.83 (27.47 – 2014.72)	-192.62 (485.35)	NS (0.31)	N=17 816.12 (620.59) 701.77 (10.64 – 2560.60)	N=17 374.31 (358.69) 226.84 (67.38 – 1227.22)	-441.81 (703.33)	0.02
11β-Prostaglandin F2α	N=31 943.48 (864.94) 707.76 (29.93 – 3380.24)	N=31 454.92 (415.03) 249.32 (0.01 – 1363.05)	-488.56 (943.07)	0.03	N=13 853.87 (760.52) 674.56 (45.24 – 2500.24)	N=13 515.68 (431.76) 449.03 (11.28 – 1363.05)	-338.19 (772.03)	NS (0.38)	N=17 1057.69 (954.38) 767.18 (29.93 – 3380.24)	N=17 376.07 (393.20) 167.40 (0.01 – 1276.7)	-681.62 (1003.91)	0.03

¹SD: Standard deviation, ^aFEV1: Forced expiratory volume in one second, ^bMEF50: Maximal expiratory flow at 50%, ^cMEF75: Maximal expiratory flow at 75%, ^dTotal IgE: total immunoglobulin E, ^eECP: Eosinophil cationic protein, TPS: Total Polyp Score, UPSIT: University of Pennsylvania Smell Identification Test, SNOT: Sino-Nasal Outcome Test, TNSS: Total Nasal Symptom Score, ACT: Asthma Control Test

Table E2. Cytokine data, showing mean, standard deviation and *p* values for total patients and split into Aspirin-intolerant and Aspirin-tolerant groups at baseline and 24 weeks.

	Total (N=31)			Aspirin-intolerant (N=13)			Aspirin-tolerant (N=17)		
	Mean (±SD)			Mean (±SD)			Mean (±SD)		
	Baseline	Week 24	<i>p</i> value	Baseline	Week 24	<i>p</i> value	Baseline	Week 24	<i>p</i> value
Eotaxin-1	604.65 (326.57)	339.29 (265.47)	p<0.001	646.03 (390.41)	366.37 (321.39)	NS (0.11)	591.55 (279.06)	332.1 (225.21)	0.001
Eotaxin-3	3161.94 (6944.9)	150.49 (107.42)	p<0.001	2528.66 (2405.85)	142.12 (87.55)	p<0.001	3680.26 (9244.51)	165.56 (119.28)	p<0.001
G-CSF	5083.04 (8733.07)	2615.67 (3521.18)	NS	6117.03 (11401.31)	2793.56 (4298.86)	NS	4568.97 (6555.58)	2618.82 (3001.96)	NS
GM-CSF	9.33 (7.63)	7.99 (4.48)	NS	11.52 (10.06)	8.06 (4.26)	NS	7.92 (5.08)	8.15 (4.8)	NS
IFN-γ	177.44 (671.33)	186.05 (540.82)	NS	66.29 (56.38)	48.27 (45.05)	NS	271.53 (906.49)	301.03 (718.16)	NS
IL-10	7.38 (7.72)	5.66 (5.86)	NS	10.2 (10.51)	4.68 (2.41)	NS	5.61 (4.05)	6.65 (7.55)	NS
IL-12p40	49.02 (47.34)	29.28 (17.47)	0.03	54.39 (48.26)	29.99 (19.55)	NS (0.13)	46.5 (48.79)	29.79 (16.27)	NS (0.21)
IL-12p70	5.01 (3.56)	5.06 (6.07)	NS	4.73 (3.34)	4.12 (4.68)	NS	5.48 (3.72)	5.9 (7.15)	NS
IL-13	43.53 (17.89)	33.8 (14.55)	0.03	41.69 (15.49)	31.9 (11.62)	NS (0.15)	45.71 (20.04)	36.12 (16.54)	NS (0.13)

IL-15	25.73 (17.26)	22.79 (13.95)	NS	26.54 (18.87)	21.86 (13.45)	NS	25.96 (16.68)	24.41 (14.52)	NS
IL-16	8274.43 (9458.6)	9236.96 (10314.08)	NS	10955.0 (12465.82)	8780.29 (8400.93)	NS	6590.28 (6319.19)	10050.08 (11906.81)	NS
IL-17A	65.33 (86.92)	33.17 (26.27)	0.04	100.13 (120.38)	35.07 (26.21)	NS (0.07)	41.85 (39.37)	33.12 (27.2)	NS (0.35)
IL-17E/IL-25	13.19 (6.56)	9.55 (7.37)	0.03	12.17 (7.2)	12.22 (6.51)	NS (1.0)	14.74 (5.26)	8.02 (7.55)	0.0044
IL-1RA	29066.16 (7360.98)	27722.82 (5734.71)	NS (0.06)	29964.26 (5844.54)	26854.85 (4456.54)	0.03	28531.05 (8637.19)	28436.05 (6750.6)	NS (0.61)
IL-1α	675.53 (1517.35)	560.84 (660.76)	NS	991.38 (2291.12)	644.0 (822.42)	NS	458.48 (487.91)	518.99 (543.44)	NS
IL-1β	2573.64 (7255.65)	4602.0 (20721.42)	NS	4892.74 (10873.38)	1057.76 (1100.06)	NS	947.77 (1569.66)	7575.68 (27987.85)	NS
IL-2	358.61 (339.38)	411.98 (375.15)	NS	447.83 (400.83)	461.77 (356.93)	NS	304.47 (286.87)	396.18 (396.34)	NS
IL-21	39.15 (36.89)	26.0 (14.69)	NS	53.03 (47.81)	25.68 (14.83)	NS	29.84 (23.61)	25.23 (15.16)	NS
IL-22	12.73 (55.95)	1.47 (0.81)	0.05	4.1 (4.71)	1.63 (1.08)	NS (0.14)	20.03 (75.68)	1.36 (0.56)	NS (0.06)
IL-27	33.46 (25.25)	26.3 (14.42)	NS	37.1 (23.62)	29.79 (14.46)	NS	31.93 (27.11)	24.16 (14.61)	NS
IL-3	117.39 (53.29)	119.38 (76.64)	NS (0.9)	97.96 (57.33)	99.59 (59.49)	NS (0.86)	128.8 (46.89)	131.19 (87.71)	NS (0.92)

IL-33	350.17 (728.43)	108.96 172.71	NS	625.84 (1046.7)	101.96 (111.13)	NS	110.6 (188.5)	102.3 (209.71)	NS
IL-4	9.35 (12.15)	11.9 (10.63)	NS	9.09 (8.82)	9.42 (6.62)	NS	10.02 (14.62)	14.43 (12.65)	NS
IL-5	736.3 (1667.52)	190.9 (210.62)	0.05	1437.03 (2437.47)	179.47 (136.9)	0.02	235.44 (264.76)	210.71 (257.67)	NS (0.85)
IL-6	499.03 (1130.93)	121.9 (195.71)	0.05	993.73 (1644.38)	157.71 (287.48)	0.04	146.96 (164.77)	100.56 (87.46)	NS (0.68)
IL-7	214.9 (136.12)	164.11 (109.8)	NS	211.96 (161.03)	164.54 (110.86)	NS	220.35 (122.75)	170.72 (111.69)	NS
IL-8	11159.01 (5785.64)	11610.46 (4198.59)	NS	9841.34 (4933.97)	11612.85 (4131.66)	NS	12291.37 (6438.25)	12024.78 (4115.77)	NS
IL-9	11.84 (12.94)	14.5 (16.44)	NS	14.82 (13.83)	16.06 (19.39)	NS	10.24 (12.34)	14.12 (14.53)	NS
CCL 17	205.06 (158.28)	98.6 (90.45)	0.001	235.67 (191.95)	118.2 (119.81)	NS (0.07)	185.21 (133.92)	87.76 (61.86)	0.01
TNF-α	20.05 (25.69)	12.65 (20.2)	0.02	26.0 (35.93)	9.43 (5.85)	NS (0.07)	16.5 (14.45)	15.47 (26.84)	NS (0.15)
TNF-β	3.26 (2.74)	2.5 (1.2)	NS	3.9 (3.62)	2.44 (0.94)	NS	2.86 (1.91)	2.64 (1.37)	NS
TSLP	42.89 (56.49)	19.93 (18.18)	0.03	55.47 (76.4)	23.66 (26.12)	NS (0.15)	31.71 (36.06)	17.81 (9.1)	NS (0.19)
VEGF	9943.77 (6885.19)	10994.2 (10197.55)	NS	9749.54 (6531.94)	11071.91 (6644.52)	NS	10114.36 (7537.63)	11416.28 (12549.39)	NS

Table E3. Missing values in clinical questionnaires.

Questionnaire (values assessed)	Missing values before imputation (n; %)	Imputed values (n; %)	Missing values after imputation (n; %)
ACT total (620)	61; 9.84%	2; 0.32%	59; 9.52%
V1 (155)	9; 5.81%	0; 0%	9; 5.81%
V2 (155)	21; 13.55%	1; 0.65%	20; 12.90%
V3 (155)	11; 7.10%	1; 0.65%	10; 6.45%
V4 (155)	20; 12.90%	0; 0%	20; 12.90%
SNOT total (2728)	106; 3.89%	32; 1.17%	74; 2.71%
V1 (682)	35; 5.13%	13; 1.91%	22; 3.23%
V2 (682)	37; 5.43%	7; 1.03%	30; 4.40%
V3 (682)	27; 3.96%	5; 0.73%	22; 3.23%
V4 (682)	7; 1.03%	7; 1.03%	0; 0%
TNSS total (496)	14; 2.82%	1; 0.2%	13; 2.62%
V1 (124)	4; 3.22%	0; 0%	4; 3.22%
V2 (124)	4; 3.22%	0; 0%	4; 3.22%
V3 (124)	5; 4.03%	0; 0%	5; 4.03%
V4 (124)	1; 0.81%	1; 0.81%	0; 0%

ACT: Excluded data from 12 visits in analysis, as referred to by the number of patients in each visit under the line plot (Figure 2E).

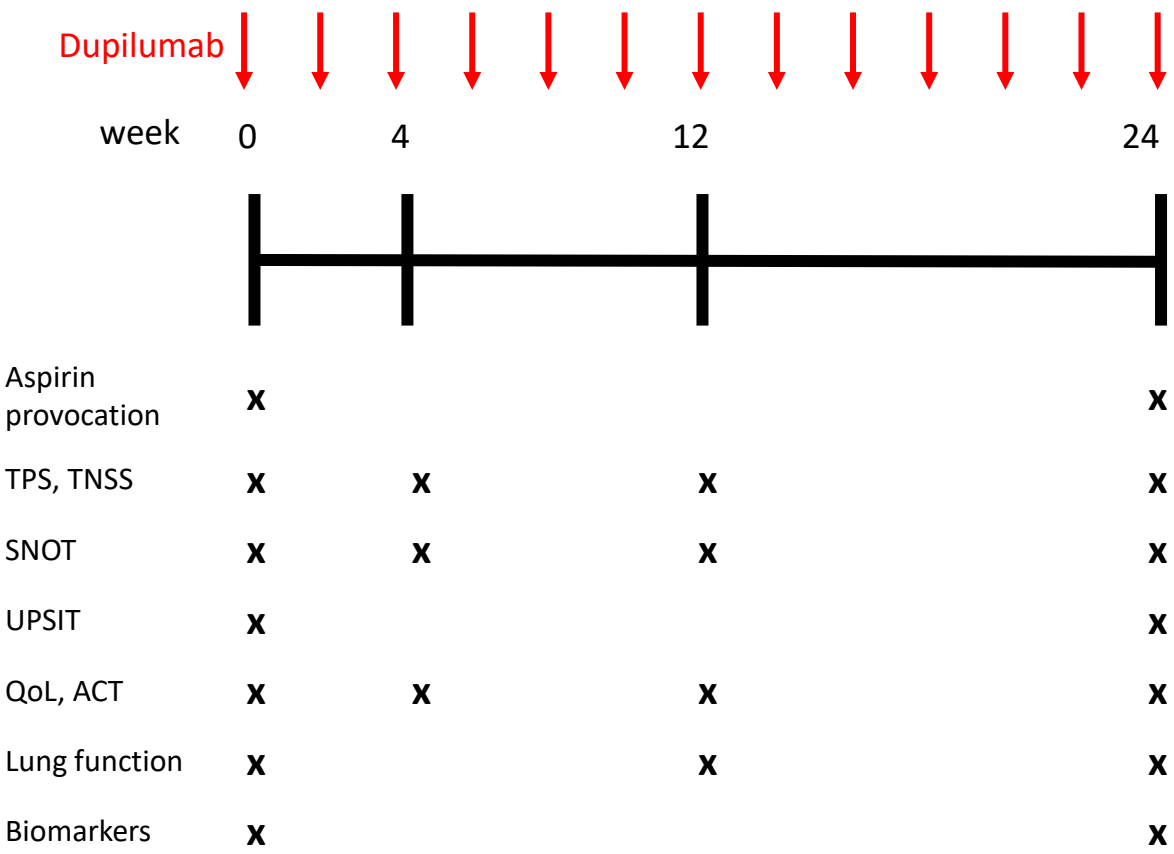
Values were imputed if questionnaire was >75% complete using a rationale and considering previous and following visits (last observation carried forward or mean of two visits).

SNOT: Excluded data from 4 visits in analysis, as referred to by the number of patients in each visit under the line plot (Figure 2C).

Values were imputed if questionnaire was >80% complete using a rationale and considering previous and following visits (last observation carried forward or mean of two visits).

TNSS: Excluded data from 3 visits. One value was imputed based on questionnaire >75% complete and using last observation carried forward.

A



B

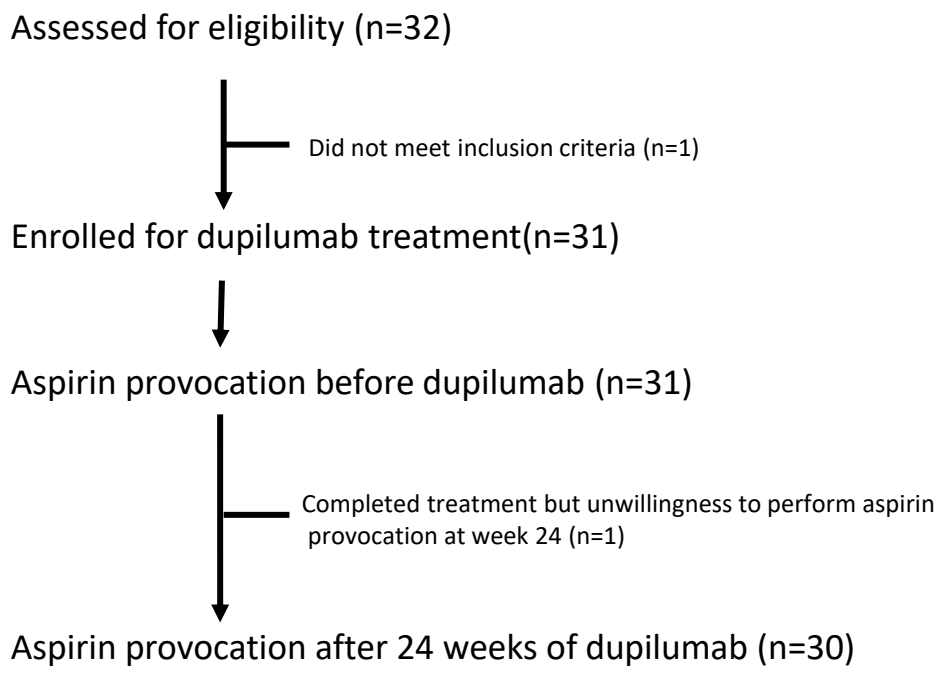


Figure E1

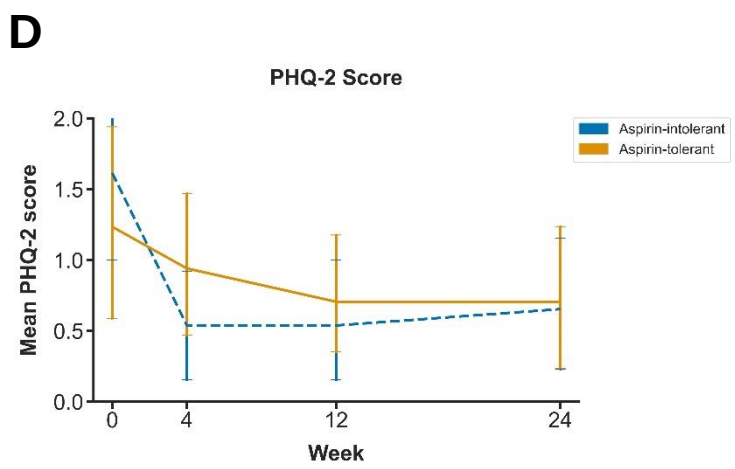
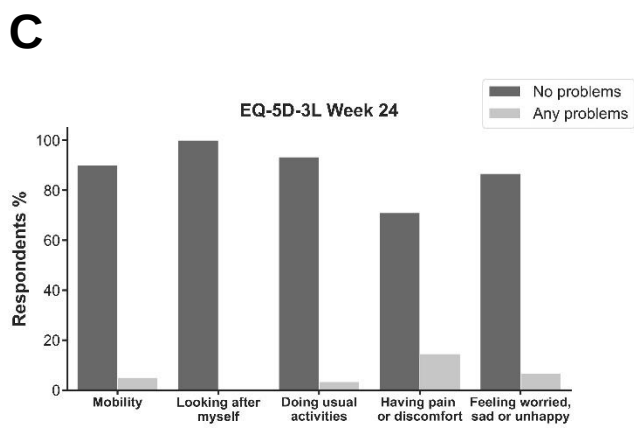
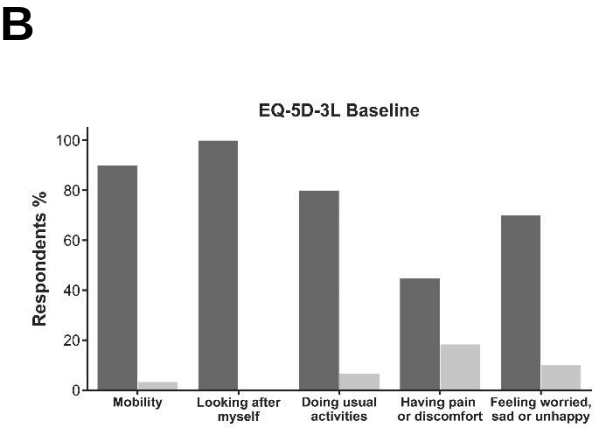
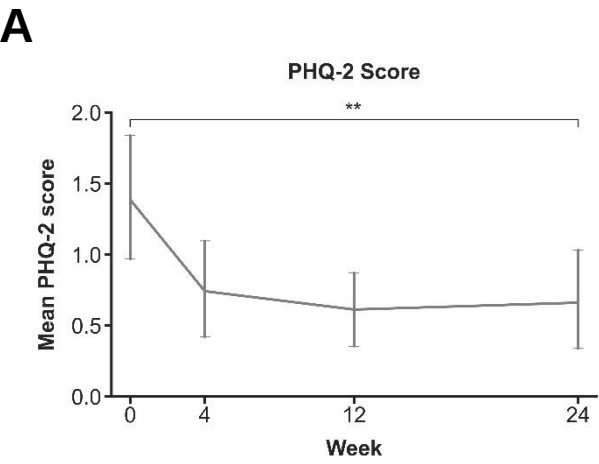


Figure E2

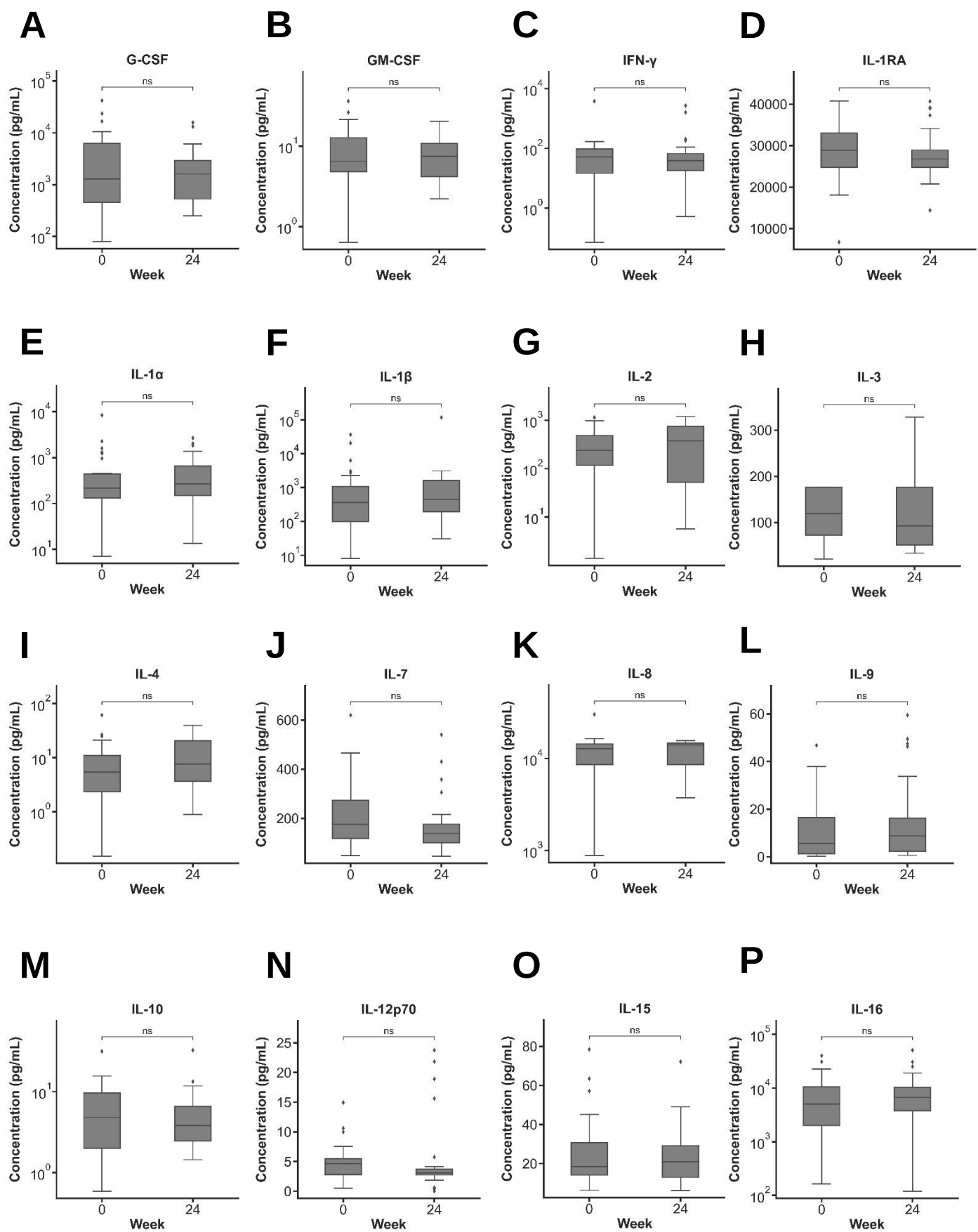


Figure E3

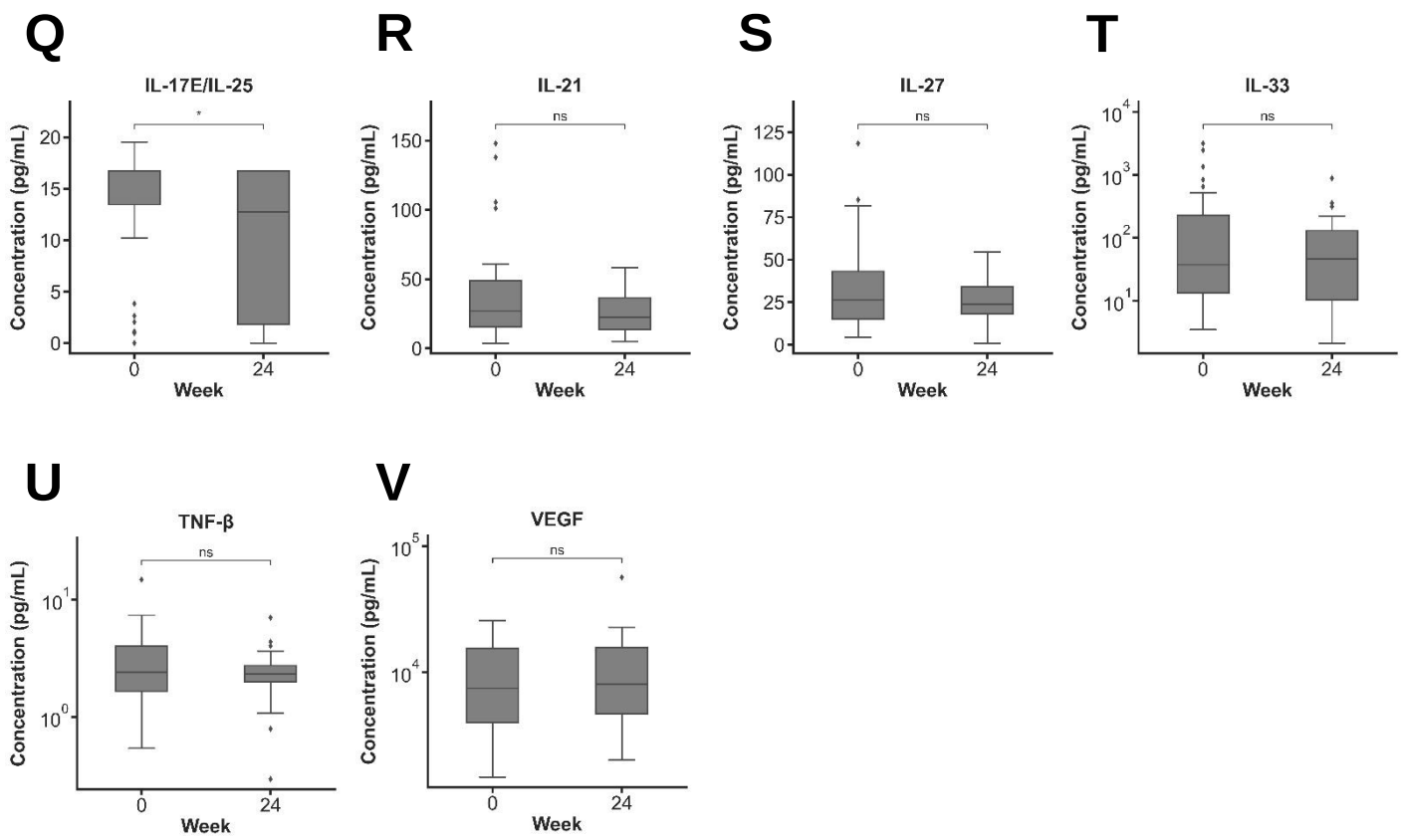


Figure E3

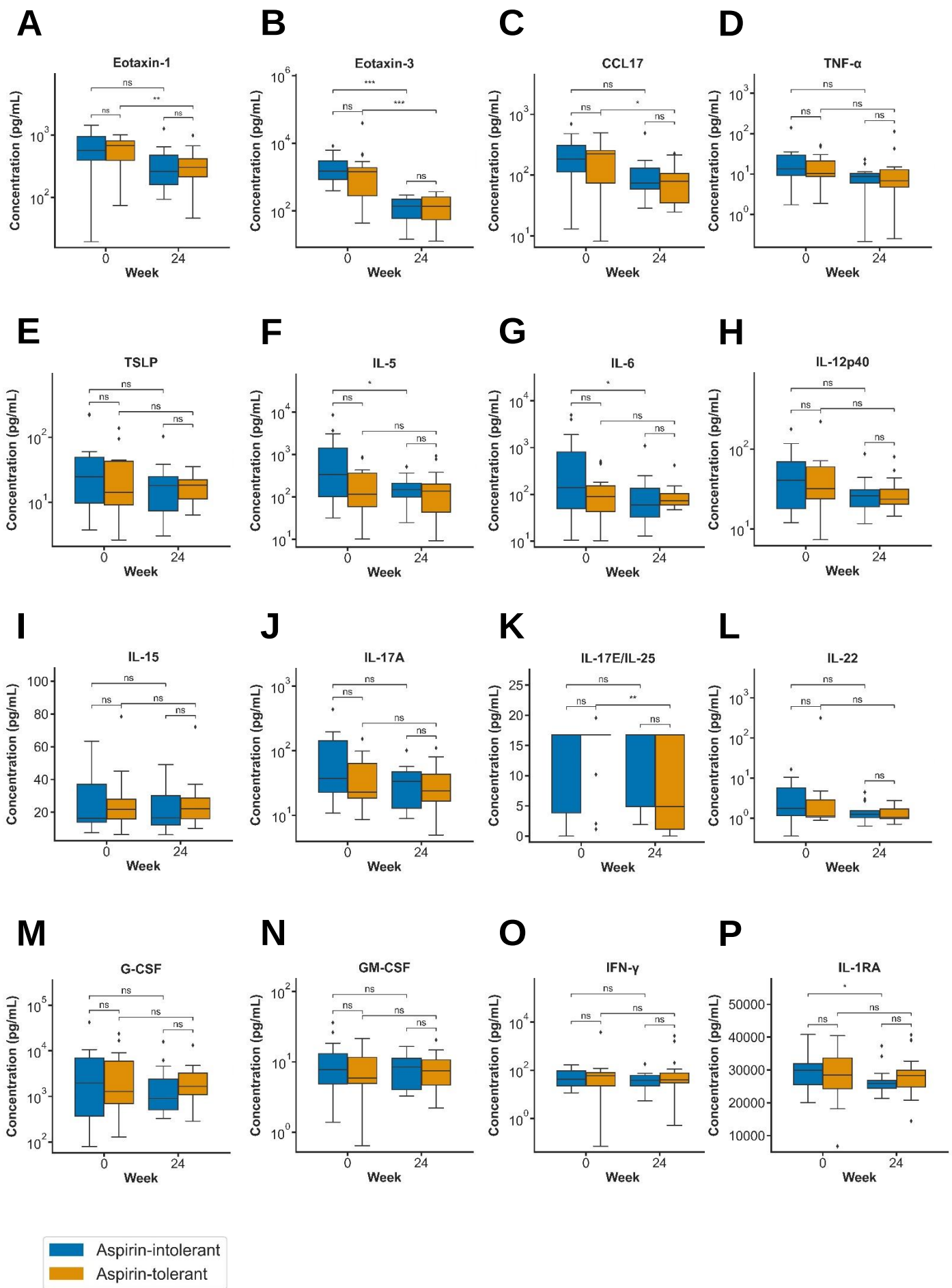


Figure E4

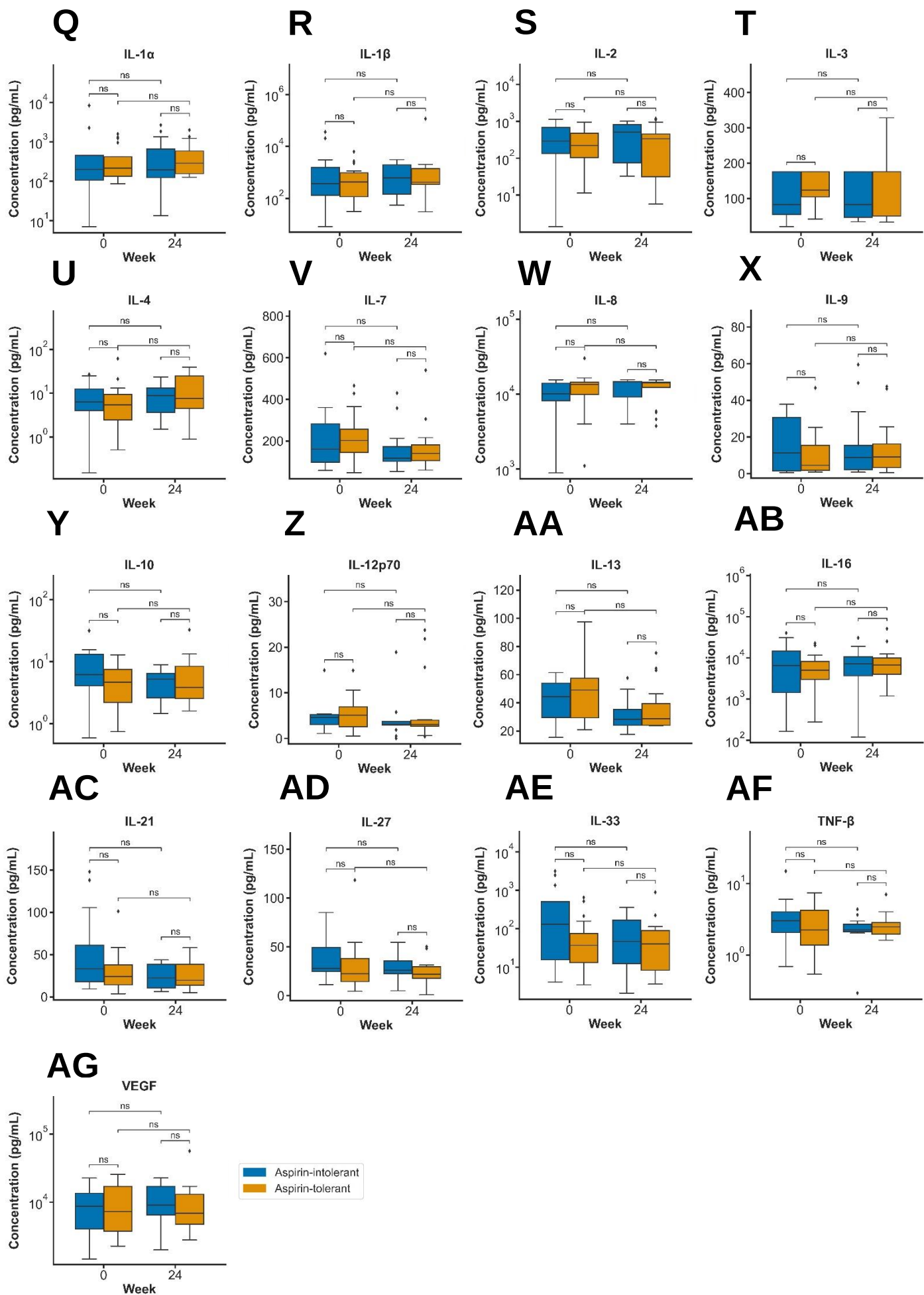


Figure E4

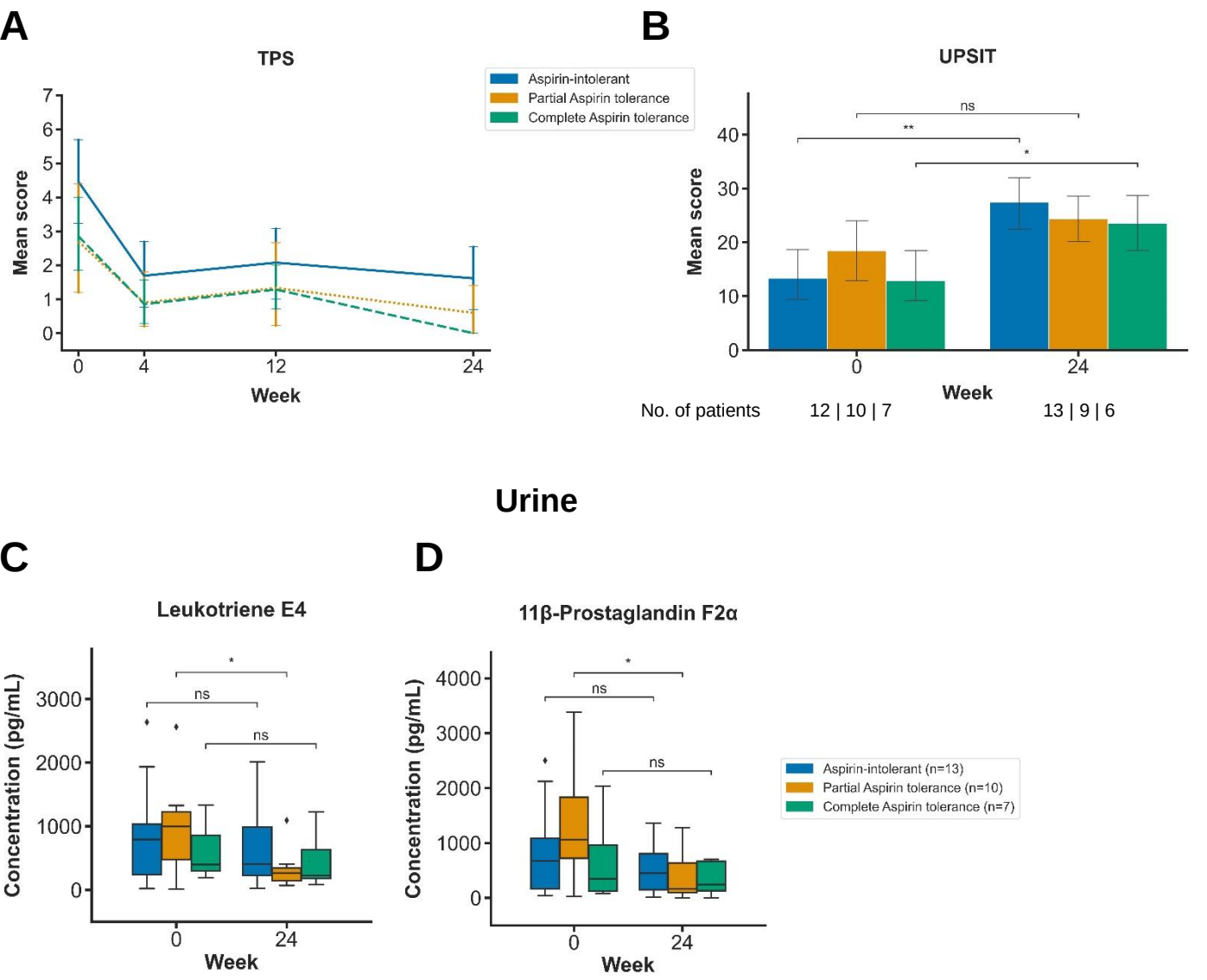


Figure E5

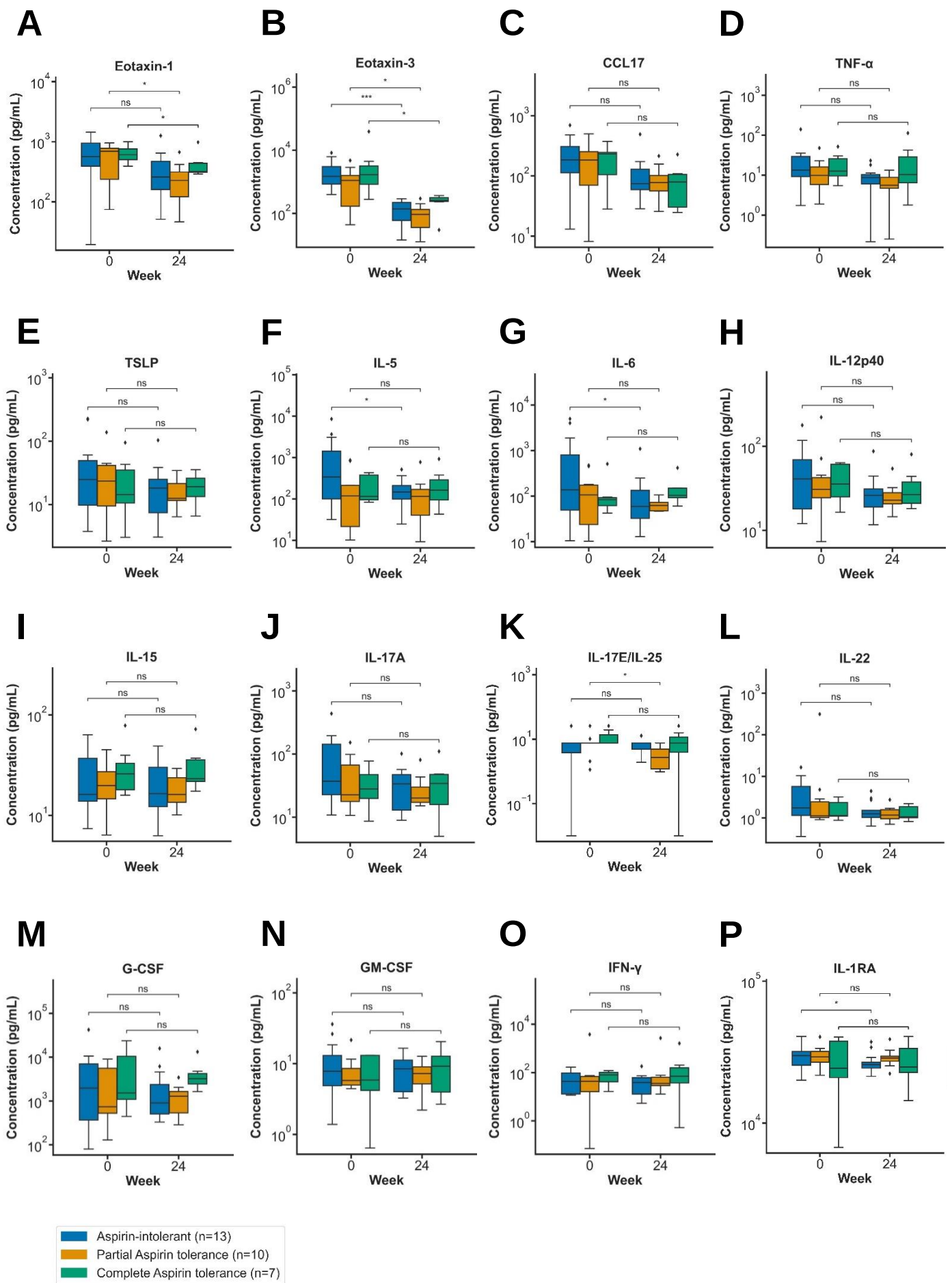


Figure E6

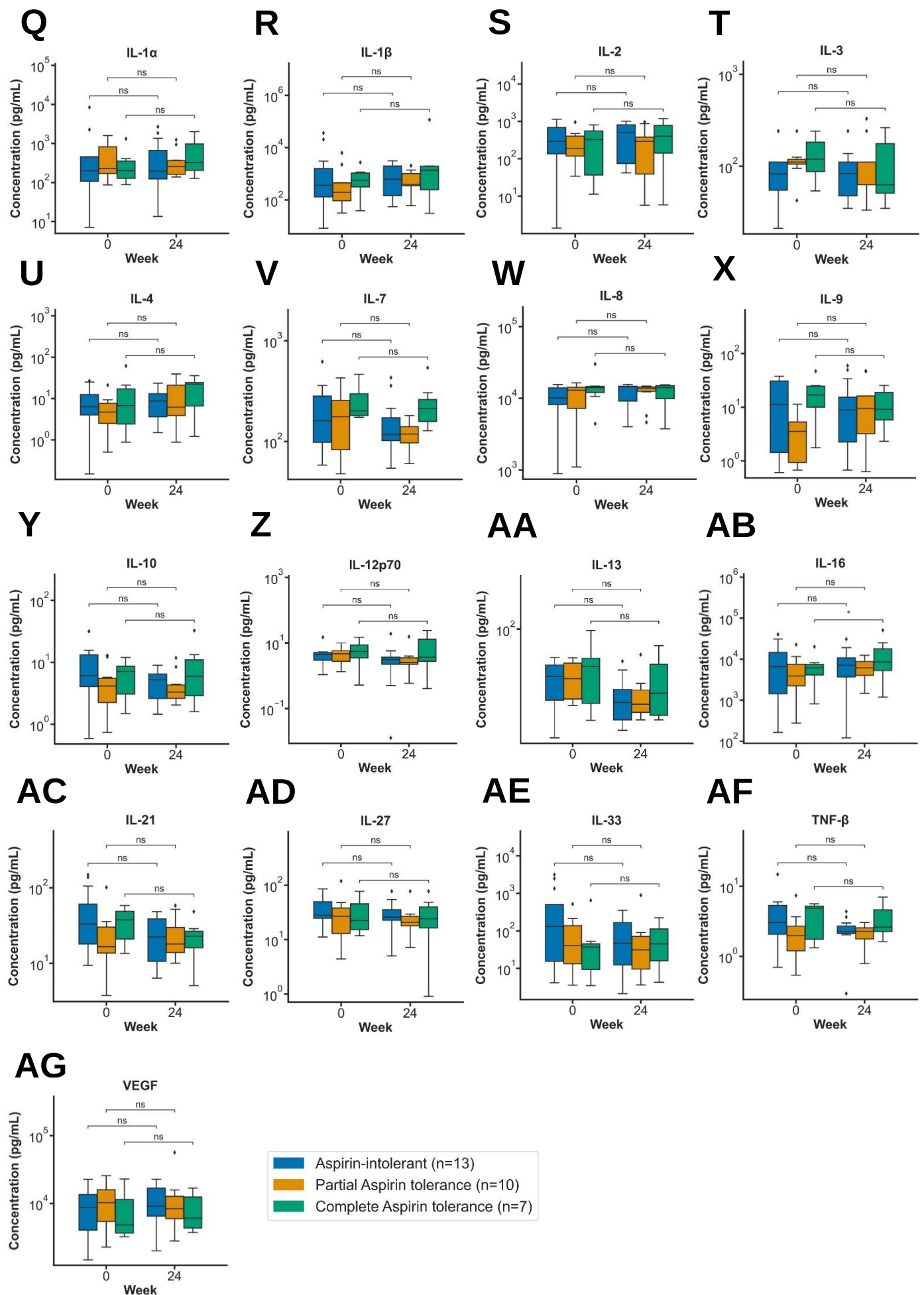


Figure E6