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Effects of anti-inflammatory drugs on distinct endotypes of chronic rhinosinusitis without nasal polyps: comparison using an *ex-vivo* model

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Abstract. – OBJECTIVE: The objectives of this study were to characterize the endotypes of different chronic rhinosinusitis without nasal polyps (CRSsNP) samples, to investigate the effects of certain anti-inflammatory drugs on these endotypes, and to investigate the effect of the same drugs on recently identified CRSsNP marker proteins.

PATIENTS AND METHODS: Initially, ethmoid tissues (ETs) from CRSsNP patients (n=12) were dissected into sections and incubated with the addition of mometasone, verapamil, cenicriviroc, and dupilumab. Cell culture media were collected after 24 hours, and the contents of the secreted proteins interferon-gamma (IFN-γ), interleukin (IL)-5, IL-17A, macrophage inflammatory protein-1 beta (MIP-1β), resistin and platelet (P)-selectin were measured using enzyme-linked immunosorbent assay (ELISA). The endotypes were characterized using the unstimulated samples. The fold changes of protein secretion caused by the analyzed substances were calculated. For each protein, the samples of the distinct endotypes were compared with the remaining samples.

RESULTS: Both single and mixed endotypes were identified within the CRSsNP samples, whereas none of the typical endotype-defining cytokines were elevated in a significant portion of the samples. All of the incubated medicaments greatly reduced the tissue secretions of IFN-γ and IL-5 in type 1 CRS while causing a lower secretion of IL-17A in all endotypes compared to the remaining samples. Among the analyzed CRSsNP marker proteins, the distinct endotypes revealed different reactions to the drugs. Dupilumab induced more effects among the examined cytokines than the marker proteins but did not stand out from the other substances overall.

CONCLUSIONS: Medications used to treat CRS may have different effects on distinct CRS endotypes.

Key Words:
Chronic rhinosinusitis, Endoscopic sinus surgery, Nasal polyps, Sinusitis.

Introduction

CRS is divided into the phenotypes CRS with nasal polyps (CRSwNP) and without nasal polyps (CRSsNP). The three underlying endotypes, type 1, type 2, and type 3 CRS, are characterized by different pathophysiological features[1]. CRS represents a high burden for patients whose medical therapy is often based on symptoms[2,3]. Consequently, many patients do not improve with medical therapy and ultimately require surgery[4-6]. These treatment failures cause immeasurable expenses every year and should, therefore, be prevented[1]. While the pathophysiology of CRSwNP is already well elucidated and many innovative treatment options exist, including the application of antibodies that specifically target CRS-associated antigens[1,7,8], research on CRSsNP is not as advanced[1].

Mometasone (MOM), verapamil (VRP), cenicriviroc (CVC), and dupilumab (DUP) are medical substances that exert their effects by different mechanisms and have anti-inflammatory properties[9-12]. While MOM is often prescribed for CRSsNP patients[13], CRSwNP patients are frequently treated by DUP[14]. VRP was also proven to be effective in this group[9,15]. CVC has not been considered for CRS treatment so far[11].

Interferon-gamma (IFN-γ), interleukin-5 (IL-5), and IL-17A are characteristic cytokines that belong to distinct Thymus-helper (Th) cell responses and participate in the pathophysiology of the different CRS endotypes[1,16]. Since there are no suitable biomarkers for CRSsNP so far, a study[17] was recently carried out in order to find such biomarkers. In this study[17], MIP-1β and resistin were found to be overexpressed, and P-selectin was found to be downregulated in CRSsNP mucus. MIP-1β and resistin are proinflammatory proteins expressed by different immune cells and the epi-

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thelium[18-20]. P-selectin is a cell adhesion molecule mainly expressed by activated platelets and endothelial cells. It plays an important role in immunity and platelet aggregation[21].

Previous research[22-24] on various diseases has already demonstrated the great importance of the endotype for therapy response. However, for CRS there is only little knowledge with regard to this phenomenon.

Therefore, the objectives of this study were to characterize the endotypes of different CRSsNP samples, to investigate the effects of certain anti-inflammatory drugs on these endotypes, and to investigate the effect of the same drugs on recently identified CRSsNP marker proteins.

Patients and Methods

Patients

This study was approved by the institutional review board of the University of Erlangen-Nürnberg (No.: 17-269_1-B), and all patients provided written informed consent. The study adheres to the Helsinki Declaration. The diagnosis of CRSsNP was based on clinical diagnostic standards from the International Consensus Statement on Allergy and Rhinology[2]. All patients underwent surgery for CRS [functional endoscopic sinus surgery (FESS), septoplasty, and turbinate reduction]. Exclusion criteria included ciliary dysfunction, autoimmune disease, cystic fibrosis, immunodeficiency, malignancies, gastroesophageal reflux disease, chronic rheumatic disease, or any other disease requiring long-term corticosteroid therapy. Figure 1 shows a flow chart of the patients who were used for this study.

Incubation of Nasal Tissues in Cell Culture Medium Under Addition of Different Drugs

Ethmoid tissue (ET) from CRSsNP patients (n=12) served as tissue samples. All tissues were used immediately after removal from the patient. For each experiment, the tissue was cut into six pieces with a visually similar size, which were weighed using the KERN 770/GS/GJ precision scale (Kern & Sohn GmbH, Balingen, Germany) and then washed briefly in EpiLife™ calcium-free, phenol red-free (CF/PRF) medium (Thermo Fisher Scientific, Bonn, Germany). As indicated in Table I, incubation media were freshly prepared before each experiment by adding different drugs to EpiLife™ CF/PRF medium. All selected medicaments had revealed effects in cell culture experiments for their respective comparable final concentrations in previous studies[25-28]. One piece of tissue was placed in one well of a CELLSTAR® cell culture plate (Carl Roth, Karlsruhe, Germany) with 1 mL of each incubation medium. Two negative controls were carried out per experiment. One consisted of pure EpiLife™ CF/PRF medium as a reference for DUP and VRP, whose solutions were prepared without using dimethyl sulfoxide (DMSO), while the other one consisted of EpiLife™ CF/PRF medium with 0.5% DMSO. This served as a reference for MOM and CVC, whose solutions were prepared by using DMSO since DMSO itself is known to affect cellular processes[29]. The tissue pieces were incubated at 37°C and a carbon dioxide (CO_2) level of 5% in the Heraeus/Kendro HERAcell Incubator (Thermo Fisher Scientific, Bonn, Germany) on a laboratory shaker (Expondo GmbH, Berlin, Germany). The media were collected after 24 hours and stored at -80°C (Figure 1).

Analysis of IFN-γ, IL-5, IL-17A, MIP-1β, Resistin, and P-selectin Protein in Cell Culture Media by ELISA

ELISA tests for IFN-γ, IL-5, IL-17A, MIP-1β, resistin, and P-selectin were conducted using the media samples of the CRSsNP ETs (n=12). All ELISAs were performed according to the manufacturer´s protocols.

For IFN-γ, the media were diluted 1:2 with Assay Dilution Buffer (included in the kit), and the

Table I. Preparation scheme of incubation solutions.

Substance	Manufacturer	Final concentration
Mometasone furoate	Cayman Chemical, Michigan, USA	30 μM
Verapamil (hydrochloride)	Cayman Chemical, Michigan, USA	93 μM
Cenicriviroc	Cayman Chemical, Michigan, USA	21.5 μM
Dupilumab (from Dupixent® 300 mg Injection Pen)	Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany	5 μM

μM, micromolar.

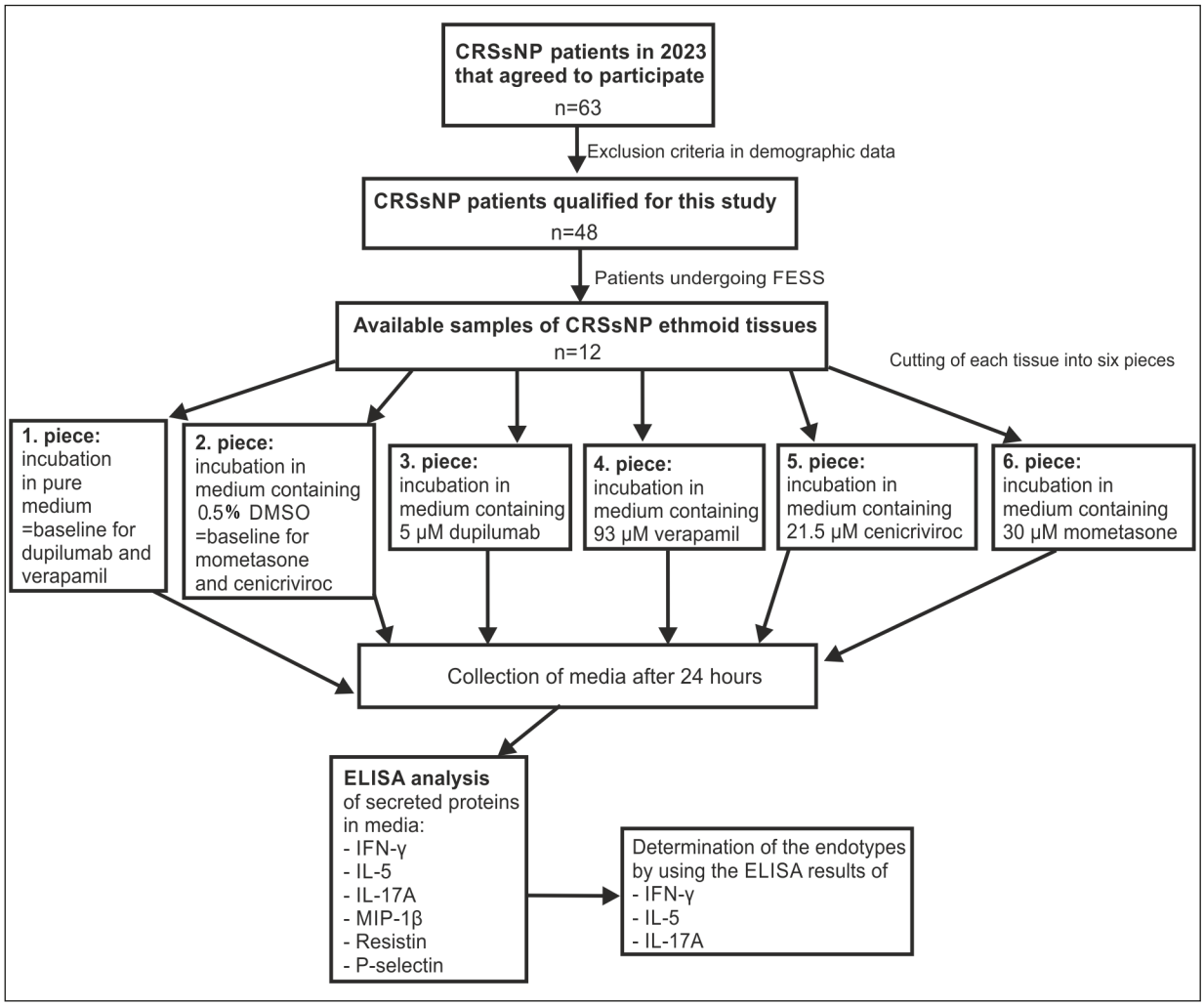

Figure 1. Flow chart of the CRSsNP patients and the analysis scheme used for this study. CRSsNP, chronic rhinosinusitis without nasal polyps; FESS, functional endoscopic sinus surgery; IFN-γ, interferon-gamma; IL, interleukin, MIP-1β, macrophage inflammatory protein-1 beta; P-selectin, platelet-selectin; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; µM, micromolar.

IFN gamma Human ProQuantum Immunoassay Kit No. A35576 (Thermo Fisher Scientific, Bonn, Germany; assay range 0.0128-5,000 pg/mL) was used. For IL-5, the media were diluted 1:2 with Assay Dilution Buffer (included in the kit), and the IL-5 Human ProQuantum Immunoassay Kit No. A35588 (Thermo Fisher Scientific, Bonn, Germany; assay range 0.0128-5,000 pg/mL) was used. For IL-17A, the media were diluted 1:2 with Assay Dilution Buffer (included in the kit), and the Human IL-17A ProQuantum Immunoassay Kit No. A35611 (Thermo Fisher Scientific, Bonn, Germany; assay range 0.32-25,000 pg/mL) was used. For MIP-1β, the media were diluted 1:2 with Assay Dilution Buffer (included in the kit), and the MIP-1 beta Human ProQuantum Immunoassay Kit No. A35597 (Thermo Fisher Scientific, Bonn, Germany; assay range 0.064-5,000 pg/mL) was used. For resistin, the media were diluted 1:2 with Reagent Diluent (R&D Systems, Minneapolis, MN, USA), and the Human Resistin DuoSet ELISA No. DY1359 (R&D Systems, Minneapolis, USA; assay range 31.2-2,000 pg/mL) was used. For P-selectin, the media were diluted 1:2 with Reagent Diluent (R&D Systems, Minneapolis, MN, USA), and the Human P-Selectin/CD62P DuoSet ELISA No. DY137 (R&D Systems, Minneapolis, MN, USA; assay range 125.0-8,000 pg/mL) was used. For the evaluation of the results of IFN-γ, IL-5, IL-17A, and MIP-1β, the ProQuan-

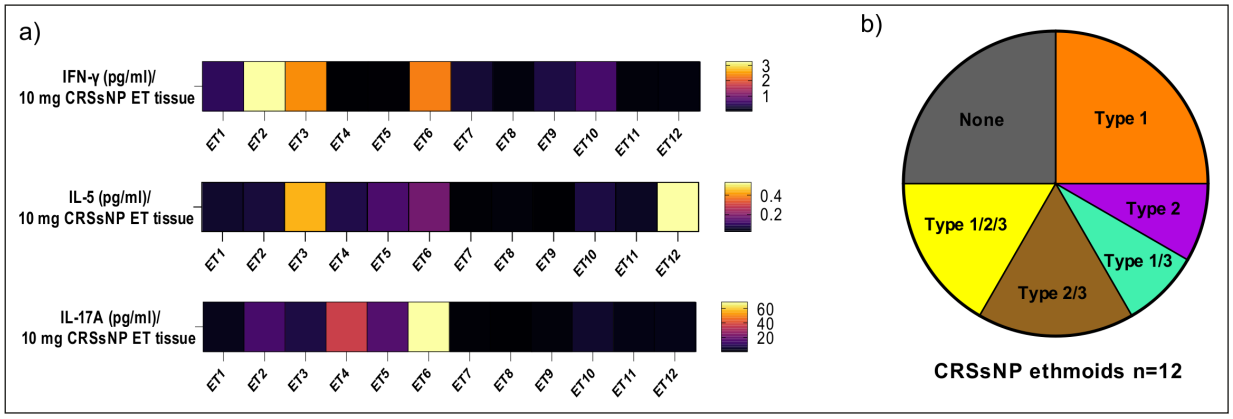

Figure 2. Heat map (**a**) of IFN-γ, IL-5, and IL-17A values in media of unstimulated pieces of CRSsNP ETs normalized to 10 mg tissue. Pie chart (**b**) of identified CRSsNP endotypes. CRSsNP, chronic rhinosinusitis without nasal polyps; ET, ethmoid; IFN-γ, interferon-gamma; IL, interleukin; pg, picogram; mg, milligram; mL, milliliter.

tum tool (Thermo Fisher Scientific, Bonn, Germany) was used. All results were calculated by generating standard curves. All values were subsequently normalized to a tissue weight of 10 mg. In order to obtain the fold changes for DUP and VRP, the values of the corresponding media were divided by the values of the pure media in which the control tissue pieces had previously been incubated. In order to obtain the fold changes for MOM and CVC, the values of the corresponding media were divided by the values of the pure media containing DMSO in which the control tissue pieces had previously been incubated.

Determination of the CRS Endotypes

Endotypes were defined by using the cell culture media values of IFN-γ, IL-5, and IL-17A of all unstimulated CRSsNP ETs (n=12) that had been previously incubated in pure cell culture media. Minimum values were defined, above which the samples were assigned to the distinct endotypes. The samples whose IFN-γ media content was >0.35 (pg/mL)/10 mg tissue were assigned to type 1 CRS, the samples whose IL-5 media content was >0.08 (pg/mL)/10 mg tissue were assigned to type 2 CRS, and the samples whose IL-17A media content was >8.0 (pg/mL)/10 mg tissue were assigned to type 3 CRS.

Statistical Analysis

All results were calculated using GraphPad Prism 9.2.0; (GraphPad Software, La Jolla, CA, USA). A false discovery rate cut-off of $p<0.05$ was used to detect significant differences. For groups displaying statistically significant differences, the fold changes between the groups were calculated by dividing the mean values. For the results of demographic data, age was compared by using the Mann-Whitney U test, while for sex, ethnicities, and comorbidity the Chi-square test was used. For the results of ELISA in cell culture media, the Mann-Whitney U test was used.

Results

Determination of the CRS Endotypes

For CRS endotype determination, the cell culture media values of the cytokines IFN-γ, IL-5, and IL-17A were determined using the unstimulated CRSsNP ETs (n=12) that had been previously incubated in pure cell culture media.

The heat map in Figure 2a shows the values of IFN-γ, IL-5, and IL-17A in media of the unstimulated CRSsNP ET pieces normalized to 10 mg tissue.

The pie chart in Figure 2b shows the distribution of the endotypes. The results revealed that the patients can be assigned to different single (type 1 CRS n=3, type 2 CRS n=1, type 3 CRS n=0) and mixed (type 1/3 CRS n=1, type 2/3 CRS n=2 and type 1/2/3 CRS n=2) endotypes. For n=3 patients, none of the analyzed cytokines were elevated.

Demographics

Table II shows demographic data. There were no significant differences in age, sex, ethnicity, or comorbidity between the distinct CRS endotypes and the remaining samples.

Quantification of IFN-γ, IL-5, and IL-17A Protein in Cell Culture Media Using ELISA and Comparison of Drug Effects on Distinct Endotypes

ELISA tests were performed to evaluate the protein content of the endotype-defining cytokines IFN-γ, IL-5, and IL-17A[1,16] in media samples of the CRSsNP ETs (n=12) after stimulation by the drugs MOM, VRP, CVC, and DUP.

Figure 3 shows the results of the secreted cytokines in the cell culture media of all samples that were assigned to a distinct endotype (pure and mixed endotypes) compared to the remaining samples after the tissues had been incubated for 24 hours.

Mometasone

Compared to the other samples, IL-5 was significantly downregulated in type 1 CRS (FC be-

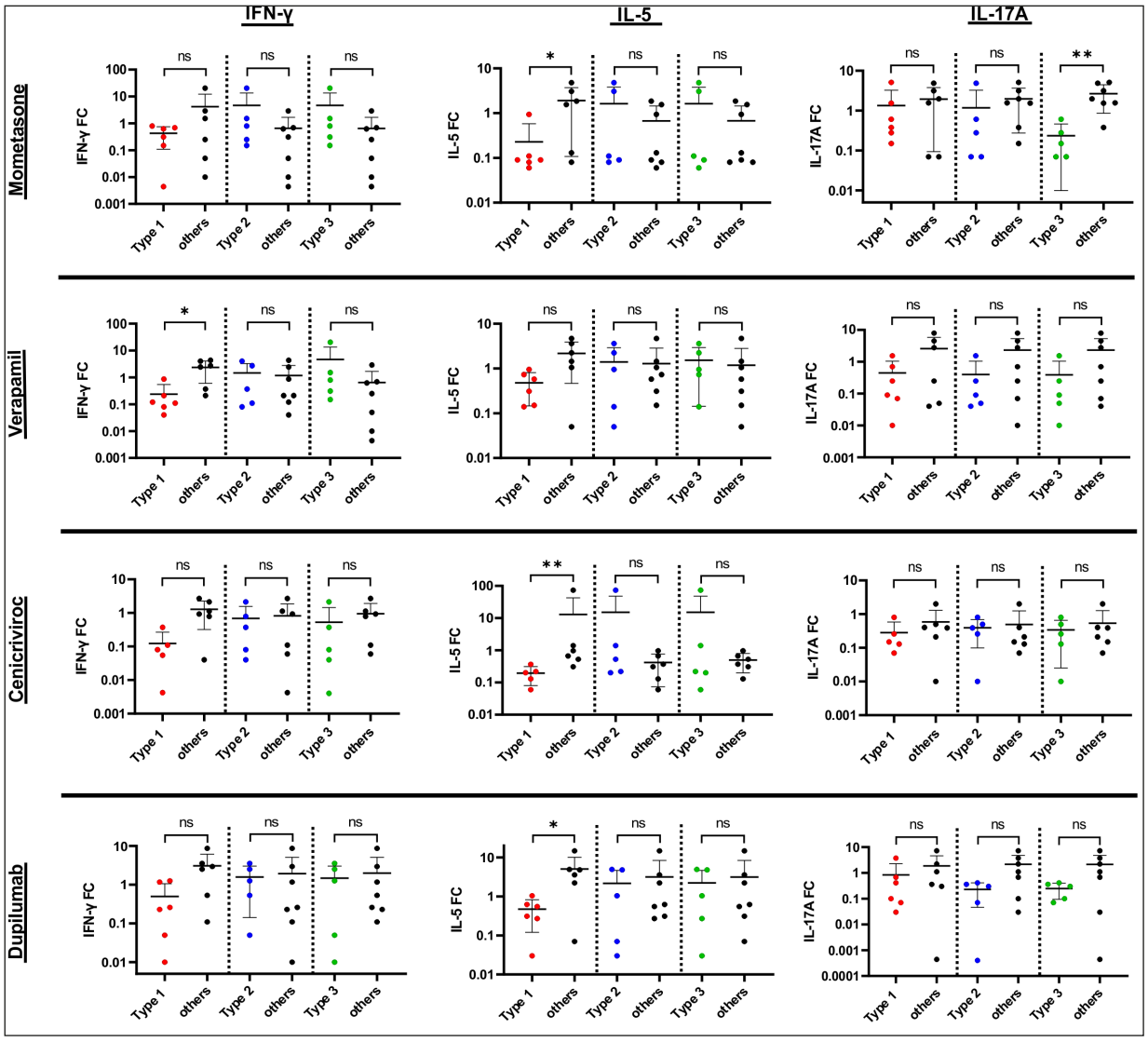

Figure 3. Scatter dot plots, including mean and SD error bars, show the results of the quantitative analysis of IFN-γ, IL5, and IL-17A by ELISA in the cell culture media. Due to the logarithmical plotting of the y-axes, some error bars are not displayed in this representation. In each panel, the type 1 CRS samples (red), the type 2 CRS samples (blue), and the type 3 CRS samples (green) are plotted against the remaining samples (black). For mometasone, statistically significant differences can be seen for both the effects on IL-5 in type 1 CRS ($p<0.05$) and on IL-17 in type 3 CRS ($p<0.01$). For verapamil a statistically significant difference can be seen for the effect on IFN-γ in type 1 CRS ($p<0.05$). For cenicriviroc a statistically significant difference can be seen for the effect on IL-5 in type 1 CRS ($p<0.01$). For dupilumab a statistically significant difference can be seen for the effect on IL-5 in type 1 CRS ($p<0.01$). *<0.05, **<0.01. FC, fold change; IFN-γ, interferon-gamma; IL, interleukin; ns, not significant.

Table II. Demographics of all CRSsNP patients that were used for this study. For each category, the type 1 CRS samples (red), the type 2 CRS samples (blue), and the type 3 CRS (green) samples are compared against the remaining samples.

Characteristics in (%)	CRSsNP patients in total (n=12)		
	CRS Type 1 (n=6) CRS Type 2 (n=5) CRS Type 3 (n=5)	others (n=6) others (n=7) others (n=7)	
Age (years), mean ± SD	41.8±17.9 45.2±15.2 45.2±15.2	30.7±11.9 29.9±13.4 29.9±13.4	ns (p=0.1429) ns (p=0.1780) ns (p=0.1780)
Sex			
Male	3/6 (50.0) 1/5 (20.0) 2/5 (40.0)	1/6 (16.7) 3/7 (42.9) 2/7 (28.6)	ns (p=0.2207) ns (p=0.4076) ns (p=0.4805)
Female	3/6 (50.0) 4/5 (80.0) 3/5 (60.0)	5/6 (83.3) 4/7 (57.1) 5/7 (71.4)	ns (p=0.2207) ns (p=0.4076) ns (p=0.4805)
Ethinicity			
Caucasian	6/6 (100) 5/5 (100) 5/5 (100)	6/6 (100) 7/7 (100) 7/7 (100)	n.a. n.a. n.a.
Comorbidity			
Asthma	0/6 (0) 0/5 (0) 0/5 (0)	0/6 (0) 0/7 (0) 0/7 (0)	n.a. n.a. n.a.
Environmental allergy	2/6 (33.3) 2/5 (40.0) 2/5 (40.0)	1/6 (16.7) 1/7 (14.3) 1/7 (14.3)	ns (p=0.5050) ns (p=0.3105) ns (p=0.3105)
AERD	0/6 (0) 0/5 (0) 0/5 (0)	0/6 (0) 0/7 (0) 0/7 (0)	n.a. n.a. n.a.

CRSsNP, chronic rhinosinusitis without nasal polyps; AERD, aspirin-exacerbated respiratory disease; SD, standard deviation; ns, not significant; n.a., not applicable.

tween the means=0.12, p=0.0476), and IL-17A was significantly downregulated in type 3 CRS (FC between the means=0.09, p=0.0051).

Verapamil

Compared to the other samples, IFN-γ was significantly downregulated in type 1 CRS (FC between the means=0.10, p=0.0108).

Cenicriviroc

Compared to the other samples, IL-5 (FC between the means=0.02, p=0.0087) was significantly downregulated in type 1 CRS.

Dupilumab

While IL-5 was slightly downregulated in type 1 CRS, the non-type 1 CRS samples revealed an upregulation (FC between the means=0.09, p=0.0411).

In general, tissue secretions of IFN-γ and IL-5 were reduced noticeably in type 1 CRS by all tested substances, while type 2 and type 3 CRS showed no or opposite effects. Secretions of IL-17A were generally more strongly reduced in all endotypes compared to the remaining samples, which might be due to the non-endotype samples. (Figure 3).

Quantifications of MIP-1β, resistin and P-selectin Protein in Cell Culture Media Using ELISA and Comparison of Drug Effects on Distinct Endotypes

ELISA tests were performed to evaluate MIP-1β, resistin, and P-selectin protein content in media samples of the CRSsNP ETs (n=12) after stimulation by the drugs MOM, VRP, CVC, and DUP. MIP-1β, resistin and P-selectin had been previously identified as novel CRSsNP marker proteins since

their expression in CRSsNP mucus differed from the controls[17]. Figure 4 shows the results of the secreted proteins MIP-1β, resistin, and P-selectin in cell culture media of all samples that were assigned to a distinct endotype (pure and mixed endotypes) compared to the remaining samples after the tissues had been incubated for 24 hours.

Mometasone

Compared to the type 2 CRS samples, MIP-1β was significantly reduced in non-type 2 CRS samples (FC between the means=15.1, p=0.0328). Resistin was slightly downregulated in type 2 CRS but, in comparison, was significantly upregulated in non-type 2 CRS (FC between the means=0.64, p=0.0442).

Verapamil

Compared to the other samples, resistin (FC between the means=0.49, p=0.0480) and P-selectin (FC between the means=0.29, p=0.0480) were significantly downregulated in type 3 CRS.

Cenicriviroc

Compared to a slight upregulation in the non-type 1 CRS samples, resistin (FC between the means=0.63, p=0.0303) was significantly downregulated in type 1 CRS. P-selectin was significantly upregulated in type 3 CRS compared to the remaining samples (FC between the means=2.82, p=0.0455).

Dupilumab

DUP revealed no significantly different effects either on MIP-1β, resistin, or P-selectin in cell culture media.

In general, MIP-1β secretion was reduced by MOM in the samples that did not belong to type 2 CRS. CVC caused a similar trend, although it was not significant. VRP and DUP hardly affected MIP-1β secretion in any endotypes. Interestingly, MOM, VRP, and CVC reduced resistin secretion in different endotypes, while DUP revealed no considerable effects. P-selectin secretion was hardly affected by MOM and DUP. VRP reduced P-selectin secretion in type 3 CRS while hardly affecting type 1 and type 2 CRS. CVC upregulated the secretion of P-selectin in type 3 CRS (Figure 4).

Discussion

CRS patients experience prolonged inflammation of the nasal mucosa lasting more than 12 weeks, significantly impacting their quality of life[3]. Current treatment options are associated with substantial annual costs[1] and are primarily symptom-based[2], leading to varying clinical outcomes and frequently proving ineffective[4-6].

Past research[22-24] on different medical conditions already suggested the importance of precision medicine for achieving therapeutic success by recording the impact of drugs on certain disease marker proteins. However, a direct comparison of the endotypes is needed to highlight their distinct responses to medications.

Therefore, the aim of our study was to examine the effects of certain anti-inflammatory drugs on the CRS endotype-defining cytokines IFN-γ, IL-5, and IL-17A[1,16] and on the recently identified CRSsNP marker proteins MIP-1β, resistin, and P-selectin[17] dependent on the CRS endotype.

Several hypotheses exist regarding the onset of CRS. Generally, foreign irritants stimulate nasal epithelial cells to produce proinflammatory factors. In CRSwNP, this response is predominantly driven by Th2 cytokines like IL-4, IL-5, and IL-13. Conversely, CRSsNP is more closely associated with Th1 cytokines such as IL-6, IL-10, and IFN-γ, as well as Th3 cytokines like IL-17A and IL-22[1,16]. However, recent findings indicate that not all defining markers of a specific Th response are altered in distinct CRS endotypes[31,32] and that the phenotypes are not necessarily associated with distinct endotypes[33]. This suggestion was confirmed by our analysis of the typical endotype-associated cytokines IFN-γ, IL-5, and IL-17A[1,16] in CRSsNP ET secretions, where we identified single endotypes as well as mixed endotypes or no endotypes at all.

MIP-1β was originally identified as being expressed by monocytes[20]. Since then, many other cell types have been identified as a source of MIP-1β during the state of inflammation[18,34]. MIP-1β was shown to be overexpressed in the mucus and tissue of CRS patients[17,34-36]. Resistin is a small hormone-like protein mainly produced by macrophages, adipocytes, and epithelial cells whose proinflammatory effects contribute to the pathophysiology of many diseases[19]. Similar to another study[37], we previously identified resistin content to be higher in CRSsNP mucus compared to the control[17]. As an adhesion molecule, P-selectin plays an important role in immunity and supports the pathogenesis of many medical conditions like thrombosis and cancer. Mainly expressed by platelets and endothelial cells, it stimulates platelet aggregation and supports leukocyte diapedesis[21]. Our past study[17] revealed a lower P-selectin content in CRSsNP nasal mucus compared to the controls.

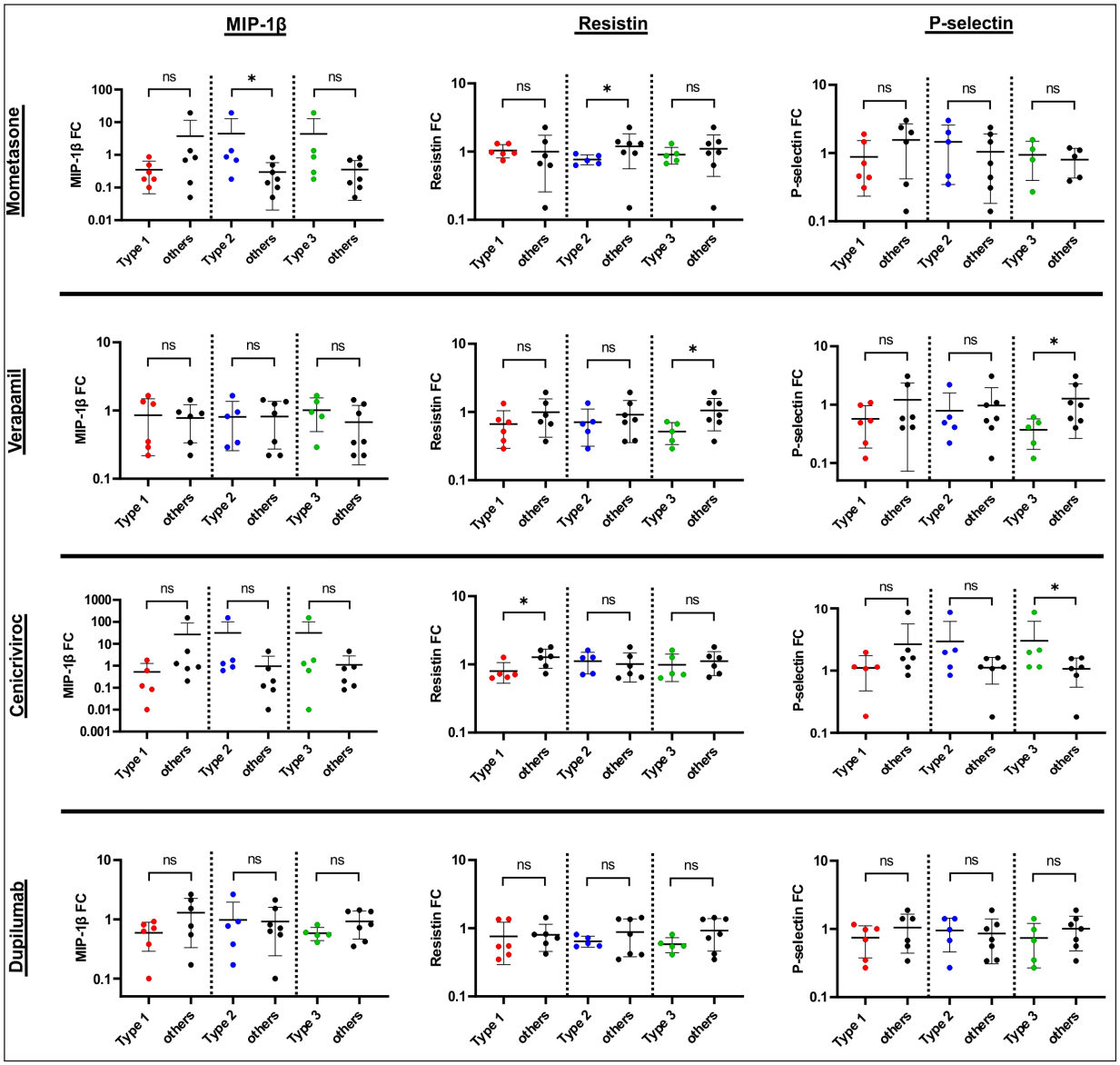

Figure 4. Scatter dot plots, including mean and SD error bars, show the results of the quantitative analysis of MIP-1β, resistin, and P-selectin by ELISA in the cell culture media. Due to the logarithmical plotting of the y-axes, some error bars are not displayed in this representation. In each panel, the type 1 CRS samples (red), the type 2 CRS samples (blue), and the type 3 CRS samples (green) are plotted against the remaining samples (black). For mometasone, statistically significant differences can be seen for both the effects on MIP-1β in type 2 CRS (p<0.05) and on resistin in type 2 CRS (p<0.05). For verapamil, statistically significant differences can be seen for both the effects on resistin in type 3 CRS (p<0.05) and on P-selectin in type 3 CRS (p<0.05). For cenicriviroc, statistically significant differences can be seen for both the effects on resistin in type 1 CRS (p<0.05) and on P-selectin in type 3 CRS (p<0.05). *<0.05, **<0.01. FC, fold change; MIP-1β, macrophage inflammatory protein-1 beta; P-selectin, platelet-selectin; ns, not significant.

The glucocorticoid (GC) MOM binds to the cytosolic GC receptor, which consequently enters the nucleolus. It regulates gene transcription, resulting in the resolution of inflammation[12,38].

Among the endotype-defining cytokines, MOM significantly reduced the secretions of IL-5 in type 1 CRS and of IL-17A in type 3 CRS. In type 2 CRS, on the other hand, there were no significant results. Past studies have shown[39] that the elevated membrane efflux pump P-glycoprotein (P-gp) in type 2 CRS contributes to GC resistance. This may explain our results. MIP-1β remained relatively unchanged in type 2 CRS but was significantly reduced in the remaining sam-

ples. Furthermore, MOM slightly but significantly upregulated resistin secretion in the samples that did not belong to type 2 CRS. This could have been triggered by so far unknown non-genomic effects[38], which could also influence proteins other than cytokines.

VRP is a blocker of long-lasting (L)-type calcium (Ca^{2+})-channels mainly used for treating cardiovascular diseases[40]. Besides that, the capability of VRP to reduce cytokine production was already reported by previous studies[9,41] and was shown to result from a disruption of the intracellular Ca^{2+} signaling network and by affecting P-gp activity and, consequently, the polarization of T lymphocytes.

VRP significantly reduced IFN-γ secretion in type 1 CRS and the secretions of resistin and P-selectin in type 3 CRS. Past studies[41-44] have already shown that VRP was capable of influencing these proteins. Veytia-Bucheli et al[41] showed that in the media of purified Thymus (T) cells, the presence of VRP induced a significant decrease in IFN-γ[41]. By administering a combination therapy of trandolapril plus VRP, Rubio-Guerra et al[42] were able to reduce circulating resistin levels in hypertensive patients with type 2 diabetes[42]. Pappone et al[43] showed that the proliferation of cultured brown fat cells was impaired by VRP[43], which is known to be a source of resistin[19]. Blaheta et al[44] demonstrated the downregulation of P-selectin expression on endothelial cells by VRP in a cell culture model[44]. However, further investigation will be needed to examine why the endotypes differ in their reaction to VRP.

CVC is a novel antagonist of the C-C chemokine receptors (CCR) type 2 and 5. These receptors and their ligands, one of which is MIP-1β, are involved in steatosis and fibrosis, cell entry of the human immunodeficiency virus (HIV), and leukocyte accumulation. CVC is therefore used for treating nonalcoholic steatohepatitis, HIV, and coronavirus disease 2019 (COVID-19)[11,45,46]. The IL-5 secretion of our CRSsNP ETs was significantly downregulated by CVC in type 1 CRS. Furthermore, resistin secretion was slightly reduced in type 1 CRS and slightly increased in the remaining samples, leading to a significant difference. CVC significantly increased P-selectin secretion in type 3 CRS, although there was a high standard deviation. Since CVC is still an experimental drug candidate[47], there is so far only little knowledge about its effects in inflammatory diseases. Mencarelli et al[48] proved that by blocking CCR5, CVC was able to reduce IL-5 expression in dextran sulfate sodium-induced murine colitis and thus had a positive effect on the mucosal inflammation[48]. Another study[49] revealed that CVC was able to reduce E-selectin in human aortic endothelial cells. Thus, CVC can have an influence on selectins. Since P-selectin was previously found to be underexpressed in CRSsNP mucus[17], the result of its upregulation by a drug is a valuable finding.

DUP is a monoclonal antibody that binds to IL-4 receptor (R)-α, a subunit of IL-4R, blocking the signaling of both IL-4 and IL-13[50]. Known for limiting the Th2 response and mitigating type 2 inflammation[51], it is used for treating nasal polyps and atopic dermatitis[50]. Our analyses of the DUP effect did not yield any significant results, except for an upregulation of the IL-5 secretion in the samples other than type 1 CRS. We speculate that this could have been caused by a compensatory mechanism[52]. In general, our result showed that DUP administration in CRSsNP patients may be unprofitable compared to the other tested drugs.

In general, although acting *via* different mechanisms, all four tested substances induced a clear reduction of IFN-γ and IL-5 in type 1 CRS, indicating that this endotype may be the most sensitive to the selected medical therapy.

In conclusion, in our study, we tested several anti-inflammatory drugs for their effects on various CRS endotype-defining cytokines[16] and on potential future CRSsNP biomarkers[17] using an *ex vivo* model.

In order to show the different impacts on the CRS endotypes, we compared them with each other. To the best of our knowledge, this is the first study to carry out a direct comparison of CRS endotypes in terms of their reaction to different medications. In addition, CVC has never been studied in the context of CRS[47] and there is currently only little information about the benefits of DUP application in CRSsNP patients[1].

However, our study had some limitations. First, the number of samples included in this study was relatively small. We applied our inclusion and exclusion criteria very strictly to ensure a homogenous cohort. That, in combination with CRSsNP patients undergoing FESS less likely in our tertiary care hospital compared to CRSwNP, reduced the number of freshly available tissues. Consequently, there were only a few samples assigned to the pure endotypes. In order to apply statistical tests, samples that were assigned to this, but also to other endotypes, i.e., were of the mixed endotypes, and were combined into one group. Com-

paring only samples of pure endotypes should be considered for future analysis in order to obtain clearer results since our comparison of the partially mixed endotypes suggests distinct reactions of the different CRS endotypes to medical treatment. Secondly, for this study, an *ex vivo* model was used in comparison to an *in vivo* model. While *in vivo* models are best to assess the individual patients' reaction[53], we wanted to acquire *in vitro* data first as some of our incubated drugs have not yet been considered for CRS treatment before or have never been applied intranasally[1,11]. However, the chosen whole tissue explant model resembles the conditions of living organisms as closely as possible[53]. Thirdly, only the secretion of the proteins was determined, which does not necessarily correspond to the tissue protein expression and the mRNA amount[54]. Future *in-vivo* tests using larger cohorts will be needed to verify our results. Some of the analyzed drugs may reveal additional effects with longer incubation time or by *in vivo* application *via* drug-eluting stents[55]. These effects have to be quantified in future studies. Nevertheless, our results indicate the importance of determining the CRS endotype and searching for marker proteins as potential targets before choosing a suitable medication for the patients.

Conclusions

This study, by showing considerable differences in the response of different CRS endotypes to selected anti-inflammatory drugs, highlights the importance of precision medicine in chronic rhinosinusitis. This study might contribute to the development of effective and innovative therapeutic strategies.

Conflict of Interest
The authors declare that they have no conflict of interest to disclose.

Informed Consent
All patients provided written informed consent for inclusion and for the usage of their tissues in research before they participated in the study.

Ethics Approval
This study was conducted in accordance with the Declaration of Helsinki of 1975 (as revised in 2013), and the protocol was reviewed and approved by the Institutional Review Board of the University of Erlangen-Nürnberg (No.: 17-269_1-B) on 11.02.2020.

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Data Availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI Disclosure
No artificial intelligence was used for the production of this manuscript, including all tables and figures.

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Authors' Contributions
V.V. Pesold: acquisition of data, analysis, and interpretation of data, drafting the article. O. Wendler: supervision, making critical revisions related to the relevant intellectual content of the manuscript. S.K. Mueller: conception and design of the study, acquisition of data, making critical revisions related to the relevant intellectual content of the manuscript, validation, and final approval of the version of the article to be published.

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