Fakultät für Medizin

From Exposure to Reaction: Expression of immune parameters under natural pollen exposure and their effect on allergic symptoms

Mehmet Gökkaya

Vollständiger Abdruck der von der Fakultät für Medizin der Technischen Universität München zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.) genehmigten Dissertation.

Vorsitz:	Prof. Dr. Carsten Schmidt-Weber
Prüfer*innen der Dissertation:	1. Prof. Dr. Claudia Traidl-Hoffmann
	2. Prof. Dr. Dietmar Zehn
	3. Prof. Mohamed Shamji, Ph.D.

Die Dissertation wurde am 30.12.2021 bei der Technischen Universität München eingereicht und durch die Fakultät für Medizin am 21.03.2023 angenommen.

1. Preface

1.1 Acknowledgement

This dissertation could not have been possible without the support from many people. Especially, I am thankful to Prof. Claudia Traidl-Hoffmann and PD Dr. Stefanie Gilles who extensively provided me with supervision, suggestions, and insightful comments to finalize this dissertation. They encouraged me to develop independent view and research skills. And thank you again Stefanie to point us in the right direction of research and navigating out of pitfalls.

My research could be presented on many congresses and helped me to expand my horizons. Hereby, I thank for all the support and finance, especially to CK-Care and GEWISA e.V. Furthermore, I acknowledge the contribution of all co-authors, through the key data and information we could already publish the results of my dissertation in peer-reviewed journals.

I also would like to thank dissertation committee members and my peers for reading my research and giving their feedbacks. In particular, I am also thankful to our study center and study participants for their help or rather participation throughout the studies. I wish to show my gratitude also to Prof. Avidan U. Neumann for close connection and bioinformatics skills.

I am very proud of the knowledge I gained through my doctoral thesis where I was able to extend the bioinformatic techniques into COVID-19 research, and thankfully resulted into two more publications in the Journal of Allergy and Clinical Immunology and The Lancet. Prof. Claudia Traidl-Hoffmann has given me opportunity that I never thought would be possible at that career stage and I hope to reach the potential that she sees in me. Her advice and encouragement will echo through my future career.

Most importantly, I owe a great debt to my parents, Nurhayat and Çapan Gökkaya for their encouragement and support all through my life. Last but not least, my deepest appreciation goes to my wife, Büşra Gökkaya who moved to Augsburg for me and had endless patience during the completion of this dissertation; I dedicate this work to her and to my newborn baby Maya.

1.2 Abstract

From Exposure to Reaction: Expression of immune parameters under natural pollen exposure and their effect on allergic symptoms

Allergic diseases occur due to a dysregulated immune system that is characterized by inflammation and the formation of specific IgE antibodies against harmless environmental antigens. Pollen exposure induces local and systemic allergic immune responses in allergic patients, but also non-allergic subjects are exposed to airborne pollen. To the best of our knowledge, to date, there is no study showing the course of symptom expression under natural pollen exposure, especially in non-allergic subjects, which are more than only a control cohort.

We followed allergic rhinitis patients and non-allergic subjects in a panel study to understand more about the humoral immune response under airborne pollen exposure and identify potential nasal biomarkers for symptom severity. Immune parameters in serum as well as in nasal fluid, such as total and specific antibodies against Bet v 1, immunoglobulin free light chains, cytokines and chemokines, were followed over the course of one year. The overarching aim was to understand the kinetic of the immune response under natural pollen exposure and its relationship to symptoms. Bioinformatic analysis on the longitudinal data reveal nasal immune variables which might be responsible for the symptom severity.

Allergic patients recorded their symptoms daily, by means of a symptom diary, and symptoms followed natural pollen concentration with a delay up to 13 days, depending on the pollen type. A subgroup of non-allergic subjects (4 out of 7 individuals) also recorded in-season symptoms. In general, the symptoms of non-allergic subjects were lower than those of allergic patients but followed airborne pollen exposure with comparable patterns. Expression of nasal CCL2, CCL22 and CCL24 were higher in allergic patients, whereas nasal IL-8 was higher in non-allergic subjects. Nasal pollen specific IgA antibodies might be protective for the pathogenesis. According to principal component analysis and Spearman correlations, nasal IL-8, IL-33 and Bet v 1-specific IgE and IgG_4 could be biomarkers for the prediction of in-season symptom severity in allergic, as well as in non-allergic subjects.

Clinical allergy diagnostics is routinely performed by serum IgE tests. Especially the specific IgE profile plays a key role for planning specific immunotherapy. We introduced a novel, non-invasive alternative to routine serum IgE diagnostics. Hereby, we evaluated nasal fluid as a new target for the allergy diagnostic.

Towards this aim, in a pilot study, nasal secretion and serum samples of adult volunteers with aeroallergen sensitizations were collected to determine IgE profiles by a molecular component based method (ISAC). Levels of specific IgE were correlated between nasal secretion and serum, per patient and for each allergen component. Receiver operating

characteristic (ROC) curve analysis was done to validate the assays and set cut-off threshold for the nasal specific IgE against birch, grass pollen and house dust mite.

Serum und nasal tests were highly similar in specificity, and the sensitivity for both tests was over 85%. The calculated threshold was set to be 0.08. Thus, nasal samples could be a new target for molecular allergy diagnostics, which is a non-invasive methods and could avoid the risk of complications and unnecessary pain to the patient. This could be validated especially in children.

The results of my thesis would help allergic patients by predicting allergic symptoms via the determination of nasal immune parameters. Also, determination of IgE profiles via non-invasive sampling could help patients as well as clinicians. The samples of our panel study were very well characterized and could be used for future projects, such as TCR repertoire analysis, to understand the diversity of the immune receptor repertoire, as well as reveal pathways of allergy development in non-allergic individuals.

1.3 Zusammenfassung

Von der Exposition zur Reaktion: Expression von Immunparametern unter natürlicher Pollenexposition und der Zusammenhang mit allergischen Symptomen.

Allergische Erkrankungen sind auf ein dereguliertes Immunsystem zurückzuführen, welche durch Entzündung und Bildung von spezifischen IgE-Antikörpern gegen harmlose Umweltantigene gekennzeichnet ist. Pollenexposition induziert lokale und systemische allergische Immunreaktionen vor allem bei allergischen Patienten, aber auch Personen ohne Allergien sind luftgetragenen Pollen ausgesetzt. Unseres Wissens nach gibt es bis heute keine Studie, die den Verlauf der Immunreaktion und der Symptomausprägung unter natürlicher Pollenexposition zeigt, insbesondere auch mit Blick auf Nichtallergiker, die bei uns nicht nur als Kontrollkohorte dienen.

Wir verfolgten allergische Rhinitis Patienten und Probanden ohne Allergien in einer Panel-Studie, um mehr über die humorale Immunantwort unter natürlichen Pollenexposition zu verstehen und um potentielle nasale Biomarker für die Symptomausprägung zu identifizieren. Die Expression von Immunparametern wie Immunglobuline, spezifische Antikörper gegen Bet v 1, freie Leichtketten von Immunglobulinen, Zytokine und Chemokine im Serum sowie im Nasensekret wurden im Verlauf eines Jahres verfolgt. Das Ziel war es, die Kinetik der Immunantwort unter natürlicher Pollenexposition zu verstehen und diese mit der Symptomschwere zu korrelieren. Bioinformatische Analysen der Längsschnittdaten filterten dabei einige nasale Immunvariablen heraus, welche mit der Symptomschwere korrelierten.

Die allergischen Symptome folgten der natürlichen Pollenkonzentration mit einer Verzögerung von bis zu 13 Tagen in Abhängigkeit von der Pollenart. Eine Untergruppe der Probanden ohne Allergien (4 von 7 Personen) zeigten ebenfalls saisonale Symptome. Im Allgemeinen waren die Symptome bei den Nichtallergiker geringer als bei den allergischen Patienten, folgten aber der Pollenexposition mit ähnlichem Verlauf. Die Expression von nasalem CCL2, CCL22 und CCL24 war bei allergischen Patienten höher, während nasales IL-8 bei Nichtallergikern dominierte. Nasales Pollen-spezifisches IgA könnte protektiv in der Pathogenese der Allergie sein. Anhand einer Hauptkomponentenanalyse und Spearman-Korrelationen konnten nasales IL-8, IL-33 und Bet v 1-spezifisches IgE und IgG_4 als Biomarker für die Vorhersage der Symptomschwere in der Pollensaison, sowohl in der allergischen als auch in der nicht-allergischen Kohorte, identifiziert werden.

Die klinische Allergiediagnostik wird routinemäßig durch Serum-IgE-Tests durchgeführt. Insbesondere die Charakterisierung des spezifischen IgE-Profils spielt eine Schlüsselrolle bei der Planung der spezifischen Immuntherapie. Wir haben eine neue, nicht-invasive Alternative zur klinischen Routinediagnostik untersucht. Hierfür evaluierten wir Nasensekrete als neues Ausgangsmaterial für die Allergiediagnostik.

In einer weiteren Studie wurden zu diesem Zweck Nasensekrete und Serumproben von sensibilisierten, erwachsenen Probanden entnommen, um spezifische IgE-Profile molekularen Komponenten basierten Methoden (ISAC) zu bestimmen. Die spezifischen IgE-Spiegel wurden zwischen Nasensekret und Serum korreliert, sowohl pro Patient als auch für jede einzelne Allergenkomponente. Grenzwertoptimierungskurven (ROC) wurden analysiert, um Schwellenwerte für die nasalen Proben festzulegen und zu validieren.

Serum Test und nasaler Test waren in der Spezifität sehr ähnlich, und die Sensitivität lag bei beiden über 85%. Der berechnete Schwellenwert wurde auf 0,08 festgelegt. Nasale Proben könnten ein neues Werkzeug für die molekulare Allergiediagnostik sein, da sie nicht-invasiv entnommen werden können und somit das Risiko von Komplikationen und unnötigen Schmerzen für den Patienten, insbesondere bei Kindern, vermeiden kann.

Die Ergebnisse meiner Arbeit könnten ein Schlüsselelement für allergische Patienten sein, um Symptome vorherzusagen. Auch die Bestimmung des spezifischen IgE-Profils über eine nicht-invasive Probenahme könnte sowohl dem Patienten als auch dem Arzt helfen. Die Proben der Panel-Studie waren sehr gut charakterisiert und könnten für zukünftige Projekte wie zum Beispiel TCR-Repertoire-Analysen zur Charakterisierung der Diversität des adaptiven Immunsystems, sowie zum Verständnis der Allergieentwicklung bei nicht-allergischen Personen verwendet werden.

1.4 Abbreviations

AID	Activation-induced cytidine deaminase
AIT	Allergen immunotherapy
APC	Antigen-presenting cells
AR	Allergic rhinitis
ASIT	Allergen-specific immunotherapy
AUC	Area under the curve
Bet v	Betula Verrucosa
BSA	Bovine serum albumin
C-region	Constant region
CC16	Clara cell protein 16
CCD	Charge-coupled Device CCD
CD	Cluster of differentiation
C_H	Heavy chain constant
COPD	Chronic obstructive pulmonary diseases
COVID-19	Coronavirus diseases 2019
CSR	Class switch recombination
CTL	Cytotoxic T-Cells
CW	Calendar week
CXC-2	C-X-C motif chemokine-2
DAMPs	Danger-associated molecular patterns
DCs	Dendritic cells
DOR	Diagnostic odds ratio
ECP	Eosinophil cationic protein
EDTA	Ethylenediamine tetraacetic acid
EtOH	Ethanol
FcεR	Fragment crystallizable epsilon receptor
FCS	Fetal bovine serum
FLC	Free light chains
FOR	False omission rate
FPR	False positive rate
GM-CSF	Granulocyte macrophage colony stimulating factor
GPCR	G protein-coupled receptor
HDM	House dust mite
HR	Hypersensitivity reaction
Ics	Immune complexes
IFN	Interferon
Ig(s)	Immunoglobulin(s)
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IHR	Immediate hypersensitivity reactions
IL	Interleukin
IRAK-1 (4)	Interleukin-1 receptor-associated kinase 1 (4)
ILCs	Innate lymphoid cells

IQR	Interquartile range
ISAAC	International Study of Asthma and Allergies in Childhood
ISAC	Immuno Solid-phase Allergen Chip
ISU-E	ISAC standardized units
LAR	Local allergic rhinitis
LPRs	Late phase reactions
LR	Likelihood ratio
MAPK	Mitogen-activated protein kinase
MC	Mast cells
MCP-1	Monocyte chemotactic protein-1
MDC	Macrophage-derived chemokine
ME	Mercaptoethanol
MIP-1ß	Macrophage inflammatory protein 1β
NA	Non-allergic
NAR	Non-allergic rhinitis
NF-κB	Nuclear factor-κB
NK	Natural killer
NPV	Negative prediction value
NS	Non-sensitized
PALMs	Pollen-associated lipid mediators
PBMCs	Peripheral blood mononuclear cells
D-PBS	Dulbecco's phosphate-buffered saline
PC1	Principal component 1
PCA	Principal component analysis
pNPP	p-Nitrophenyl phosphate
PPV	Positive prediction value
PR-10	Pathogenesis related protein family 10
ROC	Receiver operating characteristics
r_s	Spearman correlation coefficients
S region	Switch region
SAR	Seasonal allergic rhinitis
SARS-CoV-2	Severe acute respiratory syndrome-Coronavirus 2
SCIT	Subcutaneous
SD	Standard deviation
SEM	Standard error of the mean
sIg	(Bet v 1-) specific immunoglobulin
SIT	Specific immunotherapy
SLIT	Sublingual
SPT	Skin prick test
T-reg	T-regulatory
TBS-T	Tris-buffered saline containing tween-20
TCR	T-cell receptors
Tfh	T follicular-helper
T_H1	Type 1 T helper

T_H2	Type 2 T helper
TNR	True negative rate
TNSS	Total nasal symptom score
TPR	True positive rate
TSLP	Thymic stromal lymphopoietin
V	Visit
V-regions	Variable regions

2. Table of contents

1. Preface ..... - 1 -

1.1 Acknowledgement ..... - 1 -

1.2 Abstract ..... - 2 -

1.3 Zusammenfassung ..... - 4 -

1.4 Abbreviations ..... - 6 -

2. Table of contents ..... - 9 -

3. Introduction ..... - 11 -

3.1 Allergy ..... - 11 -

3.2 Different types of hypersensitivity reactions ..... - 11 -

3.3 Effector cells in type I allergic immune reactions ..... - 13 -

3.4 T_H1 and T_H2 paradigm and beyond ..... - 16 -

3.5 The humoral immune response in allergic reactions ..... - 17 -

3.6 Cytokines and Chemokines ..... - 19 -

3.7 Birch pollen and Bet v 1 – the major birch pollen allergen ..... - 21 -

3.8 Allergic rhinitis ..... - 22 -

4. Aim of the study ..... - 25 -

5. Materials and Methods ..... - 27 -

5.1 Materials ..... - 27 -

5.2 Study design ..... - 33 -

5.2.1 Panel study ..... - 33 -

5.2.2 ISAC study ..... - 35 -

5.3 Airborne pollen monitoring and pollen season ..... - 37 -

5.4 Monitoring of symptoms ..... - 38 -

5.5 Biosampling ..... - 38 -

5.5.1 Serum ..... - 38 -

5.5.2 Nasal secretion ..... - 38 -

5.5.3 Nasal curettage ..... - 38 -

5.5.4 Isolation of PBMCs ..... - 39 -

5.5.5 Isolation of PMNs ..... - 39 -

5.6 Determination of specific IgE by ISAC ..... - 40 -

5.7 Measurement of immune variables ..... - 40 -

5.8 Measurement of Bet v 1-specific immunoglobulins ..... - 41 -

5.9 Flow cytometry analysis ..... - 41 -

5.9.1 Nasal curettages ..... - 41 -

5.9.2 Nasal lavage ..... - 42 -

5.9.3 Control staining for flow cytometry ..... - 42 -

5.10 Establishment of T-cell subsets sorting ..... - 43 -
5.11 Gene expression analysis of polymeric immunoglobulin receptor ..... - 43 -
5.11.1 Total RNA Isolation ..... - 43 -
5.11.2 Real-Time Polymerase Chain Reaction ..... - 44 -
5.12 Statistical data analysis ..... - 45 -
6. Results ..... - 47 -
6.1 Panel study ..... - 47 -
6.1.1 Symptom kinetics under natural pollen exposition[#] ..... - 47 -
6.1.2 Kinetics of humoral immune responses under natural pollen exposure[#] ..... - 50 -
6.1.3 Seasonal comparison of total immunoglobulins[#] ..... - 52 -
6.1.4 Seasonal comparison of Bet v 1-specific immunoglobulins[#] ..... - 54 -
6.1.5 Pollen related nasal chemokine and cytokine levels[#] ..... - 56 -
6.1.6 Nasal biomarkers for in-season symptom severity in both cohorts[#] ..... - 56 -
6.1.7 Gene expression analysis of polymeric immunoglobulin receptor ..... - 61 -
6.1.8 Nasal flow cytometry analysis ..... - 61 -
6.1.9 Establishment of T-cell sorting ..... - 64 -
6.2 Panel ISAC Study ..... - 66 -
6.2.1 Sensitization profiles[§] ..... - 66 -
6.2.2 Inter correlation of sIgE levels in serum and nasal secretion[§] ..... - 66 -
6.2.3 Nasal specific IgE correlate with serum specific IgE[§] ..... - 69 -
6.2.4 Evaluation of nasal sampling as a diagnostic tool[§] ..... - 73 -
7. Discussion ..... - 75 -
8. Appendix ..... - 80 -
9. References ..... - 84 -
10. Figure legends ..... - 95 -
11. Table legends ..... - 98 -
12. Publications ..... - 99 -
13. Poster presentations ..... - 100 -
14. Awards ..... - 101 -

3. Introduction

3.1 Allergy

The prevalence of allergic diseases like allergic rhinitis, asthma, atopic dermatitis, food allergy and anaphylaxis, has increased over the last decades. Allergic diseases reduce the quality of life for more than 150 million Europeans (Calderon et al., 2012). The term “allergy” was introduced by the pediatrician-scientist Clemens von Pirquet in 1906 (Shulman, 2017). It is an exaggerated immune response to harmless environmental agents like pollen, house dust or food. The American allergologists Coca and Cooke proposed the term “atopy” for the propensity to produce immunoglobulin E (IgE) as a result of hypersensitivity reactions (HR) (Cohen, Dworetzky, & Frick, 2003).

3.2 Different types of hypersensitivity reactions

The commonly used term “allergy” actually refers to one of four different hypersensitivity reactions (HR), which will be briefly described in the following section (Figure 1).

HR responses of types II and III are mediated mainly by immunoglobulin G (IgG), while type IV HR are T-cell mediated. The focus of the thesis will be on IgE dependent type I hypersensitivity reactions (Murphy, Travers, Walport, & Janeway, 2008).

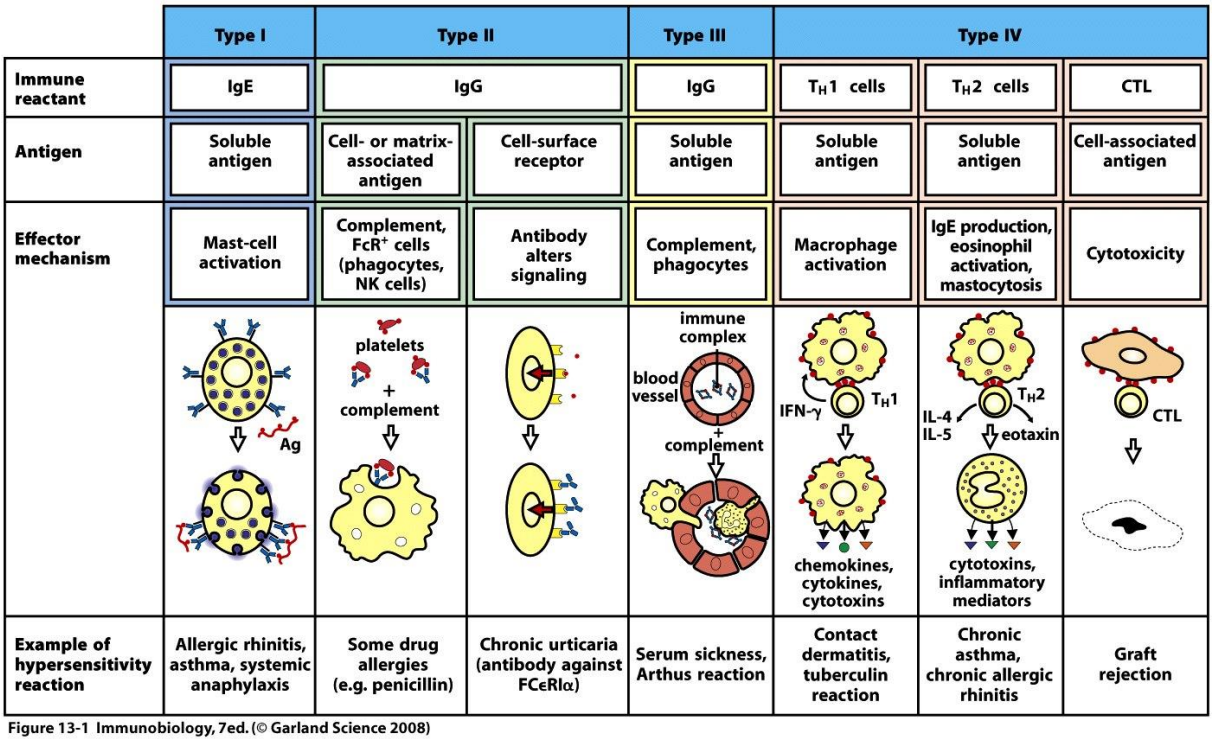


Figure 13-1 Immunobiology, 7ed. (© Garland Science 2008)

Figure 1: Different types of hypersensitivity reactions. Hypersensitivity is classified by immune reactant, antigen and effector mechanism into four main groups according to Gell & Coombs (Murphy, Travers, Walport, & Janeway, 2008).

Type I, type II and type III hypersensitivity reactions (HR) appear within 24 hours after antigen exposure (Justiz Vaillant, Vashisht, & Zito, 2020). Antibodies of IgG and IgM isotype activate the complement system in type II HR (i.e., cytotoxic reactions), which leads to recruiting and activation of immune cells and lysis of target cells. Neutrophils release enzymes and reactive oxygen species, resulting in tissue damage. Fc receptors on natural killer (NK) cells bind to the IgG constant domain and release perforin with the resultant cell lysis (Abbas, Moussa, & Akel, 2020; Actor & Actor, 2012).

Also in type III HR (i.e., immune complex reactions), IgG and IgM are involved, which form antigen-antibody complexes with soluble antigens. Such overproduced immune complexes (ICs) are deposited in blood vessel walls in various organs and trigger inflammation, leading to tissue damage (Maker, Stroup, Huang, & James, 2019).

The fourth type is a delayed type HR. In type IV HR, T-cells such as type 1 T helper (T_H1), type 2 T helper (T_H2) and cytotoxic T-Cells (CTL) are activated and secrete cytokines leading to tissue damage (Marwa & Kondamudi, 2020).

The main focus of this thesis lies on the type I HR, which are of the immediate-type and are commonly referred to when speaking of “allergy”. Soluble antigens from pollen, mites, fungi, animal dander, foods, drugs or insects are known as allergens, which mainly promote IgE production and type I HR. IgE binds to the high-affinity Fcε receptor (FcεRI) on mast cells, which results in release of histamine-containing granules, which in turn cause the inflammation associated with allergic reactions (Abbas et al., 2020), e. g. pruritus, peripheral vasodilation, erythema by extravasation of capillary blood, and edema by fluid shift into the interstitial space.

3.3 Effector cells in type I allergic immune reactions

The allergic immune reaction can be divided into two phases: sensitization and elicitation phases (Figure 2).

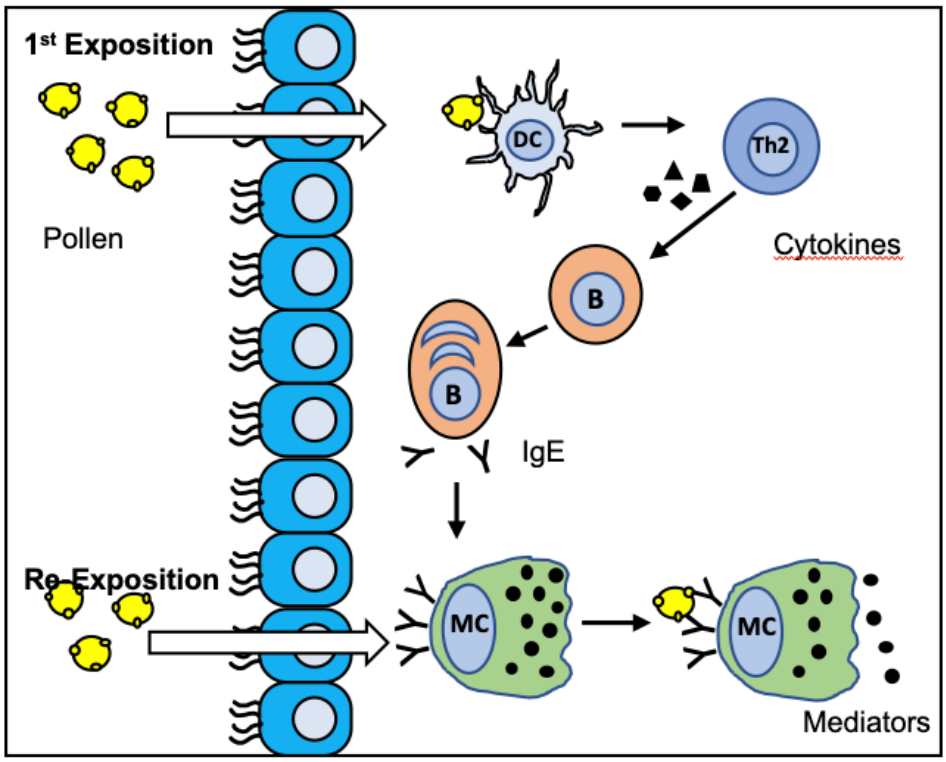


Figure 2: Basic mechanism of allergic inflammation

The allergic sensitization begins by the uptake of environmental antigens by professional antigen presenting cells (APCs), such as dendritic cells (DCs), which are situated in the barrier-forming organs of the body, e.g. epithelia. By as yet incompletely understood mechanisms – probably due to PRR engagement – DCs become activated, which means they acquire a migratory phenotype, upregulate co-stimulatory and antigen-presenting molecules, and secrete cytokines. Activated DCs then migrate to the draining lymph node, where they enter the T cell zone and present antigen-derived peptides to naïve T-cells via MHC molecules (intracellular antigens via MHC I, extracellular antigens via MHC II).

Intracellular, e.g. viral or tumor antigens, are presented on MHC I molecules to CD8 positive T-cells, whereas extracellular antigens (i.e. most allergens) are presented via MHC II molecules to CD4 positive T cells. The antigen-specific T cell becomes activated by the antigen:MHC complex, co-stimulation signals and DC-derived cytokines, and starts to proliferate and differentiate into an effector T cell.

In allergic sensitization, as yet unknown signals lead to the differentiation of T_H2 cells, which secrete IL-4, IL-5 and IL13. IL-4 derived from T_H2 cells induces an isotype switch in antigen-specific B cells, which become IgE producing plasma cells. The secreted antigen-

specific IgE enters the circulation and binds to high affinity IgE-receptors (FcεRI) on mast cells (MC), basophils and eosinophils, which are the main primary effector cells of allergies (He, Zhang, Zeng, Chen, & Yang, 2013). This process is called “allergic sensitization”.

Upon re-exposure to the same allergen, the allergen binds to IgE antibodies bound to FcεRI on mast cells in the tissue. Cross-linking of the FcεRI leads to immediate type allergic reactions - the degranulation of the mast cells, which leads to histamine, leukotriene and cytokine secretion (Figure 3). The molecules released from mast cells have broad effects on the tissues and on immune responses. Histamine is the main effector molecule of type I HR as described before and mediates responses such as vasodilation and erythema; in addition other mast cell derived factors mediate itch, recruitment of immune cells and subsequent local inflammation. This is the so-called allergic elicitation phase.

Class of product	Examples	Biological effects
Enzyme	Tryptase, chymase, cathepsin G, carboxypeptidase	Remodel connective tissue matrix
Toxic mediator	Histamine, heparin	Toxic to parasites Increase vascular permeability Cause smooth muscle contraction
Cytokine	IL-4, IL-13	Stimulate and amplify T_H2-cell response
	IL-3, IL-5, GM-CSF	Promote eosinophil production and activation
	TNF-α (some stored preformed in granules)	Promotes inflammation, stimulates cytokine production by many cell types, activates endothelium
Chemokine	CCL3	Attracts monocytes, macrophages, and neutrophils
Lipid mediator	Prostaglandins D_2, E_2 Leukotrienes B4, C4	Cause smooth muscle contraction Increase vascular permeability Stimulate mucus secretion
	Platelet-activating factor	Attracts leukocytes Amplifies production of lipid mediators Activates neutrophils, eosinophils, and platelets

Figure 13-12 Immunobiology, 7ed. (© Garland Science 2008)

Figure 3: Overview over mast cell released molecules and their biological effects (Murphy, Travers, Walport, & Janeway, 2008).

Innate lymphoid cells (ILCs) might be crucial for the induction of the T_H2 pathway (van Ree, Hummelshoj, Plantinga, Poulsen, & Swindle, 2014). A subset of ILCs, the so-called ILC2 cells, do not express T-cell receptors (TCR). Upon activation by interleukin (IL)-25, IL-33 and thymic stromal lymphopoietin (TSLP), ILC2 cells also secrete the T_H2 signature cytokines IL-

4, IL-5, IL-9 as well as IL-13 and thus promote T_H2 responses (Pasha, Patel, Hopp, & Yang, 2019).

Mast cells and T_H2 cells are not the only source of IL-5, also ILC2 cells secret large amounts of IL-5, which recruits and activates eosinophils (Stier & Peebles, 2017). Activated eosinophils can trigger tissue damage by secreting toxic proteins, e.g. ECP (Fulkerson & Rothenberg, 2013). Upon eosinophil degranulation, the derived proinflammatory mediators amplify the inflammatory response (Figure 4).

Class of product	Examples	Biological effects
Enzyme	Eosinophil peroxidase	Toxic to targets by catalyzing halogenation Triggers histamine release from mast cells
	Eosinophil collagenase	Remodels connective tissue matrix
	Matrix metalloproteinase-9	Matrix protein degradation
Toxic protein	Major basic protein	Toxic to parasites and mammalian cells Triggers histamine release from mast cells
	Eosinophil cationic protein	Toxic to parasites Neurotoxin
	Eosinophil-derived neurotoxin	Neurotoxin
Cytokine	IL-3, IL-5, GM-CSF	Amplify eosinophil production by bone marrow Cause eosinophil activation
	TGF-α, TGF-β	Epithelial proliferation, myofibroblast formation
Chemokine	CXCL8 (IL-8)	Promotes influx of leukocytes
Lipid mediator	Leukotrienes C4, D4, E4	Cause smooth muscle contraction Increase vascular permeability Increase mucus secretion
	Platelet-activating factor	Attracts leukocytes Amplifies production of lipid mediators Activates neutrophils, eosinophils, and platelets

Figure 13-13 Immunobiology, 7ed. (© Garland Science 2008)

Figure 4: Overview over eosinophil released molecules and their biological effects (Murphy, Travers, Walport, & Janeway, 2008).

Besides eosinophils, also neutrophils are contributing to allergic late phase reactions (LPRs), possibly even as APCs. Neutrophils are activated depending on the local cytokines. After allergen uptake, they may switch their ability from being first-line innate immune cells to an allergic effector unit, functioning as APCs to activate local allergen specific effector T cells during allergic LPRs (Polak et al., 2019).

3.4 T_H1 and T_H2 paradigm and beyond

Cytotoxic T cells are CD8 positive and kill infected cells or pathologically transformed cells directly. In contrast, CD4 positive T helper cells mainly contribute to adaptive immune responses by initiating and shaping the immune response. Intracellular bacteria, viruses and tumor antigens mainly induce T_H1-cell responses, whereas parasites, toxins or allergens trigger T_H2-cell responses. These two subsets of T helper cells are characterized by their distinct signature cytokine secretion, typically Interferon (IFN)-γ (T_H1) and IL-4 (T_H2) (Hirahara & Nakayama, 2016). There are also other T helper cell subsets such as T_H9, T_H17, T_H22, T follicular-helper (T_{fh}), T-regulatory (T_{reg}) cells. In this thesis, we will focus on T_H2 cells.

Pollen exposure triggers T_H2 dominated immune responses in susceptible individuals (Aglas et al., 2018). Upon re-exposure to pollen, also the subsequent T helper cell response is skewed towards T_H2 in sensitized (allergic) individuals. The T_H2-derived cytokines IL-4 and IL-5 in turn suppress T_H1 cells (Usui et al., 2006). The T_H1/T_H2 paradigm states that increased levels of T_H2 cells counteract antigen-specific T_H1/T_{reg} differentiation, which would be protective in allergy. Thus, allergen-specific T_H2 responses critically contribute to the pathogenesis of allergic diseases (Figure 5). Allergen-specific immunotherapy aims at shifting the allergen-specific T helper cell response away from pathogenic T_H2 and towards protective T_{reg} responses.

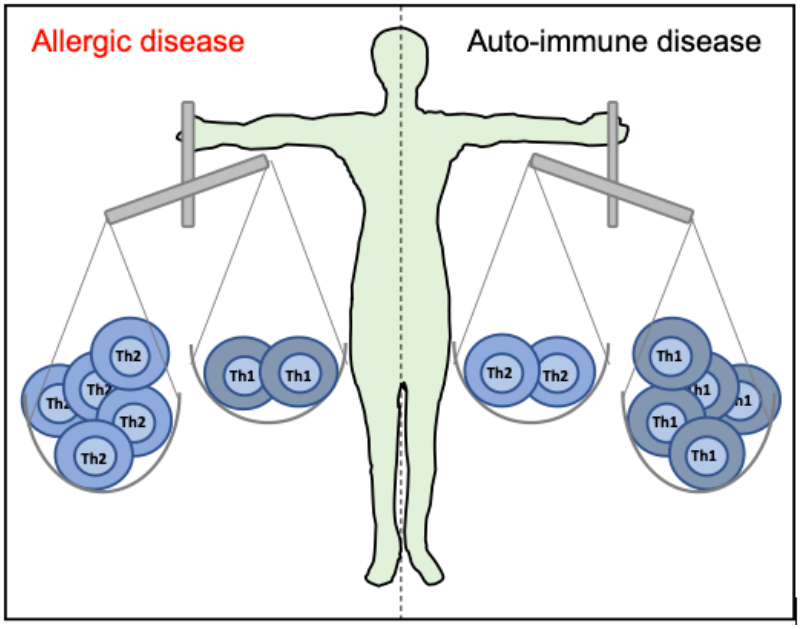


Figure 5: Th1/Th2 balance leads to different disease outcome

The signature cytokine of T_H2 cells is IL-4. Binding of IL-4 to its receptor leads to phosphorylation and dimerization of the transcription factor STAT6. Translocation of STAT6 to the nucleus initiates the transcription of GATA-3, the key T_H2-driving transcription factor. GATA-3 binds to the promoters of the genes for IL-4, IL-5 and IL-13, and thereby induces the

expression of the T_H2 effector cytokines(Maier, Duschl, & Horejs-Hoeck, 2012). These cytokines in turn, induce mucus production, contraction of smooth muscle cells, differentiation of naïve T cells to T_H2 cells, production and class-switching of immunoglobulins to the IgE isotype, as well as expansion and activation of basophils, mast cells and eosinophils (Lloyd & Snelgrove, 2018). Besides IL-4, other cytokines, including IL-33 and TSLP, are associated with T_H2 inflammation (Akasaki et al., 2016).

3.5 The humoral immune response in allergic reactions

In allergic immune responses, T_H2 cells induce B cells to differentiate into allergen specific, IgE-secreting plasma cells. Antibodies are immunoglobulin heteromeric complexes consisting of two heavy and two light chains, each of which have variable (V) and constant (C) regions. The V-region is responsible for specific antigen binding whereas the C-region exhibits distinct effector functions such as binding to Fc receptors (FcR) on phagocytes and dendritic cells. Immunoglobulin isotype classes are distinguished by their heavy chain constant (C_H) region (Schroeder & Cavacini, 2010). After V(D)J recombination, mature naïve B cells express the B cell receptor (BCR) at their surface, which corresponds to cell-bound IgM. Gene rearrangement processes enable immunoglobulin class switching of IgM to IgG, IgA, or IgE. Due to minor amino acid variances in the C_H regions, IgG and IgA are additionally divided in to IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2 (Yu & Lieber, 2019). Upon exposure to an antigen, T_H2 and B cells interact, and under the influence of T_H2-derived IL-4, class switch recombination (CSR) is initiated in the B cell. DNA double-strand breaks are generated in the switch (S) region of Cμ and Cε by the activation-induced cytidine deaminase (AID). Sμ and Sε regions are merged together by the DNA repair machinery and subsequent excision of the intervening DNA as an episomal circle (Wesemann et al., 2011). CSR is completed by IgE isotype transcription (Figure 6). Another possible mechanism by which a B cell can switch its isotype under the influence of T_H2 cytokines is sequential class switching. In this process, the B cell switches first to IgG_4 (or to IgG_1 in mice) and then, subsequently, on to IgE. In the stage between the two switches, the B cell produces IgG_4 because the gene for Cγ4 is upstream from the gene for Cε. Sequential class switching is required for the production high-affinity IgE antibodies (Aalberse, Platts-Mills, & Rispens, 2016; van Zelm, 2014; Xiong, Dolpady, Wabl, Curotto de Lafaille, & Lafaille, 2012). It is also frequent in germinal center reactions in general and plays an important role in the maturation of antibody responses. Memory B cells presumably persist in the form of IgG^+, not IgE^+ B cells, which switch to IgE only upon reactivation.

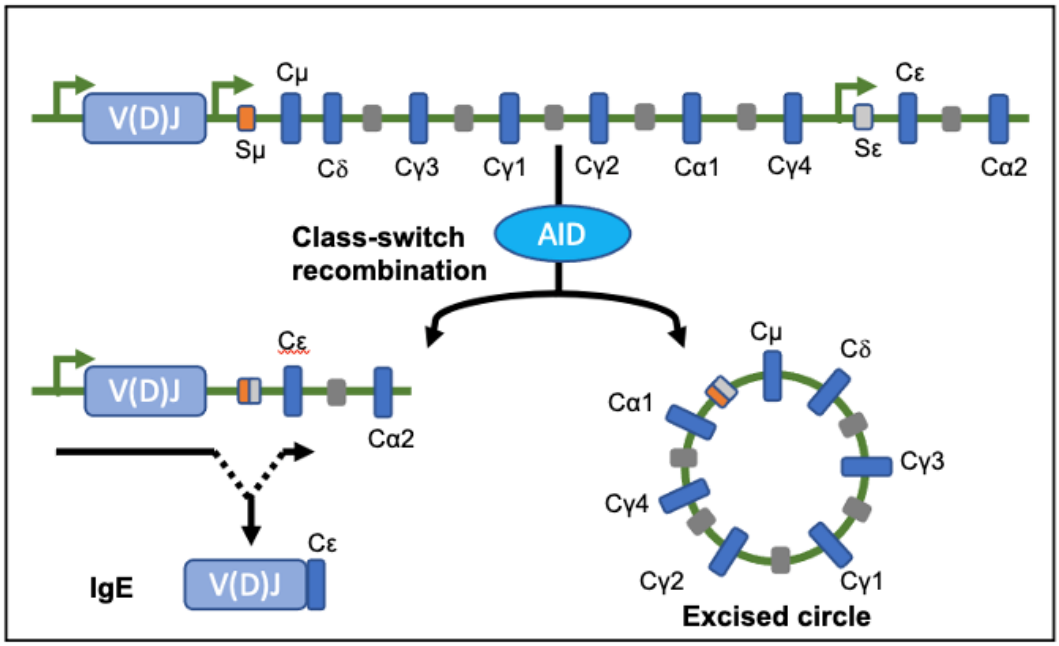


Figure 6: Overview of human immunoglobulin class switch recombination.

IL-4 is the main inducer of immunoglobulin E class switching (Junttila, 2018). A second signal, the activation of CD40 on the surface of the B cell, is required to produce IgE. The concentrations of IgE are very low compared to other antibodies. The half-life of free IgE is approximately 2 days, which is 10 times shorter than the half life of IgG (Kelly & Grayson, 2016). Once specific IgE is produced, it stimulates allergic reactions through binding to its high-affinity receptor, FcεRI on effector cells (e.g. mast cells). In contrast, IgG antibodies bind first to the antigen and then to the IgG receptors (FcγRs) on the effector cells, thus neutralizing the antigen or activating phagocytosis of IgG-opsonized microbes.

There is also a low affinity IgE receptor, FcεRII CD23, which has a unique structure among all antibody receptors. CD23 is a C-type (Ca^{2+}-dependent) lectin-like superfamily member. Trimeric or monomeric CD23 regulates IgE biosynthesis in B cells by positive or negative feedback mechanisms (Sutton, Davies, Bax, & Karagiannis, 2019).

The most abundant antibody isotype is immunoglobulin G (IgG), divided into four sub-isotypes (IgG_1, IgG_2, IgG_3 and IgG_4) with distinct effector functions. IgG subclasses 1-3 activate the classical complement cascade by binding to the C1q unit. IgG_4, which is induced by T_H2 cytokines, is expressed during some stages of allergic immune responses or under persistent allergen exposure. IgG_4 can neutralize allergens and thus reduce the interaction between allergens and specific IgE. IgG_4 binds to the FcγIIb and thus reduces allergic reactions by induction of IL-10 (Scott-Taylor, Axinia, Amin, & Pettengell, 2018). Therefore, IgG_4 levels are elevated after a successful AIT and serve as biomarker (Zissler & Schmidt-Weber, 2020). As mentioned above, IgE class switching mostly occurs as sequential switching via IgG_4

(Cameron et al., 2003). Thus IgG_4 reflects memory B cell precursors, which can easily switch on to IgE.

3.6 Cytokines and Chemokines

Several cytokines and chemokines are involved in allergic diseases. IL-4, IL-13 and IL-5 are the main T_H2 cytokines. Apart from these signature T_H2 cytokines, there are cytokines and chemokines, which also contribute to allergic immune responses.

IL-1ß promotes neutrophil chemotaxis upon LPS stimulation (Moore & Kunkel, 2019). Its secretion depends on inflammasome activation. Therefore, IL-1ß has an important role in the pathogenesis of allergic diseases. IL-1ß and IL-18 can support T_H2 responses by mediating inflammation and APC activation in the absence of IL-12 (Xu et al., 2000). It was shown in a multivariate analysis that IL-1ß correlates to the severity of persistent allergic rhinitis (Han, Kim, Oh, Kim, & Lee, 2019).

Cytokines such as IL-1, but also allergen exposure induces IL8/CXCL8. This is a pro-inflammatory chemokine which can be produced by tissue- and immune cells and which is involved in early host defense by activating neutrophils. Nasal challenges with IL-8 induced neutrophil influx in allergic patients (Bochenska-Marciniak, Kupczyk, Gorski, & Kuna, 2003). Moreover, IL-8 recruits NK-, T cells, basophils and eosinophils. IL-8 levels are increased in patients with psoriasis, asthma and chronic obstructive pulmonary diseases (COPD) (M. Akdis et al., 2016).

IL-33 controls the initial steps toward T_H2 responses (Hammad & Lambrecht, 2015). IL-33 plays an important role in ILC2 induction. IL-33 also amplifies T_H2-cell responses, but is not directly capable of differentiating T_H2 cells (Kurowska-Stolarska et al., 2008). The major source of IL-33 are epithelial cells, which constitutively express IL-33 as a “alarmin” (Imai, 2019). Additionally, IL-33 accumulates inside the cells and it is released after allergen challenges or cell death (Komai-Koma et al., 2012). The receptor for IL-33 is made up of two membrane-bound polypeptides, IL-1Rα and ST2. The IL-33:ST2 axis is related to the initial phase of allergic diseases. After the interaction of IL-33 with the receptor, an intracellular signaling cascade (Figure 7) is initiated consisting in the activation of MyD88, recruitment of IRAKs and TRAF6, activation of mitogen-activated protein kinases (MAPK) and activation of the transcription factor nuclear factor-κB (NF-κB) and activator protein (AP)-1, which initiate the expression of several proinflammatory cytokines (Takatori, Makita, Ito, Matsuki, & Nakajima, 2018). ST2 can be shed from the cell surface and in its soluble form ST2 inhibits IL-33 function by sequestering IL-33.

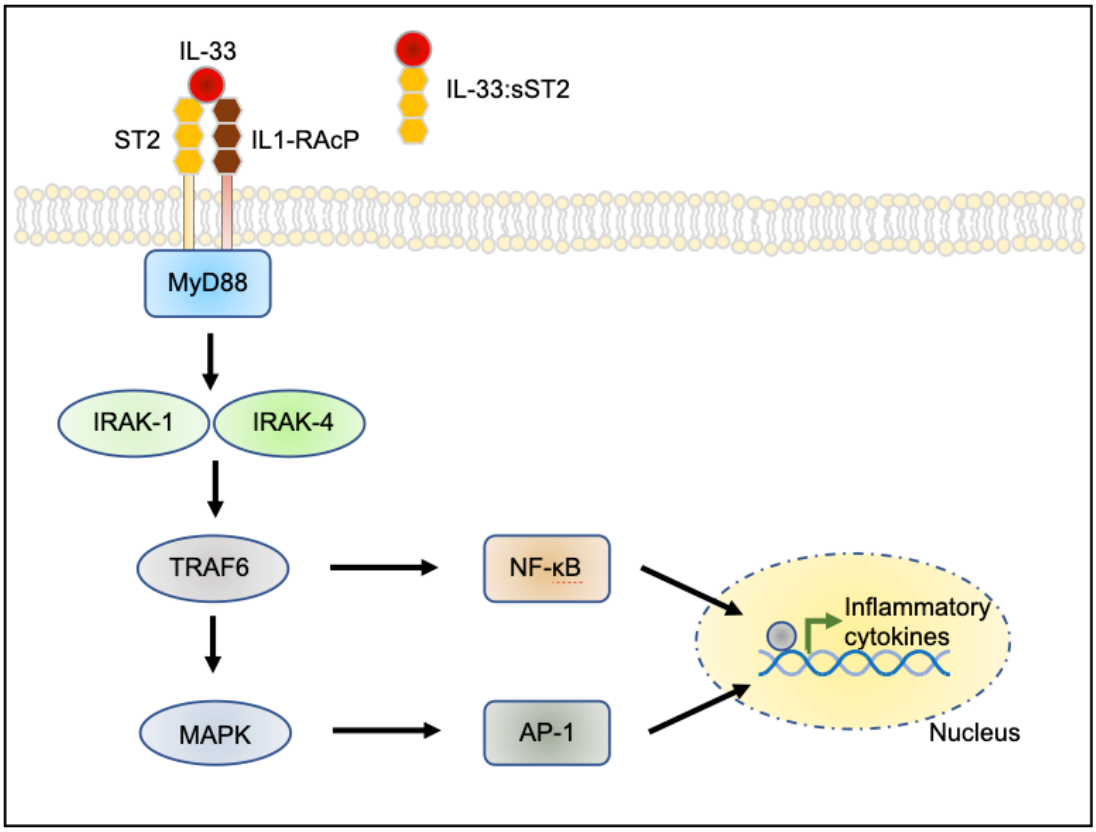


Figure 7: Intracellular signaling cascade by the interaction of IL-33 and ST2.

Another chemokine involved in allergic rhinitis is IL-16, which generally attracts T cells, IL-16 levels were shown to be significantly correlated to eosinophil numbers in peripheral blood of allergic rhinitis patients (Karaki et al., 2005).

Macrophage Chemotactic Protein-1 (MCP-1/CCL2) regulates allergic airway inflammation by stimulating IL-4 expression. It has been shown that MCP-1 deficient mice are deficient in inducing T_H2 cytokines (Gu et al., 2000), and elevated MCP-1 levels have been found in nasal secretions of SAR patients within the pollen season (Kuna, Lazarovich, & Kaplan, 1996).

Macrophage-derived chemokine (MDC/CCL22) recruits T_H2 cells, and its expression is controlled by T_H2 (IL-4, IL-5) and T_H1 (IFN-γ) cytokines. MDC is a strong chemoattractant for eosinophils. This was, among other studies, also shown in animal experiments, in which eosinophils migrated in a dose and time dependent manner towards an MDC gradient (Pinho et al., 2003).

Macrophage inflammatory protein 1β (MIP-1ß/CCL4) binds to CCR5, which mediates eosinophil chemotaxis (Kobayashi et al., 2019). On the other hand, in gene expression profiling experiments, MIP-1ß was identified to be involved in T_H1 immune responses (Dorner et al., 2002).

3.7 Birch pollen and Bet v 1 – the major birch pollen allergen

Pollen of the Betulaceae family are major causative agents of seasonal allergic rhinitis (Asam et al., 2014).

Betula verrucosa (Bet v) 1 with a molecular weight of 17 kDa belongs to the pathogenesis-related (PR)-10 protein family (Ciprandi et al., 2019). PR-10 proteins are involved in plant host defense mechanisms and are highly expressed in response to danger signals (Kofler et al., 2012) or abiotic stress (Liu & Ekramoddoullah, 2006). Apart from birch, also other tree pollen such as alder, hazel, hornbeam and oak, as well as many food plants, express PR-10 proteins which are homologous to Bet v 1. Sensitization to Bet v 1 thus often leads to IgE cross-reactivity with other plant allergens (Table 1).

Table 1: List of the Bet v 1 homologues allergens (http://www.meduniwien.ac.at; 06.03.2021).

Allergen	Source	Exposure
Aln g 1	Alnus glutinosa (European alder)	Inhalation
Bet pl PR-10	Betula platyphylla (Asian white birch)	Inhalation
Bet v 1	Betula verrucosa (Betula pendula)(European white birch)	Inhalation
Car b 1	Carpinus betulus (European hornbeam)	Inhalation
Cas s 1	Castanea sativa (Sweet chestnut)	Inhalation
Cor a 1	Corylus avellana (European hazel)	Ingestion; Inhalation
Fag s 1	Fagus sylvatica (European beech)	Inhalation
Ost c 1	Ostrya carpinifolia (European hop hornbeam)	Inhalation
Que a 1	Quercus alba (White oak)	Inhalation
Act c 8	Actinidia chinensis (Gold kiwi fruit)	Ingestion
Act d 11	Actinidia deliciosa (Kiwi fruit)	Ingestion
Act d 8	Actinidia deliciosa (Kiwi fruit)	Ingestion
Api g 1	Apium graveolens (Celery)	Ingestion
Ara h 8	Arachis hypogaea (Peanut)	Ingestion
Dau c 1	Daucus carota (Carrot)	Ingestion
Fra a 1	Fragaria ananassa (Strawberry)	Ingestion
Gly m 4	Glycine max (Soybean)	Ingestion
Jug r 5	Juglans regia (English walnut)	Ingestion
Mal d 1	Malus domestica (Apple)	Ingestion
Mor a PR-10	Morus alba (White mulberry)	Ingestion
Mor b PR-10	Morus bombycis (Chinese mulberry)	Ingestion
Pru ar 1	Prunus armeniaca (Apricot)	Ingestion
Pru av 1	Prunus avium (Sweet cherry)	Ingestion
Pru p 1	Prunus persica (Peach)	Ingestion
Pyr c 1	Pyrus communis (Pear)	Ingestion
Rub i 1	Rubus idaeus (Red raspberry)	Ingestion
Sola l 4	Solanum lycopersicum (Tomato)	Ingestion
Vig r 1	Vigna radiata (Mung bean)	Ingestion

Allergen	Source	Exposure
Vig r 6	Vigna radiata (Mung bean)	Ingestion

Bet v 1 is biochemically and immunologically well characterized. Its crystal structure reveals the ability to bind to different ligands such as fatty acids, flavonoids, cytokinin and quercetin-3-O-sophoroside (Kofler et al., 2012; Mogensen, Wimmer, Larsen, Spangfort, & Otzen, 2002; Seutter von Loetzen et al., 2014). Bet v 1 alone does not activate dendritic cells or lead to sensitization (Aglas et al., 2018). In contrast, aqueous birch pollen extracts containing Bet v 1, other proteins and over 10,000 low molecular weight (<3kDa) components, among them the pollen-associated lipid mediators (PALMs) and adenosine, are able to induce T_H2 cell polarization (Gilles et al., 2011; Gilles et al., 2010; Gilles-Stein et al., 2016; Mariani et al., 2007; C. Traidl-Hoffmann et al., 2005) and T_H2 sensitization *in vivo* (Aglas et al., 2018), which Bet v 1 alone does not. Other substances released from pollen grains, such as proteases, can degrade tight junction proteins (Runswick, Mitchell, Davies, Robinson, & Garrod, 2007), which makes allergens more accessible to subepithelial cells, and NADPH oxidases (Dharajiya, Bacsi, Boldogh, & Sur, 2007) that induce oxidant stress in the tissue, leading to inflammatory cell recruitment.

Beyond that, elevated concentrations of certain air pollutants like ozone lead to increased pollen allergenicity by influencing the Bet v 1 content (Beck et al., 2013). There are recent studies about the role of anthropogenic climate change on pollen allergenicity and pollen season (F. Kolek et al., 2021; Kolek, Plaza, Charalampopoulos, Traidl-Hoffmann, & Damialis, 2021). Comparing the time from 1990 to 2018, the overall pollen season periods have extended up to 20 days and pollen concentrations have increased up to 21% (Anderegg et al., 2021).

Studies on specific IgE reactivity underline the clinical impact of Bet v 1 sensitization in comparison to sensitization to other birch pollen allergens. According to a study, Bet v 1 sensitization in birch pollen allergic adults and children varies between 62-98% across Europe (Moverare et al., 2002).

3.8 Allergic rhinitis

More than 150 million Europeans suffer from allergic diseases induced by airborne allergen exposure (i.e. plant pollen, dust mite feces or fungal spores) (Bieber et al., 2016; Zuberbier, Lotvall, Simoens, Subramanian, & Church, 2014). The direct and indirect societal costs of allergic diseases are huge. Allergic rhinitis (AR) is among the most common chronic diseases, affecting about 23% of the European population (Bauchau & Durham, 2004).

AR is characterized by the symptoms of sneezing, nasal itching, rhinorrhea and nasal congestion. The disease is driven by allergen-specific T_H2 cells and IgE-mediated immediate-type HR to airborne allergens.

The pathophysiologic mechanism differentiates AR from non-allergic rhinitis (NAR). However, individuals with AR symptoms but negative specific serum IgE and skin prick test (SPT) often have sIgE in nasal fluid and are called local allergic rhinitis (LAR) patients (Rondon et al., 2018). Mast cell-derived mediators such as histamine are the main contributors to allergic rhinitis symptoms (Akhouri & House, 2021). Asthma often co-occurs in AR patients, and untreated, persistent or chronic AR can lead to the development of allergic asthma with the risk of exacerbations and fatal attacks (Eyerich, Metz, Bossios, & Eyerich, 2019).

Based on the allergen exposure time, AR patients are divided into seasonal or perennial AR patients (Akhouri & House, 2021). The most common causative of seasonal allergic rhinitis (SAR) is plant pollen.

AR, like other atopy-spectrum diseases, is a multifactorial disease, the susceptibility to which is governed by life-style, behavioral, nutritional and genetic factors. Mainly in childhood, a farming environment with contact to stable animals, high diversity of environmental microbes, highly diverse, natural diet rich in plant fibers and early-life contact to siblings and pets are recommended as protective factors (Sandini et al., 2011). In the International Study of Asthma and Allergies in Childhood (ISAAC), behavioral and life-style associated risk factors, such as antibiotic use, air pollution, pregnant smoking and lack of physical exercise are found to be important (Bousquet, Anto, et al., 2020). Depending on the severity of the disease, SAR patients can have an impaired quality of life including daytime sleepiness, anxiety disorders, depression, fatigue and reduced social interactions (Dass et al., 2017; Harter et al., 2019; Stuck et al., 2004).

The diagnosis of AR is based on the clinical history of allergic symptoms, a nasal examination and laboratory tests. Nasal allergen provocation tests are the “gold standard” in the diagnostic of AR (Bousquet, Anto, et al., 2020). Skin prick tests remain a common diagnostic method, even though they are invasive and may lead to incorrect diagnoses due to poorly characterized allergen extracts (Jensen-Jarolim, Jensen, & Canonica, 2017).

Sensitization to allergens can be analyzed also by blood test by detection of allergen-specific IgE antibodies. The new component-resolved methods, e.g. Immuno Solid-phase Allergen Chip (ISAC), allows analyzing for specific IgE to multiple allergens in a single drop of blood (Matricardi et al., 2016). After diagnosis, allergic patients should be treated according to the guidelines. A cross-sectional study in Bavaria reported that over 30% of allergies were not treated (Boehmer et al., 2018).

Treatment should not be limited to pharmacotherapy, e.g. with anti-histamines or corticosteroids (Bousquet, Pfaar, et al., 2019). Instead, the recommended treatment of AR according to the guidelines is allergen-specific immunotherapy (AIT), preferably early in life (BousquetSchunemann, et al., 2019; Halken et al., 2017). In Germany, only every tenth person is treated properly and only 7% of allergic rhinitis patients receive causative treatment (Claudia Traidl-Hoffmann, 2020).

AIT is a tolerance-inducing treatment administered by subcutaneous (SCIT) or sublingual (SLIT) route. Hereby, allergen-specific T_{reg} cells are induced to form an immunosuppressive milieu via secretion of IL-10, IL-35 and TGF-ß and expression of inhibitory surface costimulatory molecules, CTLA-4 and PD-1. T_{regs} induce B cells to express more IgG_4 and less IgE (Alvaro-Lozano et al., 2020).

Allergic patients are facing new challenges. Coronavirus diseases 2019 (COVID-19), induced by Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2), has spread in a global pandemic. COVID-19 infected individuals with allergic rhinitis should continue intranasal corticosteroids (Bousquet, Akdis, et al., 2020). According to current knowledge, SCIT and SLIT can be continued in asymptomatic allergic patients with negative SARS-CoV-2 test results (Klimek et al., 2020).

4. Aim of the study

The main causative of seasonal allergic rhinitis (SAR) is plant pollen. Pollen of grasses (Poaceae family) (Garcia-Mozo, 2017), birch and alder (Betulaceae) as well as hazel (Corylaceae family) (Bergmann, Heinrich, & Niemann, 2016; Panzner, Vachova, Vitovcova, Brodska, & Vlas, 2014; Stemeseder et al., 2017) are the most relevant allergenic pollen types in temperate climates of the Northern hemisphere. Most of the birch pollen allergic patients have co-sensitizations to pollen of hazel and alder (Biedermann et al., 2019; De Knop et al., 2011). Their major allergens all belong to the pathogenesis-related-10 (PR-10) protein family, which show high inter-species homology. The tree pollen season depends on temperatures and lasts typically from February to May. In experimental and natural exposure studies (A. Damialis, Traidl-Hoffmann, C., Teudler, R., 2019), a positive correlation between airborne pollen exposure and allergic symptoms in sensitized subjects has been already shown. However, due to the variable dynamics of the pollen seasons, symptom prediction in patients is complex, especially for those sensitized to birch, alder and hazel pollen (A. Damialis, Häring, F., Brunner, J., Buters, J., Traidl-Hoffmann, C., 2016). Furthermore, pollen seasons of different allergenic pollen types can be overlapping.

Local immune responses are more dynamic than the systemic immune responses to airborne pollen (Gokkaya, Damialis, et al., 2020). There is no clear information about the relationship between the complex exposure to different allergic pollen types and the kinetics of the nasal immune response, and how this correlates to allergic symptoms. Symptom severity of each individual may differ over the time of exposure because of lag-effects due to a delayed immunological response or even possible negative feedback mechanisms.

Not only allergic patients, but also non-allergic individuals are exposed to pollen. In non-allergic individuals, pollen exposure may lead to local, unspecific immune responses, e.g. by the expression of granulocyte-chemotactic factors like IL-8 and C-X-C motif chemokine-(CXC) 2 (Mattila et al., 2010). There is still an open question about which factors protect or predict non-allergic individuals from developing pollen allergies. The scientific gap motivated us to design a natural pollen exposure panel study to identify nasal biomarkers for in-season symptom severity.

In this panel study seasonal allergic rhinitis (SAR) patients and non-allergic (NA) control subjects were monitored over the course of one year. Immune parameters in serum as well as in nasal fluid were analyzed and seasonal differences under real-life exposure were studied by multivariate exploratory methods.

A precise allergy diagnosis is the first step of a successful causative treatment. To date, allergy diagnostics is routinely performed by blood test for the detection of allergen-specific immunoglobulin E (sIgE) or by skin prick test. Nowadays, molecular allergen diagnostic tests,

such as Immuno Solid-phase Allergen Chip (ISAC), are becoming more widely used for blood testing in clinical diagnostics. All these tests are invasive. Thus, in a further study, adult volunteers with allergic sensitization to at least one aeroallergen were recruited. Allergen specific IgE in serum and nasal secretion were studied to test for applicability of nasal secretion sIgE profiling as novel, non-invasive means of allergy diagnostic.

The results of this dissertation are based on two previously published first author articles in peer-reviewed journals (Gokkaya, Damialis, et al., 2020; Gokkaya, Schwierzeck, et al., 2020).

5. Materials and Methods

5.1 Materials

Table 2: List of buffers

Puffer	Ingredient	Mass	Unit
10x TBS	Tris-Base	24	g
	NaCl	87.66	g
	Adjust pH to 7.6		
	Adjust final volume ddH_20	<1000	mL
ELISA - Washing Buffer	10x TBS	100	mL
	Tween 20	5	mL
	Adjust final volume with ddH_20	<1000	mL
ELISA - Blocking Buffer	10x TBS	20	mL
	Tween 20	1	mL
	BSA	6	g
	Adjust final volume with ddH_20	<200	mL
ELISA - Dilution Buffer	10x TBS	20	mL
	Tween 20	1	mL
	BSA	1	g
	Adjust final volume with ddH_20	<200	mL
ELISA - Coating Buffer	NaHCO3	4.2	g
	Adjust to final volume with ddH_20	<1000	mL
	Adjust pH to 9.5		
ELISA - Bet v 1	Bet v 1a	1	mg
	Ampuwa	100	µl
	Solve it on shaker for 2h aliquot á 5µl		
ELISA - Coating Solution Bet v 1	ELISA - Coating Buffer	5	mL
	ELISA - Bet v 1	5	µl
Freezing Media	DMEM F12	250	mL
	DMSO	50	mL
	FCS	200	mL
2% Natriumazid	NaN_3	1	g
	D-PBS w/o Ca/Mg	50	mL
	Mix and filter through 0.22µm Filter		
FACS - Buffer	D-PBS w/o Ca/Mg	500	mL
	FCS - heat inactivated	25	mL
	EDTA (0.5 M, pH 8)	2	mL
	Na-Azid (2%)	5	mL
Cell thawing Media	RPMI 1640 (+L-Glutamine)	500	mL
	FCS	50	mL

Table 3: Mix for cDNA synthesis

Components	Volume
5x iScript reaction mix	4 µl
iScript Reverse Transcriptase	1 µl
RNA template	15 µl

Table 4: Thermal cycler protocol for cDNA synthesis

Steps	Time	Temperatur
Priming	5 min	25°C
Reverse Transcriptase	20 min	46°C
Reverse Transcriptase inactivation	1 min	95°C

Table 5: Primers for quantitative Real-Time PCR

Human	Forward primer 5'→ 3'	Reverse primer 5'→ 3'
PIGR	AGG TGC TAG ACT CTG GTT TTC GG	TCT GCT CCC ATC GGC TTG A
GAPDH	GAA GGT GAA GGT CGG AGT	GAA GAT GGT GAT GGG ATT
18S	CGT CTG CCC TAT CAA CTT TC	TTT TCG TCA CTA CCT CCC C

Table 6: Mix for quantitative Real-Time PCR

Components	Volume
2x iTaq SYBR Green Supermix	5µl
Primer	1.6 µl
cDNA template (5 ng)	3,4 µl

Table 7: Thermal cycler protocol for quantitative Real-Time PCR

Step	Time	Temperatur	Cycles
1. Activation	30 seconds	95°C	1x
2. Denaturation	5 sec	95°C	40x
3. Annealing / extension	30 seconds	60°C	

Table 8: List of reagents, consumables and instruments

Type	Name	Supplier
Reagent	2-Mercapto-Ethanol	Sigma-Aldrich, München, Germany
Instrument	Absorbance Microplate reader Sunrise™	Tecan, Männedorf, Switzerland

Type	Name	Supplier
Consumable	Absorbent filter paper	Pall GmbH, Dreieich, Germany
Reagent	AKP Goat Anti-human IgA	Sigma-Aldrich, Saint Louis, MO, USA
Reagent	AKP Mouse Anti-human IgE	BD PharmingenTM, Heidelberg, Germany
Reagent	AKP Mouse Anti-human IgG4	BD PharmingenTM, Heidelberg, Germany
Reagent	Albumin from bovine serum (BSA)	Sigma-Aldrich, München, Germany
Reagent	Alkaline Phosphatase Yellow Liquid Substrat	Sigma-Aldrich Chemie, Steinheim, Germany
Reagent	Ampuwa Aqua ad injectabilia	Laboratori Diaco Biomedicali, Trieste, Italy
Instrument	autoMACS Pro Separator	Miltenyi Biotec, Bergisch Gladbach, Germany
Reagent	autoMACS rinsing solution	Miltenyi Biotec, Bergisch Gladbach, Germany
Reagent	autoMACS running buffer	Miltenyi Biotec, Bergisch Gladbach, Germany
Consumable	BD FACSFlow™ 20L	BD Biosciences, Erembodegem, Belgium
Reagent	Bet v 1a (Bet v1.0101 EMBL: X15877 / Swissport: P15494)	AG Lorenz, Salzburg, Austria
Reagent	Bio-Plex Pro™ Human 9-Plex customized	Bio-Rad Laboratories, Hercules, CA USA
Reagent	Bio-Plex Pro™ Human Cytokine Standard 27-Plex, Group I	Bio-Rad Laboratories, Hercules, CA USA
Reagent	Bio-Plex Pro™ Human IgE Isotyping Assay	Bio-Rad Laboratories, München, Germany
Reagent	Bio-Plex Pro™ Human Isotyping Panel	Bio-Rad, München, Germany
Reagent	Bio-Plex Pro™ Human Th17 Cytokine 16-plex Standards	Bio-Rad Laboratories, Hercules, CA USA
Instrument	Bio-Plex Pro™ Wash Station Washer	Luminex Corporation, Austin, TX USA
Consumable	Bio-plex Sheath Fluid	Bio-Rad Laboratories, Newark, DE USA
Instrument	Bio-Plex® 200 system array reader	Bio-Rad Laboratories, Hercules, CA USA
Reagent	Bio-Plex® Calibration Kit	Bio-Rad Laboratories, München, Germany
Reagent	Bio-Plex® Validation Kit 4.0	Bio-Rad Laboratories, München, Germany
Instrument	BioDrop	BioDrop UK Ltd, Cambridge, U.K.
Reagent	Bovine Serum Albumin (BSA)	Carl Roth, Karlsruhe, Germany
Consumable	Butterfly needles	Dahlhausen, Köln, Germany
Reagent	CD127-PE-Cy7 (1:40)	BD Pharmingen™, Heidelberg, Germany
Reagent	CD14 FITC (1:50)	BD Biosciences, Heidelberg, Germany
Reagent	CD14 micro-beads (human)	Miltenyi Biotec, Bergisch Gladbach, Germany
Reagent	CD16-APC-Vio770 (1:50)	Miltenyi Biotec, Bergisch Gladbach, Germany
Reagent	CD19-ECD (1:20)	Beckman Coulter, Brea, CA USA
Reagent	CD25-PE (1:5)	Beckman Coulter, Brea, CA USA
Reagent	CD3-APC-Cy7 (1:40)	BD Pharmingen™, Heidelberg, Germany
Reagent	CD4-BV421 (1:40)	BD, Franklin Lakes, NJ USA
Reagent	CD45 Per-CF594 (1:200)	BD Biosciences, Heidelberg, Germany
Reagent	CD45RA-FITC (1:10)	Beckman Coulter, Brea, CA USA
Reagent	CD66b-BV421 (1:40)	BD, Franklin Lakes, NJ USA

Type	Name	Supplier
Reagent	CD8-PerCP (1:10)	BD Biosciences, Heidelberg, Germany
Consumable	CellClean®	Sysmex Europe GmbH, Nordstedt, Germany
Consumable	Cellpack®	Sysmex Europe GmbH, Nordstedt, Germany
Instrument	Centrifuge 5418	Eppendorf, Hamburg, Germany
Instrument	Centrifuge 5810	Eppendorf, Hamburg, Germany
Instrument	CFX384 Touch™ Real-time PCR Detection System	Bio-Rad, München, Germany
Instrument	CoolCell™ LX Freezing Container	BioCision, Mill Valley, CA USA
Consumable	Costar® Assay Plate, half area	Corning Inc., Corning, USA
Consumable	Costar® Spin-X®	Corning Inc., Corning, USA
Consumable	Cryo Pure 1.8 ml	Sarstedt, Nümbrecht, Germany
Reagent	CXCR5-APC (1:20)	BioLegend, San Diego, CA USA
Consumable	CytoFLEX Daily QC Fluoropheres	Beckman Coulter, Brea, CA USA
Instrument	Cytoflex LS Flow Cytometer Platform	Beckman Coulter, Brea, CA USA
Consumable	CytoFlex Sheath Fluid	Beckman Coulter, Brea, CA USA
Reagent	D-PBS w/o Ca/Mg	Life technologies, Carlsbad, CA USA
Reagent	DEPC treated water (pyrogen free)	Life technologies, Carlsbad, CA USA
Reagent	DMEM F12	Life technologies, Carlsbad, CA USA
Reagent	DMSO, cell culture grade	Applichem, Darmstadt, Germany
Reagent	DNase I	Qiagen, Hilden, Germany
Reagent	eBioscienceTM 1X RBC Lysis Buffer	Life technologies, Carlsbad, CA, U.S.A.
Reagent	EDTA (0,05 %, pH 8,0)	Life technologies, Carlsbad, CA USA
Consumable	EDTA-Monovettes	Sarstedt, Nümbrecht, Germany
Reagent	Ethanol absolute	Merck, Darmstadt, Germany
Consumable	FACS tubes	Thermo Fisher Scientific, Schwerte, Germany
Reagent	FcR-blocking Reagent, human	Miltenyi Biotec, Bergisch Gladbach, Germany
Reagent	Fetal calf serum (FCS) Hyclone™	Thermo Fisher Scientific, Schwerte, Germany
Consumable	Flowclean Cleaning Agent	Beckman Coulter Ireland, Clare, Ireland
Reagent	Heparin-Natrium 250.000 U	Ratiopharm, Ulm, Germany
Instrument	Hirst-type volumetric traps	Burkard, Hertfordshire, England
Reagent	Histopaque® 1077	Sigma-Aldrich, Saint Louis, MO, USA
Reagent	Histopaque® 1119	Sigma-Aldrich, Saint Louis, MO, USA
Reagent	Human IgA, Plasma	EMD Biosciences, San Diego, CA, USA
Reagent	Human IgG, Plasma	EMD Biosciences, San Diego, CA, USA
Reagent	ImmunoCAP ISAC 112	Thermo Fischer Scientific, Uppsala, Sweden
Reagent	ImmunoCAP Specific IgE Control M, t3	Thermo Fischer Scientific, Uppsala, Sweden
Instrument	Incubator	Binder, Tuttlingen, Germany
Consumable	Injekt® 10 mL	Braun, Melsungen, Germany
Reagent	iScript™ cDNA Synthesis Kit	Bio-Rad Laboratories, München, Germany

Type	Name	Supplier
Consumable	IsoFlow sheath Fluid (Beckman Coulter)	Beckman Coulter, Brea, CA USA
Reagent	iTaq™ Universal SYBR® Green Supermix	Bio-Rad Laboratories, München, Germany
Reagent	Live/Dead™ Fixable Aqua Dead Cell Stain Kit (1:1000)	Thermo Fisher Scientific, Schwerte, Germany
Instrument	LuxScan 10K-A	CapitalBio, Beijing, China
Reagent	Lymphoprep	Axis Shield, Oslo, Norway
Consumable	MACS Smartstrainer (100µm)	Miltenyi Biotec, Bergisch Gladbach, Germany
Instrument	Magnetic stirrer RCT basic	IKA Werke, Staufen, Germany
Consumable	Maxisorp plates (96 well)	Nunc, Roskilde, Denmark
Instrument	Micro scale Quintix 2102S	Sartorius, Göttingen, Germany
Instrument	Microplate washer 405 wash	BioTek, Winoosky, VER USA
Consumable	Microtubes 2 ml PP, sterile	Sarstedt, Nürnbrecht, Germany
Instrument	MoFlo AstriosEQ cell sorter	Beckman Coulter, Brea, CA USA
Reagent	Mouse Anti-human IgA/A_2	Sigma-Aldrich, Saint Louis, MO, USA
Reagent	Mouse Anti-human IgE	BD PharmingenTM, Heidelberg, Germany
Reagent	Mouse Anti-human IgG_4	BD PharmingenTM, Heidelberg, Germany
Instrument	Multichannel Pipettes	Sartorius, Göttingen, Germany
Reagent	Natriumazid (NaN_3)	Merck, Darmstadt, Germany
Instrument	Navios Flow Cytometer	Beckman Coulter, Brea, CA USA
Consumable	Optifit Tips	Sartorius, Göttingen, Germany
Reagent	p-nitrophenyl phosphate	Sigma-Aldrich, München, Germany
Consumable	PCR foil MicroAmp	Applied Biosystems (Life technologies) Carlsbad, CA USA
Consumable	PCR tubes	Eppendorf, Hamburg, Germany
Consumable	Perfusor syringes	Braun, Melsungen, Germany
Instrument	pH meter S210	Mettler-Toledo, Columbus, OH USA
Consumable	Pipettes (1, 5, 10, and 25 ml)	Greiner Bio-One, Frickenhausen, Germany
Instrument	Pipettes with diposable tips	Sartorius, Göttingen, Germany
Instrument	PoMo - Bioaerosol Analyzer BAA500	Hund, Wetzlar, Germany
Reagent	PromoFluor-840, maleimide	PromoCell GmbH, Heidelberg, Germany
Consumable	QIAschredder	Qiagen, Hilden, Germany
Consumable	qPCR plates 384 well I	Bio-Rad, München, Germany
Consumable	Rainbow Calibration Particles, 8 Peaks	Spherotech, Lake Forest, IL USA
Consumable	Reaction tubes (0.5; 1.5; 2 ml)	Eppendorf, Hamburg, Germany
Consumable	Reaction tubes (15 ml; 50 ml)	Sarstedt, Nürnbrecht, Germany
Consumable	Rhino-pro® Nasal Mucosal Curette	Arlington Scientific, Springville, CA USA
Reagent	RNA ProtectCell™	Qiagen, Hilden, Germany
Reagent	Rneasy Mini Kit (50)	Qiagen, Hilden, Germany
Consumable	RNeasy Mini Kit for RNA Isolation	Qiagen, Hilden, Germany

Type	Name	Supplier
Reagent	RPMI 1640 + L-Glutamine	Thermo Fisher Scientific, Schwerte, Germany
Consumable	Safety Space™ Filter Tips	Sartorius, Göttingen, Germany
Consumable	Sealing tape for 96-Well Plates	Thermo Fisher Scientific, Schwerte, Germany
Consumable	Serum S-Monovette®	Sarstedt, Nümbrecht, Germany
Reagent	Siglec 8-APC (1:20)	Miltenyi Biotec, Bergisch Gladbach, Germany
Reagent	Sodium chloride (NaCl)	Carl Roth, Karlsruhe, Germany
Reagent	Sodium hydrogen carbonate ($NaHCO_3$)	Merck, Darmstadt, Germany
Reagent	Sputolysin™	Merck, Darmstadt, Germany
Consumable	Sterile filter device (250 ml; 500 ml)	Sarstedt, Nürnbrecht, Germany
Reagent	Streptavidin-horseradish peroxidase	R&D GE Healthcare UK limited, Wiesbaden
Reagent	SuperBlock® Blocking Buffer in D-PBS	Pierce Biotechnology, Rockford, IL USA
Consumable	Syringe filter units (0.22; 0.45 µm)	Merck Millipore, Darmstadt, Germany
Instrument	Sysmex	Sysmex Corporation, Kobe, Japan
Instrument	Tecan Sunrise	Männedorf, Switzerland
Reagent	Tetramethylbenzidine (TMB)	Sigma-Aldrich, München, Germany
Instrument	Thermo Mixer C	Eppendorf, Hamburg, Germany
Reagent	Triton X	Sigma-Aldrich, München, Germany
Reagent	Trizma base	Sigma-Aldrich, München, Germany
Reagent	Trypanblue 0.4% solution	Life technologies, Carlsbad, CA USA
Reagent	Tween 20 detergent	Merck Millipore, Darmstadt, Germany
Consumable	URFP-30-2, Ultra Rainbow Fluorescent Particles	Spherotech, Lake Forest, IL USA
Instrument	Waterbath SW23	Julabo, Seelbach, Germany

5.2 Study design

5.2.1 Panel study

In the Augsburg region, adult, otherwise healthy allergic rhinitis patients and adult, healthy non-atopic control subjects were enrolled for the panel study. The study continued over the course of one year and consisted of a daily symptom diary and 15 biosampling visits from November 2015 to October 2016.

The study was approved by the local ethics committee (internal code: 19/15) and conformed to the guidelines of Helsinki. Written informed consent was obtained from all the study participants before inclusion.

The initial screening procedure consisted in a detailed anamnesis on type, severity and seasonality of symptoms, relevant co-morbidities, medication intake, other known allergies and past immunotherapies. Candidates with traveling plans during the birch pollen season, with perennial allergies (e.g. house dust mite) and with recent immunotherapies were excluded in advance. Suitable candidates underwent serum IgE test and skin prick test to confirm allergic sensitization. A thorough ENT examination was also done to exclude perennial allergic rhinitis, nasal polyps or chronic rhinosinusitis.

All included study participants were asked to keep a daily symptom diary by means of an online questionnaire (“Pollen App”; Stiftung Deutscher Polleninformationsdienst, Berlin/Germany, https://www.pollendiary.com/Phd/en/start). Whole blood, serum, nasal secretion and nasal curettages were taken at all 15 visits (Figure 8). Biosampling was repeated every 4 weeks (outside the pollen season) and every second week (during the main birch pollen season).

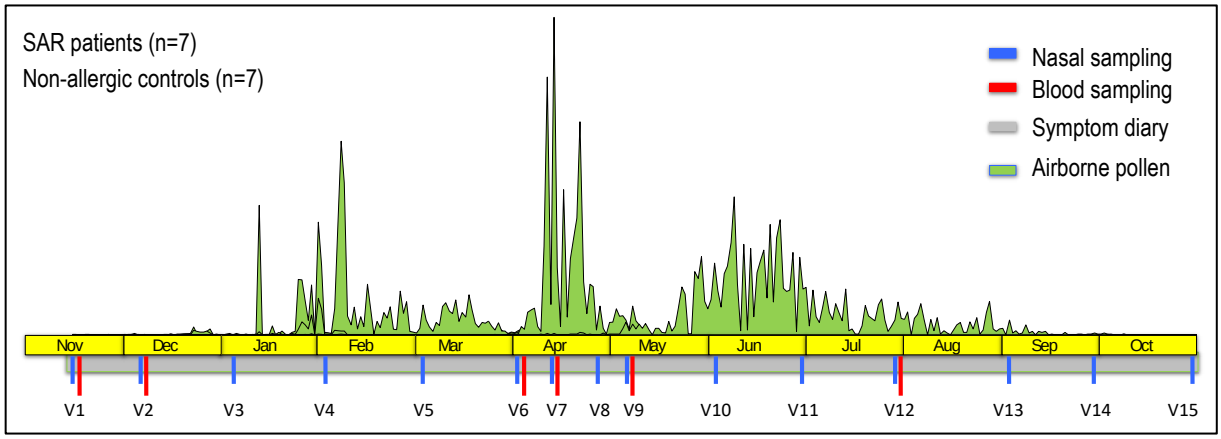


Figure 8: Timeline of the panel study

Initial cohorts of 8 seasonal allergic rhinitis (SAR) patients and 10 non-atopic (NA) subjects were included based on the screenings. One of the SAR patients had a positive SPT to birch and had described springtime allergic symptoms in the screening visit. But the patient was

asymptomatic during the birch pollen season of the study. A precise allergy diagnosis by ISAC revealed specific IgE to profilin and grass pollen, but not to Bet v 1 of PR10 group allergens. This patient was therefore excluded from the analysis.

After retrospective exclusion based on the consistency and reliability of participation (i.e. continuous presence at the study site and regular registering of symptoms, travelling during the main pollen season) 7 NA volunteers and 7 SAR subjects were kept for the analysis.

Total serum IgE levels of NA volunteers were 40.0 ± 18.1 IU/ml (mean ± SEM). NA subjects had no positive SPT to birch pollen and no aeroallergen sensitization. In contrast, all SAR patients had a positive SPT to birch pollen, total serum IgE levels of 62.5 ± 16.1 IU/ml (mean ± SEM), CAP class ≥ 1 to birch pollen, without co-sensitization against HDM (perennial allergens). The age was 35.4 ± 3.3 years (mean ± SEM). Grass pollen sensitizations were present in (3 out of 7 individuals) SAR patients (Table 9).

Table 9: Cohort characteristics of seasonal allergic rhinitis and non-atopic subjects. Serum total and specific IgE levels (IU/ml) were determined by ImmunoCAP. NA1-NA10: Non-allergic subjects. B1-B8: SAR subjects. Positive sensitization ≥0.35 IU/ml.

ID	Gender (m/f)	Age (years)	Total IgE (IU/ml)	Skin Prick Test	Birch	Hazel	Grass	HDM
B1	m	25	63.7	++++	1.3	1.0	2.1	0.2
B2	f	26	24.8	++	9.5	4.5	0.4	0
B4	f	53	29.0	+++	4.7	2.4	0	0
B5	f	54	71.2	+++	4.0	3.4	2.3	0
B6	a	25	52.4	++++	7.3	3.6	0.2	0
B7	f	39	37.2	+	5.5	3.9	0.1	0.1
B8	m	31	159.0	++++	41.6	29.0	4.8	0
NA1	f	36	46.8	Ø	0	0	0	0
NA3	f	56	7.4	Ø	0	0	0	0
NA5	m	29	5.6	Ø	0	0	0.1	0
NA6	f	21	37.8	Ø	0	0	0	0.1
NA8	f	51	17.9	Ø	0	0	0	0
NA9	f	27	12.2	Ø	0	0	0	0
NA10	f	23	152.0	Ø	0	0	0	0

Sensitization profiles of the study subjects were confirmed by an additional, component-resolved allergen diagnostic test (ImmunoCAP ISAC 112, Thermo Fisher Scientific, Uppsala, Sweden). Hereby, specific serum IgE to various airborne allergens was determined. According to the manufacturer, the cut-off for positivity of the test is 0.3. NA subjects had no specific IgE against Bet v 1, other PR-10 proteins or other pollen allergens (Table 10).

Table 10: Sensitization profile of the study participants by component resolved allergen diagnosis. Only positive results for 27 out of 112 allergens are shown.

Allergen	B1	B2	B4	B5	B6	B7	B8	NA1-NA3-NA6 - NA8-NA9-NA10	NA5
Act d 8	0.0	0.0	0.4	0.0	0.0	0.0	0.7	0	0
Aln g 1	0.0	1.1	1.3	0.0	2.1	1.3	5.1	0	0
Alt a 1	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0	0
Api g 1	0.0	0.0	0.0	0.0	0.9	0.0	0.9	0	0
Api m 1	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0	0
Ara h 8	0.0	1.4	0.8	0.7	3.4	0.6	4.0	0	0
Bet v 1	2.8	11.3	5.8	5.3	10.8	3.6	46.0	0	0
Cor a 1.0101	0.7	2.5	1.6	1.8	4.1	1.5	9.8	0	0
Cor a 1.0401	0.3	4.0	1.4	1.2	3.0	1.4	4.9	0	0
Cyn d 1	1.0	0.3	0.0	0.8	0.0	0.0	1.3	0	0
Equ c 1	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0	0
Fel d 1	0.0	0.0	0.0	2.6	1.3	0.0	0.0	0	0
Fel d 4	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0	0
Gly m 4	0.0	0.0	0.3	0.0	1.9	0.0	0.7	0	0
Hev b 5	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0	0
Hev b 8	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0	0
Jug r 3	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0	0
Mal d 1	0.6	2.6	1.1	0.8	2.5	1.0	15.3	0	0
Mer a 1	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0	0
MUXF3	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0	0
Ole e 1	0.3	0.0	0.0	0.0	0.0	1.5	3.9	0	0
Phl p 1	3.0	1.8	0.0	9.1	1.1	0.0	13.0	0	0
Phl p 11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.4
Phl p 5	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0	0
Pol d 5	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0	0
Pru p 1	0.0	2.9	1.5	0.3	4.8	1.4	5.6	0	0
Ves v 5	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0	0

5.2.2 ISAC study

The ISAC study was performed between September and October 2018, outside the main pollen season. Total of 49 adult subjects underwent an initial allergy diagnostic by ImmunoCAP to confirm eligibility for the study.

Serum and nasal fluid were collected once per subject. The ISAC Study was approved by the local ethics committee (internal code: 19/15 S) and conformed to the guidelines of Helsinki. Written informed consent was obtained from all the study participants before inclusion.

47 adult subjects with different allergic sensitization patterns, mainly to aeroallergens, and 2 non-sensitized control subjects were included. All 47 sensitized subjects had at least one aeroallergen sensitization, e.g. against birch-, hazel-, alder-, grass-pollen or HDM. The age of

the study participants was 39 ± 2.1 years (mean ± SEM) and had total serum IgE levels of 258.3 ± 66.3 IU/ml (mean ± SEM) (Table 11).

Table 11: Cohort characteristics of the study subjects. Serum total and specific IgE levels (IU/ml) were determined by ImmunoCAP (Phadia).

ID	Gender (f/m)	Age (years)	Total IgE (KU/l)	Birch	Alder	Hazel	Grass	HDM
01	f	56	6.1	0.1	0.1	0.1	0.6	0.1
02	f	80	160	5.5	4.1	5.4	1.0	2.2
03	f	31	2200	7.3	8.6	7.4	85.0	2.8
04	m	67	550	0.2	0.3	0.3	0.8	4.3
05	f	48	170	1.8	1.7	1.4	27.0	0.9
06	f	42	60	14.0	11.0	11.0	0.1	0.1
07	f	58	670	5.1	4.9	3.0	13.0	39.0
08	f	35	390	0.1	0.1	0.1	1.3	0.1
09	f	50	34	1.2	1.6	1.3	11.0	0.1
10	f	46	360	19.0	14.0	13.0	1.6	6.4
11	f	51	250	7.0	4.7	3.3	3.8	2.2
12	f	57	35	4.8	4.1	4.9	1.6	0.1
13	f	24	430	22.0	14.0	13.0	99.0	0.1
14	f	62	72	2.1	1.6	1.2	5.9	0.1
15	m	51	30	0.4	0.4	0.4	2.3	0.1
16	m	68	31	3.6	2.8	1.0	0.1	0.1
17	m	45	190	0.3	0.6	0.3	6.1	0.3
18	f	43	110	6.5	4.7	3.8	15.0	0.3
19	m	31	30	0.1	0.1	0.1	0.1	10.0
20	f	25	470	0.1	0.1	0.1	9.6	7.1
21	m	20	2500	0.3	0.2	0.2	65.0	0.3
22	m	23	230	0.1	0.1	0.1	3.3	35.0
23	f	20	93	0.6	0.7	0.6	28.0	0.1
24	f	21	210	23.0	17.0	20.0	40.0	1.2
25	f	22	80	0.1	0.1	0.1	29.0	0.4
26	m	41	25	0.1	0.1	0.1	0.5	0.1
27	m	27	76	0.1	0.1	0.1	3.2	11.0
28	m	29	180	0.1	0.1	0.1	4.2	27.0
29	f	26	480	9.3	6.7	9.0	6.6	23.0
30	f	22	70	0.3	0.2	0.4	4.8	5.6
31	f	58	240	16.0	15.0	8.9	17.0	5.8
32	f	36	20	8.0	5.2	4.0	0.1	0.1
33	m	25	11	0.1	0.1	0.1	0.5	1.3
34	f	24	34	0.1	0.1	0.1	1.5	6.3
35	f	60	81	0.2	0.2	0.2	12.0	0.1
36	f	27	570	1.4	0.1	0.4	28.0	0.2

ID	Gender (f/m)	Age (years)	Total IgE (KU/l)	Birch	Alder	Hazel	Grass	HDM
37	m	33	233	0.0	0.0	0.0	0.0	14.9
38	f	27	82	0.1	0.1	0.1	13.0	0.4
39	m	32	56	19.0	18.0	22.0	0.1	0.1
40	f	28	22	0.1	0.1	0.1	5.1	0.3
41	f	49	16	5.5	3.8	3.3	0.1	0.1
42	f	48	42	0.1	0.1	0.1	3.9	0.1
43	m	36	58	1.3	1.1	0.5	14.0	0.1
44	f	38	140	0.1	0.1	0.1	0.7	22.0
45	f	34	360	1.4	0.6	0.9	14.0	39.0
46	f	42	19	0.1	0.1	0.1	3.0	0.1
47	m	44	290	0.1	0.1	0.1	0.1	1.1
48	f	23	140	0.1	0.1	0.1	0.2	0.1
49	f	28	50	0.1	0.1	0.1	0.1	0.1

5.3 Airborne pollen monitoring and pollen season

Airborne birch pollen was measured at the Bayerisches Landesamt für Umwelt (LfU) in Augsburg. Pollen monitoring took place at ground level using an automatic Bioaerosol Analyzer BAA500 (Hund, Wetzlar, Germany). Briefly, air samples were acquired every 3 hours, impacted on a microscope slide with a sticky surface and automatically recorded with a CCD camera under a light microscope. The images were used to classify the pollen types by an image recognition algorithm (Oteros et al., 2015). Also, a conventional Hirst-type volumetric trap (Burkard, Hertfordshire, England) was used to measure the pollen. In this method, the pollen were classified and counted manually under the light microscope to validate the accuracy of the automated pollen monitoring. In both methods, daily pollen concentrations were calculated as the number of pollen grains per m^3 of air.

The main pollen season for each pollen type was set to be from 2.5% up to 97.5% of the cumulative pollen grains of the whole year (A. Damialis, Halley, Gioulekas, & Vokou, 2007). Depending on the sensitization profile, the pollen season was defined individually for each subject. The season of hazel, alder, birch and grass pollen in 2016 lasted from end of December to August. Visits V3 to V12 were defined as “in season” for NA controls and SAR patients sensitized to hazel, alder, birch and grasses. For SAR subjects sensitized only to hazel, alder, and birch but not to grass pollen, visits from V3 to V8 were defined as “in season”. For comparison of Bet v 1-specific immunoglobulins, only the V6 to V8 were defined as “in season”.

5.4 Monitoring of symptoms

Symptoms were entered daily via a pollen diary on a digital platform accessible via smartphone or computer (“Pollen App”; Stiftung Deutscher Polleninformationsdienst, Berlin/Germany, https://www.pollendiary.com/Phd/en/start). Nasal, ocular and pulmonary symptoms were recorded separately with the severity ranging from 0 to 3 (0: none, 1: mild, 2: moderate, 3: severe). Besides allergic symptoms, also general wellbeing from 1 to 10 (1: very good, 10: very bad), medication use and additional notes were recorded and used to calculate the total symptom and medication score as described previously (Bousquet et al., 2017).

5.5 Biosampling

5.5.1 Serum

Venous blood was obtained by venipuncture and drawn into a 5ml serum tube (Sarstedt, Nümbrecht, Germany). The coagulated blood was centrifuged at 1,000 x g for 10 minutes at room temperature. The serum was transferred into a clean 2mL tube and kept at -80°C until processing.

5.5.2 Nasal secretion

Nasal secretion was collected at all visits as described in (Gilles-Stein et al., 2016), with slight modifications. Briefly, a strip of absorbent filter paper (Pall GmbH, Dreieich, Germany) was unilaterally inserted into the inferior turbinate (between vestibulum and limen nasi) and held in place for 90 seconds. The filter paper strip was then removed and placed into the insert of a Costar Spin-X® (Corning Inc., Corning, USA). The nasal secretion was eluted by adding 100µl (Panel study) or 60µl (ISAC study) of double-distilled water to the strip of absorbent filter paper and subsequent centrifugation at 10,000 x g for 5 minutes at 4°C. Samples were stored at -80°C until analysis.

5.5.3 Nasal curettage

By using a nasal speculum and a disposable nasal mucosal curette (Arlington Scientific, Springville, CA USA), the mucosal surface of the nasal middle meatus was scraped. Curettages from one nostril were put into an Eppendorf tube containing 100 µl D-PBS (Life technologies, Carlsbad, CA USA) for the immune cell analysis via flow cytometry and kept on ice. Curettages from the other nostril were collected into an autoclaved Eppendorf tube containing 350µl RNA ProtectCell™ (Qiagen, Hilden, Germany) for genetic analysis and kept at -80°C until their analysis.

5.5.4 Isolation of PBMCs

100 mL of blood was drawn into heparinized perfusor syringes. The blood was diluted 1:1 in D-PBS without calcium and magnesium (Life technologies, Carlsbad, CA USA) and the diluted blood was pipetted very gently onto 10 mL separation medium lymphoprep (Axis Shield, Oslo, Norway).

After centrifugation without brake at 1,000 x g for 15 minutes at room temperature, the peripheral blood mononuclear cells (PBMCs) layer (between erythrocyte pellet and plasma) was transferred into a new tube and PBMCs were washed twice with D-PBS + 5mM EDTA and pooled (Life technologies, Carlsbad, CA USA). The pooled PBMCs were resuspended with D-PBS + 2mM EDTA and the total cell number was determined in a Neubauer chamber (BRAND GmbH + CO KG, Wertheim, Germany) or Sysmex (Sysmex Corporation, Kobe, Japan). After final centrifugation, the pellet was resuspended at $5x10^7$ cells/1.8 mL in freezing medium. After gradual cooling at -80°C, the cells were stored in liquid nitrogen until their analysis.

5.5.5 Isolation of PMNs

Polymorphonuclear (PMN) granulocytes were isolated from 20 mL EDTA whole blood. The blood was diluted in D-PBS 1:2. After pipetting 10 mL Histopaque 1119 (Sigma-Aldrich, Saint Louis, MO, USA) in a 50 mL tube, 10 mL Histopaque 1077 (Sigma-Aldrich, Saint Louis, MO, USA) were overlayed followed by diluted blood at the lowest speed using a serological pipette. The samples were centrifuged at 2200 rpm for 15 minutes at RT without break.

PBMC and granulocyte layers (PMN) transferred into two separate 50 mL tubes. Each sample was washed with D-PBS+5mM EDTA (Life technologies, Carlsbad, CA USA).

For the isolation of neutrophils, PMNs were incubated for 5 minutes at RT in red blood lysis buffer (Life technologies, Carlsbad, CA, U.S.A.). After stopping the reaction with an excess volume of D-PBS, cells were washed and total cell numbers determined in a Neubauer chamber.

For the monocyte isolation, PBMCs were resuspended in 10 mL MACS buffer and total cell number was determined in the Neubauer chamber. The cells were resuspended in 80µl of MACS buffer per 10^8 cells. CD14 Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) were added (30µL per 10^8 cells) and the cells incubated for 15 minutes at 4°C. After one wash step with MACS buffer, the pellet was resuspended in 500µl MACS buffer per 10^8 cells and labeled cells isolated with an AutoMACS Pro separator using the “Possel” program according to the manufacturer´s instruction. The $CD14^+$ monocytes were found in the positive fraction, the unlabelled PBMCs in the negative fraction.

5.6 Determination of specific IgE by ISAC

Specific IgE in serum or nasal secretion was analyzed by ImmunoCAP ISAC 112 according to the manufacturer´s instruction (Thermo Fischer Scientific, Uppsala, Sweden).

Briefly, once the ISAC chips were equilibrated with wash buffer, 30 µl of samples were pipetted on the biochip matrix, which is spotted with recombinant or native allergens. Specific IgE antibodies in the sample bind to their respective allergens. After a wash step, 30 µl of detection antibody was pipetted on the biochip. After a final wash step, the fluorescence intensity was determined by laser scanning using Luxscan™ 10K-A Microarray (CapitalBio, Beijing, China) and a calibration curve was calculated in ISAC standard units (ISU-E) by MIA and Xplain-Software (Phadia, Uppsala, Sweden).

5.7 Measurement of immune variables

Cytokines, chemokines and immunoglobulins were analyzed by Luminex multiplex assay which uses a mixture of color-coded beads, pre-coated with analyte specific capture antibodies. Total immunoglobulin isotypes IgE, IgA, IgM, IgG_1, IgG_2, IgG_3 and IgG_4 were detected in nasal secretion and serum of the panel study subjects. A customized panel, consisting of IL-33, CCL24/Eotaxin-2, CCL4/MIP-1β, CCL2/MCP-1, CCL22/MDC, CXCL8/IL-8, IL-16, G-CSF and IL-1β was used to analyze the nasal secretion samples. Free immunoglobulin light chains (FLC) were measured in nasal secretions by Enzyme-linked Immunosorbent Assay (ELISA) as previously published (Powe et al., 2010).

All multiplex magnetic bead assays were carried out according to the manufacturer´s instructions (Bio-Rad Laboratories, Hercules, CA USA) in a 96-well plate. Briefly, a serial dilution of standards was prepared. The optimal dilution of samples was determined in pre-experiments and varied for the analytes measured (Table 12).

Table 12: Dilution factor of samples according to the assay.

Single-/Multiplex	Nasal	Serum
Chemo-/Cytokine	1:8	-
Immunoglobulin panel	1:8	1:20.000
IgA	1:50	1:20.000
IgE	1:4	1:50

Plates were washed on an automated magnetic bead wash station (Luminex Corporation, Austin, TX USA). 50µl of diluted samples, standards, quality controls and blanks were pipetted into the plate. After 60 minutes incubation at room temperature, beads were washed and incubated with 25 µl of detection antibody. After incubation, beads were washed again and 50 µl of Streptavidin phycoerythrin (SA-PE)-labeled detection antibodies were added to form an

antibody-antigen sandwich. After the final wash step, the beads were resuspended in assay buffer and measured on the Bio-Plex 200 System (Bio-Rad Laboratories, Hercules, CA USA). Analysis of the measurement data was done using the Bio-Plex Manager 6.1 software (Bio-Rad Laboratories, Hercules, CA USA).

5.8 Measurement of Bet v 1-specific immunoglobulins

Bet v 1-specific immunoglobulins of the isotype IgE, IgG_4 and IgA were measured in serum and nasal fluid via ELISA as described previously (Guhsl et al., 2015). 96-well plates were coated with 1µg/ml recombinant Bet v 1(Aglas et al., 2018), in 50mM NaHCO at 4°C over night. Plates were washed in tris-buffered saline containing 0.5% tween-20 (TBS-T). For blocking, Superblock Blocking Buffer (Pierce Biotechnology, Rockford, IL USA) was used for IgE and IgG_4 ELISAs, and TBS-T + 3% BSA for IgA ELISA. After 2 hours blocking at room temperature, plates were washed and the samples diluted in TBS-T + 0.5% BSA according to the Table 13 and incubated at 4°C over night.

Table 13: Dilution factors for the ELISAs according to the initial establishment.

ELISA	Nasal NA /SAR	Serum NA	Serum SAR
Bet v 1 specific IgA	1:100	1:50	1:10
Bet v 1 specific IgG_4	1:20	1:10	1:300
Bet v 1 specific IgE	1:10	1:50	1:50

After incubation with the samples, the plates were washed and 50 µl/well of alkaline phosphatase conjugated anti-human IgE (1:750 - BD Pharmingen, Heidelberg, Germany), IgG_4 (1:500 - BD Pharmingen, Heidelberg, Germany), or IgA (1:10.000 - Sigma-Aldrich, St. Louis, MO USA) were added. After 2 hours at room temperature, the colour was detected after addition of 50 µl p-nitrophenyl phosphate (pNPP) (Sigma-Aldrich, München, Germany). Absorbance was measured at 405 nm on a microplate reader (Tecan Sunrise, Männedorf, Switzerland).

5.9 Flow cytometry analysis

Immune cells from nasal samples were analyzed by flow cytometry. Due to limited cell numbers in nasal samples, staining and gating protocols were established on PBMCs.

5.9.1 Nasal curettages

Nasal curettages were stained for neutrophils and monocytes. Samples were meshed through a 100µm MACS Smartstrainer (Miltenyi Biotec, Bergisch Gladbach, Germany) to

remove large debris and to get a single cell suspension. The cell strainer was washed to remove residual debris and cells were centrifuged for 10 minutes at 300 x g. The cell pellet was resuspended in 400µl FACS buffer and split into two wells of a 96-well U-bottom plate.

Cells were pelleted by centrifugation and the supernatant pipetted off. One of the cell pellets was stained with a Live/Dead™ Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific, Schwerte, Germany), whereas the second pellet was used as unstained control and resuspended in D-PBS. After 30 minutes incubation at 4°C in the dark, cells were washed and incubated in 10µl Fc-receptor-blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) for 10 minutes at room temperature. Subsequently, cells were mixed with 100µl antibody mix; mouse anti-human CD14 1:100 (BD Biosciences, Heidelberg, Germany), mouse anti-human CD16 1:50 (Miltenyi Biotec, Bergisch Gladbach, Germany), mouse anti-human CD45 1:750 (BD Biosciences, Heidelberg, Germany), and unstained cells with FACS-buffer, and incubated for 30 minutes at 4°C in the dark. The cells were centrifuged for a final wash step. The cells were then resuspended in 300µl FACS buffer and acquired on a Navios flow cytometer (Beckman Coulter, Brea, CA USA) according to the manufacturer´s instructions.

5.9.2 Nasal lavage

Nasal lavages from SAR patients and non-allergic control subjects were collected from March to November 2018. Briefly, each nostril were washed with 10 mL NaCl solution by using a 10 mL syringe and the lavage fluid were collected in a 50 mL tube. Nasal lavages were meshed through a cell strainer and pelleted by centrifugation at 300 x g for 10 minutes at 4°C. For FACS staining, the same protocol was used as described above, but with an extended antibody mix; mouse anti-human CD14 1:50 (BD Biosciences, Heidelberg, Germany), mouse anti-human CD16 1:50 (Miltenyi Biotec, Bergisch Gladbach, Germany), mouse anti-human CD45 1:200 (BD Biosciences, Heidelberg, Germany), mouse anti-human CD66b 1:40 BD, (Franklin Lakes, NJ USA), mouse anti-human Siglec-8 1:20 (Miltenyi Biotec, Bergisch Gladbach, Germany) to analyze neutrophils, monocytes and eosinophils. Cells were acquired on a Cytoflex LS flow cytometer (Beckman Coulter, Brea, CA USA) and analyzed in Kaluza® software (Beckman Coulter, Brea, CA USA).

5.9.3 Control staining for flow cytometry

Flow cytometry analysis is built upon the principle of gating. Fluorescence-minus-one (FMO) controls are run to differentiate between specific and background signal. Therefore, an aliquot of $CD14^+$ monocytes and $CD16^+$ neutrophils were mixed and incubated at 65°C for 10 minutes to obtain dead cells as positive control for the live/dead staining. Unstained and full stained cells and the FMO controls were prepared as described before for nasal lavage samples (Table 14), acquired on a Cytoflex LS flow cytometer (Beckman Coulter, Brea, CA

USA) according to the manufacturer´s instructions and analyzed in Kaluza® software (Beckman Coulter, Brea, CA USA).

Table 14: Antibody mix for the control staining.

	Live/Dead	PE-CF594	FITC	APC-Vio770
Unstained	-	-	-	-
Allstained	+	+	+	+
FMO Live/Dead	-	+	+	+
FMO CD45 - PE-CF594	+	-	+	+
FMO CD14 - FITC	+	+	-	+
FMO CD16 - APC-Vio770	+	+	+	-

5.10 Establishment of T-cell subsets sorting

$CD4^+$ subsets of T follicular helper cells (T_{fh}), regulatory T cells (T_{reg}) and conventional T cells (Tcon) were sorted on a MoFlo AstriosEQ cell sorter (Beckman Coulter, Brea, CA USA) according to the manufacturer´s instructions. Briefly, PBMCs were thawed and counted on a hemocytometer (Sysmex Corporation, Kobe, Japan). Each $1x10^6$ cells were stained with a live/dead stain (Thermo Fisher Scientific, Schwerte, Germany) followed by the mouse anti-human antibody mix (Table 15).

Table 15: Antibody mix for the T-cell subsets sorting

Antibody	Dyes	Dilution	Company
CD3	APC-Cy7	1:40	BD PharmingenTM, Heidelberg, Germany
CD19	ECD	1:20	Beckman Coulter, Brea, CA USA
CD4	BV421	1:40	BD, Franklin Lakes, NJ USA
CD8	PerCP	1:10	BD Biosciences, Heidelberg, Germany
CD45RA	FITC	1:10	Beckman Coulter, Brea, CA USA
CD25	PE	1:5	Beckman Coulter, Brea, CA USA
CD127	PE-Cy7	1:40	BD PharmingenTM, Heidelberg, Germany
CXCR5	APC	1:20	BioLegend, San Diego, CA USA

5.11 Gene expression analysis of polymeric immunoglobulin receptor

5.11.1 Total RNA Isolation

Total RNA was purified from nasal curettages using the RNeasy Mini Kit (Qiagen, Hilden, Germany) to determine gene expression levels of the polymeric immunoglobulin receptor (PIGR).

Briefly, the nasal curettage stored in RNA ProtectCell™ (Qiagen, Hilden, Germany) was thawed, centrifuged at 16,000 x g for 5 minutes at room temperature, and the supernatant was

discarded. The pellet was lysed by vortexing after adding 600µl RLT buffer (Qiagen, Hilden, Germany) + ß-mercaptoethanol (ME). The cell suspension was homogenized using QIAschredder (Qiagen, Hilden, Germany) to reduce viscosity. Next, 70% Ethanol (EtOH) was added to the homogenized lysate. After a wash step residual DNA was removed by DNase digestion. Several wash steps were done and total RNA was eluted in 30 µl nuclease free water. The total RNA concentration was determined on a BioDrop spectrophometer (BioDrop UK Ltd, Cambridge, UK).

5.11.2 Real-Time Polymerase Chain Reaction

The iScript cDNA synthesis Kit (Bio-Rad Laboratories, München, Germany) was used to convert RNA to cDNA. The optimal thermal cycler protocol (Table 4; materials) and the reaction mix (Table 3; materials) were tested beforehand and cDNA synthesis was performed according to the kit´s instruction manual.

Primers (Table 5; materials) were designed for the polymeric immunoglobulin receptor (PIGR) gene by using the Primer-Blast platform (Ye et al., 2012). GAPDH and 18S served as reference genes. The quantitative expression level of mRNA was determined on a Real-time PCR machine, CFX384 Touch™ (Bio-Rad, München, Germany) by using the qPCR iTaq™ Universal SYBR® Green kit. Reactions were run in triplicates. The thermal cycler protocol (Table 7; materials) was run with the reaction mix (Table 6; materials) according to the manufacturer´s instruction. A melt curve was generated to verify the single amplicons generated by RT-qPCR. The delta delta CT (ΔΔCT) method was applied to compare the relative gene expression levels in the different samples per patient. First, a delta-CT was calculated by subtracting the mean CT values of the reference genes from the CT value for the PIGR gene. This serves to normalize PIGR gene expression to the expression of the housekeeping genes. Next, the delta CT value of the pre-season visits (mean of visits 1 & 2) was subtracted from the delta-CT values of visits 3-15 to calculate the ΔΔCT. Finally, the fold gene expression ($2^{-\Delta\Delta CT}$) was calculated by the formula:.

$$\Delta Cq = Cq\ (PIGR) - Cq\ (mean(GAPDH, 18S))$$

$$\Delta\Delta Cq = \Delta Cq_{specific\ visit} - \Delta Cq_{mean(pre-season)}$$

$$Fold\ gene\ expression = 2^{-\Delta\Delta CT}$$

5.12 Statistical data analysis

Raw measurement data were entered in MS Excel (Microsoft 2016, Washington, USA). Descriptive and statistical data analyses were performed using either R (RStudio Inc., Boston, USA) or GraphPad Prism 6 (GraphPad Software, California, USA).

Cytokine and immunoglobulin measurements from the panel study were normalized per patient to their standard deviation over the 15 visits. Medians of thus normalized data were used to perform a non-parametric Mann-Whitney-U-test. The alpha-level was set to 0.05.

For principal component analysis (PCA), log data were calculated, and real zero values were set to 0.01. Immune parameters were correlated to in-season symptom scores by using Spearman correlation.

For the evaluation of flow cytometry data, specific cell numbers were determined as percentages of total acquired cells.

For the ISAC study, ISU-E values were calculated to log data, and real zero values were set to 0.01. The correlations between nasal and serum sIgE levels were tested by Spearman correlation. The level of statistical significance (alpha-error, p) was set to 0.05, and was adjusted for multiple testing to p=0.003 by Bonferroni correction. Schematic outcomes for the statistics of the data are represented in the table 16, followed by calculations based on the contingency table.

Table 16: Schematic contingency table

	Sensitization			
Results	yes	n	no	n
Positive	True Positive (TP)	a	False Positive (FP)	c
Negative	False Negative (FN)	b	True Negative (TN)	d

$$Sensitivity = \frac{a}{a+b} \qquad Specificity = \frac{d}{c+d}$$

$$Positive\ Likelihood\ Ratio = \frac{Sensitivity}{1-Specificity} \qquad Negative\ Likelihood\ Ratio = \frac{1-Sensitivity}{Specificity}$$

$$Positive\ Predictive\ Value = \frac{a}{a+c} \qquad Negative\ Predictive\ Value = \frac{d}{b+d}$$

$$Youden\ Index = \max\{sensitivity + specificity - 1\}$$

$$Accuracy = \frac{a+d}{a+b+c+d}$$

A receiver operating characteristic (ROC) curve was created for sIgE values (ISAC studies) by using Prism 6. Briefly, a ROC curve is a plot of the true positive rate in function of the false positive rate for different cut-offs to test diagnostic performance of a test (Figure 9). For each calculated point, it illustrates the sensitivity and specificity of a test at a certain

threshold. The Area Under the ROC curve (AUC) is a number that summarizes the overall performance in a single value.

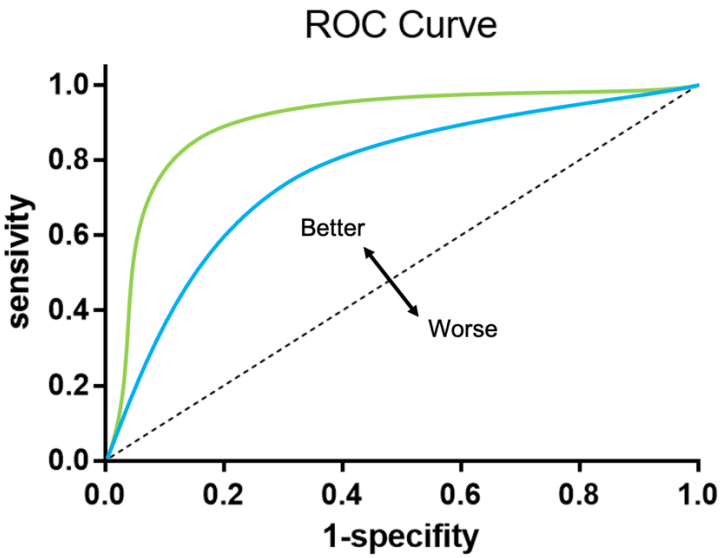


Figure 9: Graphical plot of a receiver operating characteristics (ROC) curve for two example datasets (blue, green). The blue test is less accurate than the green test. The diagonal indicates a random classifier.

6. Results

The results of the panel study (Gokkaya, Damialis, et al., 2020) and the ISAC study (Gokkaya, Schwierzeck, et al., 2020) are published in peer-reviewed journals as listed in the publication list. Therefore the following results and discussion chapters are based on one or more publications which were published before the submission of this thesis itself. To avoid multiple referring, references to “Gökkaya, Damialis, et al., 2020” are indicated in the title by “#” and the references to “Gökkaya, Schwierzeck, et al., 2020” are indicated by “§”.

6.1 Panel study

6.1.1 Symptom kinetics under natural pollen exposition#

In the panel study cohort, symptom scores were calculated for each subject. As expected, all seasonal allergic rhinitis (SAR) patients showed symptoms during the hazel, alder and birch pollen season. Surprisingly, a subset of the non-allergic (NA) control group also reported symptoms during the pollen season (Figure 10).

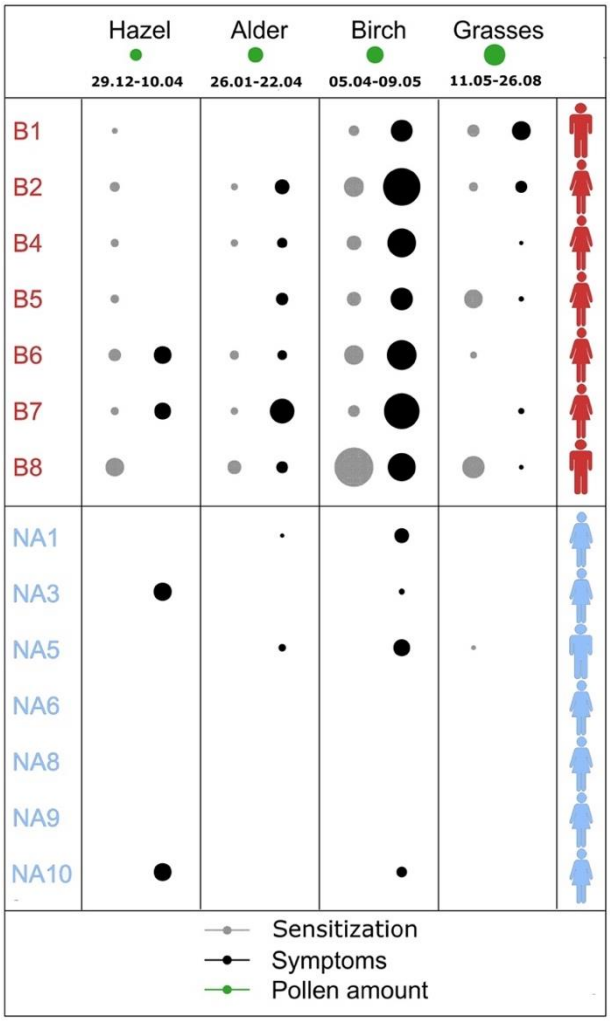


Figure 10: Sensitization and symptom profiles of the panel study cohort. SAR patients are displayed in red, NA subjects in blue. Specific IgE levels (black dots), cumulative symptom

score (grey dots), cumulative airborne pollen (green dots) are showed in relation to the dot size to create an overview of the different patient specific profiles.

Sensitization against birch pollen did not change over the time. However, during the birch pollen season, wheal and flare size, as measured by skin prick test (SPT) were much higher compared to pre-season values (Figure 11).

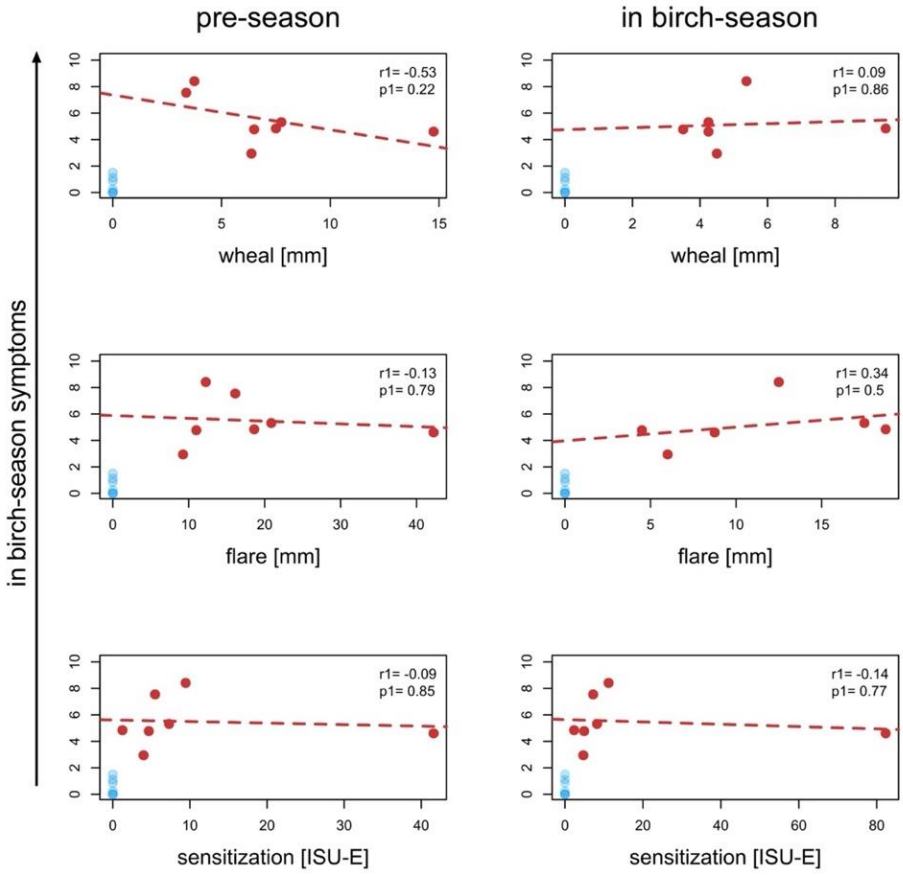


Figure 11: Association between symptoms during the birch pollen season and clinical allergy test results. Spearman correlation was tested before and during the birch pollen season. SAR patients are displayed in red, NA subjects in blue. Wheal and flare sizes were measured by skin prick test (SPT).

The kinetics of symptom scores were plotted cumulatively over the course of one year and analyzed in relation to airborne pollen concentrations (Figure 12). The non-allergic subjects were divided into two different subsets, symptomatic (NA1, NA3, NA5 and NA10) and asymptomatic subjects (NA6, NA8 and NA9) based on their reported symptom scores during the birch pollen season. Of note, the symptom severity score of the NA subjects was on a lower overall scale compared to SAR patients (Figure 12A). During the grass pollen season, only two out of seven NA subjects (NA3 and NA10) exhibited any symptoms (Figure 12B). A mean symptom score was calculated for each group to display pollen related symptoms.

Symptoms of SAR patients appeared according to airborne pollen concentrations with a lag of 3 days (hazel), 13 days (alder) and 0 days (birch) (Figure 12C; red line) and the symptom

severity almost doubled over the main birch pollen season. While the asymptomatic NA subjects (Figure 12C; dark blue line) did not display any symptoms in late winter and spring with the beginning of the Betulaceae-Corylaceae pollen seasons, the symptom score of symptomatic NA subjects (Figure 12C; dark blue line) was increased slightly, with higher lag effect but otherwise similar to the SAR cohort.

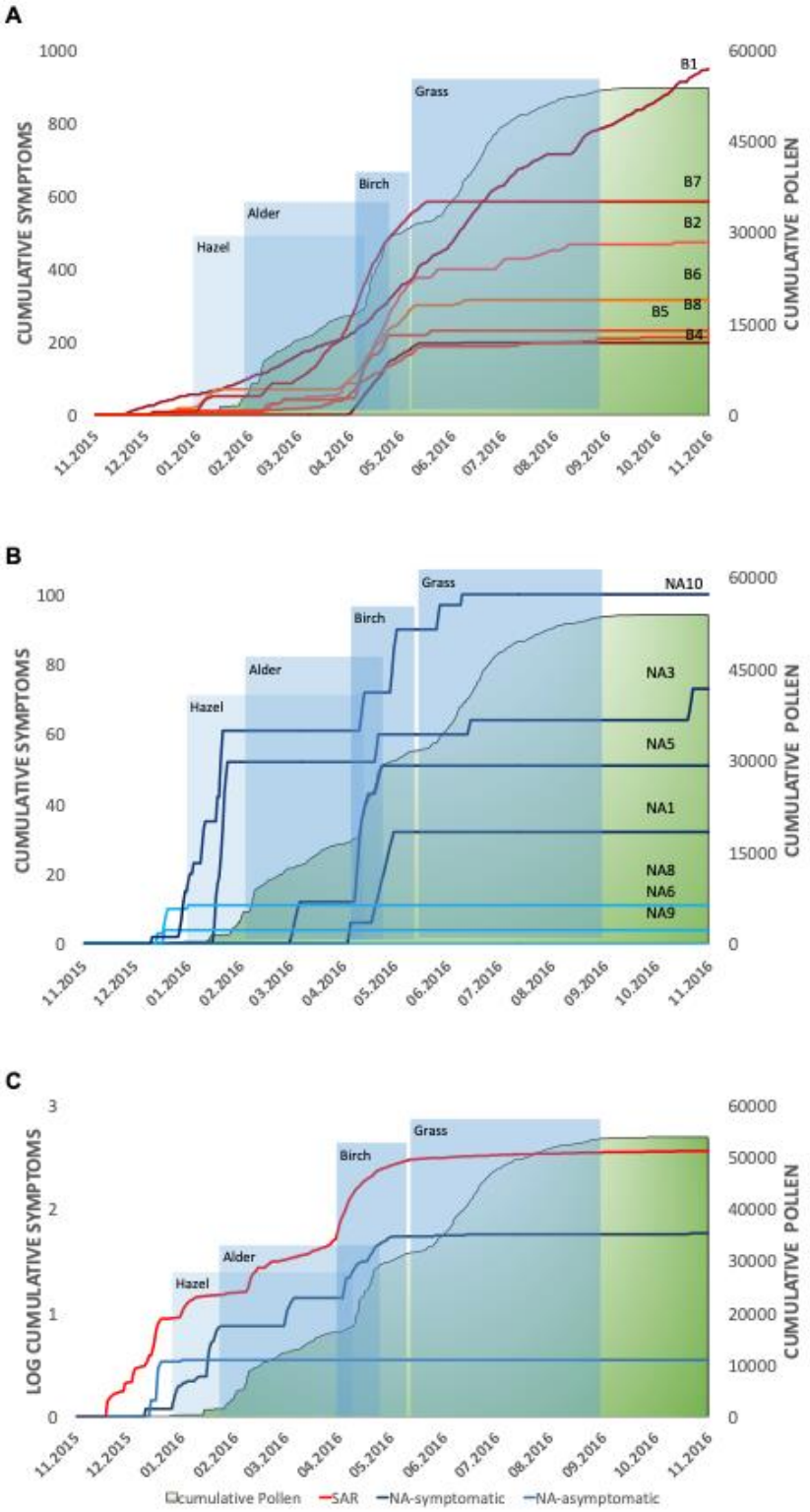


Figure 12: Plots of cumulative symptom scores in relation to airborne pollen concentrations over time (x-axis). The left y-axis indicates the log cumulative symptom score of SAR patients

(A) and NA subjects (B), whereas the right y-axis indicates the cumulative airborne pollen concentration, visualized as the green shaded area. Blue shaded areas illustrate the main pollen seasons of hazel, alder, birch and grasses (2.5-97.5% of total cumulative pollen). The red line (SAR patients), light blue line (asymptomatic) and the dark blue line (symptomatic) show the mean cumulative symptom score in log scale (C).

6.1.2 Kinetics of humoral immune responses under natural pollen exposure#

Distinct kinetics of the humoral immune responses of SAR patients and NA subjects were observed. Serum and nasal immune parameters were normalized as described in the methods to determine if there are differences across the study participants for each of the sampling time points (Figure 13).

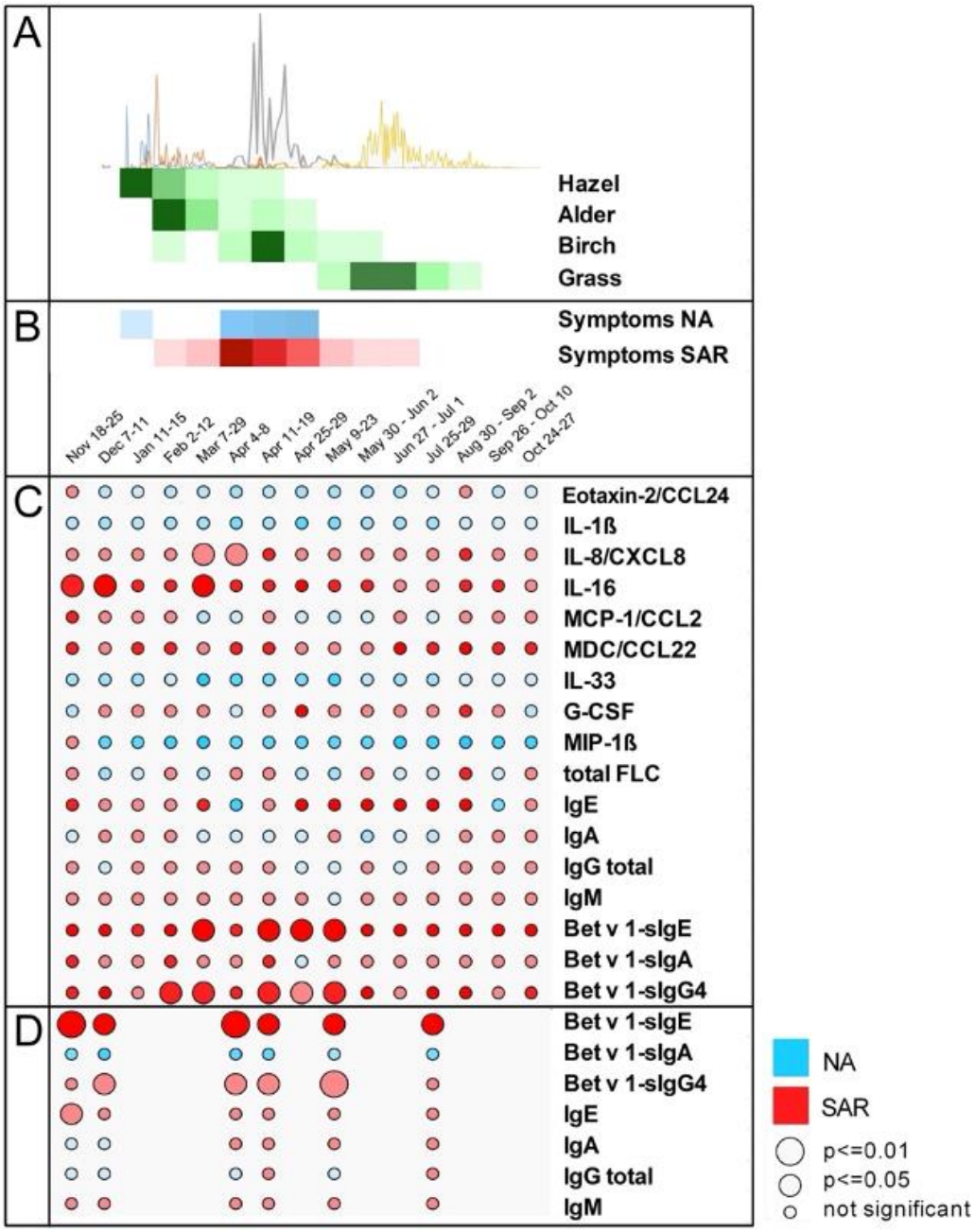


Figure 13: Cross-sectional comparison of all humoral immune variables, resolved per visit. (A) Cumulative pollen concentration per time point. Pollen concentrations for each pollen type are

normalized to total cumulative pollen during the entire study. (B) Cumulative symptoms are normalized as described in the methods. (C) Cross-sectional nasal humoral immune variables per visit. (D) Cross-sectional serum humoral immune variables per visit. The size of the dots indicates the statistical significance level between cohorts. Blue color indicates increase of immune variables in NA and red color indicates increase of immune variables in SAR. Cross-sectional differences were tested by Mann-Whitney-U-test and displayed as the intensity of each color. P values were adjusted for multiple testing by Bonferroni correction.

A cross-sectional comparison indicates specific changes in the nasal immune variables during the entire study period, although not all the variables reached statistical significance. Especially nasal immune variables such as IL-8 and IL-16 were significantly higher in SAR patients as compared to NA subjects. Throughout the birch pollen season from March to April, IL-8 was significantly increased ($p<0.05$), whereas IL-6 was significantly higher in SAR patients during the wintertime from November to December ($p<0.05$) and in March at the beginning of birch pollen season ($p<0.05$).

Nasal Bet v 1-specific IgE levels were significantly higher in SAR patients compared to NA subjects ($p<0.05$) during the birch pollen season and also overlapping into the grass pollen season in May. Similarly to sIgE levels, nasal Bet v 1-specific IgG_4 levels were significantly increased but the increase occurred earlier in February ($p<0.05$). Throughout all visits, SAR patients had significantly higher serum Bet v 1-specific IgE and IgG_4 levels than NA subjects ($p<0.01$ or $p<0.05$). There were no statistically significant differences between serum Bet v 1-specific IgA levels, however, NA subjects expressed higher levels of specific IgA at all time-points than SAR patients.

6.1.3 Seasonal comparison of total immunoglobulins#

Furthermore, serum and nasal immunoglobulin levels (IgE, IgA, IgM, IgG, IgG_1, IgG_2, IgG_3, IgG_4) and nasal immunoglobulin free light chains (FLC) were compared in and out of the pollen season. For this, immunoglobulin isotypes levels were normalized by dividing each value by the standard deviation across all measurements per subject. The medians were calculated per subject in regard to the defined pollen season.

During the pollen season, nasal immunoglobulins showed cross-sectional differences. Nasal IgA (p<0.05), IgG_1 (p<0.05) and IgG_2 (p=0.007) levels were significantly higher in NA subjects. Nasal total immunoglobulin free light chains (FLCs; κ plus λ) tended to be higher in SAR patients but this trend was not statistically significant (Figure 14).

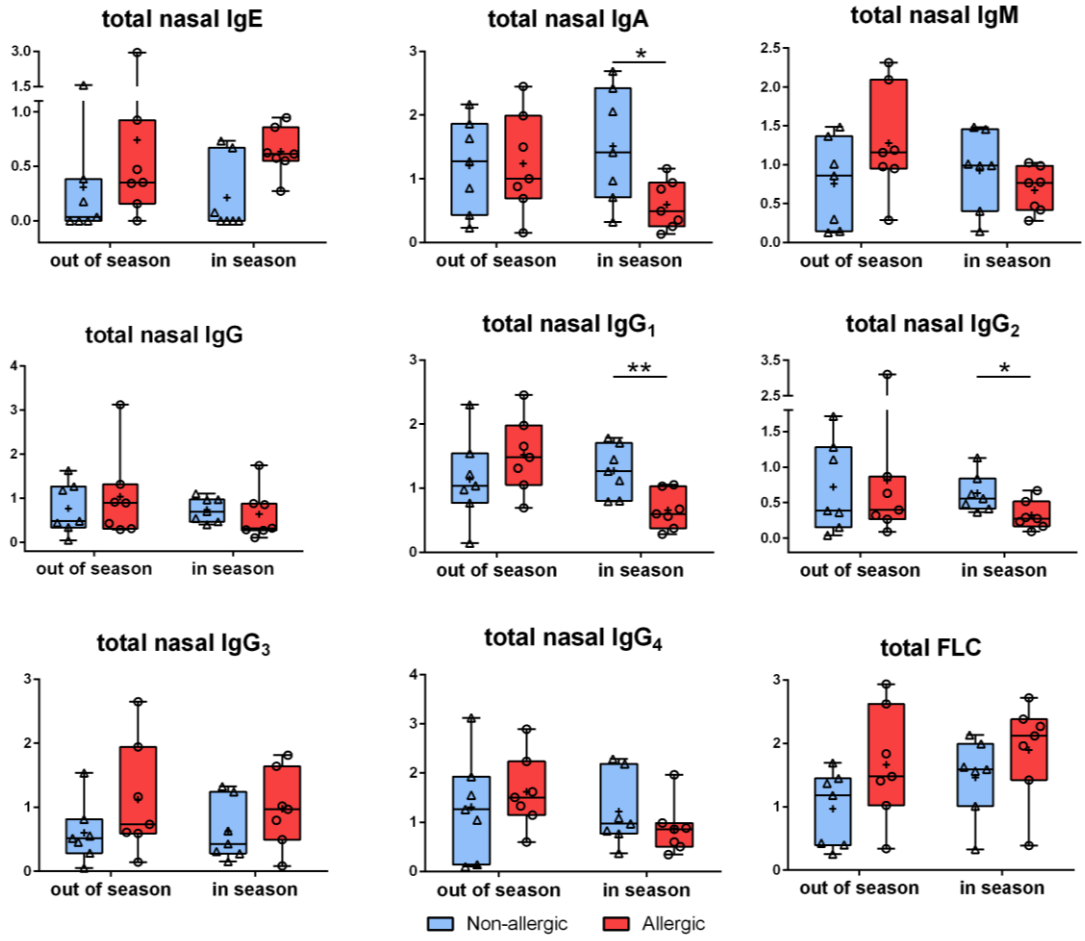


Figure 14: Cross-seasonal comparison of nasal immune variables in both cohorts. Y-axis: normalized values; "+": geometric mean. Cross-seasonal differences were tested by Mann-Whitney-U-test *: p≤0.05; **: p<0.01

There were no significant differences of serum immunoglobulin isotypes between SAR patients and NA subjects except for IgA (Figure 15). Total IgA levels were significantly higher in SAR patients in comparison to NA subjects (in season; p=0.007, out of season; p<0.05).

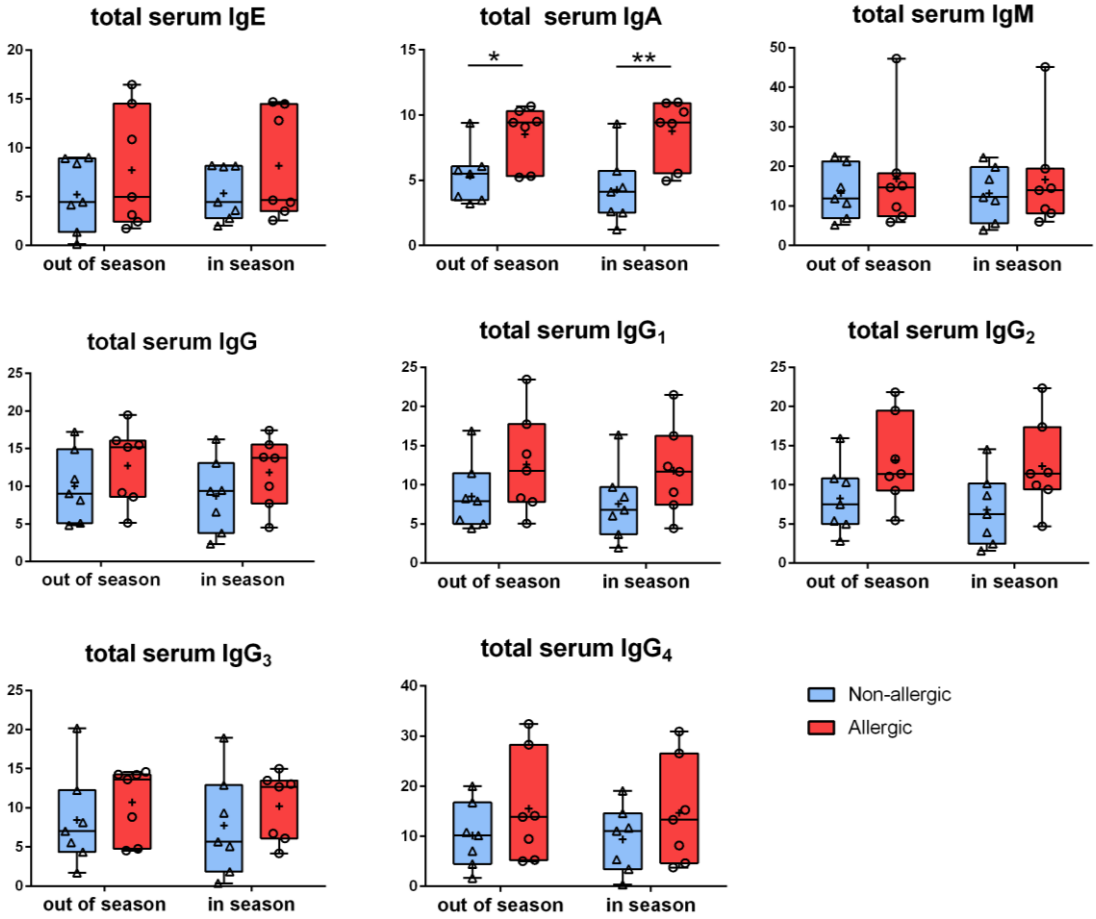


Figure 15: Cross-seasonal comparison of serum immunoglobulins in NA and SAR cohorts. Y-axis: normalized values; "+": geometric mean. Cross-seasonal differences were tested by Mann-Whitney-U-test *: p≤0.05; **: p<0.01

6.1.4 Seasonal comparison of Bet v 1-specific immunoglobulins#

The main focus of this study was the birch pollen-specific immune response. Bet v 1-specific Ig levels were determined in the pollen season (start of April to start of May 2016) and out of the pollen season. Throughout all visits SAR patients expressed nasal and serum Bet v-1 specific IgE (Figure 16). Cross-sectional differences were significant and independent of seasonality.

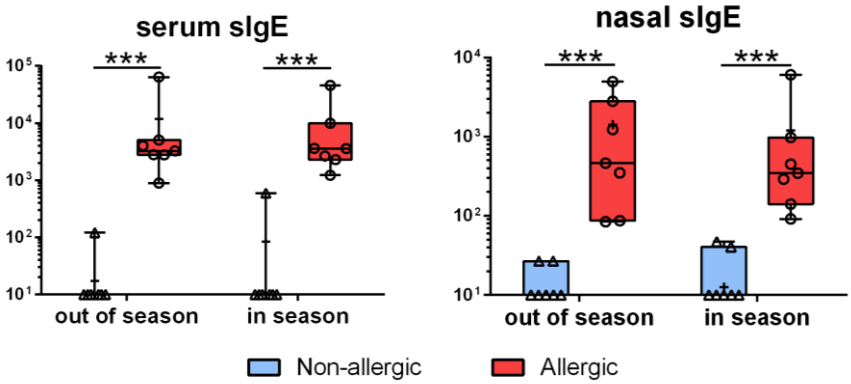


Figure 16: Cross-sectional and cross-seasonal comparison of Bet v 1-specific IgE. Median sIgE levels in serum and nasal fluid plotted as raw values (in U/mL). "+" indicates the geometric mean; *: p≤0.05; **: p<0.01; ***: p<0.005, Mann-Whitney-U-test.

The same patterns were seen for Bet v 1-specific IgG_4 (Figure 17). There were no seasonal but only cross-sectionally significant differences, which were statistically significant.

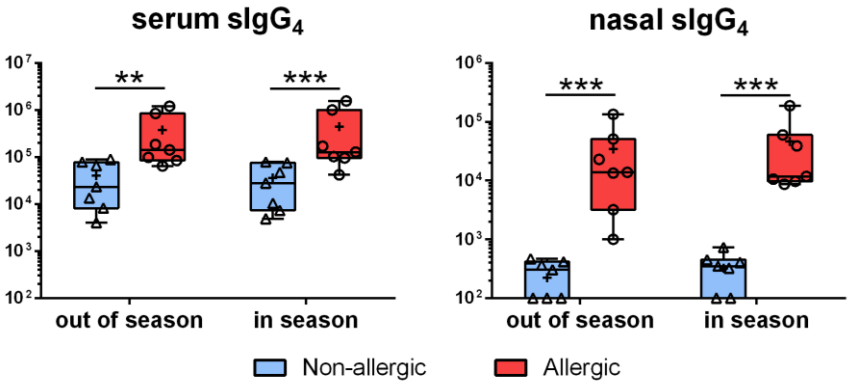


Figure 17: Cross-sectional and cross-seasonal comparison of Bet v 1-specific IgG_4. Median $sIgG_4$ levels in serum and nasal fluid plotted as raw values (in U/mL). "+" indicates the geometric mean; *: p≤0.05; **: p<0.01; ***: p<0.005, Mann-Whitney-U-test.

Bet v 1-specific IgA was detectable in serum and nasal fluid of all participants (Figure 18). Serum sIgA tended to be higher in NA subjects than in SAR patients, however the trend was not statistically significant. Similarly, nasal Bet v 1-specific IgA did not differ between the cohorts.

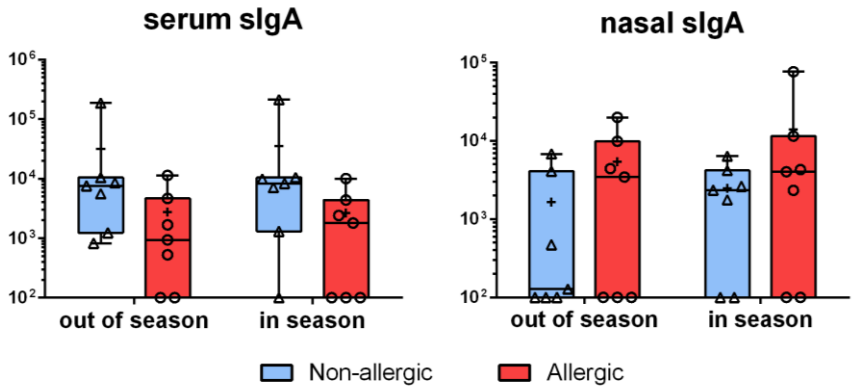


Figure 18: Cross-sectional and cross-seasonal comparison of Bet v 1-specific IgA. Median sIgA levels in serum and nasal fluid plotted as raw values (in U/mL). "+" indicates the geometric mean; *: p≤0.05; **: p<0.01; ***: p<0.005, Mann-Whitney-U-test.

6.1.5 Pollen related nasal chemokine and cytokine levels#

Chemokine and cytokine levels were compared seasonally between SAR patients and non-allergic individuals (Figure 19) to further examine the nasal immune response. Raw values were normalized by dividing each value by the standard deviation over all measurements per subject. Nasal Eotaxin-2 levels were significantly higher in SAR patients than in NA subjects in and out of pollen season ($p<0.005$). Levels of MCP-1 ($p<0.05$) and MDC ($p<0.01$) were also significantly higher in SAR patients but only outside of the pollen season, whereas nasal IL-8 was only higher in NA subjects than SAR patients only during the pollen season ($p \leq 0.05$).

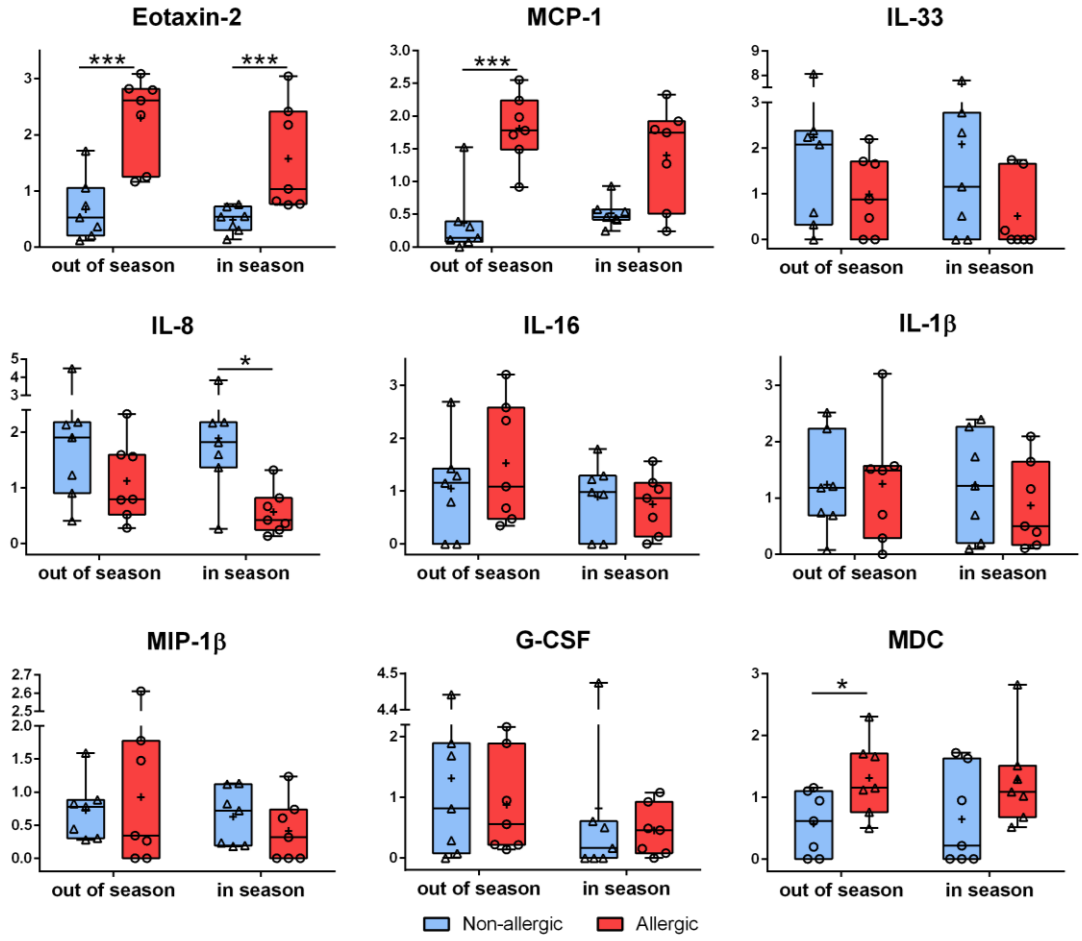


Figure 19: Comparison of nasal chemokines and cytokines in- and out of pollen season. Median chemokine and cytokines in nasal fluid are plotted as normalized values. "+" indicates the geometric mean; *: p≤0.05; **: p<0.01; ***: p<0.005, Mann-Whitney-U-test.

6.1.6 Nasal biomarkers for in-season symptom severity in both cohorts#

To show the complex data of each individual, heat maps were created. SAR patients and NA subjects show distinct patterns of nasal immune parameters (Figure 20). Especially during the birch pollen season (Visit 6, 7, and 8), NA subjects show different expression levels under natural pollen exposure. Pre-season (Visit 1 and 2) were defined to analyze the prediction in early time points.

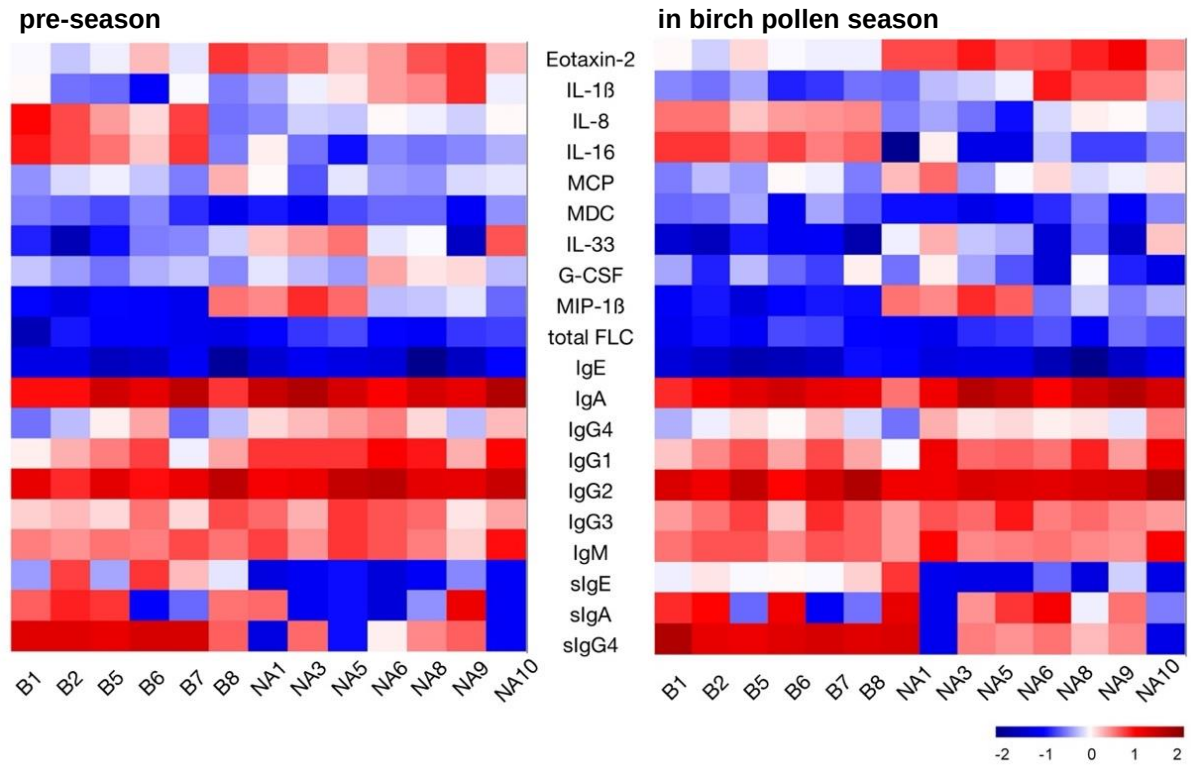


Figure 20: Heat-map of all nasal immune parameters (y-axis) for all study participants (x-axis). Left panel: in pre-season, right panel: during the birch pollen season.

Non-supervised Principal Component Analysis (PCA), performed on log-transformed nasal immune parameter concentrations, was used to identify nasal biomarkers associated with symptom severity (Figure 21). SAR patients as well as NA subjects were clustered by their symptom severity through principal component (PC) 1.

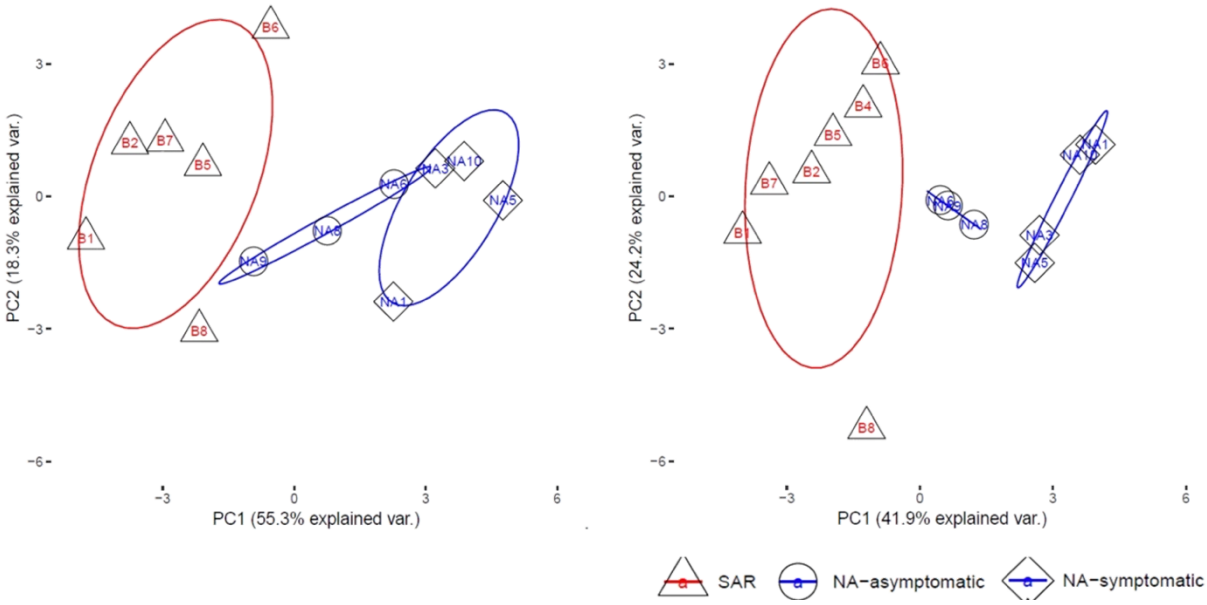


Figure 21: Non-supervised principal component analysis of nasal immune variables. Left panel: in pre-season, right panel: in birch pollen season. Nasal immune parameters were log transformed and true zero values were imputed with 0.001.

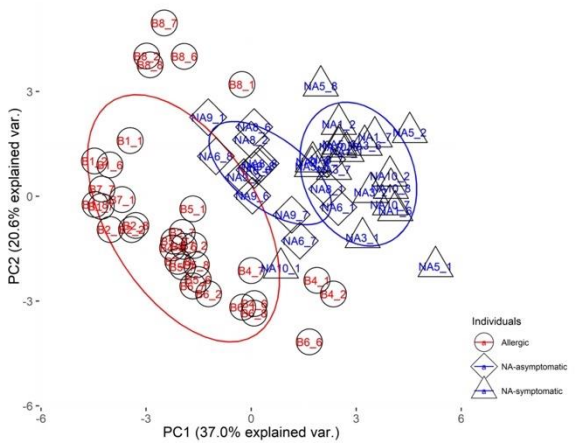
The nasal immune variables involved in PC1 of the PCA are shown in figure 22. The largest contribution to the overall variance within all nasal immune variables was made up by Bet v 1-specific IgG_4, IgA and IgE as well as IL-8, IL-16 and IL-33 levels. The evenness of these variables occurred in pre-season as well as in birch pollen season.

Pre-season	Principal component scores	in season
-0.532	Bet v 1-specific IgG_4	-0.572
-0.404	Bet v 1-specific IgE	-0.428
-0.377	IL-16	-0.387
-0.296	IL-8/CXCL8	-0.302
-0.428	Bet v 1-specific IgA	-0.243
-0.144	MDC/CCL22	-0.148
-0.112	G-CSF	-0.125
-0.125	IgE	-0.085
-0.087	IgG_2	-0.073
-0.061	total FLC	-0.067
-0.075	IgG_3	-0.052
-0.092	IgM	-0.031
-0.059	IgA	0.005
0.003	IgG4	0.025
-0.147	MCP-1/CCL2	0.025
0.008	IgG_1	0.067
-0.079	IL-1ß	0.084
-0.073	Eotaxin-2/CCL24	0.098
0.090	MIP-1ß	0.225
0.151	IL-33	0.239

Figure 22: Principal components scores within PC1 in pre-season and in birch pollen season of non-supervised PCA analysis.

Although the study cohort size was 14 participants, the granularity of the visits indicates the robustness of the PCA by an additional 1-leave-out method. For this, the PCA analysis was reanalyzed by exclusion of one immune parameter each (total 20 times) and instead of the mean data points, every single measurements were used to show the true sample size. PCA results were consistent with regard to the clusters (Figure 23).

A



B

PCA V1_2 + 6_7_8	Principal component scores	mean PCA 1-leave-out (*n* =20)
-0.548	Bet v 1-specific IgG_4	-0.437
-0.446	Bet v 1-specific IgE	-0.356
-0.346	IL-16	-0.273
-0.269	IL-8/CXCL8	-0.212
-0.429	Bet v 1-specific IgA	-0.404
-0.145	MDC/CCL22	-0.116
-0.147	G-CSF	-0.134
-0.114	IgE	-0.094
-0.057	IgG_2	-0.047
-0.068	total FLC	-0.058
-0.058	IgG_3	-0.048
-0.068	IgM	-0.055
-0.006	IgA	-0.005
0.017	IgG4	0.014
-0.076	MCP-1/CCL2	-0.066
0.020	IgG_1	0.017
-0.007	IL-1ß	-0.013
-0.010	Eotaxin-2/CCL24	-0.016
0.113	MIP-1ß	0.079
0.184	IL-33	0.146

Figure 23: Robustness testing by using the 1-leave out method. A: Non-supervised PCA analysis with all the individual visits of pre-season and in birch pollen season. B: Ranking and the values of the principal components were highly comparable to the original PCA results.

PCA analysis and Spearman correlations between in-season symptom severity and the concentration of immune variables pointed out potential predictive biomarkers. Finally, the correlation between immune parameters and birch related symptom severity was examined. In-season as well as pre-season immune parameter levels (top PC1; Bet v 1-specific IgE, IgG4, IL-8, IL16 and IL-33) were plotted against in-season symptoms for both cohorts (Figure 24).

In the NA cohort, pre-season as well as in-season nasal IL-8 levels were negatively correlated with in-season symptom severity. In comparison, Bet v 1-specific IgG_4 levels were negatively correlated only pre-season and nasal IL-33 levels were positively correlated only within the birch pollen season.

In the SAR cohort, only pre-season nasal Bet v1-specific IgE showed a significant positive correlation with in-season symptom severity. IL-16 did not show any association in either cohort.

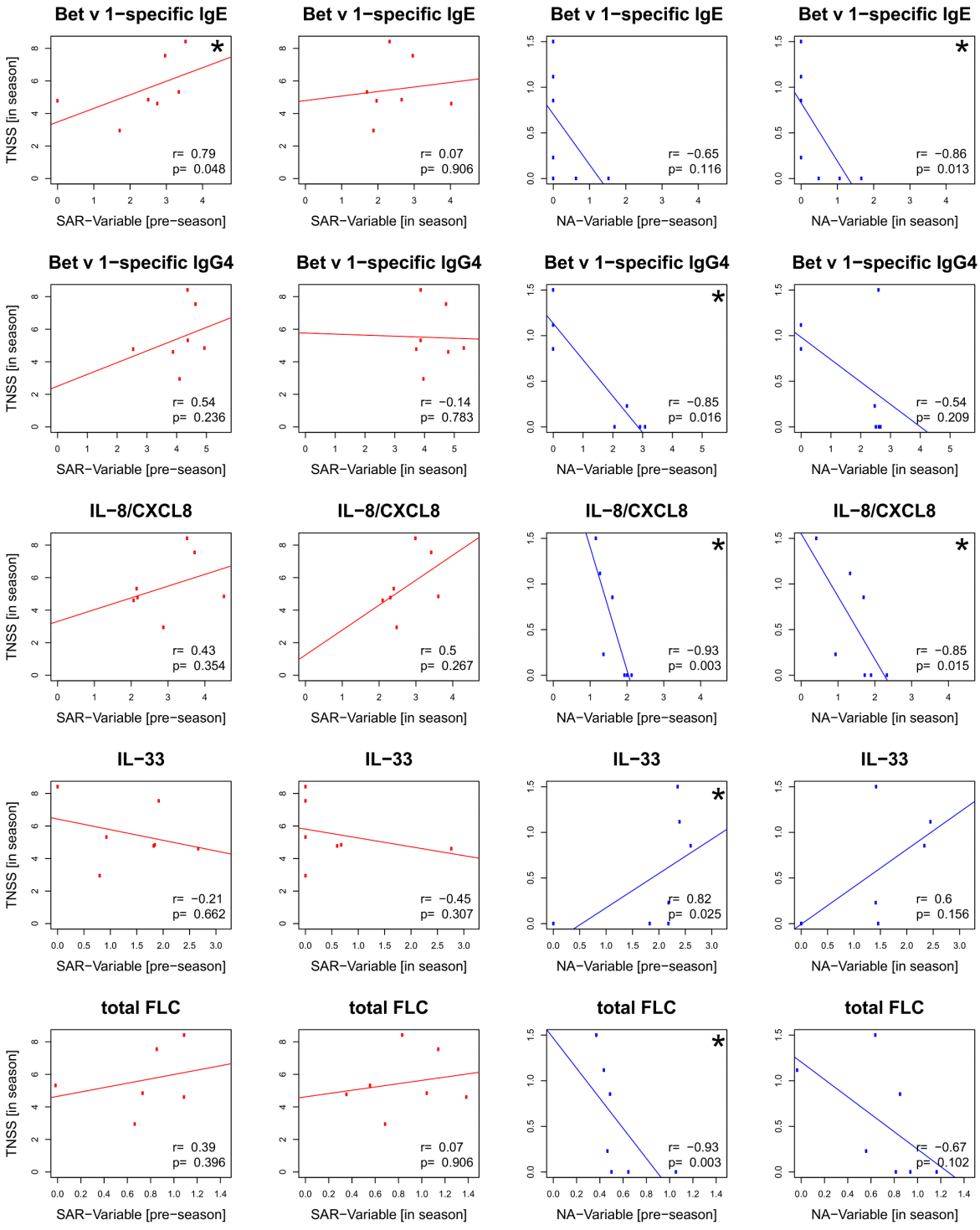


Figure 24: Correlation plots of selected immune parameters against in-season symptom severity. Y-Axis: in season symptom severity as total nasal symptom score (TNSS). X-axis: log-transformed expression levels of immune parameters. Colors indicate SAR patients (red) and NA subjects (blue). Stars in the plots indicate significance by Spearman correlation.

6.1.7 Gene expression analysis of polymeric immunoglobulin receptor

Polymeric immunoglobulin receptor (pIGR) translocate immunoglobulins from inside to outside of the epithelial barrier. To understand more about the kinetics of pIgR we analyzed the time-course of PIGR gene expression (fold change) in nasal tissue (curettages) through calculating the delta delta CT ($2^{-\Delta\Delta CT}$). Pre-season ΔCT was used as baseline for each subject. Nasal PIGR expression was increased in non-allergic individuals upon pollen exposure, whereas in allergic patients, expression peaked mainly after the birch pollen peak and dropped subsequently to the baseline.

Non-allergic individuals increased nasal PIGR expression upon pollen exposure, whereas the expression peak of allergic patients was only after the birch pollen peak with less variance across the entire study time (Figure 25).

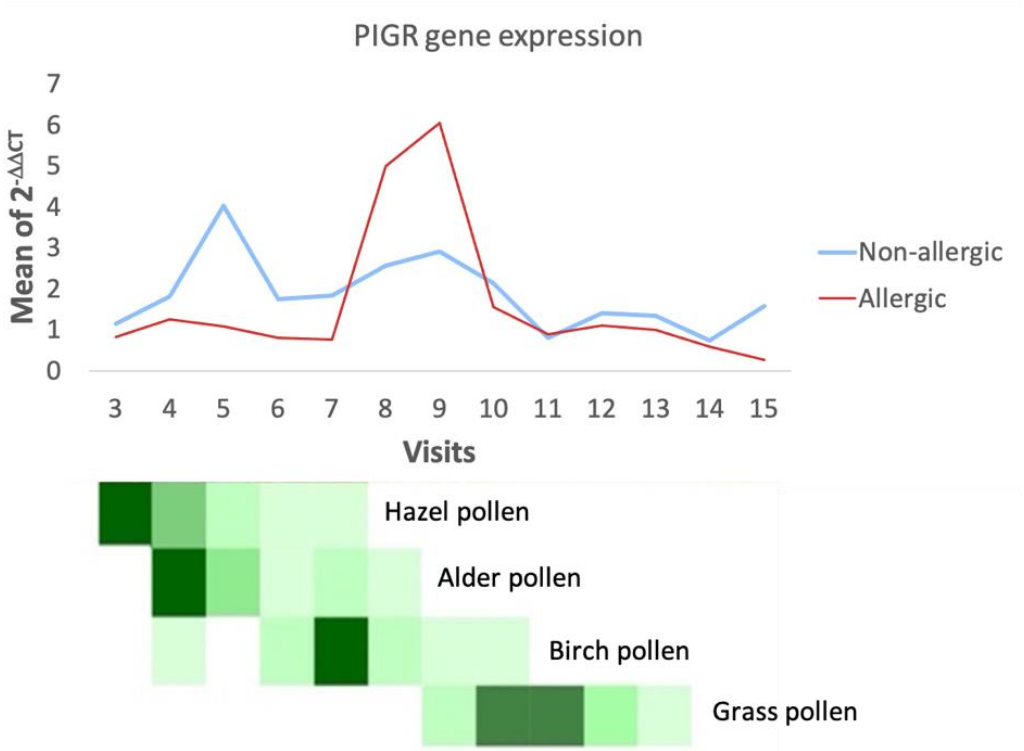


Figure 25: Kinetics of PIGR gene expression under natural pollen exposure. PIGR gene expression levels of allergic (red) and non-allergic (blue) cohort were calculated by mean $2^{-\Delta\Delta CT}$. Accordingly, pre-season (Visit 1-2) was set as baseline. The lower green panel indicates the airborne pollen concentrations.

6.1.8 Nasal flow cytometry analysis

In the panel study of 2016, cells in the nasal curettages were stained with antibodies against CD45, CD16, and CD14 to evaluate the levels of monocytes ($CD45^+CD14^+CD16^-$) and neutrophils ($CD45^+CD14^+CD16^-$) under natural pollen exposure. There were no significant changes throughout the study, neither in allergic nor in non-allergic individuals. In a follow-up panel study (2018) the staining protocol was modified to analyze not only neutrophils and monocytes but also eosinophils.

Therefore, the FACS panel was extended and fluorescence-minus-one (FMO) staining was used as a background control to compare with the full staining. Gates were set by examining spillover effects of other fluorescent labels that were detected in the channel of the non-stained antibody (Figure 26). The observed spillover effect was less than 2%.

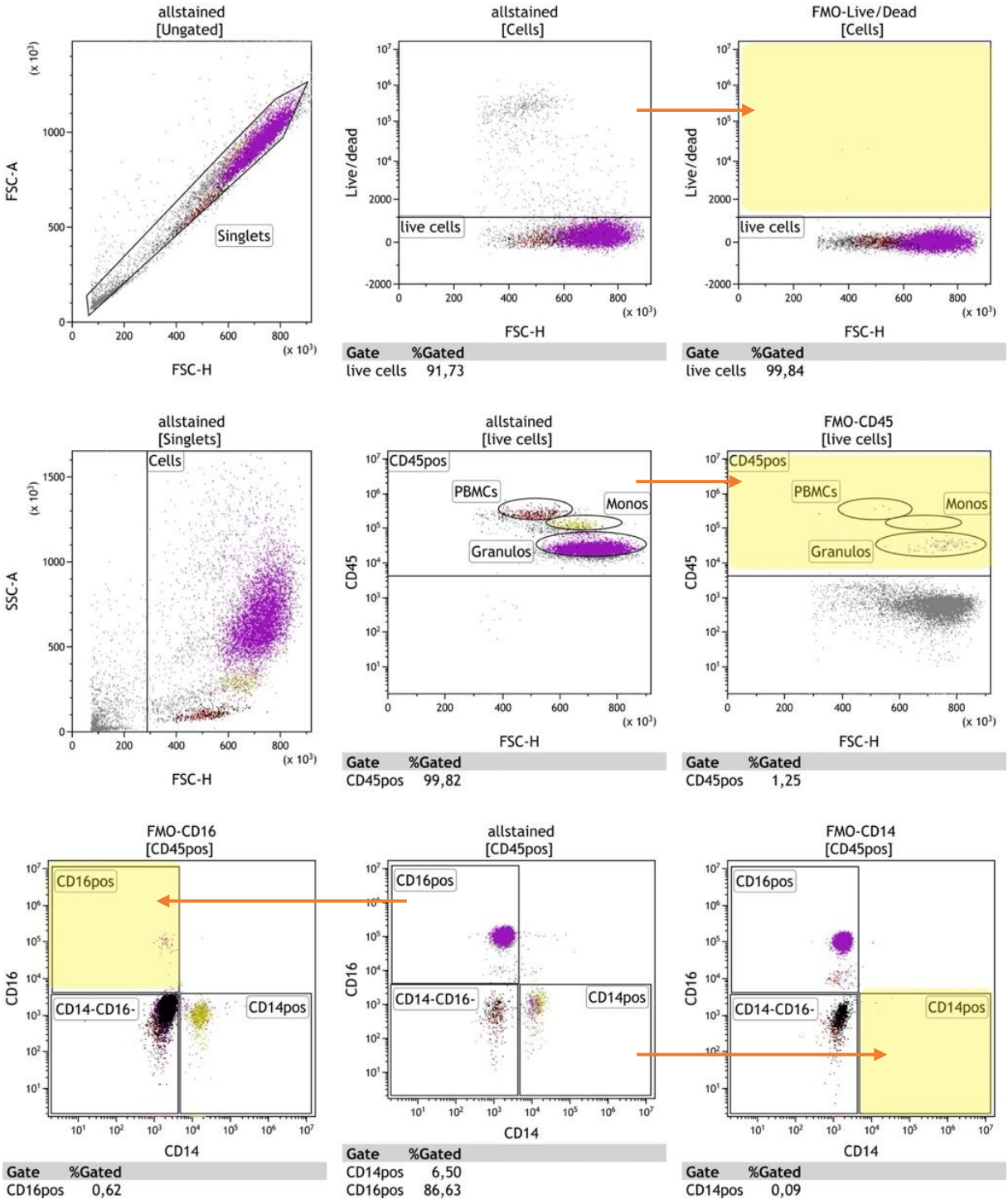


Figure 26: FMO strategy of the FACS panel. Cells were stained with all antibodies and compared to FMO controls. The plots in the middle panel are examples with all the antibodies. Plots with yellow shades are FMO control samples.

Cells in nasal lavages were stained with antibodies against CD45, CD16 and CD14 and a Live/Dead discrimination marker. Preliminary results showed eosinophils in the nasal lavage as $CD45^{+}CD14^{low}CD16^{low}$ cells (Figure 27).

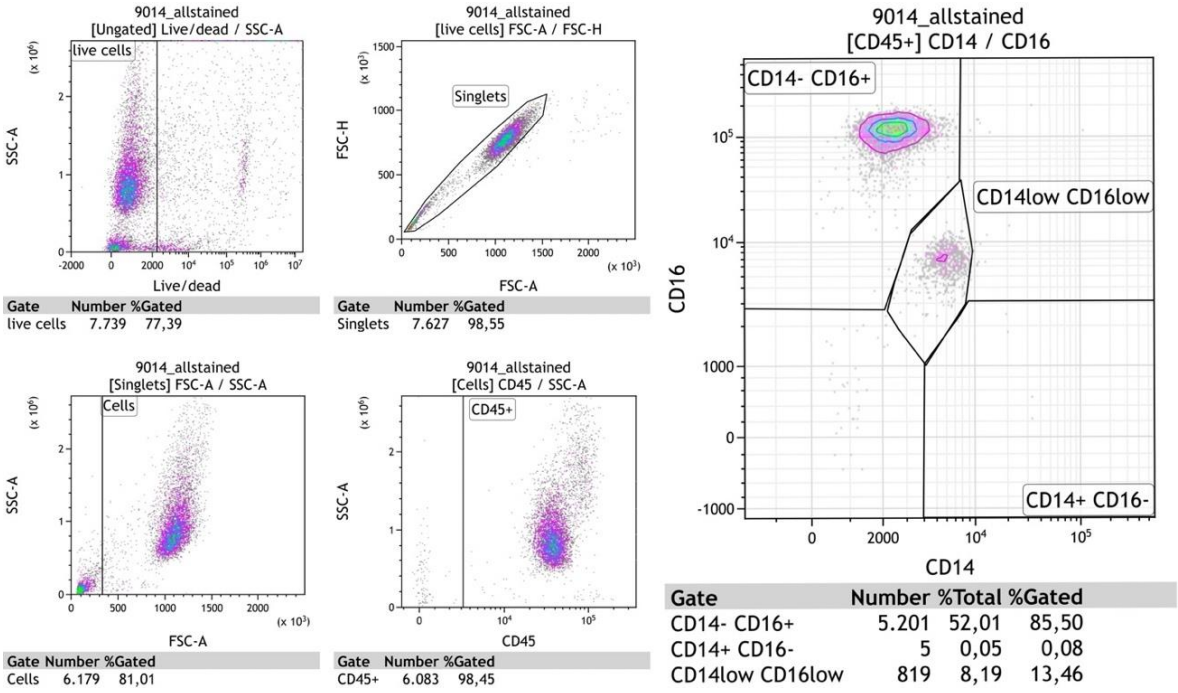


Figure 27: Gating strategy of granulocytes and monocytes. In the nasal lavage, eosinophils as $CD45^{+}CD14^{low}CD16^{low}$, neutrophils as $CD45^{+}CD14^{low}CD16^{+}$ and monocytes as $CD45^{+}CD14^{+}CD16^{-}$ are detected.

In the allergic cohort, eosinophils ($CD45^{+}CD14^{low}CD16^{low}$) increased during the pollen season. The non-allergic cohort showed similar responses in lower levels during the grass pollen season (Figure 28).

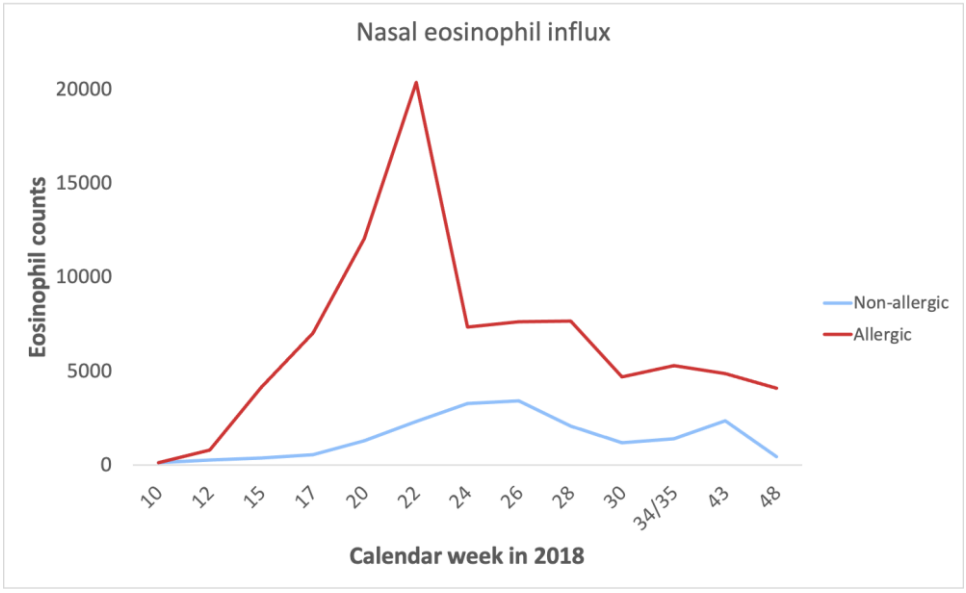


Figure 28: Nasal eosinophil influx over the study period of the panel study 2018. Allergic (red) and non-allergic (blue) subjects show similar patterns of nasal eosinophil infiltration during the grass pollen season, but the magnitude is higher in allergic subjects.

After the main pollen season a slow decline of eosinophil levels was observed but did not reach the level of baseline (Figure 29). The maximum number of eosinophils in the nasal lavage was in week 22 (28th May to 3rd of June) during the highest grass pollen peak.

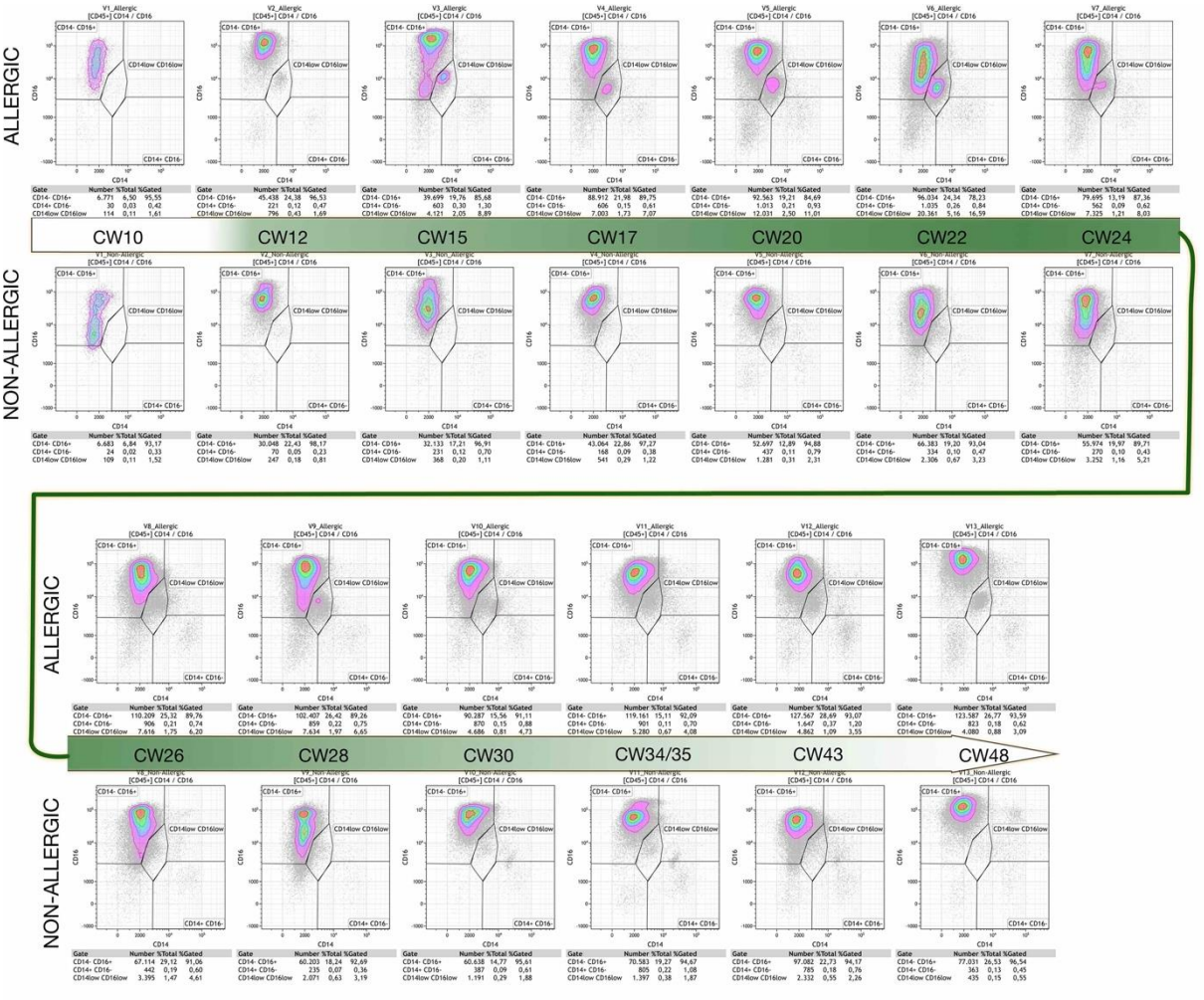


Figure 29: Nasal eosinophil influx under airborne pollen exposure. Intensity of the green bar between calendar week (CW) 10 to 48 indicates pollen counts by estimation. Eosinophils (gate in the middle of FACS plot as $CD45^{+}CD14^{low}CD16^{low}$) of allergic patients peaked during the main grass pollen season.

6.1.9 Establishment of T-cell sorting

PBMCs were stained with antibodies against CD19, CD3, CD4, CD8, CD19, CD25, CD127, CD45RA and CXCR5 for the cell sorting. After establishing the FACS staining and gating strategy (Figure 30), CD4 positive subsets such as T follicular helper cells (T_{fh}), regulatory T cell (T_{reg}) and conventional T cells (Tcon) were sorted for future projects. The sorting efficiency was over 95% and post-sort analysis also showed very high purity.

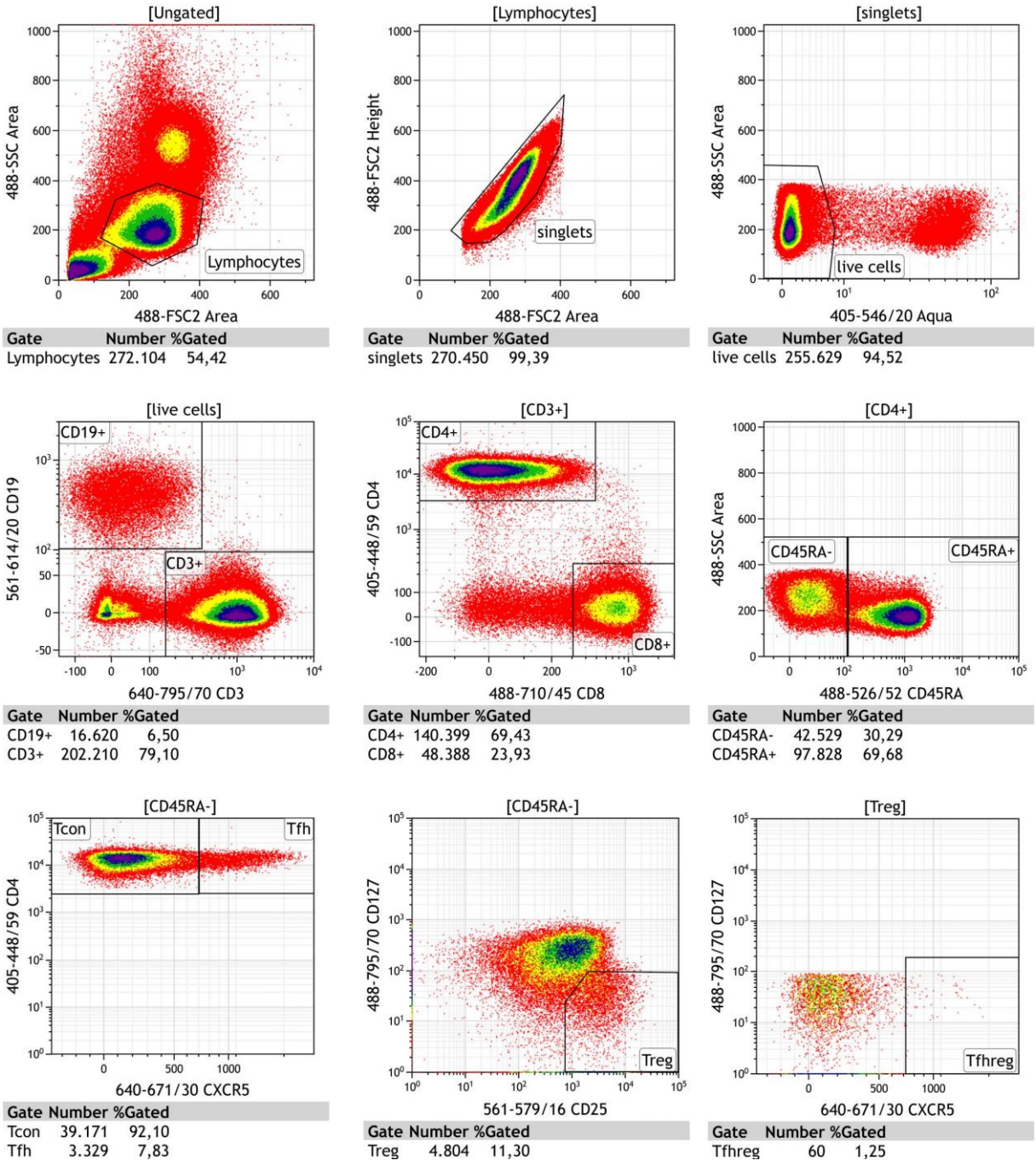


Figure 30: Gating strategy of T-cell sorting. Subset of CD19+, CD8+, CD4+CD45RA-CXCR5- (Tcon), CD4+CD45RA-CXCR5+ (Tfh), CD4+CD45RA-CD127-CD25+ (Treg) were sorted.

6.2 Panel ISAC Study

6.2.1 Sensitization profiles§

Serum specific IgE levels of the ISAC study participants against hazel- (t4), alder- (t2), birch- (t3), grass-pollen (g6) and HDM (d1) extracts were measured by ImmunoCAP. Birch pollen sensitization co-occurred with sensitization against hazel and alder pollen, all of which belong to the Betulaceae pollen family expressing homologous Bet v 1-like PR-10 proteins, and therefore frequently elicit cross-reactive IgE responses. 31 out of 49 subjects were polysensitized. The most prevalent sensitization was to grass pollen (39/49), followed by Betulaceae pollen (24/49) and HDM (24/49). None of the subjects was sensitized only against birch pollen and HDM without having also a grass-pollen sensitization (Figure 31,A).

Sensitization profiles were compared between extract based ImmunoCAP (t5, t2, t3, g6 and d1) and molecular component-based ISAC (Cor a 1, Aln g 1, Bet v 1, Bet v 2, Bet v 4, Cyn d 1, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Der p 1, Der p 2, Der f 1 and Der f 2). Both methods showed the same results for grass-pollen sensitization but differed for HDM, birch-, hazel- and alder-pollen. ISAC resulted in fewer positive tests than ImmunoCAP (Figure 31,B).

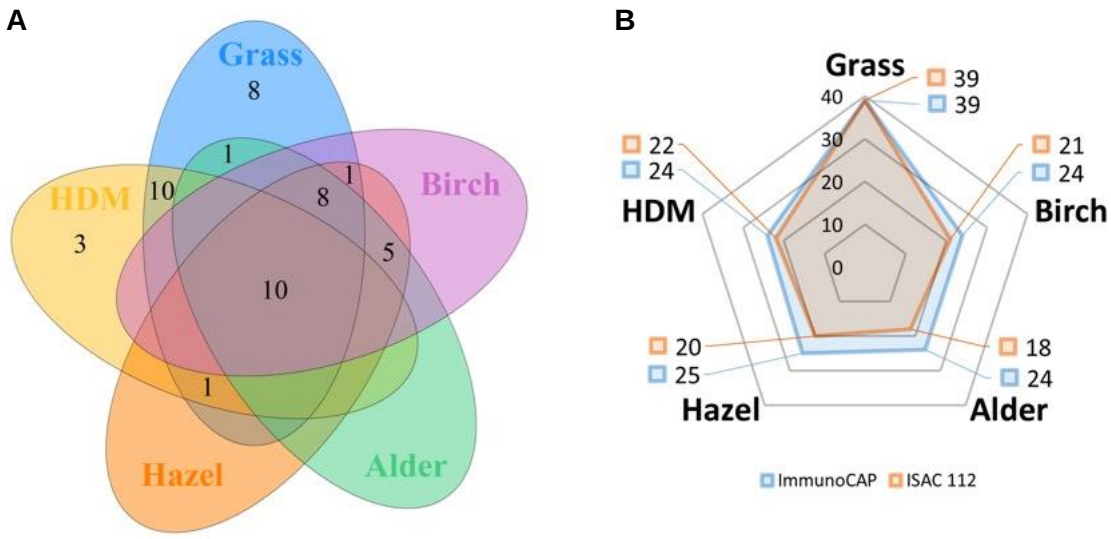


Figure 31: Sensitization profiles of ISAC study participants. (A) Venn diagram of serum sIgE sensitizations by ImmunoCAP. (B) Comparison of sensitization profiles derived from both methods (ImmunoCAP and ISAC).

6.2.2 Inter correlation of sIgE levels in serum and nasal secretion§

Next, specific IgE levels were determined against Betulaceae-, grass-pollen and HDM in serum as well as in nasal secretion. Allergen components within the same protein families were selected for PR-10 allergens (Table 20, appendix), grass pollen allergens (Table 21, appendix) and for HDM allergens (Table 22, appendix).

Molecular components of homologous aeroallergens within the same protein families were positively inter-correlated in serum (Figure 32; p<0.001) and nasal (Figure 33; p<0.001) samples.

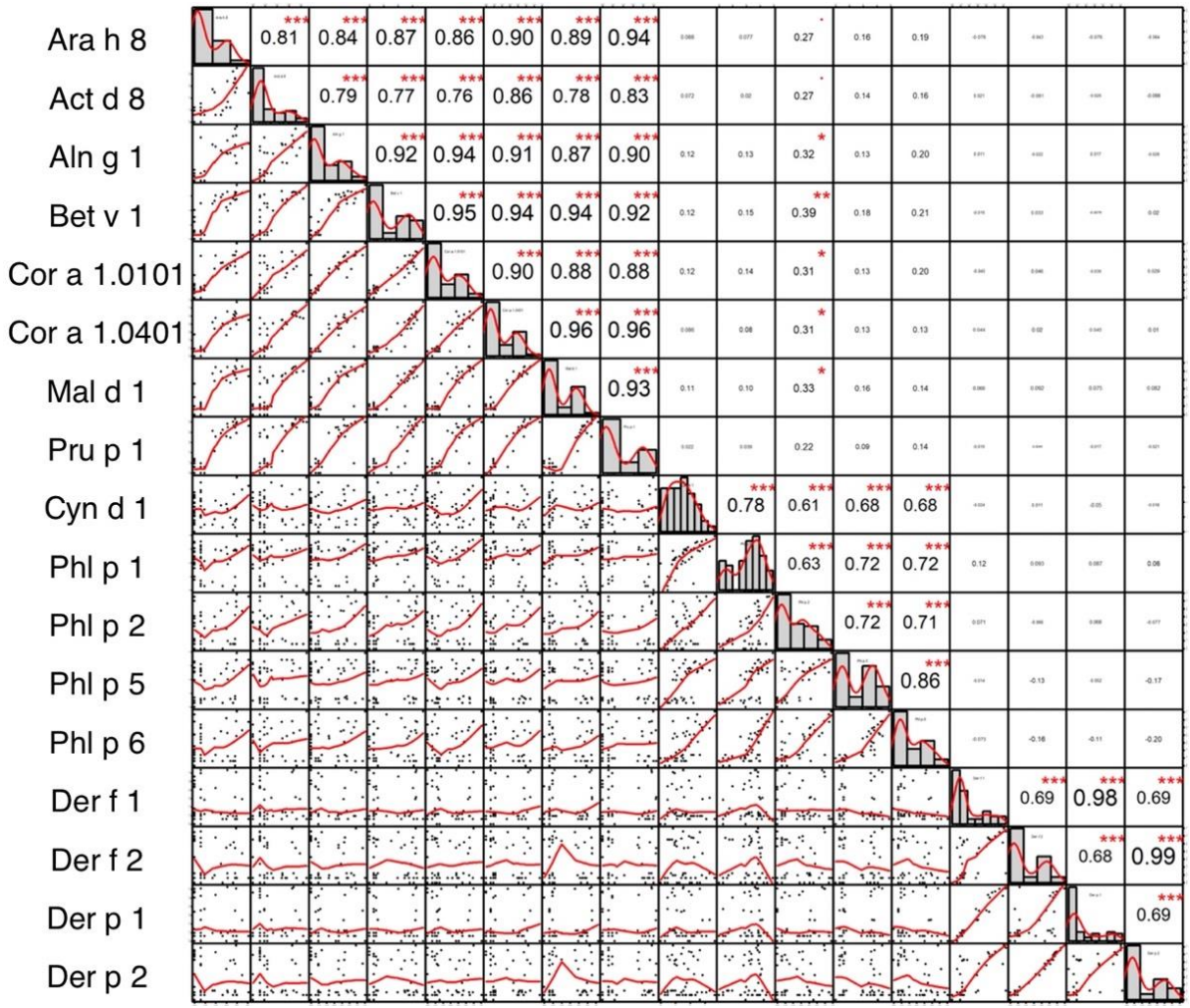


Figure 32: Correlation matrix of serum sIgE. IgE levels of serum are correlated within the allergens of the PR-10 family, grasses and HDM. Bar-plots on the diagonals indicate the distribution of the data. ***: p<0.001; **: p<0.01; **: p<0.05; Spearman correlation.

The Spearman correlation coefficients of each aeroallergen within the same protein family were marginally lower in serum (PR-10: median: 0.90; IQR 0.86, 0.93; grass: median 0.72; IQR 0.68, 0.72; HDM: median 0.69; IQR 0.69, 0.91) than in nasal fluid (PR-10: median 0.92; IQR 0.89, 0.95; grass: median 0.78; IQR 0.76, 0.83; HDM: median 0.78; IQR 0.77, 0.90).

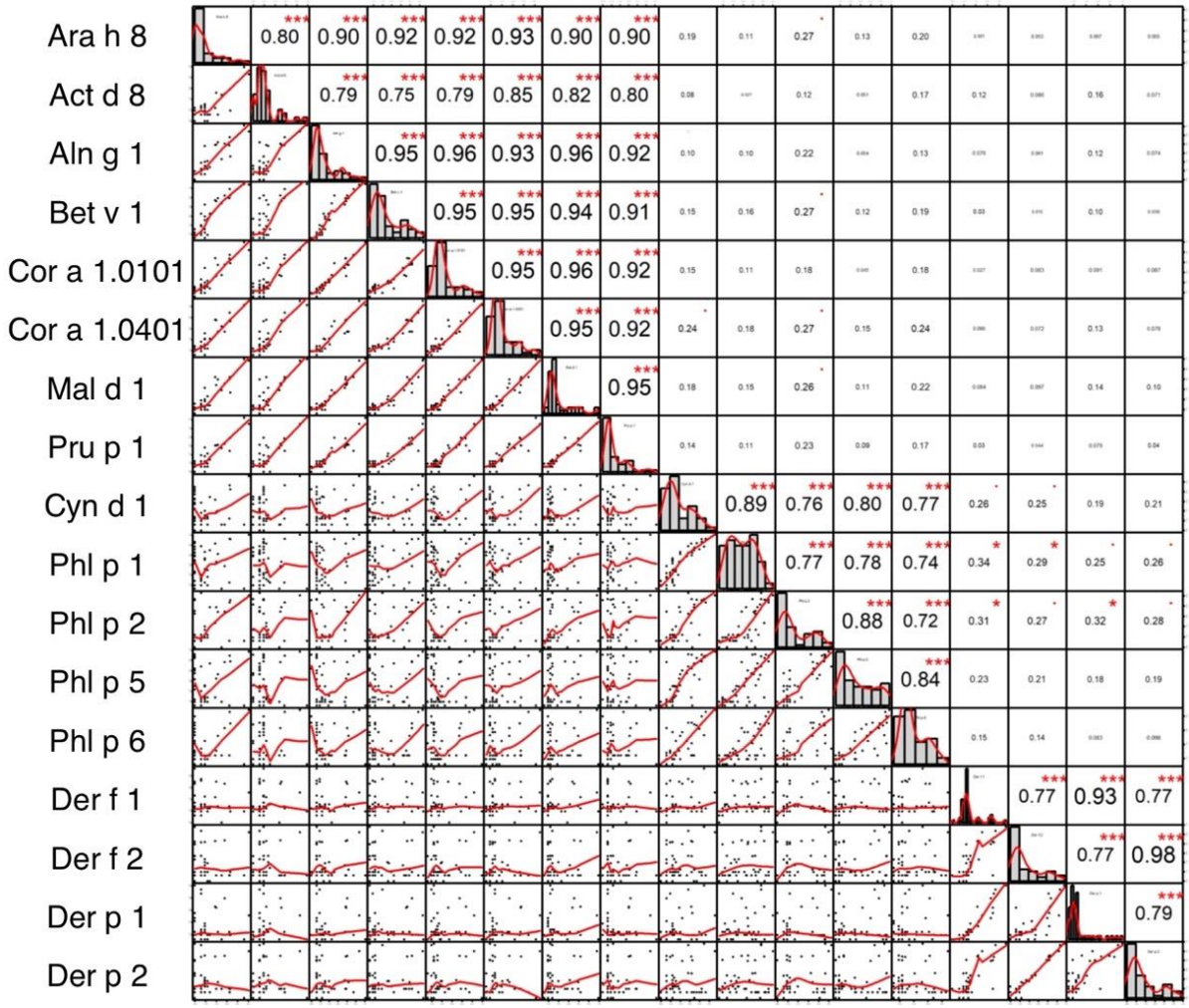


Figure 33: Correlation matrix of nasal sIgE. IgE levels of nasal fluid are inter-correlated within the allergens of the PR-10 family, grasses and HDM. Bar-plots on the diagonals indicate the distribution of the data. ***: p<0.001; **: p<0.01; **: p<0.05; Spearman correlation.

Sensitization patterns measured in serum and nasal secretion were highly comparable for allergens of the PR-10 family, grass pollen, and HDM.

6.2.3 Nasal specific IgE correlate with serum specific IgE[§]

Next, we investigated the relationship between nasal and serum specific IgE levels. IgE levels against aeroallergens were significantly (n=49; p <0.001) and positively correlated between nasal secretion and serum (Figure 34).

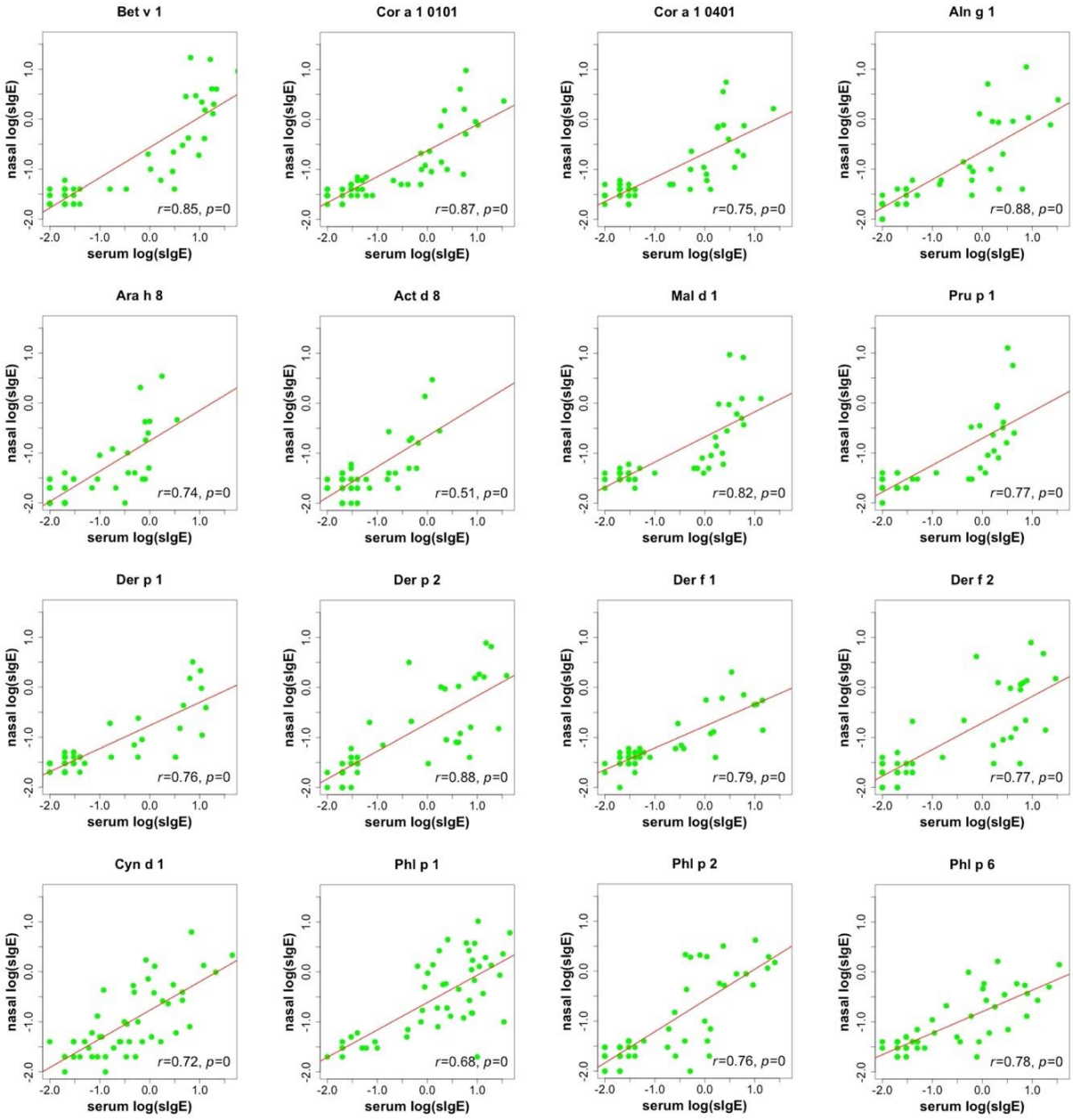


Figure 34: Correlation plot of serum and nasal sIgE levels among different aeroallergens. Serum sIgE levels on the x-axis were plotted against nasal sIgE levels on the y-axis. Each green dot indicates one of the study subjects (n=49). Fitted red lines indicate Spearman correlations.

The median of all Spearman correlation coefficients (r_s) of each aeroallergen was 0.77, with IQR 0.75 to 0.85 (Table 17). The highest correlation coefficient was calculated for Der p 2 and Aln g 1 (r_s=0.88), followed by Cor a 1 (r_s=0.87) and Bet v 1 (r_s=0.85).

Table 17: Spearman correlation coefficients for all tested aeroallergens. Allergen-specific IgE profiles measured in sera and nasal secretions.

Allergen	Correlation Coefficient R
Act d 8	0.51
Aln g 1	0.88
Ara h 8	0.74
Bet v 1	0.85
Cor a 1.0101	0.87
Cor a 1.0401	0.75
Cyn d 1	0.72
Der f 1	0.79
Der f 2	0.77
Der p 1	0.76
Der p 2	0.88
Mal d 1	0.82
Phl p 1	0.68
Phl p 2	0.76
Phl p 5	0.85
Phl p 6	0.78
Pru p 1	0.77

Sensitization profiles were determined for each study participant individually. Nasal sIgE profiles were positively and significantly correlated to serum sIgE profiles (Figure 35).

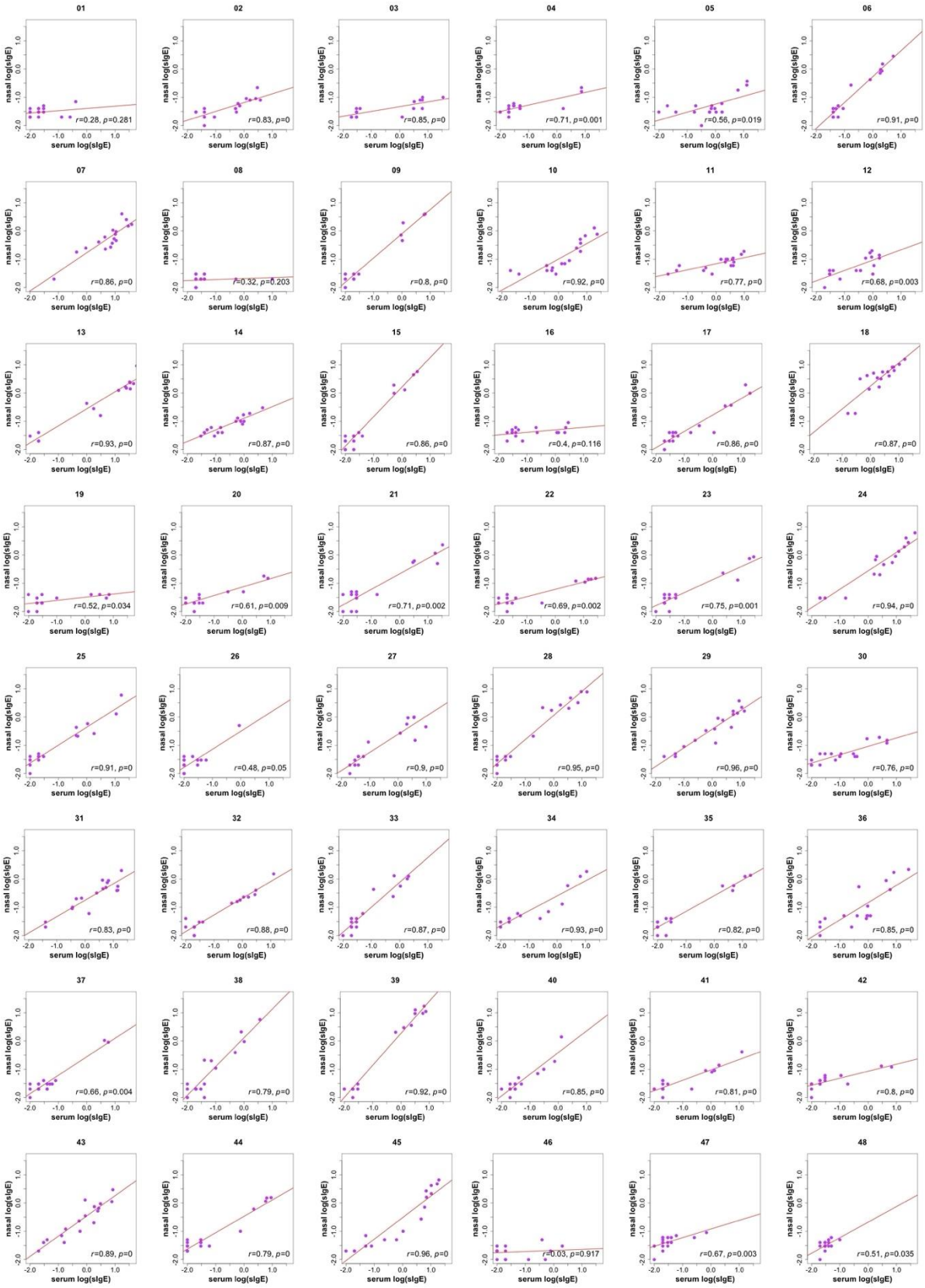


Figure 35: Correlation plots of serum and nasal sIgE levels. Serum sIgE levels on the x-axis were plotted against nasal sIgE levels on the y-axis. Each purple dot indicates one of the tested components for PR-10 proteins, grass-pollen and HDM allergens. Fitted red lines indicate Spearman correlations.

The median of all Spearman correlation coefficients (r_s) for all subjects was 0.77 with IQR 0.68 to 0.88 (Table 18). Only a few subjects (01, 08,16, 26 and 46) had lower correlation coefficients (r_s >0.5). Subjects with poly-sensitization showed the highest correlations.

Table 18: Spearman correlation coefficients for all subjects. Allergen-specific IgE profiles measured in sera and nasal secretions.

ID	Correlation coefficient R		ID	Correlation coefficient R		ID	Correlation coefficient R
45	0.96		**07**	0.86		**04**	0.71
29	0.96		**17**	0.86		**22**	0.69
28	0.95		**40**	0.85		**12**	0.68
24	0.94		**36**	0.85		**47**	0.67
13	0.93		**03**	0.85		**37**	0.66
34	0.93		**31**	0.83		**20**	0.61
39	0.92		**02**	0.83		**49**	0.57
10	0.92		**35**	0.82		**05**	0.56
06	0.91		**41**	0.81		**19**	0.52
25	0.91		**09**	0.80		**48**	0.51
27	0.9		**42**	0.80		**26**	0.48
43	0.89		**38**	0.79		**16**	0.40
32	0.88		**44**	0.79		**08**	0.32
33	0.87		**11**	0.77		**01**	0.28
18	0.87		**30**	0.76		**46**	0.03
14	0.87		**23**	0.75			
15	0.86		**21**	0.71			

6.2.4 Evaluation of nasal sampling as a diagnostic tool§

The standard allergy diagnostic test ImmunoCAP was compared to the molecular allergy diagnostic test ISAC. To validate the nasal diagnostic in comparison to the established serum diagnostic, a cut-off threshold for nasal secretion samples was assessed by a receiver operating characteristics (ROC) curve through Youden´s Index. The area under the curve (AUC) for serum (0.97) and nasal fluid (0.93) ISAC test was similar (Figure 36).

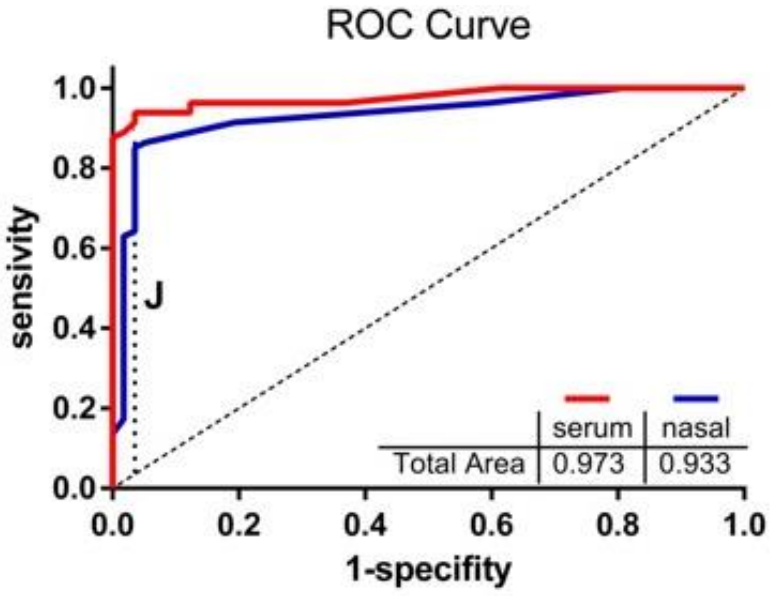


Figure 36: Receiver operating characteristic (ROC) curve for nasal (blue) and serum (red) IgE diagnosis (ISAC). Youden´s Index "J" shows the threshold for positive nasal test.

Accordingly, the nasal threshold was computed to be 0.08 ISU-E. The serum cut-off threshold for ISAC was validated to be 0.3 ISU-E by the manufacturer. All measurements above this threshold indicate sensitization. Therefore, the diagnostic capability of serum and nasal sIgE determination for birch-pollen (Bet v 1, Bet v 2 and Bet v 4), grass-pollen (Cyn d 1, Phl p 1, Phl p 2, Phl p 5 and Phl p 6) and HDM (Der f 1, Der f 2, Der p 1 and Der p 2) were analyzed by a contingency table (Table 19A, B).

Nasal secretion sampling was validated (Table 19C) in allergy diagnosis as compared to serum sampling and had similar high specificity (serum: 0.95, nasal: 0.96) and high positive prediction values (serum: 0.96, nasal: 0.97). The diagnostic sensitivity and negative prediction value of the nasal diagnostic was slightly lower than of the serum diagnostic (TPR serum: 0.94 vs. TPR nasal: 0.85; NPV serum: 0.92 vs. NPV nasal: 0.82). The diagnostic accuracy was described by likelihood ratios for nasal secretion (LR+ 24.28, LR- 0.15) and serum (LR+ 17.83, LR- 0.07).

Table 19: Comparison of ImmunoCAP and ISAC diagnostic performed on serum (B) and nasal secretion (A). Diagnostics via ImmunoCAP used as a control. C: Statistical analysis to validate performance of serum and nasal ISAC.

A

		Sensitization	
		Yes	No
Nasal Secretion	positive test	69	2
	negative test	12	55

B

		Sensitization	
		Yes	No
Serum	positive test	76	3
	negative test	5	54

C

Serum	**Statistical analysis**	**Nasal**
0.94	Sensitivity (TPR)	0.85
0.95	Specificity (TNR)	0.96
0.96	Positive prediction value (PPV)	0.97
0.92	Negative prediction value (NPV)	0.82
0.04	Type I error	0.03
0.06	Type II error	0.15
0.05	False positive rate (FPR)	0.04
0.08	False omission rate (FOR)	0.18
17.83	Likelihood ratio pos. (LR+)	24.28
0.07	Likelihood ratio neg. (LR-)	0.15
273.60	Diagnostic odds ratio (DOR)	158.13
0.95	F1 score	0.91
0.94	Accuracy	0.90
0.88	Matthews correlation coefficient	0.80
0.89	Youden´s Index	0.82
0.88	Markedness	0.79

7. Discussion

Throughout the longitudinal panel study, local humoral immune responses were compared between seasonal allergic rhinitis patients and non-allergic subjects under airborne pollen exposure.

SAR patients showed higher levels of nasal chemokines such as CCL2/MCP-1, CCL22/MDC, and CCL24/Eotaxin-2, which were related to late-phase responses in a nasal allergen challenge study (Kramer et al., 2006). Similar to our findings, also other studies showed the upregulation of CCL24 (Chae, Park, Oh, Lee, & Chung, 2005; De Corso et al., 2011; Konig et al., 2015) and CCL2 (Peric et al., 2016) in the nasal fluid of SAR patients, but in this study the recruitment was during the pollen exposure and only on a single visit.

During the pollen season, total IgA increased in the nasal fluid of non-allergic subjects. Bet v 1-specific IgA was higher in serum compared to nasal fluid. Possibly, SAR patients have an impaired trans-epithelial antibody transport or IgA exclusion by polymeric immunoglobulin receptor (pIgR). In line with the present results, other researchers have previously found that nasal IgA was correlated significantly with allergic symptoms, whereas serum IgA was not (Suzuki, Yokota, Ozaki, Matsumoto, & Nakamura, 2019).

In our study, the PIGR gene expression was investigated. The pIgR is thought to translocate IgA antibodies from the inside to the outside of the epithelial barrier (Johansen & Kaetzel, 2011). In support of this hypothesis, $PIGR^{-/-}$ mice have increased serum IgA levels due to a defective transport mechanism (Wei & Wang, 2021). A defect in IgA transepithelial transport could also be reflected in the serum IgA levels observed in our panel study, which were significantly higher in allergic patients compared to non-allergic individuals. Allergen-specific IgA plays a protective role in allergic inflammation by inhibiting the interaction between IgE and allergen after allergen-specific immunotherapy or by passive immunization (Shamji et al., 2021). A previous study showed that the ratio of Bet v 1-specific IgA to total IgA was higher in nasal fluid of SAR patients only during the pollen season, but not after experimental nasal challenge. A decrease in total IgA was observed 45 minutes after allergen challenge, whereas Bet v 1-specific IgA did not change (Keen, Johansson, Reinholdt, Benson, & Wennergren, 2005). It could be due to exposure time, which is prolonged during the pollen season. This indicates an active role of allergen-specific nasal IgA only during the pollen season.

Further gene expression analyses were planned for the nasal curettage samples of the panel study participants, however, unfortunately, RNA concentrations were too low for further analysis.

Non-supervised PCA identified nasal Bet v 1-specific IgG_4 as one of the main factors that clusters subjects in regards to their symptom severity. Nasal specific IgG_4 was positively

correlated to in-season symptom severity in SAR patients. IgG_4 levels during allergen-specific immunotherapy (ASIT) were investigated in many studies and found to be associated negatively with symptom severity (C. A. Akdis & Akdis, 2011; Eckl-Dorna et al., 2018; Gadermaier et al., 2011; Shamji & Durham, 2011). In our panel study, allergic patients had similar IgG_4 levels, while symptomatic non-allergic subjects have lower or non-detectable $sIgG_4$ levels compared to asymptomatic NA subjects. IgG_4 was found as a candidate nasal biomarker also in other studies (Shamji et al., 2019; Shamji et al., 2012).

In our study, in addition to IgG_4, also nasal IL-33 contributed as a factor to the biomarker panel that separated the subjects according to their symptom severity. Positive correlations between in-season symptom severity and IL-33 levels in NA subjects raise the question of a mechanism mediated by type-2 innate lymphoid cells (Holgado et al., 2019). Nasal IL-33 was decreased in SAR patients during the pollen season. The explanation might be that nasal IL-33 in SAR patients is mainly bound to its soluble receptor, ST2, which might inhibit its binding to specific antibodies in immunological assays. Consequently, it was not readily detected in nasal fluid of SAR patients. Baumann et al. also mentioned the lack of IL-33 levels in SAR patients under natural pollen exposure but showed a positive correlation between ST2 and the symptom severity (Baumann et al., 2013).

All SAR patients and a subset of NA subjects (4/7) recorded symptoms during natural pollen exposure. Even though the symptoms of NA subjects were on a lower scale than those of SAR patients, they were clearly associated to the airborne pollen concentrations and to the kinetics of the different tree pollen seasons. Symptoms of NA subjects started with the onset of the Corylaceae and peaked with the onset of the birch pollen season. There was a 13 days lag from the beginning of pollen season to the first symptoms. Previously, time-series analysis had shown a positive and significant cross-correlation between airborne pollen concentrations and nasal symptoms, with up to 9 days lag effect (Gilles et al., 2020).

All NA subjects underwent sensitization screening by SPT, ImmunoCAP and ISAC. Only one of the non-allergic subjects had a low-grade sensitization to a single grass pollen allergen (rPhl p 11) but had not registered any symptoms during the grass pollen season. All other NA subjects were non-reactive to any of the tested aeroallergens. There were low levels of Bet v 1-sIgE detectable in some of the nasal fluid samples of subjects NA6 and NA9. However, those were among the asymptomatic subjects. Subjects with LAR are characterized by allergic symptoms and presence of local expression of allergen specific IgE in the nose but not in serum (Rondon et al., 2018). Therefore, the diagnosis of local allergic rhinitis (LAR) in symptomatic non-allergic individuals can be excluded.

Nasal immune responses of non-allergic subjects have been examined in only a few previous studies (Gilles-Stein et al., 2016; Joenvaara et al., 2009; Mattila et al., 2010). So far,

there was no systematical research on the symptom kinetics of non-allergic subjects. The time-course of pollen-related symptom expression of NA subjects in the present study strikingly resembled that of the SAR patients. 4 out of 7 NA subjects showed very similar seasonal symptom kinetics as the SAR patients, and the nasal biomarkers for in-season symptom severity were the same both cohorts. These findings could be an indication that our symptomatic NA subjects might be prone to develop SAR at some later stage in their life.

A recent publication indicates that airborne pollen exposure affects innate antiviral immune responses of respiratory epithelial cells isolated from non-allergic and SAR donors (Gilles et al., 2019). Also immune responses like IL-8 expression and neutrophil infiltration correlate with acute respiratory viral infection and symptom severity (Henriquez, Hayney, Xie, Zhang, & Barrett, 2015). One of the main biomarkers for symptom severity of NA subjects was nasal IL-8. Both, exposure to pollen and virus infection increase IL-8 expression (Blume et al., 2013; Herz, Lacy, Renz, & Erb, 2000; Mellow et al., 2004). Therefore, especially during the pollen season, individuals could have a higher recruitment of neutrophils into the site of allergic inflammation, or have more viral infections in the nasal mucosa.

The longitudinal panel study observed only 14 subjects but had a high granularity of sampling. A validation study was conducted to reproduce the biomarker results for the symptom severity. The well-defined biomarker signature reveals the importance of early treatment for individuals, which might develop allergies in later stages of life. Nasal biomarkers in SAR patients may also be helpful in the prediction of success in allergen-specific immunotherapy.

Self-reported symptoms are always subjective and could be over-interpretated, as they might rely on co-factors such as the general well-being, even if the daily query allows the participants to compare their symptoms of the present day to those of the previous days. Based on our results, immune parameters might be an objective measure to reflect symptom severity in SAR patients.

Biomarkers for symptom severity in non-allergic subjects could help to discover a predisposition to develop pollen allergies in the future or indicate an early stage of allergic sensitization and could thus be a tool for primary prevention.

In our exploratory study, we did not observe cross-seasonal changes in total serum immunoglobulins, except for IgA. In contrast, nasal immunoglobulin levels are more variable under pollen exposure. Furthermore, our panel study results demonstrated a correlation of symptoms with nasal specific IgE but not with serum specific IgE.

In the current study, no changes were observed in the numbers of nasal infiltrating neutrophils or monocytes under natural pollen exposure. Therefore, the immune cell staining was extended in the follow-up panel study of 2018. The results clearly showed increased

eosinophil infiltration during the pollen season, which was correlated to pollen concentrations. In a previous study, nasal eosinophil cationic protein (ECP) was found to be correlated with symptom severity in allergic patients, but not with the serum ECP levels (Klimek, Riechelmann, & Amedee, 1996). In another study, nasal smear eosinophil counts were shown to mark a cut-off value for the diagnosis of allergic rhinitis (Pal, Sinha Babu, Halder, & Kumar, 2017). However, we showed that nasal eosinophil influx is dynamic over the time and depends on the pollen exposure, which should be considered when assessing nasal eosinophils as a diagnostic marker.

In our exploratory study we identified specific antibodies of IgE and IgG_4, IL-33 and IL-8 as biomarkers. Similar to our findings, also Kim et al. found nasal Clara cell protein 16 (CC16) as a nasal biomarker, which reduces T_H2 cytokines and correlates with the symptom severity (Kim et al., 2020). Currently, most of the biomarkers are based on serum samples (Zissler, Esser-von Bieren, Jakwerth, Chaker, & Schmidt-Weber, 2016). Shifting the focus on nasal biomarkers or adopting biomarkers to non-invasive samples such as nasal fluid could facilitate clinical diagnostics in the future.

Another focus of this thesis was the validation of non-invasive nasal sampling for the detection of specific IgE to airborne allergens and its correlation to the serum sIgE. Therefore, serum as well as nasal fluid specific IgE levels were measured by ISAC. Most of the subjects were sensitized not only to grass pollen (79.6%), but also to birch pollen (53.1%) as well as to HDM (53.1%). Interestingly, specific sensitization profiles were observed. Birch pollen and HDM sensitizations did not co-occur, unless there was an additional grass pollen sensitization. A previous work on a larger cohort also found no association between tree pollen and HDM (Fiocchi et al., 2015). In another study, most of the birch-pollen sensitized patients also showed alder- and hazel-pollen co-sensitization (Blankestijn et al., 2017), which is generally explained by cross-sensitization, since the major allergens of these pollen belong to the same PR-10 protein family (Worm et al., 2014) and show high inter-species amino acid sequence homology (Jacob et al., 2019). How relevant is order in which we acquire sensitizations to different allergens? Could it in a specific, one-way direction? Could one sensitization be the door opener to other sensitizations? The results obtained here suggest that these interesting questions should be addressed in future studies on sensitization patterns.

The molecular component-resolved method ISAC identified more subjects as sensitized to alder and hazel pollen compared to ImmunoCAP. The commonly used method ImmunoCAP is based on allergen extracts with largely unspecified components. More studies are needed to distinguish between genuine and cross-sensitization against birch, alder and hazel pollen. As other studies pointed out, specific patterns are related to allergic symptoms during the pollen season (Breiteneder et al., 2019). Scala et. al compared different allergy diagnostics and described ISAC as the best method compared to SPT and ImmunoCAP (Scala, Villalta,

Meneguzzi, Brusca, & Cecchi, 2020). Of note, anamnesis based on the specific symptom history of a patient is of critical importance in determining whether any given IgE sensitization is clinically relevant. If the clinical relevance remains unclear, allergen provocation tests are still the ultimate diagnostic tool.

In this study, similar specificities of ISAC for serum (0.95) and nasal secretion (0.96) were calculated. The sensitivity of nasal allergy diagnostic (0.85) was lower compared to serum (0.94). Nevertheless, previously studies on serum (specificity: >0.90, sensitivity: 68.2-93.9%) showed the same results as the nasal tests (Garcia et al., 2016). Serum and nasal tests were done according to manufacturer´s instructions. Therefore, the experimental setup (e.g. incubation times, fluorescent marker, and sampling methods) for nasal testing should be optimized more to increase the sensitivity. In particular the threshold for the positive outcome could be set to a lower value than 0.30 ISU-E without losing specificity, provided by the manufacturer. In our study, sIgE levels against aeroallergens tested in nasal fluid were significantly and positively correlated to serum with respect to proper procedure where the nasal samples should be clear and not tinted with blood due to dry nasal mucosa.

To my knowledge, none of the previous studies comparing nasal and serum diagnostic compared ISAC test results in nasal fluid and serum on a whole aeroallergen panel. They compared only single allergens of Dermatophagoides, Japanese cedar, mugwort and fungi (Ahn, Hong, & Choi, 2017; Campo et al., 2018; Meng, Lou, Wang, Wang, & Zhang, 2018; Sakaida, Masuda, & Takeuchi, 2014).

Overall, only 5 of 47 aeroallergen-sensitized subjects had lower correlations (r_s=< 0.5) between nasal and serum test results. Those subjects all showed low levels of total IgE and IgG. This could indicate improper sampling in these subjects, perhaps due to poor adsorption or too dry nasal mucosa. Recently, kits for nasal fluid collection have become commercially available (e.g. Nasosorption™ FXi, Hunt Developments Ltd, UK). Optimization of nasal fluid sampling, sample processing and sampling quality controls should be done in order to optimize the detection of specific IgE antibodies in nasal secretions.

In conclusion, nasal fluid could be useful for measurement of specific IgE and of interest for clinical diagnostics. Studies on children should be conducted to validate the correlation between serum and nasal IgE. Non-invasive nasal tests could replace the current serum test especially in children. In addition, symptom severity and nasal specific IgE as biomarkers open up new perspectives in the field of allergy research and personalized medicine by allowing patients to have their allergy phenotyped on the molecular level and receiving personalized medical advice, optimized medication and tailored allergen-specific immunotherapy.

8. Appendix

Table 20: Serum and nasal specific IgE levels. Serum (S) and nasal (N) sensitization to allergens against Betulaceae-pollen (Ara h 8, Act d 8, Aln g 1, Bet v 1, Cor a 1.0101, Cor a 1.0401, Mal d 1, Pru p 1) were tested via ISAC. Specific IgE levels, marked in bold, indicate positive sensitization (serum ≥ 0.3 ISU-E; nasal ≥ 0.08 ISU-E).

	Ara h 8		Act d 8		Aln g 1		Bet v 1		Cor a 1.0101		Cor a 1.0401		Mal d 1		Pru p 1	
ID	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
02	**0.5**	0.0	0.0	0.0	**0.6**	0.1	**3.0**	**0.2**	**1.2**	**0.1**	**0.5**	0.0	**0.7**	0.1	**0.5**	0.0
03	0.0	0.0	0.0	0.0	**6.3**	0.0	**3.1**	0.0	**5.3**	**0.1**	0.0	0.0	0.0	0.0	0.0	0.0
04	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0
05	**0.3**	0.0	0.0	0.0	**0.6**	0.0	**1.7**	0.1	**0.7**	0.1	0.2	0.1	**1.0**	0.0	**0.6**	0.0
06	**0.8**	**0.4**	0.2	**0.3**	**2.1**	**0.9**	**5.3**	**2.8**	**2.2**	**1.5**	**1.8**	**0.7**	**1.9**	**1.0**	**2.0**	**0.8**
07	**0.9**	**0.3**	**0.4**	**0.2**	**8.4**	**1.1**	**17.4**	**4.0**	**10.3**	**0.8**	**4.6**	**0.2**	**4.4**	**0.6**	**2.7**	**0.4**
08	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
09	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	**0.8**	0.0	**0.6**	0.1	**23.2**	**0.8**	**18.4**	**1.3**	**5.9**	**0.5**	**6.0**	**0.2**	**6.0**	**0.4**	**4.4**	**0.3**
11	**0.7**	0.0	**0.4**	0.1	**1.5**	**0.1**	**9.6**	**0.2**	**2.5**	**0.1**	**4.0**	**0.1**	**2.3**	**0.1**	**2.1**	**0.1**
12	**0.4**	0.0	0.1	0.0	**0.6**	**0.1**	**0.9**	**0.2**	**0.9**	**0.1**	**1.1**	0.1	**1.7**	**0.1**	**1.7**	**0.1**
13	**1.0**	**0.4**	**1.8**	**0.3**	**32.9**	**2.4**	**58.1**	**9.1**	**34.0**	**2.3**	**23.6**	**1.6**	**13.3**	**1.2**	**3.1**	**0.2**
14	0.1	0.0	0.2	0.0	0.2	0.1	**4.6**	**0.3**	**0.8**	**0.1**	**0.5**	**0.1**	**0.9**	**0.1**	0.1	0.0
15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.1	0.0	0.2	0.0	**2.2**	0.0	**2.9**	**0.1**	0.2	0.1	**1.3**	0.0	**2.4**	0.1	**1.2**	0.0
17	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	**1.8**	**3.4**	**0.9**	**1.4**	**1.3**	**5.1**	**16.3**	**15.7**	**4.6**	**4.0**	**2.7**	**5.6**	**5.9**	**8.2**	**4.1**	**5.6**
19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
24	**3.5**	**0.5**	0.2	0.0	**2.6**	**0.2**	**21.7**	**4.0**	**9.3**	**0.9**	**1.8**	**0.7**	**1.6**	**0.2**	**2.0**	**0.9**
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29	0.1	**0.1**	0.0	0.0	**1.6**	**0.9**	**11.0**	**2.2**	**5.5**	**1.6**	**2.4**	**0.8**	**5.6**	**1.2**	**0.6**	**0.3**
30	0.0	0.0	0.0	0.0	0.0	0.0	**0.3**	0.0	**0.3**	0.1	0.0	0.1	0.1	0.0	0.0	0.0
31	**0.4**	**0.1**	**0.5**	**0.2**	**4.1**	**0.9**	**19.2**	**2.0**	**0.8**	**0.2**	**6.1**	**0.8**	**5.5**	**0.5**	**2.6**	**0.3**
32	**0.8**	**0.2**	**0.7**	**0.2**	**0.4**	**0.1**	**12.8**	**1.5**	**1.1**	**0.2**	**3.0**	**0.4**	**2.8**	**0.3**	**1.6**	**0.2**
33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
34	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
36	**1.0**	0.1	0.3	0.0	0.1	0.1	**5.9**	**0.4**	**0.4**	0.1	**1.0**	**0.1**	**1.2**	0.1	**0.9**	0.1
37	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
38	0.0	0.0	0.0	0.0	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39	**0.7**	**2.0**	**1.3**	**3.0**	**7.6**	**11.1**	**6.6**	**17.0**	**6.0**	**9.5**	**2.3**	**3.6**	**3.2**	**9.4**	**3.2**	**12.6**
40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
41	0.2	0.0	0.0	0.0	**0.7**	**0.1**	**12.5**	**0.4**	**1.9**	**0.1**	**1.1**	**0.1**	**1.3**	**0.1**	**1.3**	**0.1**
42	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0

	Ara h 8		Act d 8		Aln g 1		Bet v 1		Cor a 1.0101		Cor a 1.0401		Mal d 1		Pru p 1	
ID	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N
43	0.2	**0.1**	0.0	0.0	**0.9**	**1.3**	**8.4**	**2.9**	**1.9**	**0.7**	**0.5**	**0.2**	**3.1**	**0.9**	**0.9**	**0.4**
44	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
45	0.0	0.0	0.0	0.0	0.1	0.0	**1.0**	**0.1**	0.1	0.0	0.2	0.1	**0.6**	0.1	0.0	0.0
46	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
47	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
49	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0

Table 21: Serum and nasal specific IgE levels. Serum and nasal sensitization to allergens against grass-pollen (Cyn d 1, Phl p 1, Phl p 2, Phl p 5 and Phl p 6) were tested via ISAC. Specific IgE levels, marked in bold, indicate positive sensitization (serum ≥0.3 ISU-E; nasal ≥0.08 ISU-E).

	Cyn d 1		Phl p 1		Phl p 2		Phl p 5		Phl p 6	
ID	serum	nasal	serum	nasal	serum	nasal	serum	nasal	serum	nasal
01	0.1	0.0	**0.4**	0.1	0.3	0.0	0.0	0.0	0.0	0.1
02	0.1	0.0	**1.6**	**0.1**	0.0	0.0	0.0	0.0	0.0	0.0
03	**6.3**	**0.1**	**34.2**	**0.1**	**1.2**	0.0	**6.5**	**0.1**	**3.3**	0.1
04	**1.7**	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0
05	**0.7**	0.0	**13.1**	**0.4**	0.2	0.0	**6.2**	**0.2**	**12.8**	**0.3**
06	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0
07	0.1	0.0	**7.0**	**0.3**	**9.3**	**0.5**	**25.2**	**2.5**	**8.0**	**0.4**
08	**0.5**	0.0	**10.0**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
09	**0.9**	**0.7**	**6.1**	**3.8**	**1.1**	**2.0**	**6.6**	**4.0**	**1.0**	**0.5**
10	**0.4**	0.0	**8.8**	**0.7**	**1.3**	0.1	0.0	0.0	0.0	0.0
11	**3.4**	0.1	**7.8**	**0.2**	0.0	0.0	**4.0**	0.1	**0.4**	0.0
12	0.3	0.0	**0.8**	**0.2**	0.0	0.0	**1.0**	0.0	0.0	0.0
13	**45.1**	**2.2**	**87.9**	**6.3**	**25.2**	**1.5**	**82.2**	**5.2**	**34.6**	**1.4**
14	0.1	0.1	**1.6**	**0.2**	**1.0**	**0.1**	**1.0**	**0.2**	**0.6**	**0.1**
15	**1.3**	**1.3**	**2.6**	**4.4**	**0.5**	**1.9**	**3.5**	**5.7**	**0.5**	**1.0**
16	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
17	**21.0**	**1.0**	**14.7**	**1.9**	**1.1**	0.0	**4.5**	**0.4**	**2.8**	**0.4**
18	**6.9**	**6.2**	**10.4**	**10.3**	**2.4**	**3.2**	**6.6**	**8.3**	**2.0**	**1.6**
19	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	**1.1**	0.1	**8.0**	**0.2**	0.0	0.0	**5.8**	**0.2**	**0.3**	0.1
21	**3.0**	**0.6**	**32.8**	**2.3**	**18.1**	**1.2**	**3.3**	**0.6**	**21.7**	**0.5**
22	**0.3**	0.0	**5.4**	**0.1**	0.0	0.0	0.0	0.0	0.0	0.0
23	**2.3**	**0.2**	**28.4**	**0.9**	0.0	0.0	**20.2**	**0.8**	**7.8**	**0.1**
24	**12.0**	**1.3**	**45.2**	**6.1**	**19.0**	**1.9**	**26.1**	**2.8**	**7.1**	**0.5**
25	**1.8**	**0.3**	**11.0**	**1.3**	**0.4**	**0.4**	**16.6**	**6.0**	**1.1**	**0.6**
26	0.0	0.0	**0.9**	**0.5**	0.0	0.0	0.0	0.0	0.0	0.0
27	0.1	**0.1**	**2.1**	**0.6**	0.1	0.0	**3.7**	**1.0**	**1.2**	**0.3**
28	**0.8**	**1.7**	**1.7**	**2.7**	**0.4**	**2.1**	**4.0**	**4.7**	0.2	**0.2**
29	**1.2**	**0.4**	**8.9**	**3.7**	0.3	**0.2**	0.1	0.1	0.1	0.0
30	0.1	0.1	**2.5**	**0.2**	**0.4**	0.0	**0.9**	**0.2**	0.1	0.0
31	**0.4**	**0.1**	**4.3**	**0.5**	**6.7**	**0.9**	**14.2**	**0.4**	**1.4**	0.1
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	0.1	**0.4**	**0.6**	**1.3**	0.0	0.0	0.0	0.0	0.0	0.0

	Cyn d 1		Phl p 1		Phl p 2		Phl p 5		Phl p 6	
ID	serum	nasal	serum	nasal	serum	nasal	serum	nasal	serum	nasal
34	0.3	0.0	**2.4**	**0.6**	0.0	0.0	0.1	0.1	0.0	0.0
35	**4.5**	**0.4**	**19.1**	**1.4**	**2.0**	**0.6**	**12.3**	**1.2**	**4.9**	**0.6**
36	**0.5**	**0.5**	**8.1**	**1.7**	**4.3**	**0.9**	**26.6**	**2.2**	**0.9**	0.0
37	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
38	**0.5**	**0.4**	**1.0**	**1.0**	**0.8**	**2.1**	**3.7**	**5.7**	0.1	**0.1**
39	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40	**0.3**	**0.1**	**1.3**	**1.4**	0.2	0.1	**0.8**	**0.2**	0.1	0.0
41	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42	0.2	0.0	**2.9**	**0.1**	0.0	0.0	**6.7**	**0.1**	0.1	0.1
43	**0.6**	**0.1**	**7.7**	**1.1**	**2.5**	**0.5**	**2.7**	**0.7**	**1.8**	**0.2**
44	0.1	0.0	**0.8**	**0.1**	0.0	0.0	0.0	0.0	0.0	0.1
45	**4.5**	**0.3**	**6.8**	**2.7**	**10.3**	**4.2**	**7.0**	**1.6**	0.1	0.1
46	0.1	0.0	**0.4**	0.1	**0.5**	0.0	**2.0**	0.0	**0.8**	0.0
47	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0
49	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 22: Serum and nasal specific IgE levels. Serum and nasal sensitization to allergens against house dust mite (Der f 1, Der f 2, Der p 1 and Der p 2) were tested via ISAC. Specific IgE levels, marked in bold, indicate positive sensitization (serum ≥0.3 ISU-E; nasal ≥0.08 ISU-E).

	Der f 1		Der f 2		Der p 1		Der p 2	
ID	serum	nasal	serum	nasal	serum	nasal	serum	nasal
01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
02	0.0	0.0	**2.6**	**0.1**	0.0	0.0	**3.8**	**0.1**
03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
04	0.0	0.1	**7.4**	**0.2**	0.0	0.1	**7.4**	**0.2**
05	0.0	0.1	**1.7**	0.0	0.0	0.0	**1.1**	0.0
06	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
07	**11.0**	**0.5**	**29.3**	**1.5**	**10.8**	**1.0**	**38.8**	**1.7**
08	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
09	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	**0.4**	0.1	**1.7**	0.1	**0.6**	0.0	**2.4**	**0.1**
11	0.1	0.1	**3.8**	**0.1**	0.0	0.0	**4.2**	**0.1**
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0
17	**0.3**	0.1	0.0	0.0	0.2	0.0	0.0	0.0
18	0.3	**0.2**	**0.8**	**4.1**	0.2	**0.2**	**0.4**	**3.2**
19	**1.6**	0.0	**5.7**	0.0	**3.3**	0.0	**7.0**	0.0
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
22	**14.6**	**0.1**	**18.4**	**0.1**	**11.2**	**0.1**	**26.8**	**0.2**
23	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	**0.4**	**0.2**	0.0	0.0	**0.5**	**0.2**

	Der f 1		Der f 2		Der p 1		Der p 2	
ID	serum	nasal	serum	nasal	serum	nasal	serum	nasal
26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	**9.7**	**0.5**	**3.7**	**1.0**	**4.0**	**0.2**	**2.3**	**0.9**
28	**3.4**	**2.0**	**9.4**	**7.9**	**7.2**	**3.2**	**15.1**	**7.7**
29	**1.3**	**0.1**	**7.8**	**1.4**	**4.8**	**0.4**	**13.7**	**1.6**
30	0.1	0.1	**4.7**	**0.2**	0.0	0.1	**4.6**	**0.1**
31	**14.3**	**0.6**	0.0	0.0	**13.3**	**0.4**	0.0	0.0
32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33	**1.1**	**0.6**	**2.1**	**1.3**	**0.6**	**0.2**	**1.9**	**1.0**
34	**1.5**	**0.1**	**6.5**	**1.2**	**0.5**	0.1	**10.9**	**1.8**
35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
36	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
37	0.1	0.0	**5.8**	**0.9**	0.1	0.0	**4.2**	**1.1**
38	0.0	0.0	0.0	**0.2**	0.0	0.0	0.1	**0.2**
39	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
41	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
43	0.0	0.1	0.2	0.0	0.0	0.0	0.1	0.1
44	**2.2**	**0.6**	**5.8**	**1.2**	**6.4**	**1.5**	**9.0**	**1.5**
45	**6.0**	**0.7**	**16.7**	**4.7**	**10.4**	**2.1**	**19.1**	**6.5**
46	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
47	0.3	0.1	0.0	0.1	**0.7**	**0.1**	0.0	0.1
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
49	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

9. References

Aalberse, R. C., Platts-Mills, T. A., & Rispens, T. (2016). The Developmental History of IgE and IgG4 Antibodies in Relation to Atopy, Eosinophilic Esophagitis, and the Modified TH2 Response. *Curr Allergy Asthma Rep, 16*(6), 45. doi:10.1007/s11882-016-0621-x

Abbas, M., Moussa, M., & Akel, H. (2020). Type I Hypersensitivity Reaction. In *StatPearls*. Treasure Island (FL).

Actor, J. K., & Actor, J. K. (2012). *Elsevier's integrated review immunology and microbiology* (2nd ed.). Philadelphia, PA: Elsevier/Saunders.

Aglas, L., Gilles, S., Bauer, R., Huber, S., Araujo, G. R., Mueller, G., . . . Ferreira, F. (2018). Context matters: TH2 polarization resulting from pollen composition and not from protein-intrinsic allergenicity. *J Allergy Clin Immunol, 142*(3), 984-987 e986. doi:10.1016/j.jaci.2018.05.004

Ahn, J. Y., Hong, S. J., & Choi, B. S. (2017). Clinical Evaluation of Techniques for Measuring Nasal-Specific Immunoglobulin E in Pediatric Patients. *J Korean Med Sci, 32*(12), 2005-2008. doi:10.3346/jkms.2017.32.12.2005

Akasaki, S., Matsushita, K., Kato, Y., Fukuoka, A., Iwasaki, N., Nakahira, M., . . . Yoshimoto, T. (2016). Murine allergic rhinitis and nasal Th2 activation are mediated via TSLP- and IL-33-signaling pathways. *Int Immunol, 28*(2), 65-76. doi:10.1093/intimm/dxv055

Akdis, C. A., & Akdis, M. (2011). Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol, 127*(1), 18-27; quiz 28-19. doi:10.1016/j.jaci.2010.11.030

Akdis, M., Aab, A., Altunbulakli, C., Azkur, K., Costa, R. A., Crameri, R., . . . Akdis, C. A. (2016). Interleukins (from IL-1 to IL-38), interferons, transforming growth factor beta, and TNF-alpha: Receptors, functions, and roles in diseases. *J Allergy Clin Immunol, 138*(4), 984-1010. doi:10.1016/j.jaci.2016.06.033

Akhouri, S., & House, S. A. (2021). Allergic Rhinitis. In *StatPearls*. Treasure Island (FL).

Alvaro-Lozano, M., Akdis, C. A., Akdis, M., Alviani, C., Angier, E., Arasi, S., . . . Vazquez-Ortiz, M. (2020). EAACI Allergen Immunotherapy User's Guide. *Pediatr Allergy Immunol, 31 Suppl 25*, 1-101. doi:10.1111/pai.13189

Anderegg, W. R. L., Abatzoglou, J. T., Anderegg, L. D. L., Bielory, L., Kinney, P. L., & Ziska, L. (2021). Anthropogenic climate change is worsening North American pollen seasons. *Proc Natl Acad Sci U S A, 118*(7). doi:10.1073/pnas.2013284118

Asam, C., Batista, A. L., Moraes, A. H., de Paula, V. S., Almeida, F. C., Aglas, L., . . . Valente, A. P. (2014). Bet v 1--a Trojan horse for small ligands boosting allergic sensitization? *Clin Exp Allergy, 44*(8), 1083-1093. doi:10.1111/cea.12361

Bauchau, V., & Durham, S. R. (2004). Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J, 24*(5), 758-764. doi:10.1183/09031936.04.00013904

Baumann, R., Rabaszowski, M., Stenin, I., Tilgner, L., Gaertner-Akerboom, M., Scheckenbach, K., . . . Wagenmann, M. (2013). Nasal levels of soluble IL-33R ST2 and IL-16 in allergic rhinitis: inverse correlation trends with disease severity. *Clin Exp Allergy, 43*(10), 1134-1143. doi:10.1111/cea.12148

Beck, I., Jochner, S., Gilles, S., McIntyre, M., Buters, J. T., Schmidt-Weber, C., . . . Traidl-Hoffmann, C. (2013). High environmental ozone levels lead to enhanced allergenicity of birch pollen. *PLoS One, 8*(11), e80147. doi:10.1371/journal.pone.0080147

Bergmann, K. C., Heinrich, J., & Niemann, H. (2016). Current status of allergy prevalence in Germany: Position paper of the Environmental Medicine Commission of the Robert Koch Institute. *Allergo J Int, 25*, 6-10. doi:10.1007/s40629-016-0092-6

Bieber, T., Akdis, C., Lauener, R., Traidl-Hoffmann, C., Schmid-Grendelmeier, P., Schappi, G., . . . Ring, J. (2016). Global Allergy Forum and 3rd Davos Declaration 2015: Atopic dermatitis/Eczema: challenges and opportunities toward precision medicine. *Allergy, 71*(5), 588-592. doi:10.1111/all.12857

Biedermann, T., Winther, L., Till, S. J., Panzner, P., Knulst, A., & Valovirta, E. (2019). Birch pollen allergy in Europe. *Allergy*. doi:10.1111/all.13758

Blankestijn, M., Knulst, A., Knol, E., Le, T.-M., Rockmann, H., Otten, H., & Klemans, R. (2017). Sensitization to PR-10 proteins is indicative of distinctive sensitization patterns in adults with a suspected food allergy. *Clinical and Translational Allergy, 7*. doi:10.1186/s13601-017-0177-4

Blume, C., Swindle, E. J., Dennison, P., Jayasekera, N. P., Dudley, S., Monk, P., . . . Davies, D. E. (2013). Barrier responses of human bronchial epithelial cells to grass pollen exposure. *Eur Respir J, 42*(1), 87-97. doi:10.1183/09031936.00075612

Bochenska-Marciniak, M., Kupczyk, M., Gorski, P., & Kuna, P. (2003). The effect of recombinant interleukin-8 on eosinophils' and neutrophils' migration in vivo and in vitro. *Allergy, 58*(8), 795-801. doi:10.1034/j.1398-9995.2003.00178.x

Boehmer, D., Schuster, B., Krause, J., Darsow, U., Biedermann, T., & Zink, A. (2018). Prevalence and treatment of allergies in rural areas of Bavaria, Germany: a cross-sectional study. *World Allergy Organ J, 11*(1), 36. doi:10.1186/s40413-018-0218-z

Bousquet, J., Akdis, C., Jutel, M., Bachert, C., Klimek, L., Agache, I., . . . group, A.-M. s. (2020). Intranasal corticosteroids in allergic rhinitis in COVID-19 infected patients: An ARIA-EAACI statement. *Allergy*. doi:10.1111/all.14302

Bousquet, J., Anto, J. M., Bachert, C., Baiardini, I., Bosnic-Anticevich, S., Walter Canonica, G., . . . Toppila-Salmi, S. (2020). Allergic rhinitis. *Nat Rev Dis Primers, 6*(1), 95. doi:10.1038/s41572-020-00227-0

Bousquet, J., Bewick, M., Arnavielhe, S., Mathieu-Dupas, E., Murray, R., Bedbrook, A., . . . Zuberbier, T. (2017). Work productivity in rhinitis using cell phones: The MASK pilot study. *Allergy, 72*(10), 1475-1484. doi:10.1111/all.13177

Bousquet, J., Pfaar, O., Togias, A., Schunemann, H. J., Ansotegui, I., Papadopoulos, N. G., . . . Group, A. W. (2019). 2019 ARIA Care pathways for allergen immunotherapy. *Allergy, 74*(11), 2087-2102. doi:10.1111/all.13805

Bousquet, J., Schunemann, H. J., Togias, A., Bachert, C., Erhola, M., Hellings, P. W., . . . Its Impact on Asthma Working, G. (2019). Next-generation Allergic Rhinitis and Its Impact on Asthma (ARIA) guidelines for allergic rhinitis based on Grading of Recommendations Assessment, Development and Evaluation (GRADE) and real-world evidence. *J Allergy Clin Immunol*. doi:10.1016/j.jaci.2019.06.049

Breiteneder, H., Diamant, Z., Eiwegger, T., Fokkens, W. J., Traidl-Hoffmann, C., Nadeau, K., . . . Akdis, C. A. (2019). Future research trends in understanding the mechanisms underlying allergic diseases for improved patient care. *Allergy*. doi:10.1111/all.13851

Calderon, M. A., Demoly, P., Gerth van Wijk, R., Bousquet, J., Sheikh, A., Frew, A., . . . Papadopoulos, N. (2012). EAACI: A European Declaration on Immunotherapy. Designing the future of allergen specific immunotherapy. *Clin Transl Allergy, 2*(1), 20. doi:10.1186/2045-7022-2-20

Cameron, L., Gounni, A. S., Frenkiel, S., Lavigne, F., Vercelli, D., & Hamid, Q. (2003). S epsilon S mu and S epsilon S gamma switch circles in human nasal mucosa following ex vivo allergen challenge: evidence for direct as well as sequential class switch recombination. *J Immunol, 171*(7), 3816-3822. doi:10.4049/jimmunol.171.7.3816

Campo, P., Del Carmen Plaza-Seron, M., Eguiluz-Gracia, I., Verge, J., Galindo, L., Barrionuevo, E., . . . Rondon, C. (2018). Direct intranasal application of the solid phase of ImmunoCAP(R) increases nasal specific immunoglobulin E detection in local allergic rhinitis patients. *Int Forum Allergy Rhinol, 8*(1), 15-19. doi:10.1002/alr.22039

Chae, S. C., Park, Y. R., Oh, G. J., Lee, J. H., & Chung, H. T. (2005). The suggestive association of eotaxin-2 and eotaxin-3 gene polymorphisms in Korean population with allergic rhinitis. *Immunogenetics, 56*(10), 760-764. doi:10.1007/s00251-004-0746-2

Ciprandi, G., Silvestri, M., Pistorio, A., Olcese, R., Del Barba, P., & Tosca, M. A. (2019). Bet v 1 sensitization modulates allergenic molecular immune response. *Eur Ann Allergy Clin Immunol, 51*(1), 21-31. doi:10.23822/EurAnnACI.1764-1489.73

Cohen, S., Dworetzky, M., & Frick, O. L. (2003). Coca and Cooke on the classification of hypersensitiveness. *J Allergy Clin Immunol, 111*(1), 205-210. doi:10.1067/mai.2003.106

Damialis, A., Häring, F., Brunner, J., Buters, J., Traidl-Hoffmann, C. (2016). Short seasons and nightly atmospheric circulation of allergenic pollen in Augsburg, Germany: is it really good news? . *6th European Aerobiology Symposium, Lyon, Γαλλία.*, 65-67.

Damialis, A., Halley, J., Gioulekas, D., & Vokou, D. (2007). Long-term trends in atmospheric pollen levels in the city of Thessaloniki, Greece. *Atmospheric Environment, 41*, 7011-7021. doi:10.1016/j.atmosenv.2007.05.009

Damialis, A., Traidl-Hoffmann, C., Teudler, R. (2019). *Climate change and pollen allergies* [eBook]. In M. R. Marselle, Stadler, J., Korn, H., Irvine, K., Bonn, A. (Ed.).

Dass, K., Petrusan, A. J., Beaumont, J., Zee, P., Lai, J. S., & Fishbein, A. (2017). Assessment of sleep disturbance in children with allergic rhinitis. *Ann Allergy Asthma Immunol, 118*(4), 505-506. doi:10.1016/j.anai.2016.12.022

De Corso, E., Baroni, S., Romitelli, F., Luca, L., Di Nardo, W., Passali, G. C., & Paludetti, G. (2011). Nasal lavage CCL24 levels correlate with eosinophils trafficking and symptoms in chronic sino-nasal eosinophilic inflammation. *Rhinology, 49*(2), 174-179. doi:10.4193/Rhino10.133

De Knop, K. J., Verweij, M. M., Grimmelikhuijsen, M., Philipse, E., Hagendorens, M. M., Bridts, C. H., . . . Ebo, D. G. (2011). Age-related sensitization profiles for hazelnut (Corylus avellana) in a birch-endemic region. *Pediatr Allergy Immunol, 22*(1 Pt 2), e139-149. doi:10.1111/j.1399-3038.2011.01112.x

Dharajiya, N. G., Bacsi, A., Boldogh, I., & Sur, S. (2007). Pollen NAD(P)H oxidases and their contribution to allergic inflammation. *Immunol Allergy Clin North Am, 27*(1), 45-63. doi:10.1016/j.iac.2006.11.007

Dorner, B. G., Scheffold, A., Rolph, M. S., Huser, M. B., Kaufmann, S. H., Radbruch, A., . . . Kroczek, R. A. (2002). MIP-1alpha, MIP-1beta, RANTES, and ATAC/lymphotactin function together with IFN-gamma as type 1 cytokines. *Proc Natl Acad Sci U S A, 99*(9), 6181-6186. doi:10.1073/pnas.092141999

Eckl-Dorna, J., Villazala-Merino, S., Linhart, B., Karaulov, A. V., Zhernov, Y., Khaitov, M., . . . Valenta, R. (2018). Allergen-Specific Antibodies Regulate Secondary Allergen-Specific Immune Responses. *Front Immunol, 9*, 3131. doi:10.3389/fimmu.2018.03131

Eyerich, S., Metz, M., Bossios, A., & Eyerich, K. (2019). New biological treatments for asthma and skin allergies. *Allergy*. doi:10.1111/all.14027

Fiocchi, A., Pecora, V., Petersson, C., Dahdah, L., Borres, M., Amengual, M., . . . Girolamo, F. (2015). Sensitization pattern to inhalant and food allergens in symptomatic children at first evaluation. *Italian Journal of Pediatrics, 41*. doi:10.1186/s13052-015-0204-9

Fulkerson, P. C., & Rothenberg, M. E. (2013). Targeting eosinophils in allergy, inflammation and beyond. *Nat Rev Drug Discov, 12*(2), 117-129. doi:10.1038/nrd3838

Gadermaier, E., Staikuniene, J., Scheiblhofer, S., Thalhamer, J., Kundi, M., Westritschnig, K., . . . Valenta, R. (2011). Recombinant allergen-based monitoring of antibody responses during injection grass pollen immunotherapy and after 5 years of discontinuation. *Allergy, 66*(9), 1174-1182. doi:10.1111/j.1398-9995.2011.02592.x

Garcia, B. E., Martinez-Aranguren, R., Bernard Alonso, A., Gamboa, P., Feo Brito, F., Bartra, J., . . . Sanz, M. L. (2016). Is the ISAC 112 Microarray Useful in the Diagnosis of Pollinosis in Spain? *J Investig Allergol Clin Immunol, 26*(2), 92-99. doi:10.18176/jiaci.0052

Garcia-Mozo, H. (2017). Poaceae pollen as the leading aeroallergen worldwide: A review. *Allergy, 72*(12), 1849-1858. doi:10.1111/all.13210

Gilles, S., Blume, C., Wimmer, M., Damialis, A., Meulenbroek, L., Gokkaya, M., . . . Traidl-Hoffmann, C. (2019). Pollen exposure weakens innate defense against respiratory viruses. *Allergy*. doi:10.1111/all.14047

Gilles, S., Blume, C., Wimmer, M., Damialis, A., Meulenbroek, L., Gokkaya, M., . . . Traidl-Hoffmann, C. (2020). Pollen exposure weakens innate defense against respiratory viruses. *Allergy, 75*(3), 576-587. doi:10.1111/all.14047

Gilles, S., Fekete, A., Zhang, X., Beck, I., Blume, C., Ring, J., . . . Traidl-Hoffmann, C. (2011). Pollen metabolome analysis reveals adenosine as a major regulator of dendritic cell-primed T(H) cell responses. *J Allergy Clin Immunol, 127*(2), 454-461 e451-459. doi:10.1016/j.jaci.2010.12.1082

Gilles, S., Jacoby, D., Blume, C., Mueller, M. J., Jakob, T., Behrendt, H., . . . Traidl-Hoffmann, C. (2010). Pollen-derived low-molecular weight factors inhibit 6-sulfo LacNAc+ dendritic cells' capacity to induce T-helper type 1 responses. *Clin Exp Allergy, 40*(2), 269-278. doi:10.1111/j.1365-2222.2009.03369.x

Gilles-Stein, S., Beck, I., Chaker, A., Bas, M., McIntyre, M., Cifuentes, L., . . . Traidl-Hoffmann, C. (2016). Pollen derived low molecular compounds enhance the human allergen

specific immune response in vivo. *Clin Exp Allergy, 46*(10), 1355-1365. doi:10.1111/cea.12739

Gokkaya, M., Damialis, A., Nussbaumer, T., Beck, I., Bounas-Pyrros, N., Bezold, S., . . . Gilles, S. (2020). Defining biomarkers to predict symptoms in subjects with and without allergy under natural pollen exposure. *J Allergy Clin Immunol, 146*(3), 583-594 e586. doi:10.1016/j.jaci.2020.02.037

Gokkaya, M., Schwierzeck, V., Tholken, K., Knoch, S., Gerstlauer, M., Hammel, G., . . . Gilles, S. (2020). Nasal specific IgE correlates to serum specific IgE: First steps towards nasal molecular allergy diagnostic. *Allergy, 75*(7), 1802-1805. doi:10.1111/all.14228

Gu, L., Tseng, S., Horner, R. M., Tam, C., Loda, M., & Rollins, B. J. (2000). Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature, 404*(6776), 407-411. doi:10.1038/35006097

Guhsl, E. E., Hofstetter, G., Lengger, N., Hemmer, W., Ebner, C., Froschl, R., . . . Radauer, C. (2015). IgE, IgG4 and IgA specific to Bet v 1-related food allergens do not predict oral allergy syndrome. *Allergy, 70*(1), 59-66. doi:10.1111/all.12534

Halken, S., Larenas-Linnemann, D., Roberts, G., Calderon, M. A., Angier, E., Pfaar, O., . . . Muraro, A. (2017). EAACI guidelines on allergen immunotherapy: Prevention of allergy. *Pediatr Allergy Immunol, 28*(8), 728-745. doi:10.1111/pai.12807

Hammad, H., & Lambrecht, B. N. (2015). Barrier Epithelial Cells and the Control of Type 2 Immunity. *Immunity, 43*(1), 29-40. doi:10.1016/j.immuni.2015.07.007

Han, M. W., Kim, S. H., Oh, I., Kim, Y. H., & Lee, J. (2019). Serum IL-1beta can be a biomarker in children with severe persistent allergic rhinitis. *Allergy Asthma Clin Immunol, 15*, 58. doi:10.1186/s13223-019-0368-8

Harter, K., Hammel, G., Krabiell, L., Linkohr, B., Peters, A., Schwettmann, L., . . . Traidl-Hoffmann, C. (2019). Different Psychosocial Factors Are Associated with Seasonal and Perennial Allergies in Adults: Cross-Sectional Results of the KORA FF4 Study. *Int Arch Allergy Immunol, 179*(4), 262-272. doi:10.1159/000499042

He, S. H., Zhang, H. Y., Zeng, X. N., Chen, D., & Yang, P. C. (2013). Mast cells and basophils are essential for allergies: mechanisms of allergic inflammation and a proposed procedure for diagnosis. *Acta Pharmacol Sin, 34*(10), 1270-1283. doi:10.1038/aps.2013.88

Henriquez, K. M., Hayney, M. S., Xie, Y., Zhang, Z., & Barrett, B. (2015). Association of interleukin-8 and neutrophils with nasal symptom severity during acute respiratory infection. *J Med Virol, 87*(2), 330-337. doi:10.1002/jmv.24042

Herz, U., Lacy, P., Renz, H., & Erb, K. (2000). The influence of infections on the development and severity of allergic disorders. *Curr Opin Immunol, 12*(6), 632-640. doi:10.1016/s0952-7915(00)00155-2

Hirahara, K., & Nakayama, T. (2016). CD4+ T-cell subsets in inflammatory diseases: beyond the Th1/Th2 paradigm. *Int Immunol, 28*(4), 163-171. doi:10.1093/intimm/dxw006

Holgado, A., Braun, H., Van Nuffel, E., Detry, S., Schuijs, M. J., Deswarte, K., . . . Beyaert, R. (2019). IL-33trap is a novel IL-33-neutralizing biologic that inhibits allergic airway inflammation. *J Allergy Clin Immunol*. doi:10.1016/j.jaci.2019.02.028

Imai, Y. (2019). Interleukin-33 in atopic dermatitis. *J Dermatol Sci, 96*(1), 2-7. doi:10.1016/j.jdermsci.2019.08.006

Jacob, T., von Loetzen, C. S., Reuter, A., Lacher, U., Schiller, D., Schobert, R., . . . Wohrl, B. M. (2019). Identification of a natural ligand of the hazel allergen Cor a 1. *Sci Rep, 9*(1), 8714. doi:10.1038/s41598-019-44999-2

Jensen-Jarolim, E., Jensen, A. N., & Canonica, G. W. (2017). Debates in allergy medicine: Molecular allergy diagnosis with ISAC will replace screenings by skin prick test in the future. *World Allergy Organ J, 10*(1), 33. doi:10.1186/s40413-017-0162-3

Joenvaara, S., Mattila, P., Renkonen, J., Makitie, A., Toppila-Salmi, S., Lehtonen, M., . . . Renkonen, R. (2009). Caveolar transport through nasal epithelium of birch pollen allergen Bet v 1 in allergic patients. *J Allergy Clin Immunol, 124*(1), 135-142 e131-121. doi:10.1016/j.jaci.2008.11.048

Johansen, F. E., & Kaetzel, C. S. (2011). Regulation of the polymeric immunoglobulin receptor and IgA transport: new advances in environmental factors that stimulate pIgR expression and its role in mucosal immunity. *Mucosal Immunol, 4*(6), 598-602. doi:10.1038/mi.2011.37

Junttila, I. S. (2018). Tuning the Cytokine Responses: An Update on Interleukin (IL)-4 and IL-13 Receptor Complexes. *Front Immunol, 9*, 888. doi:10.3389/fimmu.2018.00888

Justiz Vaillant, A. A., Vashisht, R., & Zito, P. M. (2020). Immediate Hypersensitivity Reactions. In *StatPearls*. Treasure Island (FL).

Karaki, M., Dobashi, H., Kobayashi, R., Tokuda, M., Ishida, T., & Mori, N. (2005). Expression of interleukin-16 in allergic rhinitis. *Int Arch Allergy Immunol, 138*(1), 67-72. doi:10.1159/000087359

Keen, C., Johansson, S., Reinholdt, J., Benson, M., & Wennergren, G. (2005). Bet v 1-specific IgA increases during the pollen season but not after a single allergen challenge in children with birch pollen-induced intermittent allergic rhinitis. *Pediatr Allergy Immunol, 16*(3), 209-216. doi:10.1111/j.1399-3038.2005.00264.x

Kelly, B. T., & Grayson, M. H. (2016). Immunoglobulin E, what is it good for? *Ann Allergy Asthma Immunol, 116*(3), 183-187. doi:10.1016/j.anai.2015.10.026

Kim, S. I., Kwon, O. E., Park, J. M., Doo, J. G., Kim, S. H., Jung, H. R., . . . Eun, Y. G. (2020). Correlation of Nasal Fluid Biomarkers and Symptoms in Patients with Persistent Allergic Rhinitis. *Ann Otol Rhinol Laryngol, 129*(6), 542-547. doi:10.1177/0003489419898717

Klimek, L., Pfaar, O., Worm, M., Bergmann, K. C., Bieber, T., Buhl, R., . . . Zuberbier, T. (2020). Allergen immunotherapy in the current COVID-19 pandemic: A position paper of AeDA, ARIA, EAACI, DGAKI and GPA: Position paper of the German ARIA Group(A) in cooperation with the Austrian ARIA Group(B), the Swiss ARIA Group(C), German Society for Applied Allergology (AEDA)(D), German Society for Allergology and Clinical Immunology (DGAKI)(E), Society for Pediatric Allergology (GPA)(F) in cooperation with AG Clinical Immunology, Allergology and Environmental Medicine of the DGHNO-KHC(G) and the European Academy of Allergy and Clinical Immunology (EAACI)(H). *Allergol Select, 4*, 44-52. doi:10.5414/ALX02147E

Klimek, L., Riechelmann, H., & Amedee, R. (1996). Eosinophil Cationic Protein in Nasal Secretions and Blood Serum in Grass-Pollen Allergic Rhinitis. *American Journal of Rhinology, 10*(5), 319-322. doi:10.2500/105065896782159738

Kobayashi, Y., Konno, Y., Kanda, A., Yamada, Y., Yasuba, H., Sakata, Y., . . . Ueki, S. (2019). Critical role of CCL4 in eosinophil recruitment into the airway. *Clin Exp Allergy, 49*(6), 853-860. doi:10.1111/cea.13382

Kofler, S., Asam, C., Eckhard, U., Wallner, M., Ferreira, F., & Brandstetter, H. (2012). Crystallographically mapped ligand binding differs in high and low IgE binding isoforms of birch pollen allergen bet v 1. *J Mol Biol, 422*(1), 109-123. doi:10.1016/j.jmb.2012.05.016

Kolek, F., Plaza, M. D. P., Leier-Wirtz, V., Friedmann, A., Traidl-Hoffmann, C., & Damialis, A. (2021). Earlier Flowering of Betula pendula Roth in Augsburg, Germany, Due to Higher Temperature, NO2 and Urbanity, and Relationship with Betula spp. Pollen Season. *Int J Environ Res Public Health, 18*(19). doi:10.3390/ijerph181910325

Kolek, F., Plaza, M. P., Charalampopoulos, A., Traidl-Hoffmann, C., & Damialis, A. (2021). Biodiversity, abundance, seasonal and diurnal airborne pollen distribution patterns at two different heights in Augsburg, Germany. *Atmospheric Environment, 267*, 118774. doi:https://doi.org/10.1016/j.atmosenv.2021.118774

Komai-Koma, M., Brombacher, F., Pushparaj, P. N., Arendse, B., McSharry, C., Alexander, J., . . . Xu, D. (2012). Interleukin-33 amplifies IgE synthesis and triggers mast cell degranulation via interleukin-4 in naive mice. *Allergy, 67*(9), 1118-1126. doi:10.1111/j.1398-9995.2012.02859.x

Konig, K., Klemens, C., Eder, K., San Nicolo, M., Becker, S., Kramer, M. F., & Groger, M. (2015). Cytokine profiles in nasal fluid of patients with seasonal or persistent allergic rhinitis. *Allergy Asthma Clin Immunol, 11*(1), 26. doi:10.1186/s13223-015-0093-x

Kramer, M. F., Jordan, T. R., Klemens, C., Hilgert, E., Hempel, J. M., Pfrogner, E., & Rasp, G. (2006). Factors contributing to nasal allergic late phase eosinophilia. *Am J Otolaryngol, 27*(3), 190-199. doi:10.1016/j.amjoto.2005.09.013

Kuna, P., Lazarovich, M., & Kaplan, A. P. (1996). Chemokines in seasonal allergic rhinitis. *J Allergy Clin Immunol, 97*(1 Pt 1), 104-112. doi:10.1016/s0091-6749(96)70288-9

Kurowska-Stolarska, M., Kewin, P., Murphy, G., Russo, R. C., Stolarski, B., Garcia, C. C., . . . Xu, D. (2008). IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol, 181*(7), 4780-4790. doi:10.4049/jimmunol.181.7.4780

Liu, J.-J., & Ekramoddoullah, A. K. M. (2006). The family 10 of plant pathogenesis-related proteins: Their structure, regulation, and function in response to biotic and abiotic stresses. *Physiological and Molecular Plant Pathology, 68*(1), 3-13. doi:https://doi.org/10.1016/j.pmpp.2006.06.004

Lloyd, C. M., & Snelgrove, R. J. (2018). Type 2 immunity: Expanding our view. *Sci Immunol, 3*(25). doi:10.1126/sciimmunol.aat1604

Maier, E., Duschl, A., & Horejs-Hoeck, J. (2012). STAT6-dependent and -independent mechanisms in Th2 polarization. *Eur J Immunol, 42*(11), 2827-2833. doi:10.1002/eji.201242433

Maker, J. H., Stroup, C. M., Huang, V., & James, S. F. (2019). Antibiotic Hypersensitivity Mechanisms. *Pharmacy (Basel), 7*(3). doi:10.3390/pharmacy7030122

Mariani, V., Gilles, S., Jakob, T., Thiel, M., Mueller, M. J., Ring, J., . . . Traidl-Hoffmann, C. (2007). Immunomodulatory mediators from pollen enhance the migratory capacity of

dendritic cells and license them for Th2 attraction. *J Immunol, 178*(12), 7623-7631. doi:10.4049/jimmunol.178.12.7623

Marwa, K., & Kondamudi, N. P. (2020). Type IV Hypersensitivity Reaction. In *StatPearls*. Treasure Island (FL).

Matricardi, P. M., Kleine-Tebbe, J., Hoffmann, H. J., Valenta, R., Hilger, C., Hofmaier, S., . . . Ollert, M. (2016). EAACI Molecular Allergology User's Guide. *Pediatr Allergy Immunol, 27 Suppl 23*, 1-250. doi:10.1111/pai.12563

Mattila, P., Renkonen, J., Toppila-Salmi, S., Parviainen, V., Joenvaara, S., Alff-Tuomala, S., . . . Renkonen, R. (2010). Time-series nasal epithelial transcriptomics during natural pollen exposure in healthy subjects and allergic patients. *Allergy, 65*(2), 175-183. doi:10.1111/j.1398-9995.2009.02181.x

Mellow, T. E., Murphy, P. C., Carson, J. L., Noah, T. L., Zhang, L., & Pickles, R. J. (2004). The effect of respiratory synctial virus on chemokine release by differentiated airway epithelium. *Exp Lung Res, 30*(1), 43-57. doi:10.1080/01902140490252812

Meng, Y., Lou, H., Wang, Y., Wang, C., & Zhang, L. (2018). The use of specific immunoglobulin E in nasal secretions for the diagnosis of allergic rhinitis. *Laryngoscope, 128*(9), E311-E315. doi:10.1002/lary.27120

Mogensen, J. E., Wimmer, R., Larsen, J. N., Spangfort, M. D., & Otzen, D. E. (2002). The major birch allergen, Bet v 1, shows affinity for a broad spectrum of physiological ligands. *J Biol Chem, 277*(26), 23684-23692. doi:10.1074/jbc.M202065200

Moore, B. B., & Kunkel, S. L. (2019). Attracting Attention: Discovery of IL-8/CXCL8 and the Birth of the Chemokine Field. *J Immunol, 202*(1), 3-4. doi:10.4049/jimmunol.1801485

Moverare, R., Westritschnig, K., Svensson, M., Hayek, B., Bende, M., Pauli, G., . . . Elfman, L. (2002). Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization. *Int Arch Allergy Immunol, 128*(4), 325-335. doi:10.1159/000063855

Murphy, K., Travers, P., Walport, M., & Janeway, C. (2008). *Janeway's immunobiology* (7th ed.). New York: Garland Science.

Oteros, J., Pusch, G., Weichenmeier, I., Heimann, U., Moller, R., Roseler, S., . . . Buters, J. T. (2015). Automatic and Online Pollen Monitoring. *Int Arch Allergy Immunol, 167*(3), 158-166. doi:10.1159/000436968

Pal, I., Sinha Babu, A., Halder, I., & Kumar, S. (2017). Nasal smear eosinophils and allergic rhinitis. *Ear Nose Throat J, 96*(10-11), E17-E22. doi:10.1177/0145561317096010-1105

Panzner, P., Vachova, M., Vitovcova, P., Brodska, P., & Vlas, T. (2014). A comprehensive analysis of middle-European molecular sensitization profiles to pollen allergens. *Int Arch Allergy Immunol, 164*(1), 74-82. doi:10.1159/000362760

Pasha, M. A., Patel, G., Hopp, R., & Yang, Q. (2019). Role of innate lymphoid cells in allergic diseases. *Allergy Asthma Proc, 40*(3), 138-145. doi:10.2500/aap.2019.40.4217

Peric, A., Sotirovic, J., Spadijer-Mirkovic, C., Matkovic-Jozin, S., Peric, A. V., & Vojvodic, D. (2016). Nonselective chemokine levels in nasal secretions of patients with perennial nonallergic and allergic rhinitis. *Int Forum Allergy Rhinol, 6*(4), 392-397. doi:10.1002/alr.21684

Pinho, V., Oliveira, S. H., Souza, D. G., Vasconcelos, D., Alessandri, A. L., Lukacs, N. W., & Teixeira, M. M. (2003). The role of CCL22 (MDC) for the recruitment of eosinophils during allergic pleurisy in mice. *J Leukoc Biol, 73*(3), 356-362. doi:10.1189/jlb.0502243

Polak, D., Hafner, C., Briza, P., Kitzmuller, C., Elbe-Burger, A., Samadi, N., . . . Bohle, B. (2019). A novel role for neutrophils in IgE-mediated allergy: Evidence for antigen presentation in late-phase reactions. *J Allergy Clin Immunol, 143*(3), 1143-1152 e1144. doi:10.1016/j.jaci.2018.06.005

Powe, D. G., Groot Kormelink, T., Sisson, M., Blokhuis, B. J., Kramer, M. F., Jones, N. S., & Redegeld, F. A. (2010). Evidence for the involvement of free light chain immunoglobulins in allergic and nonallergic rhinitis. *J Allergy Clin Immunol, 125*(1), 139-145 e131-133. doi:10.1016/j.jaci.2009.07.025

Rondon, C., Campo, P., Eguiluz-Gracia, I., Plaza, C., Bogas, G., Galindo, P., . . . Torres, M. J. (2018). Local allergic rhinitis is an independent rhinitis phenotype: The results of a 10-year follow-up study. *Allergy, 73*(2), 470-478. doi:10.1111/all.13272

Runswick, S., Mitchell, T., Davies, P., Robinson, C., & Garrod, D. R. (2007). Pollen proteolytic enzymes degrade tight junctions. *Respirology, 12*(6), 834-842. doi:10.1111/j.1440-1843.2007.01175.x

Sakaida, H., Masuda, S., & Takeuchi, K. (2014). Measurement of Japanese cedar pollen-specific IgE in nasal secretions. *Allergol Int, 63*(3), 467-473. doi:10.2332/allergolint.13-OA-0668

Sandini, U., Kukkonen, A. K., Poussa, T., Sandini, L., Savilahti, E., & Kuitunen, M. (2011). Protective and risk factors for allergic diseases in high-risk children at the ages of two and five years. *Int Arch Allergy Immunol, 156*(3), 339-348. doi:10.1159/000323907

Scala, E., Villalta, D., Meneguzzi, G., Brusca, I., & Cecchi, L. (2020). Comparison of the performance of Skin Prick and ISAC Tests in the diagnosis of allergy. *Eur Ann Allergy Clin Immunol, 52*(6), 258-267. doi:10.23822/EurAnnACI.1764-1489.135

Schroeder, H. W., Jr., & Cavacini, L. (2010). Structure and function of immunoglobulins. *J Allergy Clin Immunol, 125*(2 Suppl 2), S41-52. doi:10.1016/j.jaci.2009.09.046

Scott-Taylor, T. H., Axinia, S. C., Amin, S., & Pettengell, R. (2018). Immunoglobulin G; structure and functional implications of different subclass modifications in initiation and resolution of allergy. *Immun Inflamm Dis, 6*(1), 13-33. doi:10.1002/iid3.192

Seutter von Loetzen, C., Hoffmann, T., Hartl, M., Schweimer, K., Schwab, W., Rösch, P., & Hartl-Spiegelhauer, O. (2014). Identification of the natural ligand of Bet v 1. *Clinical and Translational Allergy, 4*(2), P3. doi:10.1186/2045-7022-4-S2-P3

Shamji, M. H., & Durham, S. R. (2011). Mechanisms of immunotherapy to aeroallergens. *Clin Exp Allergy, 41*(9), 1235-1246. doi:10.1111/j.1365-2222.2011.03804.x

Shamji, M. H., Kappen, J., Abubakar-Waziri, H., Zhang, J., Steveling, E., Watchman, S., . . . Durham, S. R. (2019). Nasal allergen-neutralizing IgG4 antibodies block IgE-mediated responses: Novel biomarker of subcutaneous grass pollen immunotherapy. *J Allergy Clin Immunol, 143*(3), 1067-1076. doi:10.1016/j.jaci.2018.09.039

Shamji, M. H., Ljorring, C., Francis, J. N., Calderon, M. A., Larche, M., Kimber, I., . . . Durham, S. R. (2012). Functional rather than immunoreactive levels of IgG4 correlate closely with clinical response to grass pollen immunotherapy. *Allergy, 67*(2), 217-226. doi:10.1111/j.1398-9995.2011.02745.x

Shamji, M. H., Valenta, R., Jardetzky, T., Verhasselt, V., Durham, S. R., Wurtzen, P. A., & van Neerven, R. J. J. (2021). The role of allergen-specific IgE, IgG and IgA in allergic disease. *Allergy*. doi:10.1111/all.14908

Shulman, S. T. (2017). Clemens von Pirquet: A Remarkable Life and Career. *J Pediatric Infect Dis Soc, 6*(4), 376-379. doi:10.1093/jpids/piw063

Stemeseder, T., Klinglmayr, E., Moser, S., Lueftenegger, L., Lang, R., Himly, M., . . . Gadermaier, G. (2017). Cross-sectional study on allergic sensitization of Austrian adolescents using molecule-based IgE profiling. *Allergy, 72*(5), 754-763. doi:10.1111/all.13071

Stier, M. T., & Peebles, R. S., Jr. (2017). Innate lymphoid cells and allergic disease. *Ann Allergy Asthma Immunol, 119*(6), 480-488. doi:10.1016/j.anai.2017.08.290

Stuck, B. A., Czajkowski, J., Hagner, A. E., Klimek, L., Verse, T., Hormann, K., & Maurer, J. T. (2004). Changes in daytime sleepiness, quality of life, and objective sleep patterns in seasonal allergic rhinitis: a controlled clinical trial. *J Allergy Clin Immunol, 113*(4), 663-668. doi:10.1016/j.jaci.2003.12.589

Sutton, B. J., Davies, A. M., Bax, H. J., & Karagiannis, S. N. (2019). IgE Antibodies: From Structure to Function and Clinical Translation. *Antibodies (Basel), 8*(1). doi:10.3390/antib8010019

Suzuki, M., Yokota, M., Ozaki, S., Matsumoto, T., & Nakamura, Y. (2019). Japanese Cedar Pollen-Specific IgA in Nasal Secretions and Nasal Allergy Symptoms. *Ann Otol Rhinol Laryngol, 128*(4), 330-337. doi:10.1177/0003489418823791

Takatori, H., Makita, S., Ito, T., Matsuki, A., & Nakajima, H. (2018). Regulatory Mechanisms of IL-33-ST2-Mediated Allergic Inflammation. *Front Immunol, 9*, 2004. doi:10.3389/fimmu.2018.02004

Traidl-Hoffmann, C. (2020). Stellungnahme zur öffentlichen Anhörung zum Thema „Allergien“ [Press release]

Traidl-Hoffmann, C., Mariani, V., Hochrein, H., Karg, K., Wagner, H., Ring, J., . . . Behrendt, H. (2005). Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. *J Exp Med, 201*(4), 627-636. doi:10.1084/jem.20041065

Usui, T., Preiss, J. C., Kanno, Y., Yao, Z. J., Bream, J. H., O'Shea, J. J., & Strober, W. (2006). T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. *J Exp Med, 203*(3), 755-766. doi:10.1084/jem.20052165

van Ree, R., Hummelshoj, L., Plantinga, M., Poulsen, L. K., & Swindle, E. (2014). Allergic sensitization: host-immune factors. *Clin Transl Allergy, 4*(1), 12. doi:10.1186/2045-7022-4-12

van Zelm, M. C. (2014). B cells take their time: sequential IgG class switching over the course of an immune response? *Immunol Cell Biol, 92*(8), 645-646. doi:10.1038/icb.2014.48

Wei, H., & Wang, J. Y. (2021). Role of Polymeric Immunoglobulin Receptor in IgA and IgM Transcytosis. *Int J Mol Sci, 22*(5). doi:10.3390/ijms22052284

Wesemann, D. R., Magee, J. M., Boboila, C., Calado, D. P., Gallagher, M. P., Portuguese, A. J., . . . Alt, F. W. (2011). Immature B cells preferentially switch to IgE with increased

direct Smu to Sepsilon recombination. *J Exp Med, 208*(13), 2733-2746. doi:10.1084/jem.20111155

Worm, M., Jappe, U., Kleine-Tebbe, J., Schäfer, C., Reese, I., Saloga, J., . . . Werfel, T. (2014). Food allergies resulting from immunological cross-reactivity with inhalant allergens - Guidelines from the DGAKI, the DDG, the AeDA and the GPA. *Allergo Journal, 23*, 16-31. doi:10.1007/s15007-014-0483-2

Xiong, H., Dolpady, J., Wabl, M., Curotto de Lafaille, M. A., & Lafaille, J. J. (2012). Sequential class switching is required for the generation of high affinity IgE antibodies. *J Exp Med, 209*(2), 353-364. doi:10.1084/jem.20111941

Xu, D., Trajkovic, V., Hunter, D., Leung, B. P., Schulz, K., Gracie, J. A., . . . Liew, F. Y. (2000). IL-18 induces the differentiation of Th1 or Th2 cells depending upon cytokine milieu and genetic background. *Eur J Immunol, 30*(11), 3147-3156. doi:10.1002/1521-4141(200011)30:11<3147::AID-IMMU3147>3.0.CO;2-J

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics, 13*, 134. doi:10.1186/1471-2105-13-134

Yu, K., & Lieber, M. R. (2019). Current insights into the mechanism of mammalian immunoglobulin class switch recombination. *Crit Rev Biochem Mol Biol, 54*(4), 333-351. doi:10.1080/10409238.2019.1659227

Zissler, U. M., Esser-von Bieren, J., Jakwerth, C. A., Chaker, A. M., & Schmidt-Weber, C. B. (2016). Current and future biomarkers in allergic asthma. *Allergy, 71*(4), 475-494. doi:10.1111/all.12828

Zissler, U. M., & Schmidt-Weber, C. B. (2020). Predicting Success of Allergen-Specific Immunotherapy. *Front Immunol, 11*, 1826. doi:10.3389/fimmu.2020.01826

Zuberbier, T., Lotvall, J., Simoens, S., Subramanian, S. V., & Church, M. K. (2014). Economic burden of inadequate management of allergic diseases in the European Union: a GA(2) LEN review. *Allergy, 69*(10), 1275-1279. doi:10.1111/all.12470

10. Figure legends

Figure 1: Different types of hypersensitivity reactions. Hypersensitivity is classified by immune reactant, antigen and effector mechanism into four main groups according to Gell & Coombs (Murphy, Travers, Walport, & Janeway, 2008). - 11 -

Figure 2: Basic mechanism of allergic inflammation - 13 -

Figure 3: Overview over mast cell released molecules and their biological effects (Murphy, Travers, Walport, & Janeway, 2008). - 14 -

Figure 4: Overview over eosinophil released molecules and their biological effects (Murphy, Travers, Walport, & Janeway, 2008). - 15 -

Figure 5: Th1/Th2 balance leads to different disease outcome - 16 -

Figure 6: Overview of human immunoglobulin class switch recombination. - 18 -

Figure 7: Intracellular signaling cascade by the interaction of IL-33 and ST2. - 20 -

Figure 8: Timeline of the panel study - 33 -

Figure 9: Graphical plot of a receiver operating characteristics (ROC) curve for two example datasets (blue, green). The blue test is less accurate than the green test. The diagonal indicates a random classifier. - 46 -

Figure 10: Sensitization and symptom profiles of the panel study cohort. SAR patients are displayed in red, NA subjects in blue. Specific IgE levels (black dots), cumulative symptom score (grey dots), cumulative airborne pollen (green dots) are showed in relation to the dot size to create an overview of the different patient specific profiles. - 47 -

Figure 11: Association between symptoms during the birch pollen season and clinical allergy test results. Spearman correlation was tested before and during the birch pollen season. SAR patients are displayed in red, NA subjects in blue. Wheal and flare sizes were measured by skin prick test (SPT). - 48 -

Figure 12: Plots of cumulative symptom scores in relation to airborne pollen concentrations over time (x-axis). The left y-axis indicates the log cumulative symptom score of SAR patients (A) and NA subjects (B), whereas the right y-axis indicates the cumulative airborne pollen concentration, visualized as the green shaded area. Blue shaded areas illustrate the main pollen seasons of hazel, alder, birch and grasses (2.5-97.5% of total cumulative pollen). The red line (SAR patients), light blue line (asymptomatic) and the dark blue line (symptomatic) show the mean cumulative symptom score in log scale (C). - 49 -

Figure 13: Cross-sectional comparison of all humoral immune variables, resolved per visit. (A) Cumulative pollen concentration per time point. Pollen concentrations for each pollen type are normalized to total cumulative pollen during the entire study. (B) Cumulative symptoms are normalized as described in the methods. (C) Cross-sectional nasal humoral immune variables per visit. (D) Cross-sectional serum humoral immune variables per visit. The size of the dots indicates the statistical significance level between cohorts. Blue color indicates increase of immune variables in NA and red color indicates increase of immune variables in SAR. Cross-sectional differences were tested by Mann-Whitney-U-test and displayed as the intensity of each color. P values were adjusted for multiple testing by Bonferroni correction. - 50 -

Figure 14: Cross-seasonal comparison of nasal immune variables in both cohorts. Y-axis: normalized values; “+”: geometric mean. Cross-seasonal differences were tested by Mann-Whitney-U-test *: p≤0.05; **: p<0.01 - 52 -

Figure 15: Cross-seasonal comparison of serum immunoglobulins in NA and SAR cohorts. Y-axis: normalized values; “+”: geometric mean. Cross-seasonal differences were tested by Mann-Whitney-U-test *: p≤0.05; **: p<0.01 - 53 -

Figure 16: Cross-sectional and cross-seasonal comparison of Bet v 1-specific IgE. Median sIgE levels in serum and nasal fluid plotted as raw values (in U/mL). “+” indicates the geometric mean; *: p≤0.05; **: p<0.01; ***: p<0.005, Mann-Whitney-U-test. ..... - 54 -

Figure 17: Cross-sectional and cross-seasonal comparison of Bet v 1-specific IgG_4. Median $sIgG_4$ levels in serum and nasal fluid plotted as raw values (in U/mL). “+” indicates the geometric mean; *: p≤0.05; **: p<0.01; ***: p<0.005, Mann-Whitney-U-test. ..... - 54 -

Figure 18: Cross-sectional and cross-seasonal comparison of Bet v 1-specific IgA. Median sIgA levels in serum and nasal fluid plotted as raw values (in U/mL). “+” indicates the geometric mean; *: p≤0.05; **: p<0.01; ***: p<0.005, Mann-Whitney-U-test. ..... - 55 -

Figure 19: Comparison of nasal chemokines and cytokines in- and out of pollen season. Median chemokine and cytokines in nasal fluid are plotted as normalized values. “+” indicates the geometric mean; *: p≤0.05; **: p<0.01; ***: p<0.005, Mann-Whitney-U-test. - 56 -

Figure 20: Heat-map of all nasal immune parameters (y-axis) for all study participants (x-axis). Left panel: in pre-season, right panel: during the birch pollen season. ..... - 57 -

Figure 21: Non-supervised principal component analysis of nasal immune variables. Left panel: in pre-season, right panel: in birch pollen season. Nasal immune parameters were log transformed and true zero values were imputed with 0.001. ..... - 57 -

Figure 22: Principal components scores within PC1 in pre-season and in birch pollen season of non-supervised PCA analysis. ..... - 58 -

Figure 23: Robustness testing by using the 1-leave out method. A: Non-supervised PCA analysis with all the individual visits of pre-season and in birch pollen season. B: Ranking and the values of the principal components were highly comparable to the original PCA results. ..... - 58 -

Figure 24: Correlation plots of selected immune parameters against in-season symptom severity. Y-Axis: in season symptom severity as total nasal symptom score (TNSS). X-axis: log-transformed expression levels of immune parameters. Colors indicate SAR patients (red) and NA subjects (blue). Stars in the plots indicate significance by Spearman correlation. - 60 -

Figure 25: Kinetics of PIGR gene expression under natural pollen exposure. PIGR gene expression levels of allergic (red) and non-allergic (blue) cohort were calculated by mean $2^{-\Delta\Delta CT}$. Accordingly, pre-season (Visit 1-2) was set as baseline. The lower green panel indicates the airborne pollen concentrations. ..... - 61 -

Figure 26: FMO strategy of the FACS panel. Cells were stained with all antibodies and compared to FMO controls. The plots in the middle panel are examples with all the antibodies. Plots with yellow shades are FMO control samples. ..... - 62 -

Figure 27: Gating strategy of granulocytes and monocytes. In the nasal lavage, eosinophils as $CD45^{+}CD14^{low}CD16^{low}$, neutrophils as $CD45^{+}CD14^{low}CD16^{+}$ and monocytes as $CD45^{+}CD14^{+}CD16^{-}$ are detected. ..... - 63 -

Figure 28: Nasal eosinophil influx over the study period of the panel study 2018. Allergic (red) and non-allergic (blue) subjects show similar patterns of nasal eosinophil infiltration during the grass pollen season, but the magnitude is higher in allergic subjects. ..... - 63 -

Figure 29: Nasal eosinophil influx under airborne pollen exposure. Intensity of the green bar between calendar week (CW) 10 to 48 indicates pollen counts by estimation. Eosinophils (gate in the middle of FACS plot as $CD45^{+}CD14^{low}CD16^{low}$) of allergic patients peaked during the main grass pollen season. ..... - 64 -

Figure 30: Gating strategy of T-cell sorting. Subset of CD19+, CD8+, CD4+CD45RA-CXCR5- (Tcon), CD4+CD45RA-CXCR5+ (Tfh), CD4+CD45RA-CD127-CD25+ (Treg) were sorted. - 65 -

Figure 31: Sensitization profiles of ISAC study participants. (A) Venn diagram of serum sIgE sensitizations by ImmunoCAP. (B) Comparison of sensitization profiles derived from both methods (ImmunoCAP and ISAC). ..... - 66 -

Figure 32: Correlation matrix of serum sIgE. IgE levels of serum are correlated within the allergens of the PR-10 family, grasses and HDM. Bar-plots on the diagonals indicate the distribution of the data. ***: p<0.001; **: p<0.01; **: p<0.05; Spearman correlation..... - 67 -

Figure 33: Correlation matrix of nasal sIgE. IgE levels of nasal fluid are inter-correlated within the allergens of the PR-10 family, grasses and HDM. Bar-plots on the diagonals indicate the distribution of the data. ***: p<0.001; **: p<0.01; **: p<0.05; Spearman correlation..... - 68 -

Figure 34: Correlation plot of serum and nasal sIgE levels among different aeroallergens. Serum sIgE levels on the x-axis were plotted against nasal sIgE levels on the y-axis. Each green dot indicates one of the study subjects (n=49). Fitted red lines indicate Spearman correlations..... - 69 -

Figure 35: Correlation plots of serum and nasal sIgE levels. Serum sIgE levels on the x-axis were plotted against nasal sIgE levels on the y-axis. Each purple dot indicates one of the tested components for PR-10 proteins, grass-pollen and HDM allergens. Fitted red lines indicate Spearman correlations. ..... - 71 -

Figure 36: Receiver operating characteristic (ROC) curve for nasal (blue) and serum (red) IgE diagnosis (ISAC). Youden´s Index “J” shows the threshold for positive nasal test. .... - 73 -

11. Table legends

Table 1: List of the Bet v 1 homologues allergens (http://www.meduniwien.ac.at; 06.03.2021). ..... - 21 -

Table 2: List of buffers ..... - 27 -

Table 3: Mix for cDNA synthesis ..... - 28 -

Table 4: Thermal cycler protocol for cDNA synthesis ..... - 28 -

Table 5: Primers for quantitative Real-Time PCR ..... - 28 -

Table 6: Mix for quantitative Real-Time PCR ..... - 28 -

Table 7: Thermal cycler protocol for quantitative Real-Time PCR ..... - 28 -

Table 8: List of reagents, consumables and instruments ..... - 28 -

Table 9: Cohort characteristics of seasonal allergic rhinitis and non-atopic subjects. Serum total and specific IgE levels (IU/ml) were determined by ImmunoCAP. NA1-NA10: Non-allergic subjects. B1-B8: SAR subjects. Positive sensitization ≥0.35 IU/ml. ..... - 34 -

Table 10: Sensitization profile of the study participants by component resolved allergen diagnosis. Only positive results for 27 out of 112 allergens are shown. ..... - 35 -

Table 11: Cohort characteristics of the study subjects. Serum total and specific IgE levels (IU/ml) were determined by ImmunoCAP (Phadia). ..... - 36 -

Table 12: Dilution factor of samples according to the assay. ..... - 40 -

Table 13: Dilution factors for the ELISAs according to the initial establishment. ..... - 41 -

Table 14: Antibody mix for the control staining. ..... - 43 -

Table 15: Antibody mix for the T-cell subsets sorting ..... - 43 -

Table 16: Schematic contingency table ..... - 45 -

Table 17: Spearman correlation coefficients for all tested aeroallergens. Allergen-specific IgE profiles measured in sera and nasal secretions. ..... - 70 -

Table 18: Spearman correlation coefficients for all subjects. Allergen-specific IgE profiles measured in sera and nasal secretions. ..... - 72 -

Table 19: Comparison of ImmunoCAP and ISAC diagnostic performed on serum (A) and nasal secretion (B). Diagnostics via ImmunoCAP used as a control. C: Statistical analysis to validate performance of serum and nasal ISAC. ..... - 74 -

Table 20: Serum and nasal specific IgE levels. Serum (S) and nasal (N) sensitization to allergens against Betulaceae-pollen (Ara h 8, Act d 8, Aln g 1, Bet v 1, Cor a 1.0101, Cor a 1.0401, Mal d 1, Pru p 1) were tested via ISAC. Specific IgE levels, marked in bold, indicate positive sensitization (serum ≥ 0.3 ISU-E; nasal ≥ 0.08 ISU-E). ..... - 80 -

Table 21: Serum and nasal specific IgE levels. Serum and nasal sensitization to allergens against grass-pollen (Cyn d 1, Phl p 1, Phl p 2, Phl p 5 and Phl p 6) were tested via ISAC. Specific IgE levels, marked in bold, indicate positive sensitization (serum ≥0.3 ISU-E; nasal ≥0.08 ISU-E). ..... - 81 -

Table 22: Serum and nasal specific IgE levels. Serum and nasal sensitization to allergens against house dust mite (Der f 1, Der f 2, Der p 1 and Der p 2) were tested via ISAC. Specific IgE levels, marked in bold, indicate positive sensitization (serum ≥0.3 ISU-E; nasal ≥0.08 ISU-E). ..... - 82 -

12. Publications

1. **Gökkaya M,** Damialis A, Nussbaumer T, Beck I, Bounas-Pyrros N, Bezold S, Amisi MM, Kolek F, Todorova A, Chaker A, Aglas L, Ferreira F, Redegeld FA, Brunner JO, Neumann AU, Traidl-Hoffmann C, Gilles S. et al. **Defining biomarkers to predict symptoms in subjects with and without allergy under natural pollen exposure.** J Allergy Clin Immunol, 146(3), 583-594 e586. doi:10.1016/j.jaci.2020.02.037

2. **Gökkaya M,** Schwierzeck V, Thölken K, Knoch S, Gerstlauer M, Hammel G, Traidl-Hoffmann C, Gilles S. et al. **Nasal specific IgE correlates to serum specific IgE: First steps toward nasal molecular allergy diagnostic.** Allergy, 75(7), 1802-1805. doi:10.1111/all.14228

3. Dorgham K*, Quentric P*, **Gökkaya M***, Marot S, Parizot C, Sauce D, Guihot A, Luyt C-E, Schmidt M, Mayaux J, Beurton A, Le Guennec L, Demeret S, Ben Salah E, Mathian A, Yssel H, Combadiere B, Combadiere C, Traidl-Hoffmann C, Burrel S, Marcelin AG, Amoura Z, Voiriot G, Neumann AU# and Gorochov G#. **Distinct cytokine profiles associated with COVID-19 severity and mortality.** Journal of Allergy and Clinical Immunology (2021), doi:10.1016/j.jaci.2021.03.047. ***Contributed equally**

4. Neumann AU, **Goekkaya M**, Dorgham K, Traidl-Hoffmann C, Gorochov G. **Tocilizumab in COVID-19 therapy: who benefits, and how?** The Lancet. 2021 Jul 24;398(10297):299-300. doi:10.1016/S0140-6736(21)01427-6.

5. Damialis A, Häring F, **Gökkaya M,** Rauer D, Reiger M, Bezold S, Bounas-Pyrros N, Eyerich K, Todorova A, Hammel G, Gilles S, Traidl-Hoffmann C. et al. **Human exposure to airborne pollen and relationships with symptoms and immune responses: Indoors versus outdoors, circadian patterns and meteorological effects in alpine and urban environments.** Sci Total Environ. 2019;653:190-199. doi:10.1016/j.scitotenv.2018.10.366

6. Gilles S, Blume C, Wimmer M, Damialis A, Meulenbroek L, **Gökkaya M,** Bergougnan C, Eisenbart S, Sundell N, Lindh M, Andersson LM, Dahl Å, Chaker A, Kolek F, Wagner S, Neumann AU, Akdis CA, Garssen J, Westin J, Van't Land B, Davies DE, Traidl-Hoffmann C. et al. **Pollen exposure weakens innate defense against respiratory viruses.** Allergy. 2020;75(3):576-587. doi:10.1111/all.14047

7. **Gökkaya, M.,** Traidl-Hoffmann, C. **Werden Nahrungsmittelallergien dermal oder oral ausgelöst?.** Pneumo News 2020; 12: 16–19. doi:10.1007/s15033-020-1964-5

8. **Gökkaya, M.,** Traidl-Hoffmann, C. **Pollenallergie: Nasale Biomarker gegen Unterversorgung.** Dtsch Arztebl International 2021; 118(7): 32-35 doi:10.3238/PersPneumo.2021.02.19.07

13. Poster presentations

2020, Jun — EAACI Digital Congress 2020. *"Nasal biomarker-profiles to distinguish between high- and low-symptomatic, non-allergic and allergic subjects in a natural pollen exposure study"*

2019, Nov — Augsburger Wissenschaftstag 2019, Augsburg, Germany. *"A non-invasive nasal fluid sampling open a new era in allergy diagnostics"*

2019, Jun — 2nd Augsburger Neurodermitis-Symposium, Augsburg, Germany. *"Changing immunoglobulin levels under natural pollen exposure: nasal IgA and IgG antibodies focused"*

2019, Mar — 46th Annual Meeting of the Arbeitsgemeinschaft-Dermatologische-Forschung (ADF), Munich, Germany. *"Changes in immunoglobulin levels under real-life pollen exposure: Role of nasal IgA and IgG antibodies"*

2018, Mar — 14th Spring School on Immunology - Deutsche Gesellschaft für Immunologie (DGfI), Ettal, Germany. *"Assessing local and systemic humoral immune responses under real-life pollen exposure using panel study cohorts"*

2018, Jan — 16th European Academy of Allergy and Clinical Immunology (EAACI) Winter School, Saas-Fee, Switzerland. *"Assessing local and systemic humoral immune responses under real-life pollen exposure using panel study cohorts"*

2017, Nov — Augsburger Wissenschaftstag 2017, Augsburg, Germany. *"From exposure to reaction - Panel study on the relationship between pollen exposure, symptoms and the local humoral immune response"*

2017, Oct — 12th Deutscher Allergiekongress (DAK), Wiesbaden, Germany. *"Von der Exposition zur Reaktion: Panelstudie zum Zusammenhang von Pollenexposition und der lokalen und systemischen Expression von Entzündungsparametern"*

2017, Mar — 31st Mainzer Allergie-Workshop - DGAKI, Mainz, Germany. *"From exposure to reaction: Panel study on the relationship between pollen exposure and the local and systemic expression of inflammatory parameter*

2017, Mar — 44th Annual Meeting of the Arbeitsgemeinschaft-Dermatologische Forschung (ADF), Göttingen, Germany. *"From exposure to reaction: Panel study on the relationship between pollen exposure and the local and systemic expression of inflammatory parameters"*

2016, Dez — Augsburger Wissenschaftstag 2016, Augsburg, Germany. *"From exposure to reaction"*

14. Awards

2020, Sept	Advancement award, „Specific Immunotherapy“, Deutsche Gesellschaft für Allergologie und klinische Immunologie e.V. (DGAKI)
2020, Sept	The Editors´ Choice by Zuhair K. Ballas – the Associate Editors of the JACI. doi:10.1016/j.jaci.2020.07.019
2019, Nov	Poster price, Dr. Wolfbauer-Stiftung
2019, Jul	Poster price, 2. Augsburger Neurodermitis-Symposium
2017, Oct	1. Poster price, Deutsche Allergie Kongress (DAK)
2017, Oct	Junior Members poster price, Deutsche Gesellschaft für Allergologie und klinische Immunologie e.V. (DGAKI)
2008 – 2014	Study scholarship - “Heinrich Böll Stiftung”
2004 – 2008	Scholarship - “Talent im Land”, Robert Bosch Stiftung & Baden-Württemberg Stiftung