OPIS

Listă 10 publicaţii relevante

Candidat pentru obţinerea atestatului de abilitare în Domeniul Medicină:

MUNTEAN IOANA ADRIANA

1. **Muntean IA,** Bocsan IC, Miron N, Buzoianu AD, Deleanu D. How Could We Influence Systemic Inflammation in Allergic Rhinitis? The Role of H1 Antihistamines. Oxid Med Cell Longev. 2018 Jun 12;2018:3718437. IF=4,936 Q2 **Pag. 3**

2. **Muntean IA,** Bocsan IC, Vesa S, Miron N, Nedelea I, Buzoianu AD, Deleanu D. Could FeNO Predict Asthma in Patients with House Dust Mites Allergic Rhinitis? Medicina (Kaunas). 2020 May 14;56(5):235. doi: 10.3390/medicina56050235. PMID: 32422966; PMCID: PMC7279291. IF=2.43 Q2 **Pag. 11**

3. **Muntean, I.A**.; Pintea, I.; Bocsan, I.C.; Dobrican, C.T.; Deleanu, D. COVID-19 Disease Leading to Chronic Spontaneous Urticaria Exacerbation: A Romanian Retrospective Study. Healthcare 2021, 9, 1144. doi: 10.3390/healthcare9091144 IF=2.645 Q2 **Pag. 21**

4. Efthymiou, D.; Panayi, P.; Feketea, G.; Pitsios, C.; **Muntean, I.A**.; Vassilopoulou, E. Alliance with the School Personnel Is Crucial for the Management of Food Allergy and Anaphylaxis in School Children. Foods 2021, 10, 2083. doi: 10.3390/foods10092083 IF=4.35 Q1 **Pag.31**

5. Bocsan IC, **Muntean IA**, Miron N, Pintea I, Dobrican CT, Ureche C, Buzoianu AD, Deleanu D. Are Markers of Allergic Inflammation in Grass Pollen Allergy Influenced by H1 Antihistamines? J Clin Med. 2021 Dec 26;11(1):113. doi: 10.3390/jcm11010113. PMID: 35011854; PMCID: PMC8745534. IF=3.9 Q2 **Pag. 39**

6. **Muntean, I.A**.; Bocsan, I.C.; Wiest, L.K.; Pintea, I.; Dobrican, C.T.; Duca, E.; Ureche, C.; Buzoianu, A.D.; Deleanu, D. Predictive Factors for Oral Immune Modulation in Cow Milk Allergy. Nutrients 2022, 14, 494. https://doi.org/10.3390/nu14030494. IF=5.719 Q1 **Pag. 48**

7. **Muntean IA**, Leru PM, Pintea I, Bocsan IC, Dobrican CT, Deleanu D. A retrospective study regarding the influence of COVID-19 disease on asthma. BMC Pulm Med. 2023 Jan

17;23(1):22. doi: 10.1186/s12890-023-02309-7. PMID: 36650490; PMCID: PMC9844196. IF=3.3 Q3 **Pag. 61**

8. Dobrican-Băruța CT, Deleanu DM, **Muntean IA**, Pintea I, Florea CM, Filip GA. IL-31-Pruritus Interleukin: Serum Values and Clinical Impact in Chronic Spontaneous Urticaria-A Romanian Retrospective Study. J Clin Med. 2023 Sep 13;12(18):5957. doi: 10.3390/jcm12185957. PMID: 37762898; PMCID: PMC10532079. IF=3.9 Q1 **Pag.70**

9. Balan RG, Deleanu DM, Pintea I, Dobrican Baruta CT, Man MA, Bocsan IC, **Muntean IA.** Managing Severe Adverse Reactions to Biologicals in Severe Asthma. Biomedicines. 2023 Nov 21;11(12):3108. doi: 10.3390/biomedicines11123108. PMID: 38137329; PMCID: PMC10740468. IF=4.7 Q1 **Pag. 87**

10. Dobrican-Băruța CT, Deleanu DM, **Muntean IA**, Nedelea I, Bălan RG, Filip GA, Procopciuc LM. The Alarmin Triad-IL-25, IL-33, and TSLP-Serum Levels and Their Clinical Implications in Chronic Spontaneous Urticaria. Int J Mol Sci. 2024 Feb 7;25(4):2026. doi: 10.3390/ijms25042026. PMID: 38396704; PMCID: PMC10889490. IF=5.6 Q1 **Pag. 100**

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2018, Article ID 3718437, 8 pages
https://doi.org/10.1155/2018/3718437

Research Article

How Could We Influence Systemic Inflammation in Allergic Rhinitis? The Role of H1 Antihistamines

Ioana Adriana Muntean,[1] Ioana Corina Bocsan,[2] Nicolae Miron,[3] Anca Dana Buzoianu,[2] and Diana Deleanu[1]

[1]*Department of Allergology and Immunology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj Napoca, Romania*
[2]*Department of Pharmacology, Toxicology and Clinical Pharmacology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj Napoca, Romania*
[3]*Department of Clinical Immunology, Sahlgrenska University Hospital, Göteborg, Sweden*

Correspondence should be addressed to Ioana Corina Bocsan; corinabocsan@yahoo.com

Received 17 February 2018; Revised 13 April 2018; Accepted 15 May 2018; Published 12 June 2018

Academic Editor: Ada Popolo

The aim of the study was the analysis of adhesion molecules' profile (ICAM-1, VCAM-1, and E-selectin) in patients with allergic rhinitis and the influence of H1 antihistamines on those markers. Seventy-nine patients with persistent allergic rhinitis (PAR) and 30 healthy volunteers were included in the study. The patients with PAR were treated with desloratadine 5 mg/day or levocetirizine 5 mg/day for 4 weeks. The clinical (rhinitis symptoms and total symptoms score (TSS), type of sensitization) and biological evaluation (total IgE, eosinophils, ICAM-1, VCAM-1, and E-selectin) as well as fractionate nitric oxide in exhaled air (FeNO) measurement was performed before and after treatment. The plasmatic levels of ICAM-1, VCAM-1, total IgE, and eosinophils and FeNO were significantly increased in patients with PAR compared to healthy volunteers. H1 antihistamines significantly improved TSS, with no differences between the investigated drugs. There was a significant decrease of eosinophils, total IgE, and FeNO after treatment. H1 antihistamines significantly decreased the plasmatic levels of ICAM-1 and E-selectin but not VCAM-1 compared to basal values. There is no difference between levocetirizine and desloratadine in the reduction of CAMs. A systemic inflammation characterized by increased levels of CAMs is present in patients with PAR. H1 antihistamines improve symptoms and reduce CAMs and FeNO levels after 1 month of treatment. H1 antihistamines might reduce the systemic inflammation which could be responsible to asthma occurrence in patients with PAR.

1. Introduction

Allergic rhinitis (AR) is an IgE-mediated immune response characterized by an inflammatory process of the nasal mucosa [1]. Now, allergic rhinitis is considered the most prevalent clinical manifestation of allergy, affecting 20–30% of the general population worldwide [1, 2]. AR is also a risk factor for asthma's occurrence; more than 25% of patients with persistent allergic rhinitis (PAR) may develop asthma over time [3].

The immune response to allergen exposure involves several cells and mediators. Immediately after allergen exposure, in the early phase of allergic inflammation there is an immediate release of mast cell products, including histamine. The released mediators generate a specific inflammatory network, which favours the expression and activation of certain cellular adhesion molecules (CAM) [4, 5]. The activation of CAMs favours the migration of proinflammatory cells such as eosinophils and neutrophils in the nasal mucosa [5, 6]. Late-phase immune response is characterized by release of various cytokines, chemokines, and other mediators, mainly produced by TH2 cells and granulocytes, which changes cellular components, with a predominant influx of TH2 cells and eosinophils [5, 6].

Vascular cell adhesion molecule 1 (VCAM-1) and intercellular cell adhesion molecule 1 (ICAM-1) belong to the immunoglobulin superfamily. Both are expressed mainly on endothelial cells [7, 8]. Proinflammatory cytokines like IL-1

and TNF-α enhance the expression of both CAMs, while Th2 cytokines significantly enhance VCAM-1 expression [9]. ICAM-1 and VCAM-1 are involved in transendothelial migration and adhesion of leukocytes, including eosinophils [6, 10], contributing in the maintenance of late immune response in the nasal mucosa.

E-selectin is a CAM expressed on the endothelial cell, mediating the rapid low-affinity adhesion of leukocytes to endothelial cells. The level of E-selectin is higher in the early stage of inflammation in the vascular endothelium [8, 9]. E-selectin is an important CAM in the initiation and organization of allergic inflammation.

H1 antihistamines are the first therapeutic option in all forms of allergic rhinitis [1]. Their main effect is related to blockade of H1 receptors, mediating their antiallergic action. Further research found that the new-generation H1 antihistamines have also an anti-inflammatory effect, decreasing the number of inflammatory cells recruited in the tissue and diminishing the expression of CAMs [11–15].

The aim of the study was the analysis of CAMs' evolution under 1-month treatment with levocetirizine and desloratadine, two H1 antihistamines from second generation in patients with PAR under continuous natural exposure to allergens. Secondarily, we also characterized the plasmatic levels of CAMs (ICAM-1, VCAM-1, and E-selectin) in patients with PAR.

2. Material and Method

2.1. Patients and Clinical Evaluation. In the present study, we performed a post hoc analysis of an initial randomized control trial (RCT) that included patients with PAR and healthy volunteers. The analyzed inflammatory markers represented secondary outcomes of the initial study [16]. Seventy-nine patients with PAR (mean age 30.44 ± 9.9 years and sex ratio $M:F = 1.02$) were included in the experimental group, while 30 healthy volunteers (mean age 28.92 ± 8.91 years and sex ratio $M:F = 1$) were included in the control one. The study protocol, inclusion and exclusion criteria, and clinical evaluation were similar to the initial study [16]. The protocol was approved by the Ethics Committee of the "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca. All patients signed the informed consent at enrollment.

The diagnosis of AR was done according to international guidelines, based on history and skin prick test (SPT) [1]. The following demographic data were noticed from anamnesis: age, sex, and living area (rural/urban). The severity of AR was established based on severity of specific symptoms: rhinorrhea, nasal congestion, sneezing, and nasal and ocular itching. The severity was analyzed on a scale from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, and 3 = severe), retrospectively, for 12 hours prior to presentation. The total symptom score (TSS) was calculated by adding the score for every symptom. A $TSS < 6$ means a mild rhinitis, while a $TSS > 6$ represents a moderate-severe form of disease.

After the baseline evaluation, the patients were randomly divided into two groups using an adaptive biased-coin randomization. The first group included 39 patients, and they received levocetirizine 5 mg/day, while the second group of 40 patients received desloratadine 5 mg/day. The treatment was recommended for 4 weeks. At the end of the four weeks, the patients were similarly evaluated.

2.2. Skin Prick Test (SPT). The diagnosis of allergy was established through skin prick test, according to international guidelines [17]. The allergen panel included international recommendation and particularities of exposure to allergens in Romania: house dust mites (*Derm. pteronyssinus* and *Derm. farinae*), grass pollens (mixed grasses), cereal pollens (cereals), Betulaceae pollens (spring trees), cat and dog epithelia, *Alternaria alternata*, and weed pollen (*Artemisia vulgaris* and *Ambrosia elatior*). Standardized allergen extracts (Hal Allergy, Netherlands) were used. SPT was done at the beginning of the study.

2.3. FeNO Measurement. The measurement of fractionated exhaled nitric oxide (FeNO) was done in accordance to international recommendations [18], using NIOX MINO® (Aerocrine, Sweden). FeNO was measured before and after 1 month of treatment with H1 antihistamines. The measured values were expressed in parts per billion (ppb). A standardized cutoff value of 25 ppb was considered a normal upper limit.

2.4. Biological Evaluation. All the biological parameters were determined before and after 1 month of treatment with H1 antihistamines. Total IgE plasmatic level was done using electrochemiluminescence immunoassay method (ECLIA). The obtained values were expressed as UI/ml. A value below 100 UI/ml was considered normal.

The eosinophils (Eo) were manually counted from the peripheral blood on a slide, and their value was expressed as %. We considered a normal value between 2–4%.

The plasmatic levels of ICAM-1, VCAM-1, and E-selectin were determined using ELISA technique (Quantikinine R&D system, USA). The blood sample of 5 ml without anticoagulant was collected and centrifuged within the 1st hour, followed by serum separation. The serum was then stored at −80°C until the determination was performed. All the aforementioned determinations were done according to the manufacturers' instructions. For each assay, samples were diluted as needed, and protein levels were calculated based on four-parameter logistic (4-PL) curve fit.

2.5. Statistical Analysis. The statistical analysis was performed using SPSS version 21 (Chicago, IL, USA). Data were labeled as nominal and expressed as percentage and continuous variables. The normal distribution for continuous variables was done using Kolmogorov-Smirnov test. Variables with normal distribution were expressed as mean and standard deviation, while variables with abnormal distribution were expressed as median and 25–75 percentiles.

The adequate statistic tests according to data distribution were chosen. The differences were assessed within groups by Wilcoxon signed-rank test and between groups by Mann–Whitney test. The influence of different parameters on CAM evolution in time was done using ANOVA test for repeating measurements. The Spearman coefficient of correlation was calculated to highlight differences between

Table 1: Patients' demographic data.

Parameter	Desloratadine ($n = 40$)	Levocetirizine ($n = 39$)	p
Age*	28.05 ± 6.32	32.89 ± 12.17	0.031
Sex^			
Male	57.5% (23)	43.6% (17)	0.263
Female	42.5% (17)	56.4% (22)	
Living area^			
Urban	85% (34)	82.1% (32)	0.770
Rural	15% (6)	17.9% (7)	
Allergic rhinitis onset (months)°	24 (6–60)	36 (7.5–68)	0.532
Allergen sensitization^			
Indoor	37.5% (15)	5.1% (2)	0.002
Outdoor	17.5% (7)	35.9% (14)	
Indoor + outdoor	45% (18)	59% (23)	
Severity^			
Mild	25% (10)	33.3% (13)	0.465
Moderate-severe	75% (30)	66.7% (26)	

*Data are expressed as mean ± SD; ^data are expressed as %, n; °data are expressed as median, 25–75th percentile. SD: standard deviation; n: number.

continuous variables. The level of statistical significance was set at $p < 0.05$.

3. Results

Patients' demographic data are presented in Table 1.

3.1. Initial Evaluation. Fifty-six (70.9%) patients presented persistent moderate-severe forms of AR. The initial TSS proved the severity of AR (median 8 (5–11)). Forty-one patients (51.9%) had multiple sensitizations to both indoor and outdoor allergens. The basal TSS was not correlated with the duration of AR but was significantly higher in patients with sensitization to pollen or multiple sensitizations ($p = 0.01$).

In patients with AR, plasmatic ICAM-1 and VCAM-1 were significantly increased compared to healthy volunteers ($p < 0.001$ and $p < 0.001$, resp.), with no differences between the groups. E-selectin was similar in healthy volunteers and patients with AR (Table 2).

The severity of AR expressed as a high value of TSS was correlated with the plasmatic level of E-selectin ($R = 0.996$, $p < 0.001$), but not with basal levels of ICAM-1 ($R = -0.051$, $p = 0.657$) or VCAM-1 ($R = -0.056$, $p = 0.622$). There is no correlation between the plasmatic level of CAM and patients' age, sex, or type of sensitization ($p > 0.05$). There is a positive correlation between the basal values of ICAM-1 and E-selectin ($R = 0.353$, $p = 0.001$).

Total IgE and Eo were significantly increased at baseline, with no differences between the groups ($p = 0.408$ and $p = 0.838$, resp.). The initial value of peripheral Eo was strongly correlated with total IgE ($R = 0.853$; $p < 0.001$). There was no correlation between basal Eo or total IgE levels and ICM-1, VCAM-1, and E-selectin ($p > 0.05$).

FeNO was increased in patients with AR (median 27 (18–46)) compared to the standardized cutoff value (25 ppb) ($p < 0.001$). There was no difference between the groups regarding the basal value of FeNO. There was no correlation between initial FeNO and severity of rhinitis' symptoms, type of sensitization, or basal values of CAM ($p > 0.05$).

3.2. One-Month Evaluation. H1 antihistamines significantly improved all the symptoms after 1 month of treatment. TSS significantly decreased after treatment (median 8 (5–11) versus median 0 (0–4), $p = 0.01$), with no differences between the investigated drugs ($p = 0.571$).

1-month evaluation revealed a significant decrease of IgE plasmatic level ($p < 0.001$), especially in patients with monosensitization either to indoor or outdoor allergens ($p = 0.05$). The reduction of total IgE was not influenced by the type of treatment; patients' age, sex, and environment; or duration of AR ($p > 0.05$) (Table 3). Total IgE significantly decreased in patients with moderate-severe forms of AR compared to those with mild disease ($p = 0.05$).

Same pattern was noticed also for peripheral Eo, with a significant reduction after treatment ($p = 0.04$). The Eo significantly decreased after 1 month of treatment especially in patients with monosensitization to indoor allergens or mixed sensitization ($p = 0.002$). Eo reduction was also significant in patients with severe forms of AR ($p = 0.04$). The reduction of Eo was not influenced by the type of treatment; patients' age, sex, and environment; or duration of AR ($p > 0.05$).

After the four-week treatment, H1 antihistamines significantly decreased the plasmatic levels of ICAM-1 ($p = 0.049$) and E-selectin ($p = 0.002$), but not VCAM-1 ($p = 0.310$) compared to basal values. There was no difference between levocetirizine and desloratadine in the reduction of adhesion molecule plasmatic levels (Table. 3). We noticed a significant reduction of CAM levels in patients with moderate-severe forms compared to patients with mild rhinitis (VCAM-1 $p = 0.037$, ICAM-1 $p = 0.001$, and E-selectin $p = 0.002$). The reduction of CAM levels was not influenced by patients'

Table 2: Plasmatic values of total IgE and adhesion molecules in healthy volunteers and patients with AR.

Parameter	Healthy volunteers ($n = 30$)	Patients with AR ($n = 79$)	p
Total IgE (UI/l)	<100	115 (45.3–169)	**<0.001**
ICAM-1 (ng/ml)	111.21 (100–206.30)	218.19 (189.13–266.65)	**0.001**
VCAM-1 (ng/ml)	557 (249–891)	1004.02 (822.32–1174.68)	**<0.001**
E-selectin (ng/ml)	32.03 (23.68–45.94)	33.81 (24.61–47.53)	0.404

Data are expressed as median, 25–75th percentile. Significance $p < 0.05$.

Table 3: Patients' biological parameters before and after treatment.

Parameter		Desloratadine ($n = 40$)	Levocetirizine ($n = 39$)	p
Total IgE	Baseline	116.5 (46.25–269)	115 (45.3–269)	0.212
	4 weeks	65 (28.32–167.5)	75 (30–150)	
Eo	Baseline	5.00 (3.20–6.50)	5.20 (2.70–7.80)	0.04
	4 weeks	4.10 (2.60–5.80)	4 (2.35–6.35)	
VCAM-1	Baseline	1037.8 (878.19–1200.82)	919.32 (818.5–1136.02)	0.202
	4 weeks	1037.98 (897.64–1193.09)	913.56 (703.58–1128.60)	
ICAM-1	Baseline	208.12 (179.95–259.04)	229.81 (195.75–275.21)	0.355
	4 weeks	205.58 (170.93–256.01)	206.13 (182.74–270.14)	
E-selectin	Baseline	33.54 (25.72–46.57)	33.81 (23.95–50)	0.459
	4 weeks	33.07 (24.46–44.89)	31.90 (22.08–49.5)	
FeNO	Baseline	38 (19–49)	23 (16.25–43)	0.05
	4 weeks	14 (11–21)	17.5 (14–22.5)	

Data are expressed as median, 25–75th percentile. Significance $p < 0.05$.

age, sex, and type of sensitization.We also analyzed the improvement of symptoms in correlation with inflammatory markers. The reduction of TSS was positively correlated with the reduction of ICAM-1 ($R = 0.238$, $p = 0.035$), but it was not correlated with VCAM-1 and E-selectin evolutions. ICAM-1 reduction was positively correlated with E-selectin ($R = 0.504$, $p < 0.001$) and VCAM-1 ($R = 0.711$, $p < 0.001$) evolutions.

FeNO was significantly reduced after 1-month treatment with AH1, desloratadine being more effective than levocetirizine (Table 3). The reduction was not influenced by patients' age, severity of allergic rhinitis or number of sensitization ($p > 0.05$), or the type of it ($p > 0.05$). FeNO had a more significant reduction in male compared to female patients ($p = 0.036$). The reduction of FeNO did not correlate with basal plasmatic levels of CAMs. The reduction of FeNO was not correlated with symptoms' improvement. The reduction of FeNO was minimal in patients sensitized to pollen compared with patients with multiple sensitization or with sensitization to indoor allergens, but the difference did not reach the level of statistical significance (median −6 (−32 to −3) versus median −12 (−35 to −1.5) versus median −11 (−35 to −2), $p > 0.05$).

4. Discussion

This study assessed the effect of H1 antihistamines from the 2nd generation, showing that both levocetirizine and desloratadine improved symptoms and reduced the level of inflammation in allergic rhinitis. We also characterized the plasmatic profile of adhesion molecules in patients with persistent allergic rhinitis.

AR is characterized by the presence of inflammation in the nasal mucosa. The exposure to allergens mediates the release of mediators from mast cells, especially histamine, which are responsible for the characteristic symptoms of AR (sneezing, nasal itching, and rhinorrhea) [19]. But these mediators will also stimulate infiltration of the nasal mucosa with inflammatory cells, including eosinophils [20]. The chronic inflammatory response with eosinophil infiltration in the nasal mucosa is the pattern of allergic inflammation [1, 19]. These cells continue to produce cytokines, chemokines, and other inflammatory mediators, which leads to persistent symptoms and tissue structural changes and damages. Thus, rhinitis progression and persistence become more dependent on mediators which promote infiltration of cells, such as eosinophils and TH lymphocytes [21]. AR is a risk factor for asthma development and may appear before or after asthma onset. Allergic inflammation is the key to understand both diseases and the mechanisms of rhinitis progression to asthma [5, 19].

The eosinophils migrate at the inflammation site due to the high expression of the adhesion molecules on the endothelial cell surfaces [22]. The role of adhesion molecules in the pathogenesis of allergic diseases was investigated in many studies [10, 23–29]. Most of them showed an increase of ICAM-1 and VCAM-1 in nasal lavage fluid, mucosa biopsies, and serum in patients with AR versus healthy subjects, after allergen challenge tests or in conditions of natural exposure [10, 23, 24, 26–28]. On the other hand, some researchers

did not observe an increased level of ICAM-1 and VCAM-1 in serum of patients with allergic rhinitis [25, 29]. In the present study, the plasmatic levels of ICAM-1 and VCAM-1 were significantly increased in patients with PAR compared to healthy volunteers ($p < 0.001$ and $p < 0.001$, resp.), with no differences between the groups. These results confirm previous published data, reflecting a systemic inflammation in patients with PAR.

The kinetics of VCAM-1 and ICAM-1 is different. In patients with AR, ICAM-1 is increased in nasal secretion in both perennial and seasonal AR from the period of onset [26]. On the other hand, the expression of VCAM-1 is upregulated in the nasal mucosa of patients with AR [28, 30], especially in the late phase of allergic response [31]. Our results are similar to those from the above aforementioned studies. Present research included patients with PAR under continuous natural exposure to allergens. The continuous exposure to indoor or/and outdoor allergens may explain a continuous production of mediators which promote eosinophil recruitments, explaining high plasmatic levels of ICAM-1 and VCAM-1.

Gorska-Ciebiada et al. [27] have shown that ICAM-1 values are significantly lower in patients with mild forms compared to those with moderate-severe rhinitis [27]. In the present study, there was no correlation between ICAM-1 and VCAM-1 and the severity of allergic rhinitis or type of sensitization. Another study showed that VCAM-1 and ICAM-1 grow during the pollen season and fall out during off-season [26]. In the present study, we did not investigate the kinetics of ICAM-1 and VCAM-1. Most of the patients have polysensitization to both indoor and outdoor allergens which may explain the high level of CAMs in serum, due to their continuous production.

Interestingly, E-selectin was similar in patients with AR and healthy volunteers. Similarly, Ural et al. found that the E-selectin value did not differ in patients with allergic and nonallergic rhinitis in nasal lavage fluid [23]. E selectin is involved in leukocyte orientation, and it is a light adhesion marker, while ICAM-1 is a leukocyte-binding adhesion marker. The basal level of E-selectin positively correlated with ICAM-1 ($R = 0.353$, $p = 0.001$). This observation might be explained by their involvement at the beginning of cell recruitments. In patients with AR, the level of E-selectin starts to increase within 15 hours after allergen exposure, [32] and it declines after 24 hours [23, 33]. These data may explain not only the low level of E-selectin in our patients but also the correlation between E-selectin and severity of symptoms ($R = 0.996$, $p < 0.001$), generated mainly by histamine release. As we mentioned before, we did not investigate the kinetics of CAMs in serum, and the patients were under natural exposure not after a controlled allergen challenge exposure. It might be interesting to investigate CAM and cytokine levels in patients with AR at different time points to analyze their kinetics in conditions of natural exposure to allergens.

IgE is the central molecule in the pathogenesis of allergic diseases. It increases after sensitization and binds to mast cells through specific receptors, but a soluble portion remains in the serum and can be determined. In different clinical studies, the IgE level did not correlate with ICAM-1 or TNF-α values, which are higher in asthmatics but not in those with AR [29]. In our study, there was a significant correlation between the Eo count and total IgE ($p < 0.001$), but the total IgE values did not correlate with other markers of inflammation, such as CAMs or FENO. Although CAMs are involved in Eo migration at the site of inflammation, the serum values of Eo did not correlate with ICAM-1 and VCAM-1 in our study. It is interesting to evaluate the level of CAMs in the nasal mucosa and to correlate with local infiltration of Eo, but in our study we did not perform such kind of investigation.

There is hypothesis suggesting that Eo recruited by CAMs can induce nitric oxide synthase in the epithelial cells of the bronchial mucosa. Nitric oxide (NO) in the exhaled air is known as the marker of eosinophilic inflammation in the lower respiratory tract. IgE-mediated inflammation results in elevated NO in the expired air [34, 35]. Studies have also shown that patients with AR during pollination have elevated NO levels in the air, even if their asthma symptoms are missing or mild [36]. Other studies showed elevated levels of exhaled NO and adenosine in patients with AR versus healthy subjects [34, 35], suggesting that a subclinical inflammation in the lower airways could exist in AR. In our group of patients, FeNO was increased in patients with AR and did not correlate with any of the studied markers. There was no correlation between the initial FeNO and the severity of rhinitis' symptoms, type of sensitization, or basal values of CAM ($p > 0.05$). But this lack of correlations cannot exclude a possible minimal inflammatory process in both the nasal and lower airway mucosa, other factors having an additional contribution to progression of inflammation in lower airways, like TNF-α-stimulation [37]. It might be interesting to monitor the evolution of patients in order to investigate if basal values of FeNo and CAMs could predict the occurrence of asthma after a period of time.

In this study, we also assessed the efficacy of H1 antihistamines, desloratadine and levocetirizine, in the therapy of AR. We also investigated a possible anti-inflammatory effect of both drugs, demonstrated by reduction of CAMs and FeNO. Several studies showed the efficacy of H1 antihistamines in allergic rhinitis [1, 2]. H1 antihistamines are now considered the first-line treatment in AR [1]. In our study, we observed that both desloratadine and levocetirizine improved nasal symptoms, reducing significantly TSS after 1 month of treatment, similar to previous published data [2, 19, 38]. TSS significantly decreased after treatment, with no differences between the investigated drugs ($p = 0.571$).

In our study, we evaluated the effect of desloratadine and levocetirizine on E-selectin, ICAM-1, and VCAM-1. After the four-week treatment, H1 antihistamines significantly decreased the plasmatic levels of ICAM-1 ($p = 0.049$) and E-selectin ($p = 0.002$), but not VCAM-1 ($p = 0.310$) compared to basal values. There was no difference between levocetirizine and desloratadine in reduction of CAM plasmatic levels. In the present study, we also noticed a significant reduction of CAM levels in patients with moderate-severe forms compared to patients with mild rhinitis (VCAM-1 $p = 0.037$, ICAM-1 $p = 0.001$ and E-selectin $p = 0.002$).

But the reduction of CAM levels was not influenced by patients' age, sex, and type of sensitization.

In vitro studies demonstrated that not all 2nd-generation H1 antihistamines had anti-inflammatory effect. Cetirizine did not influence E-selectin, ICAM-1, and VCAM-1 *in vitro* studies although the authors noted an underexpression of ICAM-1 in epithelial cells of patients with AR treated with cetirizine [11]. Loratadine was seen to influence the level of VCAM-1 but not ICAM-1 in patients with monosensitization to house dust mites [12]. Regarding fexofenadine (an active metabolite of terfenadine), several studies showed its similar efficiency to cetirizine [39], loratadine [40], desloratadine, and levocetirizine [38] in improving rhinitis symptoms. But Schäper et al. [13] also demonstrated its anti-inflammatory effect, due to reduction of ICAM-1 in nasal secretions after 14 days of treatment [13]. *In vitro* studies revealed that levocetirizine inhibited ICAM-1 [15] and downregulated the activity of P-selectins [41] and the expression of VCAM-1 [42]. Desloratadine induced downregulation of ICAM-1 [43]. In most of these studies, the used concentrations of AH1 were higher than the therapeutic ones [41]. There are also *in vivo* studies that revealed the anti-inflammatory effect of H1 antihistamines. Both desloratadine and levocetirizine reduced ICAM-1 and nasal Eo [44], similar to present results. In the present study, VCAM-1 was not reduced by AH1, only ICAM-1 and E-selectin. Probably, the expression of ICAM-1 and E-selectin, markers of initial allergic response, is related to histamine release from mast cells, while other new synthesized cytokines and chemokines are probably involved in the expression of VCAM-1.

One-month evaluation revealed a significant decrease of IgE plasmatic level ($p < 0.001$) especially in patients with monosensitization either to indoor or outdoor allergens ($p = 0.05$). Total IgE significantly decreased in patients with moderate-severe forms of AR compared to those with mild disease ($p = 0.05$). The same pattern was also noticed also for peripheral Eo, with a significant reduction after treatment ($p = 0.04$). These results are similar to previous reported data [16, 44–46], for rupatadine, levocetirizine, and desloratadine, after 2–4 weeks of treatment.

The effect of H1 antihistamines on lower airway subclinical inflammation in patients with AR has been demonstrated in few studies [19]. *In vitro* studies have shown that NO synthase activity can be downregulated by H1 antihistamine therapy [47]. Animal studies have demonstrated that histamine released by mast cell plays an important role in the production of FeNO and in the enhancement of bronchial hyperreactivity [48]. *In vivo*, it has been demonstrated that levocetirizine lowers the FeNO values after 3 months of treatment in children with mite allergy [42]. In our study, we observed that FeNO was significantly reduced after 1-month treatment with H1 antihistamines, and desloratadine was more effective than levocetirizine. This observation could be explained by the differences between investigated subgroups in relation with patient sensitization. The group treated with levocetirizine had a lower basal value of FeNO than desloratadine group, so the room for improvement of FeNO was more limited. To establish the clear role of each H1 antihistamines in reducing FeNo level, further extensive studies are required. But, this observation might open a new strategy in limiting subclinical inflammation using AH1. The continuous treatment with H1 antihistamines in patients with PAR might reduce the occurrence of asthma, confirming the previous published data [42].

There are some limitations of this study. Firstly, a small number of patients were included in the study, sensitized to both indoor and outdoor allergens. The randomization of the patients took into account the treatment, not the type of sensitization, which may explain the differences between investigated subgroups, more patients sensitized to pollen being included in levocetirizine subgroup. The study has another limitation, the lack of information regarding different anti-inflammatory effects of H1 antihistamines according to allergen exposure. It might be interesting to analyze the effect of AH1 on CAMs and other related mediators (cytokines and chemokines) and to differentiate the results according to the type of sensitization.

The present study emphasized the anti-inflammatory role of H1 antihistamines from 2nd generation, demonstrated by reduction of CAM plasmatic levels in patients with PAR. The reduction of CAMs was noticed in the plasma not in the nasal mucosa, in conditions of natural continuous exposure to allergens. Also, the research investigates two H1 antihistamines from 2nd generation in order to establish if there are significant differences between them in improving both clinical symptoms and inflammatory parameters.

5. Conclusions

Patients with PAR have high serum levels of ICAM-1 and VCAM-1. FeNO as a marker of subclinical inflammation is increased in patients with PAR. H1 antihistamines improve allergic rhinitis symptoms and reduce the markers of inflammations after 1 month of treatment. Desloratadine has a better anti-inflammatory effect in reducing FeNO. Baseline values of CAMs did not predict the response to therapy.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this article.

Authors' Contributions

Ioana Adriana Muntean and Ioana Corina Bocsan contributed equally to this work.

Acknowledgments

This work was partially funded by POSDRU Grant (no. 159/1.5/S/138776) entitled "Model colaborativ instituțional pentru translarea cercetării științifice biomedicale în practica

clinică-TRANSCENT" and by a research Grant (no. 27020/48/2011) of the Iuliu Haţieganu University of Medicine and Pharmacy, Cluj Napoca.

References

[1] J. Bousquet, N. Khaltaev, A. A. Cruz et al., "Allergic rhinitis and its impact on asthma (ARIA) 2008," *Allergy*, vol. 63, pp. 8–160, 2008.

[2] J. Bousquet, H. J. Schünemann, T. Zuberbier et al., "Development and implementation of guidelines in allergic rhinitis – an ARIA-Ga^{2}len paper," *Allergy*, vol. 65, no. 10, pp. 1212–1221, 2010.

[3] B. Leynaert, C. Neukirch, S. Kony et al., "Association between asthma and rhinitis according to atopic sensitization in a population-based study," *The Journal of Allergy and Clinical Immunology*, vol. 113, no. 1, pp. 86–93, 2004.

[4] R. H. Schwartz, "T cell anergy," *Annual Review of Immunology*, vol. 21, no. 1, pp. 305–334, 2003.

[5] M. L. Kowalski and R. Alam, "Signal transduction mechanisms as new targets for allergists," *Allergy*, vol. 56, no. 3, pp. 199–203, 2001.

[6] M. F. Kramer, T. R. Jordan, C. Klemens et al., "Factors contributing to nasal allergic late phase eosinophilia," *American Journal of Otolaryngology*, vol. 27, no. 3, pp. 190–199, 2006.

[7] H. Tachimoto, S. A. Hudson, and B. S. Bochner, "Acquisition and alteration of adhesion molecules during cultured human mast cell differentiation," *The Journal of Allergy and Clinical Immunology*, vol. 107, no. 2, pp. 302–309, 2001.

[8] B. S. Bochner, *Adhesion Molecules in Allergic Disease*, Marcel Dekker, New York, 1997.

[9] B. S. Bochner, D. A. Klunk, S. A. Sterbinsky, R. L. Coffman, and R. P. Schleimer, "IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells," *The Journal of Immunology*, vol. 154, no. 2, pp. 799–803, 1995.

[10] Y. Ohashi, Y. Nakai, A. Tanaka et al., "Soluble vascular cell adhesion molecule-1 in perennial allergic rhinitis," *Acta Oto-Laryngologica*, vol. 118, no. 1, pp. 105–109, 1998.

[11] G. M. Walsh, "The effects of cetirizine on the function of inflammatory cells involved in the allergic response," *Clinical and Experimental Allergy*, vol. 27, Supplement 2, pp. 47–53, 1997.

[12] M. Branco-Ferreira, M. C. dos Santos, M. L. Palma-Carlos, and A. G. Palma-Carlos, "Antihistamines and serum adhesion molecule levels," *Allergie et Immunologie*, vol. 33, no. 6, pp. 233–236, 2001.

[13] C. Schäper, B. Gustavus, B. Koch et al., "Effects of fexofenadine on inflammatory mediators in nasal lavage fluid in intermittent allergic rhinitis," *Journal of Investigational Allergology & Clinical Immunology*, vol. 19, no. 6, pp. 459–464, 2009, 20128420.

[14] J. Mullol, F. de Borja Callejas, M. A. Martínez-Antón et al., "Mometasone and desloratadine additive effect on eosinophil survival and cytokine secretion from epithelial cells," *Respiratory Research*, vol. 12, no. 1, p. 23, 2011.

[15] D. Tworek, M. Bocheńska-Marciniak, M. Kupczyk, P. Górski, and P. Kuna, "The effect of 4 weeks treatment with desloratadine (5mg daily) on levels of interleukin (IL)-4, IL-10, IL-18 and TGF beta in patients suffering from seasonal allergic rhinitis," *Pulmonary Pharmacology & Therapeutics*, vol. 20, no. 3, pp. 244–249, 2007.

[16] C. I. Bocşan, A. I. Bujor, N. Miron, Ş. C. Vesa, D. Deleanu, and A. D. Buzoianu, "In vivo anti-inflammatory effect of H1 antihistamines in allergic rhinitis: a randomized clinical trial," *Balkan Medical Journal*, vol. 32, no. 4, pp. 352–358, 2015.

[17] L. Heinzerling, A. Mari, K. C. Bergmann et al., "The skin prick test - European standards," *Clinical and Translational Allergy*, vol. 3, no. 1, p. 3, 2013.

[18] American Thoracic Society and European Respiratory Society, "ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 8, pp. 912–930, 2005.

[19] G. W. Canonica and E. Compalati, "Minimal persistent inflammation in allergic rhinitis: implications for current treatment strategies," *Clinical & Experimental Immunology*, vol. 158, no. 3, pp. 260–271, 2009.

[20] A. Togias, "Unique mechanistic features of allergic rhinitis," *The Journal of Allergy and Clinical Immunology*, vol. 105, no. 6, Part 2, pp. S599–S604, 2000.

[21] L. Borish, "Allergic rhinitis: systemic inflammation and implications for management," *The Journal of Allergy and Clinical Immunology*, vol. 112, no. 6, pp. 1021–1031, 2003.

[22] D. J. Adamko, S. O. Odemuyiwa, D. Vethanayagam, and R. Moqbel, "The rise of the phoenix: the expanding role of the eosinophil in health and disease," *Allergy*, vol. 60, no. 1, pp. 13–22, 2005.

[23] A. Ural, M. S. Tezer, A. Yücel, H. Atilla, and F. Ileri, "Interleukin-4, interleukin-8 and E-selectin levels in intranasal polyposis patients with and without allergy: a comparative study," *The Journal of International Medical Research*, vol. 34, no. 5, pp. 520–524, 2006.

[24] M. Turgay, G. Keskin, G. Kminkli et al., "Soluble intercellular adhesion molecule-1 (sICAM-1) in patients with allergic rhinoconjunctivitis," *Allergol Immunopathol (Madr)*, vol. 24, no. 3, pp. 129–131, 1996.

[25] C. M. Liu, C. T. Shun, and Y. K. Cheng, "Soluble adhesion molecules and cytokines in perennial allergic rhinitis," *Annals of Allergy, Asthma & Immunology*, vol. 81, no. 2, pp. 176–180, 1998.

[26] L. Håkansson, C. Heinrich, S. Rak, and P. Venge, "Priming of eosinophil adhesion in patients with birch pollen allergy during pollen season: effect of immunotherapy," *The Journal of Allergy and Clinical Immunology*, vol. 99, no. 4, pp. 551–562, 1997.

[27] M. Gorska-Ciebiada, M. Ciebiada, M. M. Gorska, P. Gorski, and I. Grzelewska-Rzymowska, "Intercellular adhesion molecule 1 and tumor necrosis factor α in asthma and persistent allergic rhinitis: relationship with disease severity," *Annals of Allergy, Asthma & Immunology*, vol. 97, no. 1, pp. 66–72, 2006.

[28] B. J. Lee, R. M. Naclerio, B. S. Bochner, R. M. Taylor, M. C. Lim, and F. M. Baroody, "Nasal challenge with allergen upregulates the local expression of vascular endothelial adhesion molecules," *The Journal of Allergy and Clinical Immunology*, vol. 94, no. 6, Part 1, pp. 1006–1016, 1994.

[29] M. Ciebiada, M. Gorska-Ciebiada, and P. Gorski, "sICAM-1 and TNF-α in asthma and rhinitis: relationship with the presence of atopy," *The Journal of Asthma*, vol. 48, no. 7, pp. 660–666, 2011.

[30] Y. Li, Y. Wang, and Q. Zhang, "Expression of cell adhesion molecule and nitric oxide synthase in nasal mucosa in allergic

rhinitis," *Lin Chuang Er Bi Yan Hou Ke Za Zhi*, vol. 20, no. 7, pp. 315–318, 2006.

[31] T. R. Jordan, E. Pfrogner, G. Rasp, and M. F. Kramer, "Clinical symptoms and mediators in the allergic early and late phase reaction," *Laryngo- Rhino- Otologie*, vol. 85, no. 2, pp. 113–123, 2006.

[32] T. Matsui, K. Asakura, H. Shirasaki, A. Kataura, and T. Himi, "Relationship between infiltrating cells and adhesion molecules in the nasal mucosa of patients with allergic rhinitis," *Acta Oto-Laryngologica*, vol. 120, no. 8, pp. 973–980, 2000.

[33] R. M. Naclerio, "Pathophysiology of perennial allergic rhinitis," *Allergy*, vol. 52, Supplement 36, pp. 7–13, 1997.

[34] D. Aronsson, E. Tufvesson, and L. Bjermer, "Allergic rhinitis with or without concomitant asthma: difference in perception of dyspnoea and levels of fractional exhaled nitric oxide," *Clinical & Experimental Allergy*, vol. 35, no. 11, pp. 1457–1461, 2005.

[35] C. Gratziou, M. Lignos, M. Dassiou, and C. Roussos, "Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis," *The European Respiratory Journal*, vol. 14, no. 4, pp. 897–901, 1999.

[36] R. Mösges and L. Klimek, "Today's allergic rhinitis patients are different: new factors that may play a role," *Allergy*, vol. 62, no. 9, pp. 969–975, 2007.

[37] H. Widegren, J. Erjefält, M. Korsgren, M. Andersson, and L. Greiff, "Effects of intranasal TNFalpha on granulocyte recruitment and activity in healthy subjects and patients with allergic rhinitis," *Respiratory Research*, vol. 9, no. 1, p. 15, 2008.

[38] C. Bachert, "A review of the efficacy of desloratadine, fexofenadine, and levocetirizine in the treatment of nasal congestion in patients with allergic rhinitis," *Clinical Therapeutics*, vol. 31, no. 5, pp. 921–944, 2009.

[39] P. H. Howarth, M. A. Stern, L. Roi, R. Reynolds, and J. Bousquet, "Double-blind, placebo-controlled study comparing the efficacy and safety of fexofenadine hydrochloride (120 and 180 mg once daily) and cetirizine in seasonal allergic rhinitis," *The Journal of Allergy and Clinical Immunology*, vol. 104, no. 5, pp. 927–933, 1999.

[40] Van Cauwenberge, Juniper, and The STAR study investigating group, "Comparison of the efficacy, safety and quality of life provided by fexofenadine hydrochloride 120 mg, loratadine 10 mg and placebo administered once daily for the treatment of seasonal allergic rhinitis," *Clinical and Experimental Allergy*, vol. 30, no. 6, pp. 891–899, 2000.

[41] D. K. Agrawal, "Anti-inflammatory properties of desloratadine," *Clinical and Experimental Allergy*, vol. 34, no. 9, pp. 1342–1348, 2004.

[42] F. Marcucci, L. Giovanna Sensi, P. Abate et al., "Anti-inflammatory activity and clinical efficacy of a 3-month levocetirizine therapy in mite-allergic children," *Inflammation & Allergy Drug Targets*, vol. 10, no. 1, pp. 32–38, 2011.

[43] J. L. Brozek, C. E. Baena-Cagnani, S. Bonini et al., "Methodology for development of the allergic rhinitis and its impact on asthma guideline 2008 update," *Allergy*, vol. 63, no. 1, pp. 38–46, 2008.

[44] M. Ciebiada, M. Barylski, and M. G. Ciebiada, "Nasal eosinophilia and serum soluble intercellular adhesion molecule 1 in patients with allergic rhinitis treated with montelukast alone or in combination with desloratadine or levocetirizine," *American Journal of Rhinology & Allergy*, vol. 27, no. 2, pp. 58–62, 2013.

[45] R. Maiti, J. Rahman, J. Jaida, U. Allala, and A. Palani, "Rupatadine and levocetirizine for seasonal allergic rhinitis: a comparative study of efficacy and safety," *Archives of Otolaryngology – Head & Neck Surgery*, vol. 136, no. 8, pp. 796–800, 2010.

[46] A. Muntean, I. C. Bocsan, and D. M. Deleanu, "The effect of H1-antihistamines on allergic inflammation in patients with allergic rhinitis," *Human & Veterinary Medicine*, vol. 8, no. 4, pp. 161–165, 2016.

[47] J. Králová, L. Račková, M. Pekarová et al., "The effects of H1-antihistamines on the nitric oxide production by RAW 264.7 cells with respect to their lipophilicity," *International Immunopharmacology*, vol. 9, no. 7-8, pp. 990–995, 2009.

[48] L. Bennedich Kahn, L. E. Gustafsson, and C. Olgart Höglund, "Nerve growth factor enhances neurokinin A-induced airway responses and exhaled nitric oxide via a histamine-dependent mechanism," *Pulmonary Pharmacology & Therapeutics*, vol. 21, no. 3, pp. 522–532, 2008.

medicina

MDPI

Article

Could FeNO Predict Asthma in Patients with House Dust Mites Allergic Rhinitis?

Ioana Adriana Muntean [1], Ioana Corina Bocsan [2,*,†], Stefan Vesa [2,†], Nicolae Miron [3], Irena Nedelea [1], Anca Dana Buzoianu [2] and Diana Deleanu [1]

[1] Department of Allergology and Immunology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400162 Cluj Napoca, Romania; Adriana.Muntean@umfcluj.ro (I.A.M.); irena.nedelea@umfcluj.ro (I.N.); diana.deleanu@umfcluj.ro (D.D.)

[2] Toxicology and Clinical Pharmacology, Department of Pharmacology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400337 Cluj Napoca, Romania; stefan.vesa@umfcluj.ro (S.V.); abuzoianu@umfcluj.ro (A.D.B.)

[3] Department of Clinical Immunology, Sahlgrenska University Hospital, 41346 Göteborg, Sweden; nicolae.miron@vgregion.se

* Correspondence: bocsan.corina@umfcluj.ro

† These authors contributed equally to this work.

Received: 13 April 2020; Accepted: 11 May 2020; Published: 14 May 2020

Abstract: *Background and Objectives*: The evolution of allergic rhinitis to asthma is a part of "atopic march". The aim of this study was to analyze possible predictive markers for asthma occurrence in patients with allergic rhinitis to house dust mites (HDM). *Materials and Methods*: Fifty-eight patients with persistent allergic rhinitis (PAR) were included. The clinical, biological evaluation and fractionated exhaled nitric oxide (FeNO) measurement were performed at enrolment. The patients were clinically evaluated after one year to determine asthma occurrence. *Results:* The severity of rhinitis symptoms, levels of total immunoglobulin E (IgE), ICAM-1, VCAM-1, E-selectin and IL-6, but not IL-8 and TNF-α were higher in patients with allergic rhinitis who developed asthma compared to non-asthmatics, but the differences were not significant to considered them as predictive factors for asthma occurrence. The risk of asthma was independently influenced by patients aged over 30 years ((OR-3.74; CI95% 0.86–16.31; $p = 0.07$), a duration of allergic rhinitis over 12 months ((OR-4.20; CI95% 0.88–20; $p = 0.07$) and a basal FeNO over 28 parts per billion (pbb) ((OR-18.68; CI95% 3.79–92.05; $p < 0.001$). *Conclusion*: Clinical and biological parameters may predict asthma occurrence in patients with persistent allergic rhinitis to HDM. Adult patients with a longer duration of rhinitis symptoms and a high level of FeNO have a greater risk to develop asthma.

Keywords: allergic rhinitis; allergic inflammation; asthma; FeNO; house dust mites

1. Introduction

Allergic rhinitis is the most frequent IgE-mediated disease and its prevalence is increasing in the last decades [1,2]. Allergic rhinitis is a risk factor for asthma development and may be clinically relevant before or after asthma diagnosis [3]. Allergic inflammation is the key of understanding these diseases and the evolution of allergic rhinitis to asthma [2,4,5].

Allergen exposure leads to mast cell degranulation in nasal mucosa and the release of mediators, mainly histamine and leukotrienes. Cytokines released from Th2 lymphocytes are responsible for inflammatory cells recruitment in affected tissues via adhesion molecules, like E-selectin, ICAM and VCAM. The recruited cells, eosinophils, neutrophils and Th2 lymphocytes, are responsible for producing more proinflammatory cytokines (IL-1β, TNF-α, IL-3, IL-4, IL-5, IL-6 and IL-8), augmenting the inflammation in airways and injury through formation of toxic reactive nitrogen species [5–8].

The evolution from allergic rhinitis to asthma was reported in several studies, and it is a part of "atopic march" [2,9–11]. Allergic rhinitis is a risk factor for developing asthma [12], especially if the onset is severe and occurs in childhood [13]. Those two diseases often coexist and represent "a single airways allergic disease" [2,9]. This concept of "united airway disease" raises the question "Which patients with allergic rhinitis will develop asthma?"

The relationship between upper and lower airway inflammation is not completely understood yet. Genetic studies show that asthma and allergic rhinitis partly coexist because they share many genetic risk variants that dysregulate the expression of immune-related genes [14]. But not only genetic factors are important, environmental ones (allergens exposure) might also contribute to this evolution. The concept of a "united airway disease" could be explained through the migration of inflammatory cells and mediators from nasal secretions to lower airways, by inhalation and aspiration, acting as triggers of inflammation in the lower part [9,15,16]. Other additional mechanisms along with inflammation might contribute to asthma occurrence, including nasal bronchial reflex and alteration of physical filter function of the nose, which can induce bronchial hyperreactivity even in non-atopic patients [16–18].

The clinical aspect of asthma is variable, from a classical description (chest tightness, dyspnea, wheezing and cough) to only chronic cough or dyspnea to mild physical effort [7,15]. Some patients with allergic rhinitis may present rare, mild asthma symptoms, which are not related to rhinitis severity and could be actually a matter of perception of asthma symptoms, like dyspnea [7].

FeNO is a marker of lower eosinophilic inflammation in allergic diseases, especially in asthma. FeNO measurement is used for asthma diagnosis, to differentiate its phenotype and to monitor treatment response [19,20]. In asthmatic patients, a high nitric oxide is more correlated with the risk of having an asthmatic access rather than a predisposition to have asthma [21]. In patients with allergic rhinitis, measurement of FeNO might also indicate the presence of eosinophilic inflammation and might predict the development of lower airway symptoms [19,22].

The aim of this study was to investigate the risk of asthma development in patients with persistent allergic rhinitis to house dust mites after one year and the role of inflammatory cytokines, adhesion molecules and FeNO in predicting asthma occurrence.

2. Materials and Methods

2.1. Study Design, Site and Ethical Approval

The present study is a post-hoc analysis of an initial randomized control trial (RCT) that included patients with persistent allergic rhinitis [23]. The present research analyzed clinical and biological factors that might predict asthma in patients with allergic rhinitis to HDM. A diagnosis of allergic rhinitis was established according to international guidelines, based on history, clinical evaluation and the skin prick test (SPT) [24].

Fifty-eight patients with persistent allergic rhinitis to HDM (median age 27.5 (23–37) years and sex ratio M:F = 1:1), that were evaluated in Allergology Department, were included in the present analysis. The study protocol was approved by University of Medicine and Pharmacy Ethics Committee (approval no. 535/02.09.2009) and all patients signed the informed consent before enrollment. The study protocol and clinical evaluation was similar to initial RCT [23]. The exclusion criteria were as follows: the presence of asthma or nasal polyps, acute and chronic upper respiratory infections, administration of intranasal or systemic corticosteroids or H1 antihistamines in the previous 30 days. The initial evaluation was performed between February 2009 and November 2011.

2.2. Clinical Evaluation

From anamnesis, we noted the following demographic data: age, sex, living area (urban/rural) and the duration of allergic rhinitis symptoms prior enrollment. Retrospectively, for 12 h, we evaluated the allergic rhinitis' symptoms (rhinorrhea, nasal congestion, sneezing, nasal and ocular itching), and their

severity on a scale from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe). At the end we calculated the total symptoms score (TSS). Based on TSS values we differentiated between mild allergic rhinitis (TSS < 6) and moderate–severe allergic rhinitis (TSS 6).

As we previously mentioned, patients that presented low airways symptoms (dyspnea, cough and wheezing) associated to specific symptoms of allergic rhinitis were excluded from the present analysis. The patients also completed an ENT examination to exclude a possible nasal obstruction of other cause or nasal polyps. Patients with nasal polyps or another ENT disease were also excluded.

After one year, we repeated the clinical evaluation to determine the possible development of asthma. We noticed the occurrence of asthma symptoms (cough, wheezing, dyspnea) or an asthma exacerbation that required specific treatment in this period of time.

Spirometry was performed at enrollment in order to exclude a possible impaired lung function due to asthma presence and after one year. We considered asthma development if one of these clinical or functional criteria were present in the period of one year.

2.3. Skin Prick Tests (SPT)

The atopy diagnosis was established through a skin prick test at enrollment, according to international guideline [25]. The skin prick test included the following panel of allergens: house dust mites (Derm. Pteronyssinus (Der p) and Derm. Farinae (Der f)), pollens (grasses, cereals, birch and weeds), animal dander (cat and dog) and molds (Alternaria alternata). Standardized allergen extracts (Hal Allergy, Netherlands) were used. The value in mm was recorded as a medium diameter wheal size.

2.4. FeNO Measurement

Fractionated exhaled nitric oxide (FeNO) was measured at enrollment, according to international recommendations [26], using NIOXMINO® (Aerocrine, Sweden). The measured values were expressed in parts per billion (ppb). A standardized value of 25 ppb was considered as normal upper limit.

2.5. Biological Evaluation

All the biological parameters were determined at the beginning of the study. Total IgE plasma level was done using the electrochemiluminescence immunoassay method (ECLIA). The obtained values were expressed as UI/mL, considering a normal value <100 UI/mL. The eosinophils (Eo) were manually counted from peripheral blood on a slide and their value was expressed as %. We considered a normal value between 2%–4%.

Plasma levels of adhesion molecules (ICAM-1, VCAM-1 and E-selectin) and cytokines (TNF-α, IL-6 and IL-8) were determined at the initial visit. Five milliliters of blood sample was collected and centrifuged within the first hour, followed by serum separation. The obtained serum was stored at −80 °C until the determination was performed. The plasmatic levels of all inflammatory markers were determined by sandwich ELISA technique using an ELISA reader StatFax 303. All the aforementioned determinations were done according to the manufacturers' instructions, using ELISA kits from Quantikinine R&D system, USA. For each assay, samples were prepared according to instructions and protein levels were calculated based on four-parameter logistic (4-PL) curve fit.

2.6. Statistical Analysis

Statistical analysis was carried out using the MedCalc Statistical Software version 18.10 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018). Quantitative data were evaluated for normality of distribution and variables with abnormal distribution were characterized by median and 25–75 percentiles. Qualitative data were expressed as frequency and percent. Comparisons between groups were performed using the Mann–Whitney (for quantitative data) and chi-square tests (for qualitative data). Spearman rho coefficient was used for examining the correlation between variables. ROC curves were used in order to find out cut-off values for quantitative variables that could

discriminate between patients with asthma and those without. A multivariate binary logistic regression was used for assessment of independent contribution of variables that achieved statistical significance in univariate analysis for asthma onset. A p value < 0.05 was considered statistically significant.

3. Results

From the entire group of patients with allergic rhinitis to HDM, 21 patients (36.2%) developed asthma after one year of surveillance.

Patients' demographic, clinical and biological data are presented in Table 1.

Table 1. Comparison between patients based on asthma diagnosis at one-year follow-up.

Variable		Total (n = 58)	Patients with Asthma (n = 21)	Patients without Asthma (n = 37)	p
Age (Years) *		27.5 (23–37)	33 (24.5–40)	26 (22–31)	0.014
Sex ^	M	50% (29)	57.1% (12)	45.9% (17)	0.5
	F	50% (29)	42.9% (9)	54.1% (20)	
Living area ^	U	82.8% (48)	85.7% (18)	81.1% (30)	0.7
	R	17.2% (10)	14.3% (3)	18.9% (7)	
Onset of AR symptoms (months) *		24 (6–60)	36 (15–66)	12 (3–48)	0.04
Total symptom score *		8.5 (5–11)	9 (5.5–13)	8 (5–11)	0.2
FeNO (ppb) *		24 (16–46)	45 (30.5–68)	19 (16–28)	<0.001
Total IgE (UI/l) *		106.5 (44.55–201.5)	118 (35.4–293)	104 (49.8–233)	0.9
Eosinophils *		0.05 (0.026–0.071)	0.05 (0.02–0.08)	0.04 (0.02–0.06)	0.5
E selectin (ng/mL) *		3.28 (2.35–4.69)	3.45 (2.26–4.81)	2.32 (2.43–4.64)	0.9
ICAM (ng/mL) *		21.53 (18.88–26.55)	22.98 (20.02–27.18)	20.87 (18.39–24.32)	0.1
VCAM (ng/mL) *		48.53 (40.95–59.50)	56.38 (41.34–60.55)	46.81 (40.93–55.95)	0.1
TNF-α (pg/mL) *		1.78 (1.22–2.33)	1.62 (1.01–2.11)	1.93 (1.34–2.41)	0.2
IL-6 (pg/mL) *		1.05 (0.75–1.70)	1.25 (0.71–1.84)	1.05 (0.78–1.65)	0.6
IL-8 (pg/mL) *		5.32 (3.33–9.38)	5.05 (2.35–8.20)	5.32 (3.95–9.51)	0.2
Wheal size of Der p allergen at prick test (mm)		7.23 ± 2.92	7.89 ± 3.24	6.57 ± 2.61	0.052

Data are expressed as * median; 25–75th percentile; ^ Data are expressed as %, n; Tests used: Mann–Whitney (for quantitative data) and chi-square tests (for qualitative data); Significance $p < 0.05$. Abbreviations: AR, allergic rhinitis; F, female; FeNO, fractional exaled nitric oxide; M, male; R, rural; TSS, total symptoms score; U, urban.

Analyzing demographic data, we noticed that asthma occurrence is correlated with patients' age, but not with their gender or living area (see Table 1). More male patients developed asthma compared to females, but the difference was not statistically significant.

Forty patients (68.9%) presented persistent moderate–severe forms of allergic rhinitis, proved by an initial TSS over 6 (median 8.5 (5–11)). The development of asthma was not correlated with a moderate–severe form of allergic rhinitis (p = 0.5), even if more patients with asthma had previously moderate–severe allergic rhinitis (76.2% vs. 64.9%). Initial TSS of allergic rhinitis was higher in patients with asthma, but the difference was not statistically significant. Initial was not correlated with the duration of AR and was not influenced by patients' sex or living area ($p > 0.05$). The duration of allergic rhinitis is significantly higher in patients with asthma after one year of surveillance.

The markers of allergic inflammation, total IgE and eosinophils were higher in patients with asthma after one year, compared with patients with allergic rhinitis without asthma, but the differences were not statistically significant ($p > 0.05$). We noticed similar results for adhesion molecules (ICAM-1, VCAM-1 and E-selectin) and IL-6, but not for TNF-α and IL-8. Only FeNO was significantly higher in patients with allergic rhinitis and asthma compared to those without asthma (see Table 1). The initial values of biological markers were not influenced by patients' age, sex and living area, duration or severity of allergic rhinitis ($p > 0.05$).

Thirty-seven patients (63.8%) were polysensitized to both indoor and outdoor allergens, but the symptoms of rhinitis were present after exposure to HDM, while 21 patients (36.2%) were sensitized only to HDM. All the patients were sensitized to Der p, while 86.20% (50 patients) were sensitized also to Der f. The wheal size of Der p sensitization was higher in patients who developed asthma compared with those without asthma. The asthma development was not correlated with number or type of sensitizations to other allergens, except HDM ($p > 0.05$).

In patients with allergic rhinitis to HDM, we observed a moderate positive correlation between baseline values of TNF-α and IL-8 (r = 0.327, $p = 0.01$), IL-6 and IL-8 (r = 0.437, $p = 0.001$), weak negative correlation between VCAM-1 and IL-8 (r = −0.290, $p = 0.03$) and a weak positive one between TNF-α and IL-6 (r = 0.260, $p = 0.04$).

The ROC curve for patients' age, duration of allergic rhinitis and FeNO were analyzed and the cut-off values were calculated for these parameters in relation with asthma onset after one year of the inclusion visit. The cut off values, AUC, sensitivity and specificity are presented in Table 2.

Table 2. ROC curve analysis for asthma diagnosis at 1-year follow-up.

Parameter	AUC	Cut-Off Value	Sensitivity	Specificity	*p*
Age	0.696 (95%CI 0.56–0.81)	>31 years	61.90% (95%CI 38.4–81.9%)	78.38% (95%CI 61.8–90.2%)	0.007
Duration of AR	0.659 (95%CI 0.52–0.77)	>12 months	76.19% (95%CI 52.8–91.8%)	54.05% (95%CI 36.9–70.5%)	0.02
FeNO	0.79 (95%CI 0.66–0.88)	>28 ppb	85.71% (95%CI 63.7–97.0%)	78.38% (95%CI 61.8–90.2%)	<0.001

Abbreviations: AR, allergic rhinitis; AUC, aria under de curve; CI, interval of confidence; FeNO, fractional exhaled nitric oxide; parts per billion.

In order to find out which parameter was independently associated with asthma' occurrence in patients with allergic rhinitis to HDM, we used a multivariate logistic regression (Table 3). Variables which achieved statistical significance in univariate analysis were introduced in the regression. Our model explained 36.2% of asthma prevalence. Age and allergic rhinitis duration were very close to statistical significance, probably due to the small number of patients. FeNO > 28 ppb was the only independent variable that predicted the onset of asthma at a one-year follow-up (Table 3).

Table 3. Multivariate analysis for asthma occurrence in patients with allergic rhinitis to HDM.

Variables	B	*p*	OR	95% C.I. for OR	
				Min	Max
Age > 31 yo	1.321	0.07	3.746	0.860	16.311
Duration of AR > 12 months	1.437	0.07	4.209	0.885	20.008
FeNO > 28 ppb	2.928	<0.001	18.682	3.791	92.057
Constant	−0.825	0.04	0.438		

Abbreviations: AR, allergic rhinitis; B, CI, interval of confidence; FeNO, fractional exhaled nitric oxide; HDM, house dust mites; Max, maximum; Min, minimum; male; OR, odds ratio; ppb, parts per billion. Test used: binary logistic regression.

4. Discussion

The present study showed a significant association of asthma symptoms in patients with persistent allergic rhinitis to HDM after one year of surveillance, 36.2% of them presenting asthma. Clinical (duration, patient age) and biological data (inflammatory markers) may predict asthma development. FeNO was an independent variable that predicted the onset of asthma at one-year follow-up.

Allergic rhinitis and asthma are considered a single respiratory disease involving two parts of the airways [2,9]. In the present study, the authors found a prevalence of asthma of 36.2% after a one-year follow-up. Similar data were already reported in the literature, with a variable prevalence between 20% and 50% of patients with allergic rhinitis [9,10]. The prevalence found in the present research was higher compared to the study published in 1998, where the prevalence of asthma in patients with allergic rhinitis was lower (21.3%) after 23 years of follow up [27]. But Greisner et al. [27] included all patients with allergic rhinitis at the same age (first year of faculty), not only patients with persistent forms and different ages. The patients from the present research had a median age of 27.5 year, higher than the age of patients from the Greisner study. A similar prevalence of asthma was also established in children (30% in 13–14 years group and 35% in 6–7 years group) [28], but they did not follow prospectively their patient and the prevalence was established retrospectively.

The current diagnose of rhinitis relies on combination of three types of data: historical, clinical examination, and allergy diagnostic testing, which allows differentiation into three subgroups: allergic, infectious, and non-allergic non-infectious rhinitis [24,29]. Bronchial hyperreactivity is commonly present in patients with persistent moderate severe allergic rhinitis and should be suspected if other risk factors are present (allergen and viral exposures, indoor and outdoor pollution, allergic rhinitis duration and severity) [30–32]. The validation of some clinical and biological factors will permit to phenotype and endotype allergic rhinitis in order to find a form of "asthma risk" allergic rhinitis. Maybe a different approach of allergic rhinitis according to its phenotype and endotype could be done in order to prevent asthma development.

In this study, the clinical, biological and inflammatory markers that might influence the appearance of asthma in patients with allergic rhinitis to HDM were evaluated. The authors included only patients with persistent allergic rhinitis in this study, knowing that duration of symptoms and their severity are risk factors for asthma development [30,32]. A duration of allergic rhinitis over 12 months was considered a risk factor for asthma occurrence similar to other previous studies, in both adults and children [3,27]. The severity of disease was not correlated with asthma occurrence in this research. In Bousquet et al.'s and del Curvillo et al.'s studies [30,32], the severity of rhinitis was correlated with asthma development, but they included patients with both intermittent and persistent allergic rhinitis, while in the present research all the patients had persistent forms.

Rhinitis phenotypes were also described in relationship to sensitization pattern [33]. Sensitization to HDM is a risk factor for asthma development because they are perennial allergens [9]. In this study, only patients with sensitization to HDM were included. In addition, the sensitization may influence in different degree the severity of allergic rhinitis and the evolution to asthma, as Vidal's study already mentioned [33]. Vidal et al. reported that severe allergic respiratory disease was associated with higher levels of both total IgE and specific IgE to HDM. The presence of sIgE to both Der p 1 and Der p 2 was associated with asthma among HDM-allergic patients [33]. Our results revealed an almost significant association ($p = 0.052$) in univariate analysis between the size of the skin prick test to Der p and the occurrence of asthma. The mean size of wheal to asthmatic patient was 7.89 mm, which may confirm the presence of clinically manifested symptoms as for both asthma and rhinitis as in Haahtela's study [34]. The authors of this research did not determine the level of sIgE to HDM; they confirmed the sensitization based only on the skin prick test. Probably a large number of patients may give more information regarding the role of wheal size in assessing rhinitis or asthma symptoms.

An important step in implementing the precision medicine in patients with allergic rhinitis is to identify possible biomarkers which characterize the endotypes and may guide us to different therapeutic approaches. In asthma research, the endotypes are already described, based on different biomarkers

(cytokines, cells). Allergic rhinitis might have complex endotypes and the current understanding of cellular and molecular processes may lead to identify certain biomarkers that characterize the endotypes, but studies are still required to confirm them. There are several modulators of endotype expression, such as environment, microbiome, lifestyle and nasal anatomy. In several studies, the following endotypes of rhinitis were proposed: type 2 immune response rhinitis, type 1 immune response rhinitis, neurogenic rhinitis, epithelial dysfunction [26,35,36].

However, in the present study, the authors focused only on type 2 immune response allergic rhinitis with HDM sensitization alone or with other co-sensitizations. Previous studies revealed that some markers are increased in patients with allergic rhinitis to HDM: total serum IgE, blood and intranasal eosinophils, various types of cytokines [18,37] and adhesion molecules [6,38,39]. In this study, the comparative univariate analysis of patients with allergic rhinitis with or without asthma after a one-year surveillance showed that mean values of adhesion molecules and IL-6 were increased in the first subgroup compared to patients without asthma, but the differences did not reach the statistical significance and, because of this, they were not included in multivariate analysis. The plasmatic values of cytokines are extremely variable and more patients should be included in order to confirm them as biomarkers. Additionally, their plasmatic level should be correlated with their level in nasal or bronchial lavage. A previous study in children reported that a cytokine imbalance predicted asthma occurrence after three years of surveillance [40], but they concluded that respiratory viral infections may play a significant role in this imbalance, not only atopy. Therefore, these inflammatory markers should be evaluated in different population subgroups, adults and children, and in conjunction with other risk factors, in order to confirm their potential role as biomarkers.

As a biologic marker, only FeNO was significantly higher in patients with allergic rhinitis and asthma compared to those without asthma. Patients with allergic rhinitis to HDM that developed asthma have an increased systemic inflammation, which was correlated with subclinical inflammation in the lower airways. FeNO is a biomarker of atopy and eosinophilic airway inflammation [41]. Previous studies found a correlation of FeNO level and type of sensitization, in both adults and children. Jouaville et al. found that FeNO level increased with the number of positive skin prick tests (SPTs) in both asthmatics and non-asthmatic subjects, but in cases of an equal level of positive SPTs the FeNO was higher in asthmatic than in non-asthmatic, reflecting its role in asthma diagnoses [42]. The exact role of FeNO in the prediction of asthma in patients with allergic rhinitis is still unclear.

In the present study, patients with allergic rhinitis to HDM with continuous symptoms for more than 12 months and an elevated FeNO at presentation over 28 ppb had 18-fold higher risk for asthma after one year. Malinovschi et al. used FeNO in evaluation of allergic rhinitis persistence and severity in general population of adolescents, but the author did not calculate a cut off value which may predict the persistence of rhinitis [43]. Additionally, they evaluate the persistence of allergic rhinitis symptoms, not the occurrence of asthma ones. Di Cara et al. found that children with allergic rhinitis and elevated values of FeNO over 35 ppb developed asthma after five years of monitoring [44]. Similar results were also reported in children and adults [45,46]. The cut off value in our study was lower than in the Di Cara study [44], but only adults were included, while the Di Cara study included only children.

The factors that lead to asthma in patients with allergic rhinitis are multiple. Further studies are still needed to evaluate the evolution of allergic inflammation. However, from the clinical practice point of view, it is important to evaluate all patients with allergic rhinitis for asthma symptoms [47]. Patients with allergic rhinitis and other risk factors for asthma should be carefully evaluated for the presence of subclinical inflammation in the lower airways, which is the substrate for bronchial hyperreactivity.

This study has a strong value because it emphasized the role of FeNO, a marker of lower eosinophilic inflammation in allergic rhinitis in order to predict the evolution of disease to other manifestations. There are some limitations of this study. Firstly, a small number of patients were included in the study. Secondly, the surveillance period is short and some of the investigated markers may not be able to predict the asthma development so soon. It could be interesting to also evaluate the modulation of these biological markers under different treatments that are recommended in allergic

rhinitis. The authors evaluated the markers in patients with rhinitis induced by HDM, but they could not compare patients with monosensitization with the ones polysensitized because of the small number of subjects included in the present analysis.

5. Conclusions

Clinical and biological parameters may predict asthma occurrence in patients with persistent allergic rhinitis to house dust mites. Adult patients with a longer duration of rhinitis symptoms and a high level of FeNO over 28 have a greater risk to develop asthma. The severity of symptoms and the serum inflammatory cytokines and adhesion molecules are not correlated with asthma occurrence after one year of monitoring. FeNO could become a useful biomarker in predicting a specific endotype of allergic rhinitis with high risk to develop asthma.

Author Contributions: Conceptualization, I.A.M., I.C.B., A.D.B. and D.D.; Data curation, I.A.M., I.C.B. and I.N.; Formal analysis, I.A.M., I.C.B. and S.V.; Investigation, I.C.B., N.M. and I.N.; Methodology, I.A.M., I.C.B., S.V. and N.M.; Project administration, I.A.M., I.C.B. and N.M.; Resources, A.D.B. and D.D.; Software, S.V.; Supervision, S.V., A.D.B. and D.D.; Validation, D.D.; Writing—original draft, I.A.M., I.C.B. and N.M.; Writing—review & editing, A.D.B. and D.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work was partially funded a research grant [27020/48/2011] of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj Napoca, Romania.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kay, A.B. Allergy and allergic diseases first of two parts. *N. Engl. J. Med.* **2001**, *344*, 30–37. [CrossRef] [PubMed]
2. Khan, D.A. Allergic rhinitis and asthma: Epidemiology and common pathophysiology. *Allergy Asthma Proc.* **2014**, *35*, 357–361. [CrossRef] [PubMed]
3. Navarro, A.; Valero, A.; Juli, B. Coexistence of Asthma and Allergic Rhinitis in Adult Patients Attending Allergy Clinics: ONEAIR Study. *J. Investig. Allergol. Clin. Immunol.* **2008**, *18*, 233–238. [PubMed]
4. Kowalski, M.L.; Alam, R. Signal transduction mechanisms as new targets for allergists. *Allergy* **2001**, *56*, 199–203. [CrossRef] [PubMed]
5. Canonica, G.W.; Complati, E. Minimal persistent inflammation in allergic rhinitis: Implications for current treatment strategies. *Clin. Exp. Immunol.* **2009**, *158*, 260–271. [CrossRef] [PubMed]
6. Christodoulopoulos, P.; Cameron, L.; Durham, S.; Hamid, Q. Molecular pathology of allergic disease II: Upper airway disease. *J. Allergy Clin. Immunol.* **2000**, *105*, 211–223. [CrossRef]
7. Aronsson, D.; Tufvesson, E.; Bjermer, L. Allergic rhinitis with or without concomitant asthma: Difference in perception of dyspnoea and levels of fractional exhaled nitric oxide. *Clin. Exp. Allergy* **2005**, *35*, 1457–1461. [CrossRef]
8. Bergmann-Hug, K.; Wirth, R.; Henseler, M.; Helbling, A.; Pichler, W.J.; Schnyder, B. Effect of natural seasonal pollen exposure and repeated nasal allergen provocations on elevation of exhaled nitric oxide. *Allergy* **2009**, *64*, 1629–1634. [CrossRef]
9. Bousquet, J.; Vignola, A.M.; Demoly, P. Links between rhinitis and asthma. *Allergy* **2003**, *58*, 691–706. [CrossRef]
10. Leynaert, B.; Neukirch, C.; Kony, S.; Guénégou, A.; Bousquet, J.; Aubier, M.; Neukirch, F.; European Community Respiratory Health Survey. Association between asthma and rhinitis according to atopic sensitization in a population-based study. *J. Allergy Clin. Immunol.* **2004**, *113*, 86–93. [CrossRef] [PubMed]
11. Spergel, J.M. From atopic dermatitis to asthma: The atopic march. *Ann. Allergy Asthma Immunol.* **2010**, *105*, 99–106. [CrossRef] [PubMed]
12. Rochat, M.K.; Illi, S.; Ege, M.J.; Lau, S.; Keil, T.; Wahn, U.; von Mutius, E.; Multicentre Allergy Study (MAS) Group. Allergic rhinitis as a predictor for wheezing onset in school-aged children. *J. Allergy Clin. Immunol.* **2010**, *126*, 1170–1175.e2. [CrossRef] [PubMed]

13. Kurukulaaratchy, R.J.; Zhang, H.; Patil, V.; Raza, A.; Karmaus, W.; Ewart, S.; Arshad, S.H. Identifying the heterogeneity of young adult rhinitis through cluster analysis in the Isle of Wight birth cohort. *J. Allergy Clin. Immunol.* **2015**, *135*, 143–150. [CrossRef] [PubMed]
14. Ferreira, M.A.; Vonk, J.M.; Baurecht, H.; Marenholz, I.; Tian, C.; Hoffman, J.D.; Helmer, Q.; Tillander, A.; Ullemar, V.; Lu, Y.; et al. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nat. Genet.* **2017**, *49*, 1752–1757. [CrossRef]
15. Schwartz, R.H. T cell anergy. *Ann. Rev. Immunol.* **2003**, *21*, 305–334. [CrossRef]
16. Passalacqua, G.; Ciprandi, G.; Pasquali, M.; Guerra, L.; Canonica, G.W. An update on the asthma-rhinitis link. *Curr. Opin. Allergy Clin. Immunol.* **2004**, *4*, 177–183. [CrossRef]
17. McLane, M.L.; Nelson, J.A.; Lenner, K.A.; Hejal, R.; Kotaru, C.; Skowronski, M.; Coreno, A.; Lane, E.; McFadden, E.R., Jr. Integrated response of the upper and lower respiratory tract of asthmatic subjects to frigid air. *J. Appl. Physiol.* **2000**, *88*, 1043–1050. [CrossRef]
18. Ciprandi, G.; Cirillo, I.; Vizzaccaro, A.; Civardi, E.; Barberi, S.; Allen, M.; Marseglia, G.L. Desloratadine and Levocetirizine improve nasal symptoms, airflow, and allergic inflammation in patients with perennial allergic rhinitis: A pilot study. *Int. Immunopharmacol.* **2005**, *5*, 1800–1808. [CrossRef]
19. Haldar, P.; Pavord, I.D.; Shaw, D.E.; Berry, M.A.; Thomas, M.; Brightling, C.E.; Wardlaw, A.J.; Green, R.H. Cluster analysis and clinical asthma phenotypes. *Am. J. Respir. Crit. Care Med.* **2008**, *178*, 218–224. [CrossRef]
20. Verini, M.; Consilvio, N.P.; Di Pillo, S.; Cingolani, A.; Spagnuolo, C.; Rapino, D.; Scaparrotta, A.; Chiarelli, F. FeNO as a Marker of Airways Inflammation: The Possible Implications in Childhood Asthma Management. *J. Allergy* **2010**, *2010*, 691425. [CrossRef]
21. Buhl, R.; Korn, S.; Menzies-Gow, A.; Aubier, M.; Chapman, K.R.; Canonica, G.W.; Picado, C.; Donica, M.; Kuhlbusch, K.; Korom, S.; et al. Prospective, single-arm, longitudinal study of biomarkers in real-world patients with severe asthma. *J. Allergy Clin. Immunol. Pract.* **2020**. [CrossRef]
22. Cirillo, I.; Pistorio, A.; Tosca, M.; Ciprandi, G. Impact of allergic rhinitis on asthma: Effects on bronchial hyperreactivity. *Allergy* **2009**, *64*, 439–444. [CrossRef]
23. Bocsan, C.I.; Bujor, A.I.; Miron, N.; Vesa, Ş.C.; Deleanu, D.; Buzoianu, A.D. In vivo anti-inflammatory effect of H1 antihistamines in allergic rhinitis: A Randomized Clinical Trial. *Balkan Med. J.* **2015**, *32*, 352–358. [CrossRef]
24. Bousquet, J.; Schünemann, H.J.; Zuberbier, T.; Bachert, C.; Baena-Cagnani, C.E.; Bousquet, P.J.; Brozek, J.; Canonica, G.W.; Casale, T.B.; Gerth van Wijk, R.; et al. Development and implementation of guidelines in allergic rhinitis—An ARIA-Ga2len paper. *Allergy* **2010**, *65*, 1212–1221. [CrossRef] [PubMed]
25. Heinzerling, L.; Mari, A.; Bergmann, K.C.; Bresciani, M.; Burbach, G.; Darsow, U.; Durham, S.; Fokkens, W.; Gjomarkaj, M.; Bom, A.T.; et al. The skin prick test—European standards. *Clin. Transl. Allergy* **2013**, *3*, 3. [CrossRef] [PubMed]
26. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am. J. Respir. Crit. Care Med.* **2005**, *171*, 912–930. [CrossRef] [PubMed]
27. Greisner, W.A., 3rd; Settipane, R.J.; Settipane, G.A. Co-existence of asthma and allergic rhinitis: A 23-year follow-up study of college students. *Allergy Asthma Proc.* **1998**, *19*, 185–188. [CrossRef]
28. Ibanez, M.D.; Navarro, A.; Sanchez, M.C.; Rondón, C.; Montoro, J.; Matéu, V.; Lluch-bernal, M.; Fernández-Parra, B.; Valero, A.; Conde, J.; et al. Rhinitis and its association with asthma in patients under 14 years of age treated in allergy departments in Spain. *J. Investig. Allergol. Clin. Immunol.* **2010**, *20*, 402–406.
29. Muraro, A.; Lemanske, R.F., Jr.; Hellings, P.W.; Akdis, C.A.; Bieber, T.; Casale, T.B.; Jutel, M.; Ong, P.Y.; Poulsen, L.K.; Simon, H.U.; et al. Precision medicine in patients with allergic diseases: Airway diseases and atopic dermatitis-PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. *J. Allergy Clin. Immunol.* **2016**, *137*, 1347–1358. [CrossRef]
30. Bousquet, J.; Anto, J.M.; Akdis, M.; Auffray, C.; Keil, T.; Momas, I.; Postma, D.S.; Valenta, R.; Wickman, M.; Haahtela, T.; et al. Paving the way of systems biology and precision medicine in allergic diseases: The MeDALL success story: Mechanisms of the Development of ALLergy; EU FP7-CP-IP; Project No: 261357; 2010–2015. *Allergy* **2016**, *71*, 1513–1525. [CrossRef]

31. Bousquet, J.; Annesi-Maesano, I.; Carat, F.; Leger, D.; Rugina, M.; Pribil, C.; El Hasnaoui, A.; Chanal, I. Characteristics of intermittent and persistent allergic rhinitis: DREAMS study group. *Clin. Exp. Allergy* **2005**, *35*, 728–732. [CrossRef] [PubMed]
32. del Cuvillo, A.; Montoro, J.; Bartra, J.; Valero, A.; Ferrer, M.; Jáuregui, I.; Dávila, I.; Sastre, J.; Mullol, J. Validation of ARIA duration and severity classifications in Spanish allergic rhinitis patients—The ADRIAL cohort study. *Rhinology* **2010**, *48*, 201–205. [CrossRef] [PubMed]
33. Vidal, C.; Lojo, S.; Juangorena, M.; Gonzalez-Quintela, A. Association between Asthma and Sensitization to Allergens of Dermatophagoides pteronyssinus. *J. Investig. Allergol. Clin. Immunol.* **2016**, *26*, 304–309. [CrossRef] [PubMed]
34. Haahtela, T.; Burbach, G.J.; Bachert, C.; Bindslev-Jensen, C.; Bonini, S.; Bousquet, J.; Bousquet-Rouanet, L.; Bousquet, P.J.; Bresciani, M.; Canonica, G.W.; et al. Clinical relevance is associated with allergen-specific wheal size in skin prick testing. *Clin. Exp. Allergy* **2014**, *44*, 407–416. [CrossRef] [PubMed]
35. Akdis, C.A.; Bachert, C.; Cingi, C.; Dykewicz, M.S.; Hellings, P.W.; Naclerio, R.M.; Schleimer, R.P.; Ledford, D. Endotypes and phenotypes of chronic rhinosinusitis: A PRACTALL document of the European Academy of Allergy and Clinical. *J. Allergy Clin. Immunol.* **2013**, *131*, 1479–1490. [CrossRef]
36. Papadopoulos, N.G.; Bernstein, J.A.; Demoly, P.; Dykewicz, M.; Fokkens, W.; Hellings, P.W.; Peters, A.T.; Rondon, C.; Togias, A.; Cox, L.S. Phenotypes and endotypes of rhinitis and their impact on management: A PRACTALL report. *Allergy* **2015**, *70*, 474–494. [CrossRef]
37. Resende, E.P.; Todo-Bom, A.; Loureiro, C.; Pinto, A.M.; Oliveiros, B.; Mesquita, L.; Silva, H.C. Asthma and rhinitis have different genetic profiles for IL13, IL17A and GSTP1 polymorphisms. *Rev. Port. Pneumol.* **2017**, *23*, 10–16. [CrossRef]
38. Castano, R.; Maghni, K.; Castellanos, L.; Trudeau, C.; Malo, J.L.; Gautrin, D. Proinflammatory mediators in nasal lavage of subjects with occupational rhinitis. *Otolaryngol. Head Neck Surg.* **2010**, *143*, 301–303. [CrossRef]
39. Muntean, I.A.; Bocsan, I.C.; Miron, N.; Buzoianu, A.D.; Deleanu, D. How Could We Influence Systemic Inflammation in Allergic Rhinitis? The Role of H1 Antihistamines. *Oxid. Med. Cell. Longev.* **2018**, *2018*, 3718437. [CrossRef]
40. Lemanske, R.F., Jr. The childhood origins of asthma (COAST) study. *Pediatr. Allergy Immunol.* **2002**, *13*, 38–43. [CrossRef]
41. Ricciardolo, F.L.M.; Sorbello, V.; Ciprandi, G. A pathophysiological approach for FeNO: A biomarker for asthma. *Allergol. Immunopathol. (Madr)* **2015**, *43*, 609–616. [CrossRef] [PubMed]
42. Jouaville, L.F.; Annesi-Maesano, I.; Nguyen, L.T.; Bocage, A.S.; Bedu, M.; Caillaud, D. Interrelationships among asthma, atopy, rhinitis and exhaled nitric oxide in a population-based sample of children. *Clin. Exp. Allergy* **2003**, *33*, 1506–1511. [CrossRef] [PubMed]
43. Malinovschi, A.; Alving, K.; Kalm-Stephens, P.; Janson, C.; Nordvall, L. Increased exhaled nitric oxide predicts new-onset rhinitis and persistent rhinitis in adolescents without allergic symptoms. *Clin. Exp. Allergy* **2012**, *42*, 433–440. [CrossRef] [PubMed]
44. Di Cara, G.; Marcucci, F.; Palomba, A.; Milioni, M.; Pecoraro, L.; Ciprandi, G.; Buttafava, S.; Frati, F.; Verrotti, A. Exhaled nitric oxide in children with allergic rhinitis: A potential biomarker of asthma development. *Pediatr. Allergy Immunol.* **2015**, *26*, 85–87. [CrossRef]
45. Singer, F.; Luchsinger, I.; Inci, D.; Knauer, N.; Latzin, P.; Wildhaber, J.H.; Moeller, A. Exhaled nitric oxide in symptomatic children at preschool age predicts later asthma. *Allergy* **2013**, *68*, 531–538. [CrossRef]
46. Muntean, A.; Bocsan, I.C.; Deleanu, D.M. The effect of H1-antihistamines on allergic inflammation in patients with allergic rhinitis. *HVM Bioflux* **2016**, *8*, 161–165.
47. Canonica, G.W. Introduction to nasal and pulmonary allergy cascade. *Allergy* **2002**, *57*, 8–12. [CrossRef]

healthcare

MDPI

Article

COVID-19 Disease Leading to Chronic Spontaneous Urticaria Exacerbation: A Romanian Retrospective Study

Ioana Adriana Muntean [1], Irena Pintea [1,*], Ioana Corina Bocsan [2], Carmen Teodora Dobrican [1,*] and Diana Deleanu [1]

1 Department of Allergology and Immunology, "Octavian Fodor" Institute of Gastroenterology and Hepatology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400462 Cluj Napoca, Romania; Adriana.Muntean@umfcluj.ro (I.A.M.); diana.deleanu@umfcluj.ro (D.D.)
2 Department of Pharmacology, Toxicology and Clinical Pharmacology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400462 Cluj Napoca, Romania; bocsan.corina@umfcluj.ro
* Correspondence: irena.nedelea@umfcluj.ro (I.P.); dobrican.carmen@umfcluj.ro (C.T.D.)

Abstract: (1) Background: The COVID-19 pandemic has resulted in the exacerbation of various chronic diseases. Due to the potential impact of SARS-CoV-2 on mast cells, we aimed to analyze the relevance of COVID-19 disease on chronic spontaneous urticaria (CSU) clinical presentation and biological profile. (2) Methods: This study is a retrospective case series of patients with CSU diagnosed and treated in the Allergy Department of the Professor Doctor Octavian Fodor RIGH, (Cluj-Napoca, Romania). Patients were assessed for disease activity and level of control with the weekly urticaria activity score and the visual analogue scale. Results were correlated with COVID-19 severity and with nonspecific markers of inflammation during and after the SARS-CoV-2 infection. (3) Results: SARS-CoV-2 impacted a significant proportion (33%) of the CSU patients, of which 71% developed a moderate-severe form of COVID-19. Most of the patients (68%) had moderate-severe forms of CSU and 65% took AH1 treatment (one dose, two-fold dose or four-fold dose). The rest of them (35%) received the second-line treatment (40.3% Omalizumab, 53% Prednisolone and 4.8% Cyclosporine). In Omalizumab treated group of UCS patients we observed that COVID-19 disease was not severe. We established a positive correlation between the severity of the infection and that of the CSU clinical presentation, with most bothersome symptoms of urticaria being experienced by moderate to severe COVID-19 CSU patients (47%). Inflammatory markers were positively correlated (p = 0.01) with a more severe clinical profile of CSU, in accordance with our hypothesis that the level of inflammation triggered by COVID-19 disease has a role in CSU exacerbation. The non-specific inflammatory markers, such as CRP, were positively associated with the UAS7 score (R2 = 0.363; p = 0.001). An increased rate of exacerbation of CSU was observed in moderate-severe COVID-19 infection. 4) Conclusions: COVID-19 disease can result in the exacerbation of chronic spontaneous urticaria, more likely in moderate to severe forms of infection.

Keywords: COVID-19; chronic spontaneous urticaria; SARS-CoV-2 infection; mast cells

Citation: Muntean, I.A.; Pintea, I.; Bocsan, I.C.; Dobrican, C.T.; Deleanu, D. COVID-19 Disease Leading to Chronic Spontaneous Urticaria Exacerbation: A Romanian Retrospective Study. *Healthcare* **2021**, *9*, 1144. https://doi.org/10.3390/healthcare9091144

Academic Editor: Roberta Ferrucci

Received: 23 July 2021
Accepted: 30 August 2021
Published: 1 September 2021

1. Introduction

Chronic spontaneous urticaria (CSU) is a disease characterized by the recurrence of pruritic wheals, occurring on most days of the week, for longer than six weeks, and accompanied by angioedema in more than 50% of the cases. In some patients, angioedema is the only clinical feature of the disease [1]. Although CSU is a limited disorder in most cases, with an average duration of disease of two to five years, active and/or refractory, difficult to treat cases pose a difficult challenge to patients and clinicians alike [1,2]. Active chronic spontaneous urticaria is a debilitating disorder, which has a major impact on the quality of life of affected individuals and is a substantial global burden [3].

The current CSU treatment algorithm follows a stepwise approach, starting with standard doses of second-generation non-sedative H1 antihistamines as the first line treatment. Up-dosing of up to a 4-fold increase of H1 antihistamines in cases non-responsive after 2–4 weeks of first line treatment, or earlier. In antihistamine-resistant patients, treatment with Omalizumab and, if this fails, Cyclosporine is the current guideline-recommended therapy of choice [1–3].

There are several theories regarding the pathogenesis of CSU, mainly involving autoinflammation and mast cell (MCs) mediator release. MC degranulation is a central event in the development of CSU cutaneous lesions, and histamine levels are elevated in biopsy skin samples [3]. MCs are strategically placed at sites that interface with our external environment such as the skin, lung, and intestines. These locations allow them to act as sentinels for tissue damage and pathogen invasion. The association between MCs and blood vessels is optimal to enhance the rapid recruitment of effector cells out of the bloodstream and into neighboring tissues [4].

Since the beginning of 2020, severe acute respiratory coronavirus 2 (SARS-CoV-2) has spread rapidly across the globe, causing the "coronavirus disease 2019" (COVID-19) pandemic, the worst global public health crisis. The pathology of severe COVID-19 is characterized by elevated levels of proinflammatory cytokines, mainly tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-1β, granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemokine (C-C-motif) ligand 2 (CCL2), many of which are produced and released by MCs [4–6]. There is evidence for SARS-CoV-2 and MCs' activation. MCs, key effector cells in CSU, along with other immune cells such as basophils, neutrophils, monocytes/macrophages, and natural killer cells are involved in cytokine storms triggered by SARS-CoV-2 severe infection [7,8]. In addition, MCs can recognize and respond to viruses through several different receptors, including toll-like receptors, retinoic acid–inducible gene-I–like receptors, FcεRI, complement, and IL-1 receptors. Engagement of these receptors results in MCs' activation and degranulation. This process is facilitated by the MCs' rapid production of proinflammatory cytokine mediators such as TNF and IL-1β that activate endothelium, leukotrienes and prostaglandins that facilitate vasodilatation, as well as a range of chemokines that promote selective recruitment of specific subsets of effector cells [5]. Moreover, recent studies show that human mast cells can be synergistically stimulated by the peptide substance P and IL-33 to release impressive amounts of vascular endothelial growth factor, IL-1β or tumor necrosis factor again without secretion of histamine or tryptase. In addition, mast cells express the renin-angiotensin system, the ectoprotease angiotensin-converting enzyme 2 required for SARS-CoV-2 binding, and serine proteases, including TMPRSS2, required for priming of the corona spike protein [9].

Recent studies bring arguments in favor of CSU exacerbation in the context of COVID-19 [10]. Due to the potential impact of the acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on inflammation in general and on mast cells, the key effector cells in chronic spontaneous urticaria (CSU) we analyzed the relevance of SARS-CoV-2 infection on CSU clinical presentation and biological profile.

2. Materials and Methods

2.1. Study Design

This study is a retrospective case series of patients (n = 218) with CSU diagnosed and treated in the Allergy Department of the Professor Doctor Octavian Fodor Regional Institute of Gastroenterology and Hepatology, (Cluj-Napoca, Romania), between February 2017 and April 2021, since January 2020 this group of patients were evaluated for the presence of COVID-19 disease and UCS exacerbations. The study protocol was approved by the "Octavian Fodor" Institute of Gastroenterology and Hepatology Ethics Committee, and all patients signed the informed consent before any assessments were done. Inclusion criteria were the presence of CSU recent onset or personal history of CSU

in the last 5 years. The exclusion criteria were as follows: known food allergies, inadequate control of chronic thyroid diseases (normal TSH level at initial evaluation), and concomitant chronic inflammatory disease: asthma, COPD, systemic autoimmune diseases, chronic infections (e.g., viral B hepatitis, viral C hepatitis which continue to show an increased prevalence in Romania) [11].

2.2. Patients and Clinical Evaluation

Diagnosis of CSU was done according to the EAACI/GA2LEN/EDF/WAO guideline. Diagnosis was based on history and typically symptoms: wheals and itch associated with or without angioedema. Age, sex, and region (rural/urban) and urticaria related symptoms were recorded. Patients were assessed for disease activity, impact, and level of control with the weekly urticaria activity score (UAS7), and the Visual Analog Scale (VAS). Interviews with the patients were made by phone or online and face to face during a clinical exam in severe symptomatic patients.

Urticaria Activity Score (UAS7) evaluated the number of wheals and itching. The number of wheals was counted by the patients and analyzed on a scale from 0 to 3 (0 = absent, 1 ≤ 20 wheals over 24 h, 2 = 20–50 wheals over 24 h, 3 ≥ 50 wheals over 24 h for), for 7 days. Itching was analyzed on a scale from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe), retrospectively for 24 h, for 7 days. Patients with UAS7 values that were higher than 16 were included in the moderate-severe CSU group. Also, VAS scale was used to assess the QL (quality of life), and a VAS value over 7 points indicated patients with moderate/severe form of CSU. Visits were performed via e-mail or via telephone, and patients completed the VAS and UAS7 monthly, at home, and sent the results by text message or e-mail. Some of the patients required a face-to-face consultation following COVID-19 disease, due to CSU severity, to increase the step of CSU treatment.

2.3. COVID-19 Disease

COVID-19 disease severity was defined by using the scale provided by the World Health Organization, according to data from previous presentations at the Infectious Disease Hospital in Cluj-Napoca. Clinical direct evaluation was performed after the 14 days of quarantine required for SARS-CoV-2 infection. All the patients were called and advised to inform in case they contracted the virus.

In all CSU patients who contracted the virus, the first line of treatment for CSU consisted of AH1 up to 4-fold dose and a prolonged course of prednisolone of 2–4 weeks, which was sufficient to control the symptoms of CSU, with only one non-responder, who was introduced on Omalizumab 300 mg monthly after the 4 weeks of conventional treatment failed to improve/control the disease. This patient responded well to the anti-IgE therapy and remission of CSU was obtained 4 months after the initiation of the biological therapy.

2.4. Biological Evaluation

Blood tests performed in all patients were: complete blood count, CRP, LDH, Troponin, TSH. These tests were done in a laboratory with national accreditation. A jeun blood samples were collected. The samples were collected on days 10 to 15 after the positive SARS-CoV-2 rtPCR.

2.5. Statistical Analysis

The statistical analysis was performed using Microsoft Excel and SPSS version 21 (Chicago, IL, USA). Data were labeled as nominal, expressed as continuous variables. Variables with normal distribution were expressed as mean and standard deviation. The differences were placed in groups using the Wilcoxon Signed Rank test and between groups using the Mann Whitney test. The Spearman' coefficient of correlation was

calculated to highlight differences between continuous variables. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Patients' Demographic Data

In Table 1 we analyzed demographic data of our patient's group. Median age was 48.3 (23–75) years and the sex ratio M:F was 1:3. 68% had moderate-severe forms of CSU and in this group the medium age was significantly higher ($p = 0.01$). Patients with a UAS7 score greater than 16 were included in moderate-severe forms of CSU. UAS7 and VAS were positively associated and statistically significant (R = 0.138, $p = 0.01$). Also, treatment with Omalizumab, Cyclosporine and Prednisone is found only in moderate/severe CSU patients.

Table 1. Demographic characteristics of the patients in our study group.

Parameter		Mild CSU ($n = 69$)	Moderate/Severe CSU ($n = 149$)	p
Age *		38.05 ± 6.2	59.89 ± 9.7	0.01
Sex	male	34.7% (24)	20.1% (30)	0.2
	female	65.3% (65)	79.9% (99)	
Living area	urban	78.2% (54)	68.4% (102)	0.7
	rural	21.8% (15)	31.6% (27)	
CSU onset (months)		124 (3–160)	186 (4.5–268)	0.5
CSU scores	UAS7	≤15	≥16	0.01
	VAS	≤6	≥6	0.01
Treatment	AH1	10% (7)	2.6% (4)	0.05
	2xAH1	37.6% (26)	5.9% (10)	0.8
	4xAH1	37.6% (26)	36.2% (54)	0.05
	* Omalizumab	0% (0)	16.7% (25)	0.01
	* Cyclosporine	0% (0)	2% (3)	0.01
	* Prednisone	0% (0)	22.1% (33)	0.01
CSU in remission	No treatment	21.8% (15)	16.7% (25)	0.6
COVID 19 infection	Not present	69.5% (48)	65.7% (98)	0.7
	Present	31.5% (21)	34.3% (51)	0.8

*Data are expressed as mean ± SD. Significance $p < 0.05$.

3.2. The Treatment in Our Group with CSU.

The CSU treatment options for patients during the SARS-CoV-2 pandemic (February 2020–April 2021) under our care are presented in Figure 1. Most of the patients (68%) had moderate-severe forms of CSU and 65% took AH1 treatment (one dose, two-fold dose or four-fold dose). The rest of them (35%) received the second-line treatment. Treatment with omalizumab and cyclosporine is not supported by the national healthcare system in Romania. Due to high costs, some of the patients who suffer from refractory CSU, and are thus non-responsive to AH1, cannot afford to pay for the therapy. These patients received a prednisone course. Patients with uncontrolled CSU during SARS-CoV-2 pandemic received treatment according to the CSU guideline (40.3% Omalizumab, 53% Prednisolone, and 4.8% Cyclosporine). Prednisone was the most frequent drug used in

managing CSU flares during SARS-CoV-2 pandemic. The dose of Omalizumab was 300 mg/month followed by a 6 month course of treatment. Cyclosporine was given with a dose of 3 mg/body weight for 2 months, 2 mg/body weight for 1 month, and 1 mg/body weight for 3 months [10], while the Prednisone course started with 0.5 mg/kg for 1 week, then the dose was tapered with 5 mg/day until a dose of 10 mg/day was reached and maintained for one week, followed by 10 mg every 2 days for a month or more in nonresponsive patients who couldn't afford/refused the omalizumab or cyclosporine therapy.

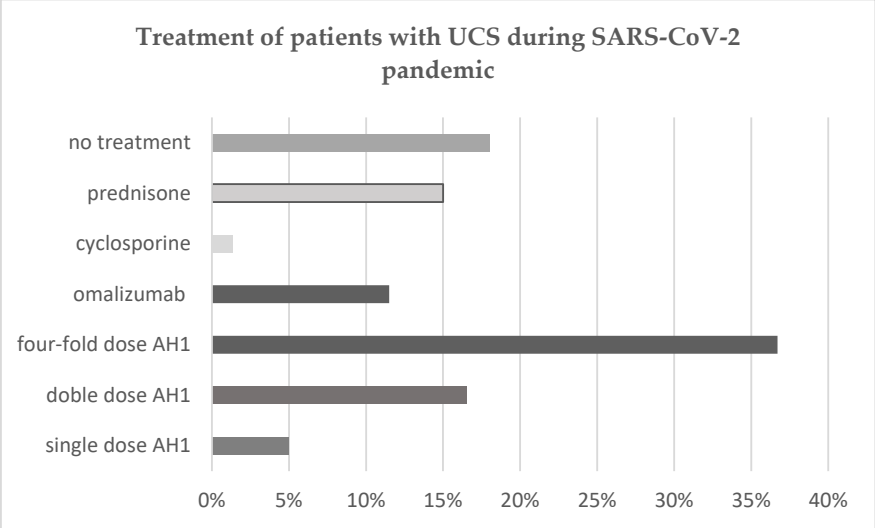

Figure 1. treatment in patients with CSU during SARS-CoV-2 pandemic, represented as percent.

3.3. CSU and COVID-19 Disease

Only 33% of patients from the CSU group went through COVID-19, of which 71% developed a moderate-severe form of disease. During SARS-CoV-2 infection, the patients were in different states of treatment for CSU: no treatment, single dose of AH1, double dose of AH1, four-fold dose of AH1 and omalizumab, cyclosporine and prednisone as add-on treatment to four-fold AH1, as described in Table 2.

Table 2. CSU treatment during COVID-19 mild or moderate-severe type of disease, represented as percent and number of patients.

CSU Treatment	Mild COVID-19 Infection (*n* = 21)	Moderate/Severe COVID-19 Infection (*n* = 51)
Single dose AH1	14.2% (3)	1.9% (1)
Double dose AH1	42.8% (9)	29.4% (15)
Four-fold dose AH1	33.3% (7)	39.1% (20)
Omalizumab	0% (0)	19.6% (10)
Cyclosporine	0% (0)	0% (0)
Prednisone	0% (0)	3.9% (2)
No treatment	9.7% (2)	9.1% (5)

During and after COVID-19 disease, the course of CSU symptoms modified, such that: 44% of infected patients had an increased severity of the disease, more frequently seen in moderate-severe COVID infection—47%), but CSU did not influence the severity of COVID infection, as described in Table 3. Most likely the high degree of inflammation in moderate-severe COVID-19 disease plays an important role in CSU exacerbation.

Table 3. CSU course during COVID-19 mild or moderate-severe infection represented as percent and number of patients.

CSU Course	Mild COVID-19 Infection (n = 21)	Moderate/Severe COVID-19 Infection (n = 51)
Better	4.7% (1)	9.8% (5)
No change	61.9% (13)	43.1% (22)
Worse	33.4% (7)	47% (24)

The non-specific inflammatory markers, such as CRP, were positively associated with the UAS7 score (R2 = 0.363; p = 0.001), and all the markers were significantly increased in moderate-severe COVID-19 infection group of patients, as described in Table 4, with an increased rate of exacerbation of CSU in moderate-severe COVID-19 infection.

In the Omalizumab group, which received 4-fold dose AH1 daily and 300 mg Omalizumab monthly; the rate of CSU exacerbation was low: 1 case from the 10 infected (1:10).

In the AH1-only treatment and no treatment groups the rate of CSU exacerbation was 28 cases from the 62 infected (1:2).

Table 4. Inflammation markers in CSU patient during COVID-19 mild or moderate-severe infection.

Parameter	Mild COVID-19 Infection	Moderate/Severe COVID-19 Infection	p
Leucocytes * 10^3/μL	7.54 (±9.7)	3.89 (±2.9)	0.03
CRP* (mg/dl)	2.9 (±6.1)	49.8 (±52.5)	0.01
Troponin* (μg/L)	0.1 (±0.2)	2.3 (±1.8)	0.02
LDH* (U/l)	438 (±195.6)	657 (±185.8)	0.05
UAS7	23 (±5.2)	38 (±3.8)	0.03
VAS	7 (±1.4)	9 (±0.8)	0.02

*Data are expressed as mean±SD.

4. Discussion

The severe acute respiratory syndrome coronavirus 2, known as coronavirus disease 2019 (COVID-19), is associated with high morbidity and mortality mostly in adults, owing to systemic symptoms as well as to the exacerbation of several chronic diseases, CSU included [4].

CSU is a chronic condition that often comes as a fluctuating disease with activity and remissions, negatively impacting the QL of patients. Since more than 50% of the patients have insufficient treatment response to antihistamines, CSU proves to be a debilitating disease and a challenge to affected individuals and clinicians alike [1,2].

The pathological findings associated with COVID-19 seem to result from the release of numerous proinflammatory molecular mediators. Mast cells, key effector immune cells in the secretion of such cytokines and chemokines, are ubiquitous in the body, especially the skin, and are critical for CSU pathology [12]. Both COVID-19 disease and CSU triggered an important inflammatory process in the whole organism.

Mast cells are typically activated by allergic triggers, but they can also be triggered by pathogen-associated molecular patterns via activation of toll-like receptors. In CSU, MCs mediators' release has various triggers: acute infections, fever, NSAIDs, histamine containing food, physical factors, such as pressure, cold, heat, exercise or sun exposure, alcohol, premenstrual or ovulatory phase, to name a few. Thus, symptoms associated to mast cell degranulation may also appear in some viral infections [1]. Mast cells (MCs) play an important role in the immune response because when they recognize viral products, they are activated and synthesize many chemokines and cytokines [13]. In addition, some

cytokines secreted by other cells such as T cells, damaged epithelial and endothelial cells or even by themselves stimulated MC activation as Hermans et al. suggested (2019) [14]. The role of MCs in coronavirus-induced disease have been discussed since the beginning of The Toll-like receptor 3 detection of viral double-stranded ribonucleic acid (RNA), viral sphingosine-1-phosphate (S1P) binding to S1P receptors, and retinoic acid-induced gene I (RIG-I) recognition of uncapped viral RNA) [6], they also express angiotensin converting enzyme 2 (ACE2), now known as the principal receptor for SARS-CoV-2, thus defining a route by which MCs could also become hosts for this virus [9].

It is well known that most SARS-CoV-2 positive patients are asymptomatic or have mild symptoms. However, increasing evidence suggests that many patients who either recovered from or had mild symptoms after COVID-19 exhibit diffuse, multiorgan symptoms months after the infection, known as the adult multisystem inflammatory syndrome [15]. The clinical picture includes symptoms that vary within wide ranges, from malaise, myalgias, chest tightness, brain fog to neuropsychiatric symptoms that are similar to those of patients with mast cell activation syndrome. [16]

The present study aimed to analyze the impact of COVID-19 disease on CSU patients in our care. Our study included a number of 218 patients with CSU found in our care, in the context of the SARS-CoV-2 pandemic. 18% of our CSU patients were in remission of the disease, and those were contacted only for COVID-19 disease evaluation. Majority of our patients were on four-fold AH1 dose (36%) which differs from other studies where sedating AH1 were used and in non-responsive patients Omalizumab was recommended, similarly to AWARE-study [17].

During the pandemic, the use of AH1 in our patients is high, and most of the patients used those drugs, due to drug availability, as well as reduced access to costly biological therapy. However, there was one patient in whom we successfully administered Omalizumab in CSU exacerbated by COVID-19, which rapidly improved the UAS7 and VAS scores. Recent studies bring arguments in favor of the clinical benefit of anti-IgE therapy in individuals with CSU exacerbated by COVID-19 [18–20]. Moreover, in our study, the most severe exacerbations of CSU were particularly seen in patients treated with AH1 alone and in those who had no treatment need for CSU prior to COVID-19 disease (in half of patients in this group CSU was exacerbated by COVID-19 disease). We hypothesize that this is due to an uncontrolled subclinical systemic inflammation in patients treated with AH1 alone, triggered by infection. Although current data prove that AH1 exhibits a mild systemic anti-inflammatory effect [21], this effect is too low in the case of viral infections triggering CSU when short courses of systemic corticosteroids are frequently used, as in our study. Also, systemic corticosteroids are used in moderate/severe COVID-19 disease [19], so that could be the reason for some patients feeling better (9.8%) and some having an unchanged course of CSU (43.1%). Although, analyzing the main treatment of COVID-19 infected patients was not our objective for this study, we may hypothesize that in COVID-19 disease treated with systemic or oral corticosteroids may also improve CSU due to the common anti-inflammatory mechanism, effective in both diseases.

All these data plead for the complex interplay between the SARS-CoV-2 and the mast cells of the host, and probably only severe disease will have an augmentation of the systemic inflammation leading to CSU exacerbation; in our study we observed that 70% of patients had a moderate to severe form of COVID-19 disease.

According to our findings, the pandemic severely impaired CSU patient care and evolution. Only 33% of the patients from the CSU group went through COVID-19 disease of which 71% developed a moderate-severe form of disease. During and after COVID-19 disease, a significant proportion of patients (44%) experienced a more severe clinical profile of CSU. The severity of CSU did not influence the course of COVID-19 disease as other studies showed [10], despite the treatment of CSU. During SARS-CoV-2 infection the patients were in different states of treatment for CSU: no treatment, single dose of AH1, double dose of AH1, four-fold dose of AH1 and omalizumab, cyclosporine and

prednisone as add-on treatment to four-fold AH1, as described in Table 2. Since in our COVID-19 disease patients' group there was none with cyclosporine treatment, which is an important immunosuppressor drug, we cannot make any comments on this situation. In the omalizumab group: this group received four-fold dose AH1 daily and 300 mg omalizumab monthly; the rate of CSU exacerbation was low: 1 case from the 10 infected (1:10). In the AH1-only treatment and no treatment groups the rate of CSU exacerbation was 28 cases from the 62 infected (1:2). We may hypothesize that maybe omalizumab has a better effect on inflammation and a protective role for exacerbation in CSU [22,23]. In our patients with CSU treated with omalizumab, the course of COVID-19 disease was not observed to be more severe, as described in recent study of Passante M. et al. [24].

Moreover, we established a positive correlation between the severity of the infection and that of the CSU clinical presentation, with most bothersome symptoms of urticaria being experienced by moderate to severe COVID-19 CSU patients (47%), which was proved by the UAS7 scores and VAS values. Although VAS is a tool used for assessing the QoL in Allergic Rhinitis corelated with RQLQ (Rhinitis Quality of Life Questionnaire), it is very simple to evaluate the impact of a chronic diseases [25] and it could also be an efficient tool to assess CSU impact on QoL. In our study UAS7 and VAS were positively and statistically significant correlated ($R = 0.138$, $p = 0.01$). But to validate VAS as a tool in CSU a multicenter study with higher number of patients is necessary.

In addition, inflammatory markers were positively correlated with the UAS7 score in moderate to severe SARS-CoV-2 infected group of CSU patients, in compliance with our hypothesis that the level of inflammation triggered by COVID-19 disease has a role in CSU exacerbation. Inversely, the severity of CSU did not influence that of COVID infection. No COVID-19 death was reported in our group of CSU patients. This is consistent with recent studies showing preponderance for CSU exacerbation in the context of COVID-19 disease [10,26]

Our study shows that systemic inflammation evaluated by nonspecific markers is statistically significantly higher in moderate to severe COVID-19 than in mild forms of disease. The markers we used were CRP, troponin, LDH, which are involved in urticaria exacerbation, as shown by other studies [27]. CRP was positively associated with the UAS7 score ($R2 = 0{,}363$; $p = 0{,}001$), so the more inflammation there is, the more impairment of CSU symptoms patients experience. In addition, some data plead for the value of CRP as a biomarker that correlates with the activity of CSU, which is consistent with our result.

Limitations of our study consist in the fact that patients with mild COVID-19 infection had inflammatory markers evaluated only after 14 days of quarantine, while in severe patients that had multiple blood samples determinations from which we took a medium value in this analysis.

The value of the study results from analyzing the impact of COVID-19 disease in a small group of CSU patients from a region not included in European Analysis. It is the first study showing the possibility to use VAS as a QoL measurement in CSU.

5. Conclusions

Mast cells, key effector immune cells in chronic spontaneous urticaria, could also contribute to the pathogenesis of COVID-19 and any post infectious inflammatory syndromes through the release of proinflammatory mediators. Thus, COVID-19 disease can result in the exacerbation of chronic spontaneous urticaria, more likely in moderate to severe forms of infection, which may lead to high health and financial costs associated with CSU. Given these data, we plead in favor of blocking mast cells and the action of their mediators both prophylactically and symptomatically during the COVID-19 pandemic in susceptible patients, CSU included.

Author Contributions: Conceptualization D.D.; methodology, I.A.M. and I.P.; formal analysis, I.C.B., C.T.D. and I.A.M.; investigation, I.A.M. and I.P.; writing—original draft preparation: I.A.M. and I.P.; writing—review and editing, I.A.M. and I.C.B.; supervision D.D.; project administration,

I.A.M., I.P, C.T.D. and D.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive external funding.

Institutional Review Board Statement: The study was approved by "Octavian Fodor" Institute of Gastroenterology and Hepatology Ethics Committee from Cluj-Napoca, no. 364/11.01.2021.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study

Data Availability Statement: Data are available at Allergology Department, Octavian Fodor" Institute of Gastroenterology and Hepatology, Cluj Napoca.

Acknowledgments: We would like to thank you to Cavaşi Adriana from "Octavian Fodor" Institute of Gastroenterology and Hepatology, Cluj Napoca for her contribution regarding the facilitation of this work.

Conflicts of Interest: The authors declare no conflict of interest

References

1. Zuberbier, T.; Aberer, W.; Asero, R.; Bindslev-Jensen, C.; Brzoza, Z.; Canonica, G.W.; Church, M.K.; Ensina, L.F.; Giménez-Arnau, A.; Godse, K.; et al. The EAACI/GA2LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria. *Allergy* **2018**, *73*, 1393–1414.
2. Deleanu, D.; Nedelea, I.; Petricau, C.; Leru, P.; Dumitrascu, D.; Muntean, A. Clinical impact of omalizumab in refractory chronic urticaria: One centre experience. *Exp. Ther. Med.* **2019**, *18*, 5078–5081.
3. Guillén-Aguinaga, S.; Jáuregui Presa, I.; Aguinaga-Ontoso, E.; Guillén-Grima, F.; Ferrer, M. Updosing nonsedating antihistamines in patients with chronic spontaneous urticaria: A systematic review and meta-analysis. *Br. J. Dermatol.* **2016**, *175*, 1153–1165.
4. Theoharides, T.C. Potential association of mast cells with coronavirus disease 2019. *Ann. Allergy, Asthma Immunol.* **2020**, *126*, 217–218, https://doi.org/10.1016/j.anai.2020.11.003.
5. Marshall, J.S.; Portales-Cervantes, L.; Leong, E. Mast Cell Responses to Viruses and Pathogen Products. *Int. J. Mol. Sci.* **2019**, *20*, 4241, https://doi.org/10.3390/ijms20174241.
6. Criado, P.R.; Pagliari, C.; Criado, R.F.J.; Marques, G.F.; Belda, W., Jr. What the physicians should know about mast cells, dendritic cells, urticaria, and omalizumab during COVID-19 or asymptomatic infections due to SARS-CoV-2? *Dermatol. Ther*. 2020, *33*, e14068.
7. Kempuraj, D.; Selvakumar, G.P.; Ahmed, M.E.; Raikwar, S.P.; Thangavel, R.; Khan, A.; Zaheer, S.A.; Iyer, S.S.; Burton, C.; James, D.; et al. COVID-19, Mast Cells, Cytokine Storm, Psychological Stress, and Neuroinflammation. *Neuroscientist* **2020**, *26*, 402–414.
8. Kritas, S.K.; Ronconi, G.; Caraffa, A.; Gallenga, C.E.; Ross, R.; Conti, P. Mast cells contribute to coronavirus-induced inflammation: New anti-inflammatory strategy. *J. Biol. Regul. Homeost. Agents* **2020**, *34*, 9–14.
9. Theoharides, T.C. Covid-19, pulmonary mast cells, cytokine storms, and beneficial actions of luteolin. *Biofactors* **2020**, *46*, 306–308.
10. Kocatürk, E.; Salman, A.; Cherrez-Ojeda, I.; Criado, P.R.; Peter, J.; Comert-Ozer, E.; Abuzakouk, M.; Agondi, R.C.; Al-Ahmad, M.; Al-trichter, S.; et al. The global impact of the COVID-19 pandemic on the management and course of chronic urticaria. *Allergy* **2021**, *76*, 816–830.
11. Paquet, C.; Babes, V.T.; Drucker, J.; Sénémaud, B.; Dobrescu, A. Viral hepatitis in Bucharest. *Bull. World Health Organ.* **1993**, *71*, 781–786.
12. Azkur, A.K.; Akdis, M.; Azkur, D.; Sokolowska, M.; van de Veen, W.; Bruggen, M.C. Immune response to SARS-CoV-2 and mecha-nisms of immunopathological changes in COVID-19. *Allergy* **2020**, *75*, 1564–1581.
13. Afrin, L.B.; Weinstock, L.B.; Molderings, G.J. Covid-19 hyperinflammation and post-Covid-19 illness may be rooted in mast cell activation syndrome. *Int. J. Infect. Dis.* **2020**, *100*, 327–332, https://doi.org/10.1016/j.ijid.2020.09.016.
14. Hermans, M.A.W.; Van Lennep, J.E.R.; Van Daele, P.L.A.; Bot, I. Mast Cells in Cardiovascular Disease: From Bench to Bedside. *Int. J. Mol. Sci.* **2019**, *20*, 3395, https://doi.org/10.3390/ijms20143395.
15. Feldstein, L.R.; Rose, E.B.; Horwitz, S.M. Multisystem inflammatory syndrome in U.S. children and adolescents. *N. Engl. J. Med*. **2020**, *383*, 334–346.
16. Akin, C.; Valent, P.; Metcalfe, D.D. Faculty Opinions recommendation of Mast cell activation syndrome: Proposed diagnostic criteria. *J. Allergy. Clin. Immunol.* **2010**, *126*, 1099–1104.
17. Maurer, M.; Staubach, P.; Raap, U.; Richter-Huhn, G.; Bauer, A.; Ruëff, F.; Jakob, T.; Yazdi, A.S.; Mahler, V.; Wagner, N.; et al. H1-antihistamine-refractory chronic spontaneous ur-ticaria: it's worse than we thought—First results of the multicenter real-life AWARE study. *Clin. Exp. Allergy* **2017**, *47*, 684–692.
18. Ayhan, E.; Öztürk, M.; An, I.; Bekçibaşi, M. COVID-19 infection under omalizumab therapy for chronic spontaneous urticaria: Three cases. *Int. J. Dermatol.* **2020**, *60*, 253–254, https://doi.org/10.1111/ijd.15379.

19. Msc, A.A.; Goldust, M.; Vestita, M. Comment on "Chronic spontaneous urticaria exacerbation in a patient with COVID-19, rapid and excellent response to omalizumab". *Int. J. Dermatol.* **2020**, *59*, 1417–1418, https://doi.org/10.1111/ijd.15215.
20. Criado, P.R.; Criado, R.F.J.; Pincelli, T.P.; Yoshimoto, T.A.; Naufal, G.G.A.; Abdalla, B.M.Z. Chronic spontaneous urticaria exacerbation in a patient with COVID-19, rapid and excellent response to omalizumab. *Int. J. Dermatol.* **2020**, *59*, 1294–1295, https://doi.org/10.1111/ijd.15134.
21. Muntean, I.A.; Bocsan, I.C.; Miron, N.; Buzoianu, A.D.; Deleanu, D. How Could We Influence Systemic Inflammation in Allergic Rhinitis? The Role of H1 Antihistamines. *Oxidative Med. Cell. Longev.* **2018**, *2018*, 1–8, https://doi.org/10.1155/2018/3718437.
22. Chilamakuri, R.; Agarwal, S. COVID-19, Characteristics and Therapeutics. *Cells* **2021**, *10*, 206, https://doi.org/10.3390/cells10020206.
23. Bumbăcea, R.; Deaconu, C.; Berghea, E.C. Management problems in severe chronic inducible urticaria: Two case reports. *Exp. Ther. Med*. **2019**, *18*, 960–963.
24. Passante, M.; Napolitano, M.; Dastoli, S.; Bennardo, L.; Fabbrocini, G.; Nisticò, S.P.; Patruno, C. Safety of Omalizumab Treatment in Patients with Chronic Spontaneous Urticaria and Covid-19. *Dermatol. Ther*. **2021**, in press, doi:10.1111/dth.15111.
25. Bousquet, J.; Bedbrook, A.; Czarlewski, W.; Onorato, G.L.; Arnavielhe, S.; Laune, D.; Mathieu-Dupas, E.; Fonseca, J.; Costa, E.; Lourenço, O.; et al. Guidance to 2018 good practice: ARIA digitally-enabled, integrated, person-centred care for rhinitis and asthma. *Clin. Transl. Allergy* **2019**, *9*, 1–19, https://doi.org/10.1186/s13601-019-0252-0.
26. Erdem, Y.; Ekinci, A.P.; Altunay, I.K.; Sivaz, O.; Inal, S.; Gokalp, M.O.; Pehlivan, G.; Özkaya, E. The impact of COVID-19 pandemic on the management of patients with chronic urticaria: An observational two-center study from Turkey. *Dermatol. Ther.* **2020**, *34*, e14652. https://doi.org/10.1111/dth.14652.
27. Deza, G.; Ricketti, P.A.; Giménez-Arnau, A.M.; Casale, T.B. Emerging Biomarkers and Therapeutic Pipelines for Chronic Sponta-neous Urticaria. *J. Allergy Clin. Immunol. Pract*. **2018**, *6*, 1108–1117.

foods

MDPI

Communication

Alliance with the School Personnel Is Crucial for the Management of Food Allergy and Anaphylaxis in School Children

Dimitris Efthymiou [1], Persefoni Panayi [2], Gavriela Feketea [3,4], Constantinos Pitsios [2], Ioana Adriana Muntean [5,*] and Emilia Vassilopoulou [6]

1 2nd Department of Psychiatry, Division of Neurosciences, Medical School, Aristotle University Thessaloniki, 54124 Thessaloniki, Greece; dimitrisefthy@gmail.com
2 Allergy Outpatient Clinic, Medical School, University of Cyprus, P.O. Box 2537, 1678 Nicosia, Cyprus; panayi.persefoni@ucy.ac.cy (P.P.); pitsios.contantinos@ucy.ac.cy (C.P.)
3 PhD School, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400337 Cluj-Napoca, Romania; g.feketea@karamandaneio.gov.gr
4 Pediatric Allergy Outpatient Clinic, Department of Pediatrics, "Karamandaneio" Children Hospital, 26331 Patras, Greece
5 Department of Allergology and Immunology, Institute of Gastroenterology and Hepatology "O Fodor", "Iuliu Hatieganu" University of Medicine and Pharmacy, 400162 Cluj-Napoca, Romania
6 Department of Nutritional Sciences and Dietetics, International Hellenic University, 57400 Thessaloniki, Greece; vassilopoulouemilia@gmail.com
* Correspondence: Adriana.Muntean@umfcluj.ro or adrianamuntean77@gmail.com

Abstract: Background: School nurses play an important role in coping with food allergy (FA) in schoolchildren, but in schools with no school nurse, the school personnel should be prepared to manage health emergencies. This study aimed to evaluate allergy management competences in primary schools in Cyprus. Methods: The study was conducted September 2016 to May 2017 in 11/13 primary schools, selected by stratified random sampling. Information was collected from a principal/designated teacher using a questionnaire from the EuroPrevall Project, adapted for Cypriot teachers. Results: An average of six children with FA per school was reported in the preceding three years. Protocols for the management of chronic diseases, including allergies, were in place in 8/11 schools. Regarding recognition of FA, 8/11 respondents knew some of the signs and symptoms. In an allergic emergency, 9/11 would call the child's parent/caregiver first and 2/11 emergency medical support. Epinephrine auto-injector (EIA) was reported by 2/11 respondents to be available in the school, but only one reported training in its use. Conclusions: The preparedness of primary schools in Cyprus did not meet safety standards regarding the preparedness of school personnel to cope with an allergic reaction in children with FA, including the use of EIA.

Keywords: food allergy; anaphylaxis; school; school nurses; epinephrine

Citation: Efthymiou, D.; Panayi, P.; Feketea, G.; Pitsios, C.; Muntean, I.A.; Vassilopoulou, E. Alliance with the School Personnel Is Crucial for the Management of Food Allergy and Anaphylaxis in School Children. *Foods* **2021**, *10*, 2083. https://doi.org/10.3390/foods10092083

Academic Editor: Andreas L. Lopata

Received: 18 June 2021
Accepted: 1 September 2021
Published: 3 September 2021

1. Introduction

Food allergy (FA) is a common pediatric emergency and constitutes a significant concern for the personnel of preschool facilities and schools. Its prevalence varies in different parts of the world, and in Europe it affects about 6% of primary school children [1–3]. Even when children with FA are trained to avoid the offending foods, accidental FA reactions may occur, about 18% of these at school [4–6]. Cow's milk is the most common allergen causing reactions in preschool children, and peanuts in schoolchildren, but culprit allergens are related to the eating habits affected by the varied diets consumed in different countries [6–8]. FA and anaphylaxis related policy in schools, and the legislation concerning treatment planning, both vary considerably from country to country, and even from school to school in the same country [9]. Even though strict avoidance of the offending food is the main form of management for FA, an individualized emergency plan, including use of

the epinephrine auto-injector (EAI), is necessary for the treatment of food anaphylaxis [10]. Emergency plans are usually put in place after a confirmed diagnosis of FA by allergists and pediatricians, both of whom can play an important role in collaboration with school personnel [11]. Proactive parents train their children to eat only home-provided food, and they communicate the diagnosis and the emergency plans to the children's school nurses, teachers and/or principals. By minimizing the risk of anaphylaxis at school, a normal life can be offered to the child with FA, with no deprivation of any of the school activities [1].

Unfortunately, the current situation regarding the prevention and management of anaphylaxis by school personnel is not ideal. Even though school nurses can have an important role in promoting the health and well-being of school-aged children, they are not employed in the state schools in most countries [12]. The school personnel are usually not sufficiently trained to recognize allergic reactions and to help children with FA [13]. The willingness of school personnel to cooperate, to be trained and to acquire confidence in handling children suffering from FA, and to cope with the legislation framework, are parameters that need to be cultivated. School personnel are important stakeholders, and they can and should be prepared to manage health emergencies in children [14]. We conducted the present study as part of the first epidemiological study on FA in Cypriot primary school children [2,3], with the purpose of evaluating the allergy management competences in primary schools in Cyprus, and to explore the knowledge and beliefs of schoolteachers about FA.

2. Materials and Methods

The study was conducted between September 2016 and May 2017 in 11 of the 13 primary schools invited to participate, with approximately 300 pupils attending each school. The selection of the schools was based on the diverse geographical location and number of local schools, in both urban and rural areas of Cyprus. Schools from the four larger cities (Nicosia, Limassol, Larnaca and Paphos), and from the provincial areas of both mountain and coastal villages of the island, were included by stratified random sampling, as previously described [2]. This investigation was conducted in parallel with a survey on food-hypersensitivity in the pupils attending the schools under question. The age range of the schoolchildren in the study schools was 5.5–12 years (mean 8.7 $\pm$ 1.7 years) [3]. The school principal of each school, or a schoolteacher designated by the school principal as the most appropriate person to provide the required information on the school preparedness for FA was assigned to participate in the study. The semi-structured questionnaire used in the EU-funded multidisciplinary Integrated Project EuroPrevall, previously translated and adapted for use in Greek populations, was used [15]. The study was approved by the Cyprus National Bioethics Committee (EEBK 2017.01.47) and the Department of Education of the Cyprus Ministry of Education, Culture, Sport and Youth.

The questionnaire (Table 1) is a comprehensive tool, consisting of 42 questions, mainly focusing on the awareness of the school personnel of FA reactions and the plans for the management of medical emergencies in the school where the principal or teacher is employed. Most of the questions are open-ended and they were addressed to the respondent as the authorized member of the school personnel. The respondents were assured of the anonymity of the research data, and the protection of personal details was ensured by the interviewer.

Table 1. Questionnaire on food allergy for teachers.

Questionnaire on Food Allergy for Teachers
1. What is your position in the school?
2. How many people work at this school?
3. Do you know if any schoolchildren have presented a food allergic reaction during the past three years?
4. If the previous answer is YES, how many children?
5. Do you know in what food allergic reactions have occurred?
6. Is there a school protocol regarding children with anamnesis of chronic medical conditions, like allergies?
7. How are food-allergic children identified?
8. Do you keep records of them?
9. If the previous answer is YES, who is authorized to access in such information?
10. Do you update the records?
11. What type of data do the records include?
12. Are you familiar with the symptoms of food allergy?
13. If the previous answer is YES, can you list them?
14. Are teachers and the rest of the personnel informed and trained to recognize food allergens and symptoms? Has an educational program been offered to them?
15. What was the nature of the offered educational program and in how many sessions?
16. What is the school protocol for the treatment of a severe anaphylactic reaction?
17. What would your first action be? (A) contact the parents (B) remain to see how it goes (C) contact child's family doctor (D) call emergency (E) administrate Anapen (since Anapen is the only available EIA in Cyprus, the brand name was used)?
18. If you choose more than one of the above actions, can you place them in a row from the first to the last?
19. Do you have Anapen available at school for severe food allergy reactions?
20. If the previous answer is YES, where do you store it?
21. What is your estimation on the percentage of teachers and non-teaching personnel that are familiar with Anapen's availability?
22. Does the school personnel know how to use Anapen?
23. Is there a specially trained person to administrate Anapen in the case of anaphylaxis?
24. Has the Cyprus Ministry of Education, Culture, Sport and Youth provided Directions on how to treat anaphylaxis or other food-related clinical features?
25. Do you have any instructions on children to avoid sharing cutlery, glasses, home-prepared meals or snacks they buy from the school canteen?
26. If YES, what is your estimation on the percentage of school personnel that know these instructions?
27. Are children trained to follow such instructions?
28. Do the parents know about that school instructions?
29. Do you believe that school rules, forbiting to share things/foods, can negatively affect children's personality and relationship?
30. Is the school personnel informed on food labels and questionable ingredients?
31. If the previous answer is YES, in what percentage?
32. Do children get informed about food labelling as a part of their school education?
33. Do you consider that you focus a lot on that health issue and thus resulting in a group of foods get excluded?
34. Do you know any food allergy awareness organization?
35. What is its name?
36. Does the school get in contact with that organization?
37. If the previous answer is YES, how much useful is?
38. On a scale of 0 to 100, how much do you worry about the incidence with food allergies at school?
39. If it near 100/or lower of 50, why?
40. Do you give more or less sententiousness compared with other health problems?
41. Do you think that this interview will motivate you to reconsider the risk of food allergy reactions?
42. What type of actions would you propose specialists to offer at your school in order to raise awareness on food allergy?

The data were analyzed with descriptive statistics using SPSS 22.

3. Results

The school principal was the designated interviewee in 8/11 schools, and a teacher was appointed to be interviewed as school representative in 3/11 schools. Each of the participating schools had an average of 25 teachers in the personnel.

The majority (10/11) of the participating schools (90.9%) had been informed by parents, in the preceding three years, that one or more pupils had a medical history of FA. An

average of six children with FA per school was estimated. Specifically, 68 cases of FA had been reported, but only 10 of these were reported during the last 3 years. The foods most commonly listed by the parents as causing the allergic reaction were (Figure 1): cereals including wheat, barley, rye, maize, rice and oats (43.48%), fruits including orange, strawberry, cherry and peach (26.09%), egg (8.7%), chocolate (4.35%), tree nuts (4.35%) and vegetables (4.35%). Protocols for the management of chronic diseases, such as allergies, were reported to be in place in 8/11 schools, and to be lacking in 3/11.

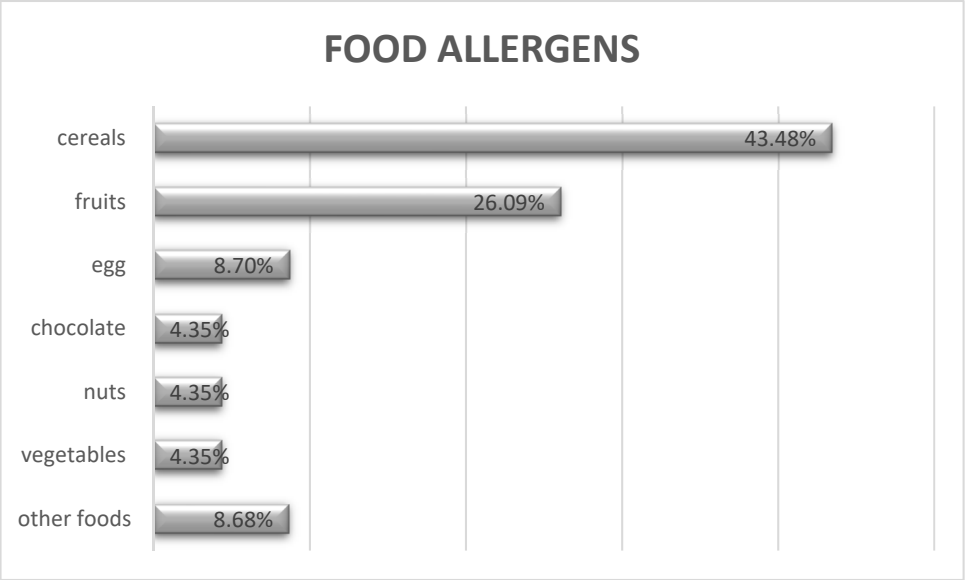

Figure 1. Food allergic reactions per food allergen, according to the school medical card, as reported by parents/carers (N = 68).

Regarding the ways used to identify the children with FA, 8/11 respondents replied that they were informed through the standard mandatory school child's medical card, which is completed yearly by the parents/caregivers [16]. The additional use of medical identifications (IDs), such as medical alert bracelets worn by children, was reported in one school. A list of the children with FA, updated every year, recording the medical history and the culprit allergens, was reported to be kept in 2/11 schools. All members of the school personnel have access to this list.

Regarding recognition of the signs and symptoms of FA, 8/11 respondents stated that they know some of the signs of FA. They mentioned seven types of signs and symptoms: wheals (4/11), itching (2/11), airway obstruction (2/11), wheezing (2/11), dyspnoea (1/11), abdominal pain (2/11) and oedema (1/11), but 3/11 could not recall any symptom. The personnel had received training relevant to allergies and allergic symptoms, and were prepared to manage an allergic reaction in a child, in only 2/11 schools (Figure 2).

In these two schools, relevant educational seminars had been provided for the whole school personnel, twice in the preceding three years, by an allergist and a dietitian.

The respondents agreed that an emergency protocol, determined by the Cyprus Ministry of Health, for the provision of first aid by teachers, including the management of severe allergic reactions in the school environment [16] is vital. As a first step in the case of an allergic emergency, 9/11 respondents would first call the child's parent/caregiver and 2/11 would first call for emergency medical support. EIA (Anapen) was reported to be available in the school by 2/11 respondents, but they doubted whether all the school staff knew of the availability of Anapen in the school, and only one stated that any of the personnel was trained to use EIA.

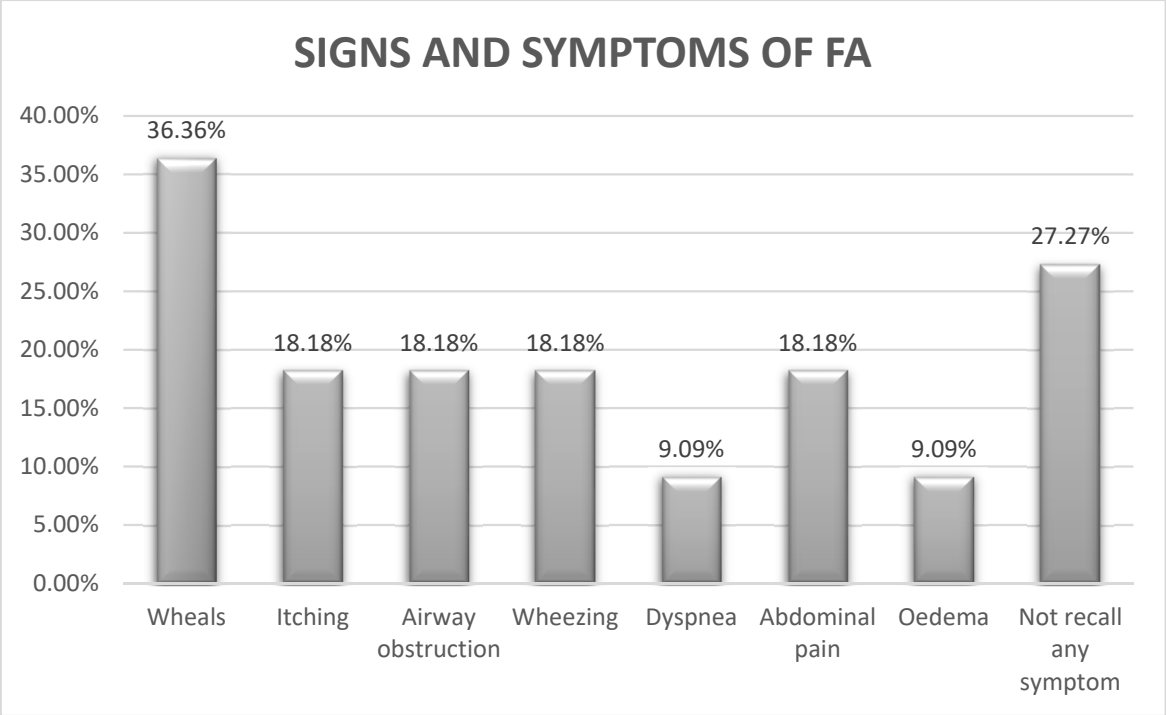

Figure 2. Different types of signs and symptoms of FA stated by teachers during an allergic reaction.

Directions issued by the Ministry of Health on how to treat FA and anaphylaxis [16], were reported in 8/11 schools, but such directions were absent from, or were not used in 3/11 schools. Only 1/11 school enforced rules restricting the sharing of cutlery, glass and foods, in order to prevent accidental allergic reactions. All the respondents reported that most members of the personnel ignore such rules and that, therefore, there is a risk of accidental exposure to food allergens. All the respondents, however, agreed that such a protocol would not affect the development of the personality and generosity of children but, on the contrary, it would raise their awareness of on how severe an allergic reaction can be.

In 3/11 schools, regular personnel training is provided by an allergist and a dietitian on food labeling, and the pupils also receive this type of training, integrated into their school program. In these three schools it was also reported that the rules of allergen labeling are followed strictly in their cafeterias, and that certain foods had been excluded, because of the absence of appropriate labeling, with the ingredients not clearly indicated according to the relevant legislation rules (Regulation EU No 1169/2011).

Of the respondents, 4/11 were greatly concerned about the possibility of food-induced allergic reactions at school, scoring > 70 on a 0–100 scale. The main reasons for their concern were their unfamiliarity with the symptoms and severity of food anaphylaxis, and their lack of confidence in managing such a reaction in the school environment. Almost all stated the need of an educational program for school staff and parents, aimed at raising awareness about FA reactions and competence in coping with them, and 5/11 considered the study interview a motivation to learn more about this medical issue. The different skills and knowledge of teachers regarding FA children is depicted in Figure 3.

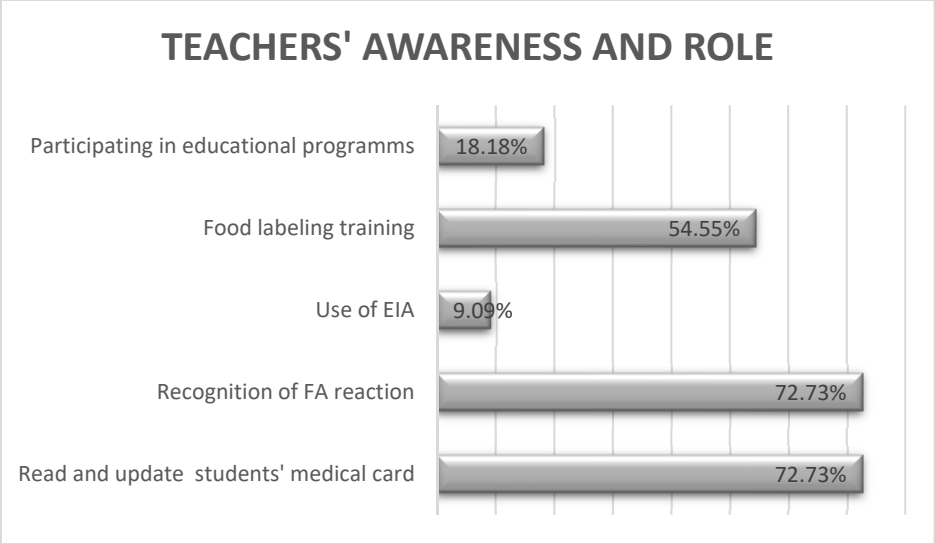

Figure 3. Percent of teachers that have different skills and knowledge about FA children in the school environment.

4. Discussion

Management of FA at school constitutes a challenge to the personnel. It includes the prompt recognition of the signs and symptoms of an allergic reaction, and rapid application of the appropriate treatment, following a specified emergency plan and using the prescribed emergency set [17]. In one relevant study performed in six schools in Houston, 59% of teachers failed to identify an allergic reaction or to follow an emergency plan [5]. The prompt recognition of signs of anaphylaxis and the implementation of the prescribed management plans are crucial. Most of the respondents in the participating Cypriot primary schools reported knowledge of the symptoms of food allergies. In an Icelandic study on children in preschool, only 55% of preschool facilities with children with severe allergy reported that all of their staff members knew the symptoms related to anaphylaxis [18].

The literature supports the high incidence (16–18%) of FA reactions in the school environment [6,19]. The preparedness of school personnel for intervention, including the use of EIA, is indispensable in the case of an anaphylactic reaction in a child at school. Despite the guidelines provided for the provision of first aid, including for anaphylaxis, by teachers [16], a gap in the awareness of the use of EIA in Cypriot primary schools emerged from our study, as has been reported in other studies on the awareness of relevant school procedures on the part of the school personnel and parents [20].

The Cypriot legislative framework states that providing first aid to children with chronic diseases that may present an acute exacerbation, is a moral duty of teaching personnel and a principle of school rules. Teachers should be well informed on the health problems of pupils and instructed on the steps to be followed in relevant health emergencies. Medication may be administered by a trained teacher on the physician's instruction or with the written consent of the parent (Ministry of Health 21.1.07.2). In spite of the legislation, we noted lack of access to appropriate medical treatment (i.e., EIA) and/or education in its administration, and absence of standard protocols on how to react in the case of an emergency. The respondents in our survey reported that in the event of an allergic reaction they would contact the parents first, rather than the medical emergency services.

These gaps in the implementation of guidelines at school are common. Parents were described as the primary drivers of FA guidelines implementation in an Australian study [21]. In a survey in Japan, school staff members reported attending courses about FA, but on being questioned before and after the course, only 34.5% of school teachers and principals knew the indications for using adrenaline in children [22]. An Italian study reported that while the majority of school teachers and principals (65.4%) knew that

"adrenaline" is the best medication for anaphylaxis, only 34.5% knew the indications for using it [23].

Teachers need to know that, in a child with FA, a reaction is probable with even minimal exposure, so that sharing food with a child who has FA [17], or letting a child who is allergic to egg use egg-containing finger paints, can be extremely dangerous [24]. Labelling of processed food is crucial, since a hidden or misinterpreted ingredient can trigger an anaphylactic reaction, which, in combination with the deficiency of knowledge of EIA of the school personnel can be life-threatening [25]. The majority of the respondents in our study reported lack of a school policy on avoiding the sharing of cutlery, glasses and foods, but also lack of training on food labelling.

Educational seminars for school personnel on FA, including first aid courses, part of which is the treatment of allergic emergencies, are of utmost importance [15]. Such intervention increases the competence and the confidence of the personnel and can lead to the timely treatment of allergic reactions in schoolchildren with FA, including the appropriate use of EIA.

The authors acknowledge the low number of participating schools, but the selection was random and stratified according to the different areas of the island. Despite this limitation, our results highlight, for the first time in Cyprus, the lack of compliance with legislation, and the inconsistencies among the different schools, the absence of standard protocols in schools for management of anaphylactic episodes, and inadequacy of appropriate training of the personnel, particularly in EIA use, but also regarding preventive measures for FA, such as sharing food and food labelling.

5. Conclusions

According to this study, the preparedness of primary school personnel in Cyprus did not meet safety standards regarding food-induced allergic reactions in schoolchildren with FA. The most frequently reported food allergens in schoolchildren were cereals, fruits, egg, chocolate and nuts, and most commonly recognized symptoms were wheals, itching, airway obstruction and wheezing. A gap in the knowledge about and the use of EIA in schools was revealed. There is a need for a written medical protocol to improve the management of allergic reactions by the school personnel, and training of primary school personnel in the recognition and management of FA reactions is recommended.

Author Contributions: Conceptualization, E.V.; methodology, D.E. and E.V.; formal analysis, P.P., G.F., C.P., D.E., I.A.M. and E.V.; investigation, D.E. and E.V.; writing—original draft preparation: E.V., P.P. and D.E.; writing—review and editing, all; supervision, E.V. and D.E.; project administration, E.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was approved by Cyprus National Bioethics Committee (EEBK 2017.01.47) and the Dept of Education of Cyprus Ministry of Education, Culture, Sport and Youth.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available upon request to E.Vassilopoulou.

Acknowledgments: Authors wish to thank Christodoulos Nikolaou for his contribution regarding the existing legal frame on first aid provision at Cyprus schools.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Muraro, A.; Clark, A.; Beyer, K.; Borrego, L.M.; Borres, M.; Lødrup Carlsen, K.C.; Carrer, P.; Mazon, A.; Rancè, F.; Valovirta, E.; et al. The management of the allergic child at school: EAACI/GA2LEN Task Force on the allergic child at school. *Allergy* **2010**, *65*, 681–689. [CrossRef]
2. Vassilopoulou, E.; Christoforou, C.; Andreou, E.; Heraclides, A. Effects of food allergy on the dietary habits and intake of primary schools' Cypriot children. *Eur. Ann. Allergy Clin. Immunol.* **2017**, *49*, 181–185. [CrossRef]

3. Vassilopoulou, E.; Vardaka, E.; Efthymiou, D.; Pitsios, C. Early life triggers for food allergy that in turn impacts dietary habits in childhood. *Allergol. Immunopathol.* **2021**, *49*, 146–152. [CrossRef] [PubMed]
4. Gonzalez-Mancebo, E.; Gandolfo-Cano, M.M.; Trujillo-Trujillo, M.J.; Mohedano-Vicente, E.; Calso, A.; Juarez, R.; Melendez, A.; Morales, P.; Pajuelo, F. Analysis of the effectiveness of training school personnel in the management of food allergy and anaphylaxis. *Allergol. Immunopathol.* **2019**, *47*, 60–63. [CrossRef]
5. Canon, N.; Gharfeh, M.; Guffey, D.; Anvari, S.; Davis, C.M. Role of Food Allergy Education: Measuring Teacher Knowledge, Attitudes, and Beliefs. *Allergy Rhinol.* **2019**, *10*, 2152656719856324. [CrossRef]
6. Nowak-Wegrzyn, A.; Conover-Walker, M.K.; Wood, R.A. Food-allergic reactions in schools and preschools. *Arch. Pediatr. Adolesc. Med.* **2001**, *155*, 790–795. [CrossRef] [PubMed]
7. Loh, W.; Tang, M.L.K. The Epidemiology of Food Allergy in the Global Context. *Int. J. Environ. Res. Public Health* **2018**, *15*, 2043. [CrossRef]
8. Perkin, M.R.; Logan, K.; Tseng, A.; Raji, B.; Ayis, S.; Peacock, J.; Brough, H.; Marrs, T.; Radulovic, S.; Craven, J.; et al. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. *N. Engl. J. Med.* **2016**, *374*, 1733–1743. [CrossRef] [PubMed]
9. Eigenmann, P.A.; Ebisawa, M.; Greenhawt, M.; Hourihane, J.O.; Perry, T.T.; Remington, B.C.; Wood, R.A. Addressing risk management difficulties in children with food allergies. *Pediatr. Allergy Immunol.* **2021**, *32*, 658–666. [CrossRef]
10. Muraro, A.; Agache, I.; Clark, A.; Sheikh, A.; Roberts, G.; Akdis, C.A.; Borrego, L.M.; Higgs, J.; Hourihane, J.O.; Jorgensen, P.; et al. EAACI food allergy and anaphylaxis guidelines: Managing patients with food allergy in the community. *Allergy* **2014**, *69*, 1046–1057. [CrossRef]
11. Polloni, L.; Baldi, I.; Lazzarotto, F.; Bonaguro, R.; Toniolo, A.; Celegato, N.; Gregori, D.; Muraro, A. School personnel's self-efficacy in managing food allergy and anaphylaxis. *Pediatr. Allergy Immunol.* **2016**, *27*, 356–360. [CrossRef]
12. Council on School Health. Role of the School Nurse in Providing School Health Services. *Pediatrics* **2016**, *137*, e20160852. [CrossRef]
13. Kilger, M.; Range, U.; Vogelberg, C. Acute and preventive management of anaphylaxis in German primary school and kindergarten children. *BMC Pediatr.* **2015**, *15*, 159. [CrossRef] [PubMed]
14. White, M.V.; Hogue, S.L.; Bennett, M.E.; Goss, D.; Millar, K.; Hollis, K.; Siegel, P.H.; Wolf, R.A.; Wooddell, M.J.; Silvia, S. EpiPen4Schools pilot survey: Occurrence of anaphylaxis, triggers, and epinephrine administration in a U.S. school setting. *Allergy Asthma Proc.* **2015**, *36*, 306–312. [CrossRef] [PubMed]
15. Le, T.M.; Kummeling, I.; Dixon, D.; Barreales Tolosa, L.; Ballmer-Weber, B.; Clausen, M.; Gowland, M.H.; Majkowska-Wojciechowska, B.; Mustakov, T.; Papadopoulos, N.G.; et al. Low preparedness for food allergy as perceived by school staff: A EuroPrevall survey across Europe. *J. Allergy Clin. Immunol. Pract.* **2014**, *2*, 480–482.e1. [CrossRef]
16. Cyprus Ministry of Education, Culture, Sports and Youth. Available online: http://www.moec.gov.cy/dde/exp_dimotiki_engrafes_metengrafes.html#simeio5 (accessed on 11 June 2021).
17. Portnoy, J.M.; Shroba, J. Managing Food Allergies in Schools. *Curr. Allergy Asthma Rep.* **2014**, *14*, 467. [CrossRef]
18. Thrastardottir, A.R.; Thordardottir, F.R.; Torfadottir, J. Protocols Related to Food Allergies and Intolerances in Preschools in Reykjavik, Iceland. *Laeknabladid* **2018**, *104*, 11–17. [CrossRef]
19. Sicherer, S.H.; Furlong, T.J.; DeSimone, J.; Sampson, H.A. The US Peanut and Tree Nut Allergy Registry: Characteristics of reactions in schools and day care. *J. Pediatr.* **2001**, *138*, 560–565. [CrossRef]
20. Cicutto, L.; Julien, B.; Li, N.Y.; Nguyen-Luu, N.U.; Butler, J.; Clarke, A.; Elliott, S.J.; Harada, L.; McGhan, S.; Stark, D.; et al. Comparing school environments with and without legislation for the prevention and management of anaphylaxis. *Allergy* **2012**, *67*, 131–137. [CrossRef]
21. Lawlis, T.; Bakonyi, S.; Williams, L.T. Food allergy in schools: The importance of government involvement. *Nutr. Diet* **2017**, *74*, 82–87. [CrossRef]
22. Ebisawa, M. Management of food allergy in Japan "food allergy management guideline 2008 (revision from 2005)" and "guidelines for the treatment of allergic diseases in schools". *Allergol. Int.* **2009**, *58*, 475–483. [CrossRef] [PubMed]
23. Polloni, L.; Lazzarotto, F.; Toniolo, A.; Ducolin, G.; Muraro, A. What do school personnel know, think and feel about food allergies? *Clin. Transl. Allergy* **2013**, *3*, 39. [CrossRef]
24. Leo, H.L.; Clark, N.M. Addressing food allergy issues within child care centers. *Curr. Allergy Asthma Rep.* **2012**, *12*, 304–310. [CrossRef] [PubMed]
25. Devetak, I.; Devetak, S.P.; Vesel, T. Future Teachers' Attitudes and Knowledge Regarding the Management of the Potential Students' Life-threatening Allergic Reactions in Slovenian Schools. *Slov. J. Public Health Zdr. Varst.* **2018**, *57*, 124–132. [CrossRef] [PubMed]

Journal of
Clinical Medicine

MDPI

Article

Are Markers of Allergic Inflammation in Grass Pollen Allergy Influenced by H1 Antihistamines?

Ioana Corina Bocsan [1], Ioana Adriana Muntean [2,3,*], Nicolae Miron [4], Irena Pintea [2,3], Carmen Teodora Dobrican [2,3], Corina Ureche [5,*], Anca Dana Buzoianu [1] and Diana Deleanu [2,3]

1 Department of Pharmacology, Toxicology and Clinical Pharmacology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400012 Cluj-Napoca, Romania; bocsan.corina@umfcluj.ro (I.C.B.); abuzoianu@umfcluj.ro (A.D.B.)
2 Department of Allergology and Immunology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400012 Cluj-Napoca, Romania; irena.nedelea@umfcluj.ro (I.P.); dobrican.carmen@umfcluj.ro (C.T.D.); diana.deleanu@umfcluj.ro (D.D.)
3 Allergology Department, "Octavian Fodor" Institute of Gastroenterology and Hepatology, 400162 Cluj-Napoca, Romania
4 Department of Clinical Immunology, Sahlgrenska University Hospital, 41346 Gothenburg, Sweden; nicolae.miron@vgregion.se
5 First Internal Medical Department, "George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology, 540136 Târgu Mureș, Romania
* Correspondence: Adriana.Muntean@umfcluj.ro (I.A.M.); corina.ureche@umfst.ro (C.U.)

Abstract: Soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular adhesion molecule-1 (VCAM-1) play important roles in allergic rhinitis (AR). Treatment with H1 antihistamines improves AR symptoms and in vitro reduces the levels of adhesion molecules. The aim of the study was to evaluate serum levels of ICAM-1 and VCAM-1 in patients with AR to grass pollen and their response to different H1 antihistamines. Material and methods: A total of 50 patients with grass pollen AR were clinically and biologically evaluated. ICAM-1 and VCAM-1 serum levels were evaluated during pollen season before and after treatment with levocetirizine and desloratadine through the ELISA method. Results: ICAM-1, VCAM-1, eosinophils, and total IgE were elevated in patients with AR, compared with healthy subjects. Both antihistamines improved specific symptoms of AR and increased patients' quality of life during pollen season after one month of treatment. H1 antihistamines reduced VCAM-1, ICAM-1, and total IgE after one-month treatment but not significantly. Patients with increased baseline values tend to remain with increased values after one-month AH1 treatment. Conclusions: ICAM-1 and sVCAM-1 levels are higher in patients with grass pollen-induced AR than healthy controls during pollen exposure. Their serum levels tend to remain at high values during pollen season despite antihistaminic therapy.

Keywords: soluble adhesion molecules; pollen allergy; eosinophils; antihistamines

Citation: Bocsan, I.C.; Muntean, I.A.; Miron, N.; Pintea, I.; Dobrican, C.T.; Ureche, C.; Buzoianu, A.D.; Deleanu, D. Are Markers of Allergic Inflammation in Grass Pollen Allergy Influenced by H1 Antihistamines? *J. Clin. Med.* **2022**, *11*, 113. https://doi.org/10.3390/jcm11010113

Academic Editor: Anna Maria Zicari

Received: 6 December 2021
Accepted: 24 December 2021
Published: 26 December 2021

1. Introduction

Allergic rhinitis is a common disease affecting 20–30% of the general population in industrialized countries. In central Europe, grass pollen is one of the major allergens during late spring and summer, responsible for symptoms of allergic rhinitis accompanied or not by allergic conjunctivitis and/or asthma [1–3]. Allergic rhinitis is characterized by an IgE-mediated immune response due to exposure to pollen and several cells and mediators could be identified. After allergen exposure, an early phase of allergic inflammation might occur, releasing immediately specific mediators from mast cells, including histamine. These mediators generate a specific inflammatory response, activating cellular adhesion molecules (CAMs) that are involved in eosinophil's migration in the nasal mucosa [4–7]. Vascular cell adhesion molecule 1 (VCAM-1) and intercellular cell adhesion molecule 1 (ICAM-1), which belong to the immunoglobulin superfamily, are expressed on endothelial cells. Adhesion

molecules are an important part of the inflammatory network in allergic diseases, involved in persistent inflammation in the upper and lower airways [1–8]. IgE and eosinophils increase during pollen season, because of continuous allergen exposure [9–11].

H1 antihistamines (AH1) are the most frequent pharmacological agents used in both intermittent and persistent forms of allergic rhinitis, although more than 50% of the patients did not respond to monotherapy with AH1 [1]. Their anti-allergic effect is related to the blockade of H1 receptors. Research from the last two decades found that the second-generation H1 antihistamines have also an anti-inflammatory effect, decreasing the number of inflammatory cells accumulated in the nasal mucosa and the expression of CAMs [12–19].

The aim of the study was to evaluate the effect of H1 antihistamines during natural exposure to grass pollen and their effects on clinical symptoms, biologic markers, and CAMs. The secondary objective was to identify if there are any differences between 2 commonly used AH1, levocetirizine, and desloratadine.

2. Materials and Methods

2.1. Study Design

In total, 50 patients with grass pollen allergic rhinitis (median age 27.3 (23–37) years and sex ratio M:F = 1:1) that were evaluated in the Allergology Department, were included in the present study. In addition, 30 healthy volunteers were also included in the control group. The first evaluation was carried out in the middle of grass pollen season in Romania, from 15 May to 15 June (2012–2015). The study protocol was approved by the University of Medicine and Pharmacy "Iuliu Haţieganu" Ethics Committee (Approval No. 535/2 September 2011), and all patients signed the informed consent before enrollment. The study protocol and clinical evaluation were performed according to the initial RCT [19], but only allergic patients to grass pollen were included in the present analysis. The intranasal eosinophils were used as a local marker of inflammation. The exclusion criteria were nasal polyps, acute and chronic upper respiratory infections, other systemic inflammations, autoimmune diseases, cardiovascular diseases, administration of intranasal, inhaled, or systemic corticosteroids or H1 antihistamines within the previous 30 days, and administration of immunosuppressive agents.

2.2. Patients' Clinical Evaluation

Diagnosis of AR was conducted according to the ARIA guideline [1], based on history, typically symptoms at pollen exposure, and skin prick test (SPT). From clinical history, the following demographic data were recorded: age, gender, and living area (rural/urban), symptoms (presence and severity). The severity of AR was assessed using the total symptoms score (TSS) and visual analog scale (VAS). Total symptoms score included rhinorrhea, nasal congestion, sneezing, nasal, and ocular itching, the severity of which symptom was assessed on a scale from 0 (absent) to 3 (severe), retrospectively, for 12 h before presentation. TSS was calculated by adding the score for each symptom. It was considered mild rhinitis if TSS was below 6, while a TSS $\geq$ 6 represents a moderate-to-severe form of the disease. VAS scale was also evaluated in order to assess the quality of life (QL), VAS value over 6 points meaning patients with a moderate-to-severe form of AR.

After the baseline evaluation, patients were randomly divided into two groups using adaptive biased-coin randomization. In total, 26 patients formed the first groups, and they were treated with levocetirizine 5 mg/day, while the second group of 24 patients received desloratadine 5 mg/day. The treatment was recommended for 4 weeks, until the end of the pollen season in Romania. The second clinical and biological evaluation was performed at the end of the four weeks of treatment.

The presence of asthma symptoms was assessed after 1.5 years, as previously described [19].

2.3. Skin Prick Tests (SPTs)

Diagnosis of allergy was established through skin prick test, according to international guidelines [20]. The allergen panel included international recommendations and particularities of exposure to allergens in Romania: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, grass pollens mix (*Agrostis stolonifera, Anthoxanthum odoratum, Dactylis glomerata, Lolium perenne, Arrhenatherum elatius, Festuca rubra, Poapratensis, Secale cereale, Holcus lanatus, Phleum pretense*), cereals pollen, birch pollen, hazel pollen, *Artemisia vulgaris,* and *Ambrosia elatior,* cat and dog dander, and *Alternaria Alternata*. Standardized allergen extracts (Hal Allergy, The Netherlands) were used. SPTs were performed at the beginning of the study.

2.4. Biological Evaluation

All the biological parameters were determined before and after 1 month of treatment with H1 antihistamines. The plasmatic level of total IgE was analyzed using the electrochemiluminescence immunoassay method (ECLIA). The obtained values were expressed as UI/mL. A value below <100 UI/mL was considered normal. The intranasal and plasmatic eosinophils (Eos) were manually counted from a slide using May–Grunwald Giemsa staining, and their value was expressed as %. We considered a normal value less than 10% in nasal secretion and less than 4% in the blood.

The serum levels of ICAM-1 and VCAM-1 were determined by the ELISA technique (Quantikinine R&D system, USA). Five ml of blood sample was collected in a tube without anticoagulant and centrifuged within the first hour, followed by serum separation. All the determinations were carried out according to the manufacturers' instructions.

All blood samples were taken on fasting between 8 a.m. and 11 a.m.

2.5. Statistical Analysis

The statistical analysis was performed using SPSS version 21 (Chicago, IL, USA). Data were labeled as nominal, expressed as a percentage, and used continuous variables. The normal distribution for continuous variables was achieved using Kolmogorov–Smirnov test. The influence of different parameters on the evolution of CAM after 1 month was investigated using the ANOVA test for repeating measurements. The Spearman coefficient of correlation was calculated to highlight differences between continuous variables. The level of statistical significance was set at $p < 0.05$.

3. Results

Patients' demographic data are presented in Table 1. There were no statistically significant differences between the two treatment groups.

Table 1. Demographic data.

Parameter		Desloratadine (n = 24)	Levocetirizine (n = 26)	p
Age *		28.05 ± 6.32	29.89 ± 12.17	0.031
Sex ^	male	50% (12)	46.1% (12)	0.263
	female	50% (12)	63.9% (14)	
Living area ^	urban	87.5% (21)	88.5% (23)	0.770
	rural	12.5% (3)	11.5% (3)	
Onset of AR (months) °		24 (6–60)	36 (7.5–68)	0.532
Sensitization ^ to grass pollen	monosensitization	29.2% (7)	23.1% (6)	0.258
	polysensitization	70.8% (17)	76.9% (20)	
Severity ^	mild	25% (6)	23.1% (6)	0.465
	moderate-to-severe	75% (18)	76.9% (20)	
Personal history of allergy		46% (11)	73% (19)	0.5
Familial history of allergy		29.2% (7)	42.3% (11)	0.774

* Data were expressed as mean and standard deviation. ^ Data were expressed as percentage. ° Data were expressed as median and percentiles. Significance $p < 0.05$.

In patients with AR to grass pollen plasmatic levels of ICAM-1 and VCAM-1 were significantly increased compared with the control group during pollen season ($p < 0.001$, respectively $p < 0.001$). Additionally, total IgE, blood, and intranasal eosinophils were increased at baseline towards healthy volunteers (Table 2).

Table 2. Nasal and blood eosinophils, plasmatic values of total IgE and adhesion molecules in healthy volunteers and patients with AR.

Parameter	Healthy Volunteers (n = 30)	Patients with AR (n = 50) Baseline	p
Total IgE (UI/l)	<100	255.8 (56.3–599)	<0.001
Nasal Eo (%)	<10	24 (10–46)	<0.001
Blood Eo (%)	<4	8 (2–15)	0.003
ICAM-1 (ng/mL)	111.21 (100–206.3)	235.11 (209.1–276.6)	<0.001
VCAM-1 (ng/mL)	557 (249–891)	996.19 (832.8–1098.2)	<0.001

Significance $p < 0.05$.

Genetic predisposition for allergic diseases and asthma was evaluated using the accurate family history of the patients. Overall, 19 patients reported a positive familial history of allergy (asthma, allergic rhinitis, or atopic dermatitis). Patients with a family history of asthma had higher values of inflammatory markers, such as blood eosinophils (median value: 5.5% vs. 9%) than patients with no asthma history, but the group of patients was too small to calculate a statistical significance.

Both investigated H1 antihistamines significantly improved all symptoms of AR and increased patients' quality of life during pollen season after one month of treatment. TSS significantly decreased after treatment (median 8.5 (5–12) vs. median 4.2 (0–6), $p = 0.01$), with no differences between levocetirizine and desloratadine ($p = 0.571$) (Table 3). A similar reduction was noticed for VAS. The one-month evaluation revealed a reduction in total IgE level ($p = 0.08$), but this was not statistically significant. The reduction in total IgE was not influenced by the type of treatment, patients' age, sex, living area, or duration of AR ($p > 0.05$).

Table 3. Nasal and blood eosinophils, plasmatic values of total IgE and adhesion molecules initially and after 1 month of AH1 treatment in patients with AR.

Parameter	Patients with AR Baseline (n = 50)	Patients with AR after 1 Month-AH1 Treatment (n = 50)	p
Total IgE (UI/l)	255.8 (56.3–599)	198.7 (49.5–482)	0.08
Nasal Eo (%)	24 (10–46)	18 (10–29)	0.03
Blood Eo (%)	8 (2–15)	5.5 (1–7)	0.03
ICAM-1 (ng/mL)	235.11 (209.1–276.6)	195.42 (124.45–239.89)	0.06
VCAM-1 (ng/mL)	996.19 (832.8–1098.2)	783.19 (689.7–1005.3)	0.09
TSS (score)	8.5 (5–12)	4.2 (0–6)	0.001
VAS (cm)	8.9 (5–10)	3.8 (0–7)	0.001

Significance $p < 0.05$.

The same pattern was also observed after a four-week treatment with H1 antihistamines for plasmatic levels of ICAM-1 ($p = 0.06$) and VCAM-1 ($p = 0.09$), compared with basal values, without reaching the level of statistical significance. The reduction in ICAM-1 and VCAM-1 was observed in 42% and 40%, respectively, while in 14 patients (28%), their levels increased despite the treatment.

There was no difference between levocetirizine and desloratadine in the reduction in CAM plasmatic levels. We observed a significant reduction in VCAM-1 and ICAM-1 levels in patients with moderate-to-severe forms, compared with patients with mild rhinitis ($p = 0.03$, $p = 0.01$, respectively). The reduction in CAM levels was not influenced by patients' age, sex, and type of sensitization. Patients with increased values at baseline tend to remain with increased values after 1-month AH1 treatment ($p = 0.01$).

Intranasal and blood Eo, were significantly reduced after 1-month treatment with AH1 ($p = 0.03$). The reduction in Eo was not influenced by the type of treatment, patients' age, sex, environment, or duration of AR ($p > 0.05$).

After 1.5 years, 10 patients (20%) had asthma symptoms. The evolution of ICAM-1 and VCAM-1 was also retrospectively assessed in these patients. Nine patients had an increased or stationary evolution of ICAM-1 and VCAM-1 during treatment with H1 antihistamines.

4. Discussion

The present study showed a mild anti-inflammatory role of second-generation H1 antihistamines as monotherapy for four weeks of treatment, demonstrated by a reduction in intranasal eosinophils but not on the CAM plasmatic levels in patients with AR to grass pollen during the pollen season. Both desloratadine and levocetirizine improved nasal symptoms and patients' quality of life if they were administered during pollen season in patients with AR. Both TTS and VAS significantly decreased after treatment, with no differences between the investigated drugs.

AR to grass pollen is characterized by the presence of inflammation in the nasal mucosa during the pollen season under allergen exposure. Allergen exposure induces mast cells degranulation and the release of mediators such as histamine, which are responsible for producing the characteristic symptoms of AR (sneezing, nasal and ocular itching, rhinorrhea, and nasal obstruction) [21,22]. In addition to histamine, other mediators are released from mast cells, such as interleukins 4 and 5 (IL-4, IL-5), leukotriene D4, and E4 (LTD4 and LTE4) [23,24]. Those mediators stimulate infiltration of the nasal mucosa with inflammatory cells, mainly eosinophils, which migrate via CAM in the nasal mucosa [25]. The pattern of chronic allergic inflammatory response is represented by eosinophils infiltration in nasal mucosa [17,26]. Commonly, patients with grass pollen allergy have also other types of sensitization (other types of pollens, house dust mites, molds, animal dander), which may induce also an IgE triggered inflammation outside of grass pollen season, maintaining a minimal specific inflammation in the airway. These cells continue to produce other inflammatory mediators, such as cytokines, chemokines leading to persistent symptoms and tissue damages with structural changes [27–29]. Thus, rhinitis persistence and aggravation become more dependent on mediators, which promote infiltration of cells, such as eosinophils and TH2 lymphocytes [21]. AR to grass pollen is a risk factor for asthma development and may appear before or after asthma onset, or during thunderstorm-related asthma [1,2,30–32]. In the present study, 20% of the patients developed asthma symptoms within the next 1.5 year follow-up.

Adhesion molecules such as ICAM-1 and VCAM-1 are surface molecules with immunoglobulin-like structure, involved in intercellular adhesion through interaction with the B2 integrin LFA-1. Their importance resides due to cell-to-cell interaction and eosinophil migration in the nasal mucosa in pollen allergic patients [33,34]. In our study, we evaluated the soluble CAM in patients with AR to grass pollen. In grass pollen allergic patients, the inflammation assessed by CAM is higher than in healthy individuals. After the 4-week treatment, H1 antihistamines decreased the plasmatic levels of ICAM-1 compared to basal values, but the reduction was not significant. Similar results were noticed in patients with perennial allergen exposure to house dust mites [35]. The reduction was not significant probably because traces of pollen remained in the atmosphere even if the 4-week evaluation was performed at the end of the pollen season. Grass pollen is the most common pollen encountered in the temperate area where the center is located. The most abundant allergenic grass pollen in many temperate regions originates from tall grasses, such as *Phleum pratense*, *Dactylis glomerata*, and *Arrhenatherum elatius* [36,37]. It is well known the allergenic cross-reactivity between the members of the Pooideae subfamily grasses of temperate regions (*Lolium perenne, Phleum pratense, Poa pratensis*). A study published by a research group from the same country reported an increased level of grass pollen in the air in May, June, and mid of July in some years [38], while the peak of the season for ragweed and mugwort pollens is noticed in late summer and autumn in Romania [39]. The

patients were evaluated in the middle of grass pollen in Romania, and according to the previous measurement of pollen level in the air, the symptoms corresponding to allergic inflammation were generated by exposure to grass pollen, not to other pollen allergens that are not encountered in the air in the same period of time.

The reduction in CAMs followed the same pattern as in perennial exposure to house dust mites, as Lee et al previously described [35]. Indeed, the treatment that modified pathological mechanism of disease, including the levels of ICAM-1 and VCAM-1 during pollen season is allergen immunotherapy, not H1 antihistamines, which are more symptomatic and pathogenic therapy than an etiologic one [25]. However, long-term treatment with AH1 might contribute to reducing nasal inflammation through inhibition of cytokines and adhesion molecules production and functions. Treatment with H1 antihistamines for one month in this study during the two-month grass pollen season in Romania may not be sufficient in duration to see a statistically significant change in plasma ICAM and VCAM levels. Patients with increased basal values during pollen season tend to remain with increased values despite AH1 treatment, as long as the allergen exposure persists.

There was no difference between investigated compounds, levocetirizine, and desloratadine in the reduction in CAM plasmatic levels. In the present study, CAMs' reduction was more significant in patients with moderate-to-severe AR, compared with patients with mild rhinitis. Other studies that investigated the role of AH1 in other forms of AR induced by mite allergy, showed a decrease in mediators released by systemic and intranasal eosinophils after levocetirizine treatment, but CAM levels were not evaluated [34].

Although CAMs are involved in cells migration, including eosinophils to the site of inflammation, the values of intranasal Eo did not correlate with the serum levels of ICAM-1 and VCAM-1 in the present study. Similar to previous studies [40,41], we found that treatment with H1 antihistamines significantly decreased intranasal eosinophils. Due to difficulty obtaining intranasal CAMs, our study focused on plasmatic CAMs as a proxy [27]. Further studies are needed to investigate levels of CAMs in the nasal mucosa and correlate these levels with local infiltration of Eo.

IgE is the primary molecule in the pathogenesis of allergic diseases. Its synthesis and its level are increased after sensitization and it binds to high-affinity Fcε1 specific receptors expressed on mast cells. Part of it remains free in the serum and can be determined. Total serum IgE is increased in a variety of diseases, and in allergic subjects may remain also normal. In different clinical studies, IgE levels did not correlate with inflammatory markers such as ICAM-1 or TNF-α values, which are higher in asthmatics but not in those with AR [29]. Usually, IgE also increased during pollen season, and decreases after the season, if the patient is not sensitized to perennial allergens. The bound IgE is responsible for recurrent symptoms and inflammation during the pollen season. In the present study, patients with clinical manifestations induced by grass pollen were included, even if some of them were sensitized to several allergens. The inflammatory response in pollen allergy may differ toward house dust mite allergic patients, due to different aspects of the allergens (perennial, dimensions, enzymatic properties) [42–45], but this hypothesis needs further investigation. Another reason for the low reduction in total IgE could be related to polysensitization of the patients, even if they did not report clinical manifestation during the grass pollen season.

The main strength of the present study resides in investigating both clinical and pathophysiological effects of two antihistamines in patients with AR, during natural exposure to an elicited allergen. There are also some limitations of this study. Firstly, a small number of patients were included in the study. Secondly, the count of pollens was not performed in the investigated area, and the level of inflammatory markers could not be correlated with the level of exposure. The third limitation resided in the lack of CAM analysis in the nasal secretion, a determination that could not be accomplished due to technical reasons. It might be interesting to correlate the effect of H1 antihistamines on both nasal and blood CAM and eosinophils.

5. Conclusions

Patients with AR to grass pollen, during the pollen season, have high intranasal eosinophils levels and high serum levels of ICAM-1 and VCAM-1. H1 antihistamines improve symptoms of AR and reduce intranasal eosinophils. Baseline values of CAMs tend to remain higher during pollen exposure and they were not changed significantly despite AH1 treatment.

Author Contributions: Conceptualization, I.C.B., I.A.M. and D.D.; methodology, I.C.B., I.A.M.; C.U. and I.P.; software, I.C.B., I.A.M., N.M. and C.T.D. validation, I.C.B., N.M. and A.D.B.; formal analysis, I.A.M., I.P. and C.U.; investigation, I.C.B., I.A.M. and N.M.; resources, I.C.B., I.A.M. and D.D.; data curation, I.C.B., I.A.M., N.M., C.U. and C.T.D.; writing—original draft preparation, I.C.B., I.A.M., I.P., C.U. and C.T.D.; writing—review and editing, I.C.B., I.A.M., D.D. and A.D.B.; visualization, I.C.B., I.A.M. and A.D.B.; supervision, D.D.; project administration I.C.B., I.A.M. and A.D.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study protocol was approved by the University of Medicine and Pharmacy "Iuliu Haţieganu" Ethics Committee (Approval No. 535/2 September 2011).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All the records are available at the Department of Allergology and Immunology "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Conflicts of Interest: The authors declare no conflict of interest regarding the publication of this article. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Bousquet, J.; Khaltaev, N.; Cruz, A.A.; Denburg, J.; Fokkens, W.J.; Togias, A.; Zuberbier, T.; Baena-Cagnani, C.E.; Canonica, G.W.; van Weel, C.; et al. Allergic rhinitis and its impact on asthma. *Allergy* **2008**, *63*, 7–160. [CrossRef]
2. Bousquet, J.; Schünemann, H.J.; Zuberbier, T.; Bachert, C.; Baena-Cagnani, C.E.; Bousquet, P.J.; Brozek, J.; Canonica, G.W.; Casale, T.B.; Demoly, P.; et al. Development and implementation of guidelines in allergic rhinitis—An ARIA-Ga2len paper. *Allergy* **2010**, *65*, 1212–1221. [CrossRef]
3. Leynaert, B.; Neukirch, C.; Kony, S.; Guénégou, A.; Bousquet, J.; Aubier, M.; Neukirch, F.; On behalf of the European Community Respiratory Health Survey. Association between asthma and rhinitis according to atopic sensitization in a population-based study. *J. Allergy Clin. Immunol.* **2004**, *113*, 86–93. [CrossRef]
4. Schwartz, R.H. T cell anergy. *Ann. Rev. Immunol.* **2003**, *21*, 305–334. [CrossRef] [PubMed]
5. Kowalski, M.L.; Alam, R. Signal transduction mechanisms as new targets for allergists. *Allergy* **2001**, *56*, 199–203. [CrossRef] [PubMed]
6. Gao, S.; Yu, L.; Zhang, J.; Li, X.; Zhou, J.; Zeng, P.; Zhang, X. Expression and clinical significance of VCAM-1, IL-6, and IL-17A in patients with allergic rhinitis. *Ann. Palliat. Med.* **2021**, *10*, 4516–4522. [CrossRef] [PubMed]
7. Kramer, M.F.; Jordan, T.R.; Klemens, C.; Hilgert, E.; Hempel, J.M.; Pfrogner, E.; Rasp, G. Factors contributing to nasal allergic late phase eosinophilia. *Am. J. Otolaryngol.* **2006**, *27*, 190–199. [CrossRef]
8. Tachimoto, H.; Hudson, S.A.; Bochner, B.S. Acquisition and alteration of adhesion molecules during cultured human mast cell differentiation. *J. Allergy Clin. Immunol.* **2000**, *107*, 302. [CrossRef] [PubMed]
9. Bochner, B.S. *Adhesion Molecules in Allergy. Disease*; Dekker, M., Ed.; CRC Press: New York, NY, USA, 1997.
10. Bochner, B.S.; Klunk, D.A.; Sterbinsky, S.A.; Coffman, R.L.; Schleimer, R.P. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J. Immunol.* **1995**, *154*, 799–803. [PubMed]
11. Ohashi, Y.; Nakai, Y.; Tanaka, A.; Kakinoki, Y.; Ohno, Y.; Washio, Y.; Masamoto, T.; Kato, A.; Sakamoto, H. Soluble vascular cell adhesion molecule-1 in perennial allergic rhinitis. *Acta Otolaryngol.* **1998**, *118*, 105–109.
12. Walsh, G.M. The effects of cetirizine on the function of inflammatory cells involved in the allergic response. *Clin. Exp. Allergy* **1997**, *27* (Suppl. 2), 47–53. [CrossRef]
13. Branco-Ferreira, M.; dos Santos, M.C.; Palma-Carlos, M.L.; Palma-Carlos, A.G. Antihistamines and serum adhesion molecule levels. *Allerg. Immunol.* **2001**, *33*, 233–236.
14. Schäper, C.; Gustavus, B.; Koch, B.; Ewert, R.; Hanf, G.; Kunkel, G.; Noga, O.; Gläser, S. Effects of fexofenadine on inflammatory mediators in nasal lavage fluid in intermittent allergic rhinitis. *J. Investig. Allergol. Clin. Immunol.* **2009**, *19*, 459–464. [PubMed]

15. Mullol, J.; de Borja Callejas, F.; Martínez-Antón, M.A.; Méndez-Arancibia, E.; Alobid, I.; Pujols, L.; Valero, A.; Picado, C.; Roca-Ferrer, J. Mometasone and desloratadine additive effect on eosinophil survival and cytokine secretion from epithelial cells. *Respir. Res.* **2011**, *12*, 23. [CrossRef] [PubMed]
16. Tworek, D.; Bocheńska-Marciniak, M.; Kupczyk, M.; Górski, P.; Kuna, P. The effect of 4 weeks treatment with desloratadine (5mg daily) on levels of interleukin (IL)-4, IL-10, IL-18 and TGF beta in patients suffering from seasonal allergic rhinitis. *Pulm. Pharmacol. Ther.* **2007**, *20*, 244–249. [CrossRef]
17. Muntean, I.A.; Bocsan, I.C.; Miron, N.; Buzoianu, A.D.; Deleanu, D. How Could We Influence Systemic Inflammation in Allergic Rhinitis? The Role of H1 Antihistamines. *Oxidative Med. Cell. Longev.* **2018**, *2018*, 3718437. [CrossRef] [PubMed]
18. Canonica, G.W.; Complati, E. minimal persistent inflammation in allergic rhinitis: Implications for current treatment strategies. *Clin. Exp. Immunol.* **2009**, *158*, 260–271. [CrossRef]
19. Bocsan, C.I.; Bujor, A.I.; Miron, N.; Vesa, Ş.C.; Deleanu, D.; Buzoianu, A.D. In vivo anti-inflammatory efect of H1 antihistamines in allergic rhinitis: A Randomized Clinical Trial. *Balk. Med. J.* **2015**, *32*, 352–358. [CrossRef]
20. Heinzerling, L.; Mari, A.; Bergmann, K.; Bresciani, M.; Burbach, G.; Darsow, U.; Durham, S.; Fokkens, W.; Gjomarkaj, M.; Haahtela, T.; et al. The skin prick test—European standards. *Clin. Transl. Allergy* **2013**, *3*, 3. [CrossRef] [PubMed]
21. Adamko, D.J.; Odemuyiwa, S.O.; Vethanayagam, D.; Moqbel, R. The rise of the phoenix: The expanding role of the eosinophil in health and disease. *Allergy* **2005**, *60*, 13–22. [CrossRef]
22. Ural, A.; Tezer, M.S.; Yücel, A.; Atilla, H.; Ileri, F. Interleukin-4, interleukin-8 and E-selectin levels in intranasal polyposis patients with and without allergy: A comparative study. *J. Int. Med. Res.* **2006**, *34*, 520–524. [CrossRef] [PubMed]
23. Turgay, M.; Keskin, G.; Kminkli, G.; Sentürk, T.; Tutkak, H.; Duman, M.; Tokgöz, G. Soluble intercellular adhesion molecule-1 (sICAM-1) in patients with allergic rhinoconjunctivitis. *Allergol. Immunopathol.* **1996**, *24*, 129–131.
24. Liu, C.M.; Shun, C.T.; Cheng, Y.K. Soluble adhesion molecules and cytokines in perennial allergic rhinitis. *Ann. Allergy Asthma Immunol.* **1998**, *81*, 176–180. [CrossRef]
25. Håkansson, L.; Heinrich, C.; Rak, S.; Venge, P. Priming of eosinophil adhesion in patients with birch pollen allergy during pollen season: Effect of immunotherapy. *J. Allergy Clin. Immunol.* **1997**, *99*, 551–562. [CrossRef]
26. Gorska-Ciebiada, M.; Ciebiada, M.; Gorska, M.M.; Gorski, P.; Grzelewska-Rzymowska, I. Intercellular adhesion molecule 1 and tumor necrosis factor alpha in asthma and persistent allergic rhinitis: Relationship with disease severity. *Ann. Allergy Asthma Immunol.* **2006**, *97*, 66–72. [CrossRef]
27. Lee, B.J.; Naclerio, R.M.; Bochner, B.S.; Taylor, R.M.; Lim, M.C.; Baroody, F.M. Nasal challenge with allergen upregulates the local expression of vascular endothelial adhesion molecules. *J. Allergy Clin. Immunol.* **1994**, *94*, 1006–1016. [CrossRef]
28. Ciebiada, M.; Gorska-Ciebiada, M.; Gorski, P. sICAM-1 and TNF-α in Asthma and Rhinitis: Relationship with the Presence of Atopy. *J. Asthma* **2011**, *48*, 660–666. [CrossRef] [PubMed]
29. Li, Y.; Wang, Y.; Zhang, Q. Expression of cell adhesion molecule and nitric oxide synthase in nasal mucosa in allergic rhinitis. *Lin Chuang Er Bi Yan Hou Ke Za Zhi J. Clin. Otorhinolaryngol.* **2006**, *20*, 315–318.
30. Matsui, T.; Asakura, K.; Shirasaki, H.; Kataura, A.; Himi, T. Relationship between infiltrating cells and adhesion molecules in the nasal mucosa of patients with allergic rhinitis. *Acta Otolaryngol.* **2000**, *120*, 973–980.
31. Mösges, R.; Klimek, L. Today's allergic rhinitis patients are different: New factors that may play a role. *Allergy* **2007**, *62*, 969–975. [CrossRef]
32. Bachert, C. A review of the efficacy of desloratadine, fexofenadine, and levocetirizine in the treatment of nasal congestion in patients with allergic rhinitis. *Clin. Ther.* **2009**, *31*, 921–944. [CrossRef] [PubMed]
33. Agrawal, D.K. Anti-inflammatory properties of desloratadine. *Clin. Exp. Allergy* **2004**, *34*, 1342–1348. [CrossRef] [PubMed]
34. Ciprandi, G.; Pronzato, C.; Ricca, V.; Bagnasco, M.; Canonica, G.W. Evidence of intercellular adhesion molecule-1 expression on nasal epithelial cells in acute rhinoconjunctivitis caused by pollen exposure. *J. Allergy Clin. Immunol.* **1994**, *94*, 738–746. [CrossRef] [PubMed]
35. Lee, C.F.; Sun, H.L.; Lu, K.H.; Ku, M.S.; Lue, K.H. The comparison of cetirizine, levocetirizine and placebo for the treatment of childhood perennial allergic rhinitis. *Pediatr. Allergy Immunol.* **2009**, *20*, 493–499. [CrossRef]
36. Popescu, F.D. Molecular biomarkers for grass pollen immunotherapy. *World J. Methodol.* **2014**, *26*, 26–45. [CrossRef]
37. D'Amato, G.; Cecchi, L.; Bonini, S.; Nunes, C.; Annesi-Maesano, I.; Behrendt, H.; Liccardi, G.; Popov, T.; Van Cauwenberge, P. Allergenic pollen and pollen allergy in Europe. *Allergy* **2007**, *62*, 976–990. [CrossRef] [PubMed]
38. Leru, P.M.; Eftimie, A.M.; Anton, V.F.; Thibaudon, M. Five-Year Data on Pollen Monitoring, Distribution and Health Impact of Allergenic Plants in Bucharest and the Southeastern Region of Romania. *Medicina* **2019**, *55*, 140. [CrossRef]
39. Bocsan, I.C.; Muntean, I.A.; Ureche, C.; Pop, R.M.; Neag, M.A.; Sabin, O.; Deleanu, D.; Buzoianu, A.D. Characterization of Patients with Allergic Rhinitis to Ragweed Pollen in Two Distinct Regions of Romania. *Medicina* **2019**, *55*, 712. [CrossRef] [PubMed]
40. Marcucci, F.; Sensi, L.G.; Abate, P.; Allocca, G.; Ugolini, E.; Di Cara, G.; Cristoforo, I. Anti-inflammatory activity and clinical efficacy of a 3-month levocetirizine therapy in mite-allergic children. *Inflamm. Allergy Drug Targets* **2011**, *10*, 32–38. [CrossRef]
41. Bautista, A.P.; Eisenlohr, C.P.; Lanz, M.J. Nasal nitric oxide and nasal eosinophils decrease with levocetirizine in subjects with perennial allergic rhinitis. *Am. J. Rhinol. Allergy* **2011**, *25*, 383–387. [CrossRef]
42. Ciebiada, M.; Barylski, M.; Gorska Ciebiada, M. Nasal eosinophilia and serum soluble intercellular adhesion molecule 1 in patients with allergic rhinitis treated with montelukast alone or in combination with desloratadine or levocetirizine. *Am. J. Rhinol. Allergy* **2013**, *27*, e58–e62. [CrossRef] [PubMed]

43. Maiti, R.; Rahman, J.; Jaida, J.; Allala, U.; Palani, A. Rupatadine and levocetirizine for seasonal allergic rhinitis: A comparative study of efficacy and safety. *Arch. Otolaryngol. Head Neck Surg.* **2010**, *136*, 796–800. [CrossRef] [PubMed]
44. Muntean, I.A.; Bocsan, I.C.; Vesa, S.; Miron, N.; Nedelea, I.; Buzoianu, A.D.; Deleanu, D. Could FeNO Predict Asthma in Patients with House Dust Mites Allergic Rhinitis? *Medicina* **2020**, *56*, 235. [CrossRef] [PubMed]
45. Gilain, L.; Guichard, C.; Beaujon, G.; Mom, T.; Monneyron, E.; Saleh, H.; Advenier, D.; Caillaud, D. Taux d'ICAM-1 soluble nasal dans les rhinites allergiques. *Ann. d'oto-Laryngol. Chir. Cervico-Faciale* **2000**, *117*, 91–97. [PubMed]

nutrients

MDPI

Article

Predictive Factors for Oral Immune Modulation in Cow Milk Allergy

Ioana Adriana Muntean [1,2,†], Ioana Corina Bocsan [3,*,†], Lena Katharina Wiest [4], Irena Pintea [1,2], Carmen Teodora Dobrican [1,2], Emanuela Duca [5], Corina Ureche [6], Anca Dana Buzoianu [3] and Diana Deleanu [1,2]

1 Department of Allergology and Immunology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400012 Cluj Napoca, Romania; Adriana.Muntean@umfcluj.ro (I.A.M.); irena.nedelea@umfcluj.ro (I.P.); dobrican.carmen@umfcluj.ro (C.T.D.); diana.deleanu@umfcluj.ro (D.D.)
2 Department of Allergology, "Octavian Fodor" Institute of Gastroenterology and Hepatology, 400162 Cluj Napoca, Romania
3 Department of Pharmacology, Toxicology and Clinical Pharmacology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400337 Cluj Napoca, Romania; abuzoianu@umfcluj.ro
4 Department of Anesthesiology, Intensive Care, Emergency and Pain Medicine, Hospital of Villingen-Schwenningen, 78056 Villingen-Schwenningen, Germany; lenaboehme@web.de
5 Department of Pediatric Pneumology, Pediatric Hospital, 400371 Cluj Napoca, Romania; emma_floca@yahoo.com
6 First Internal Medical Department, "George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology, 540139 Târgu Mureș, Romania; corina.ureche@umfst.ro
* Correspondence: bocsan.corina@umfcluj.ro
† These authors contributed equally to this work.

Abstract: Aim: The present study analyzed clinical and biological factors that might predict achievement of tolerance in patients with IgE-mediated cow milk allergy (CMA). Method: Seventy patients with IgE-mediated CMA (44.24 ± 24.16 months) were included in the study. The patients were evaluated clinically through skin prick test and sIgE to whole milk, casein, beta-lactoglobulin and alpha-lactalbumin. An eviction diet of 6 months was established, followed by oral food challenge test (OFC) and oral immunotherapy (OIT) with baked milk for 6 months. The tolerance was assessed after 2 years follow up. Results: Thirty percent of patients presented anaphylaxis of different degrees of severity as first manifestation of CMA. Sixty-two patients followed OIT or an accelerated reintroduction of milk. Ten patients (14.28%) did not obtain tolerance to milk within 2 years. A larger wheal in SPT and higher sIgE to milk, casein and betalactoglobulin were noted in patients with positive OFC. A basal level of <2.5 kU/l for sIgE to milk and <11.73 kU/l for sIgE to caseins predicted the occurrence of tolerance in patients with all types of clinical manifestations, including anaphylaxis. Conclusion: Basal levels of sIgE to milk and casein may help to identify patients that could become tolerant to milk.

Keywords: casein; cow milk allergy; oral immunotherapy; oral tolerance

Citation: Muntean, I.A.; Bocsan, I.C.; Wiest, L.K.; Pintea, I.; Dobrican, C.T.; Duca, E.; Ureche, C.; Buzoianu, A.D.; Deleanu, D. Predictive Factors for Oral Immune Modulation in Cow Milk Allergy. *Nutrients* **2022**, *14*, 494. https://doi.org/10.3390/nu14030494

Academic Editor: Francisco J. Pérez-Cano

Received: 4 December 2021
Accepted: 19 January 2022
Published: 23 January 2022

1. Introduction

Cow milk allergy (CMA) is the most common food allergy in children, with an estimated prevalence of 0.5% to 3% in the pediatric population below 1 year old [1]. Self-reported incidence of CMA is much higher than confirmed allergy in both children and adults [2]. The incidence of self-reported allergy varies between 1.2% to 17%, while the rate of prevalence for milk allergy confirmed by an oral food challenge test is lower, between 0% and 3% [3]. The sensitization to milk is less than 1% in the general population, varying throughout Europe, [4,5]. When the confirmation of the allergy is obtained through skin prick test and specific IgE, the prevalence of CMA is between 2–9% [4–6].

The term cow milk allergy refers to an immune-mediated reaction induced by exposure to cow milk and includes three categories of diseases: IgE-mediated, non-IgE-mediated and

combined (produced by IgE and non-IgE mechanisms). An IgE-mediated cow milk allergy is a type I hypersensitivity reaction, and the clinical manifestations occur within minutes to 2 h after milk ingestions. This form represents almost 60% of CMA cases, but this estimation could vary according to patient age and geographical area [1,7].

The clinical manifestations are variable from acute urticaria or exacerbation of atopic dermatitis to the most severe presentation, which is anaphylaxis [1]. CMA is responsible for 10–19% of all food-induced anaphylactic cases, being the third cause of anaphylactic reactions induced by foods, after peanuts and tree nuts [8]. A positive diagnosis algorithm starts with a careful clinical evaluation, followed by skin prick test and laboratory findings [8]. Skin prick test with fresh milk or standardized extract represents a fast method to detect sensitization but not the allergy [9]. Measurement of specific IgE to cow milk through ELISA, RAST or CAP-FEIA technique is a diagnostic approach with high sensitivity, but sometimes, it may deliver false positive results; thus, they should be analyzed in the clinical history context. A basophil activation test is another useful diagnostic tool for CMA in combination with sIgE, especially in children with atopic dermatitis [10], but it is not frequently used in clinical practice. There are still no equal test assay systems for serum sIgE, which makes for a difficult comparison between studies and techniques. Several studies have tried to describe the predictive values of IgE levels for clinical reactivity [8], but the differences are quite significant mainly due to various selection criteria, age of the patient or different criteria for analyzing the clinical reactivity [9,11,12].

The first measure in the management of CMA is allergen avoidance. A diet without milk and dairy products is recommended until clinical tolerance is induced [2,6,13]. The oral tolerance to cow milk is reached in almost half of the patients by an age of 5 years, increasing the rate up to 75% until teenage years [6,14], but some patients remain with persistent CMA [13,15]. However, the experience of the last 20 years has shown that the natural history of food allergy is changing and that less individuals become tolerant and that a longer time to resolution is needed [13].

Oral immunotherapy (OIT) has shown some promising results in improving patients' quality of life in CMA. It is a therapeutic method that can also be used in young children. Adverse reactions including anaphylaxis may occur during OIT, especially during the escalation phase. The rate of desensitization is variable, with 20–30% of patients remaining with persistent CMA despite OIT [6]. The tolerance to cow milk induced by OIT or achieved naturally may vary from country to country, and it is influenced by the genetic inheritance and the microbiota from the gut [16]. OIT may permit achievement of a rapid tolerance to milk, which allows the children to have normal activities without any restrictions. Standardized protocols of OIT with validated optimal dose and ideal duration, data regarding degree of protection, safety, and efficacy in different ages and populations need to be established [17–19]. There is also an urgent need to establish standardized outcome measures to be applied in food allergy studies, for both prediction of tolerance and for monitoring of OIT [20]. This may allow for a better harmonization of data resulting from different clinical trials.

The aim of the present study was to identify possible clinical and biological predictive factors for achievement of tolerance after OIT in a cohort of patients with IgE-mediated CMA. The second objective was to establish the effectiveness of a modified protocol for oral immunotherapy to milk in obtaining oral tolerance.

2. Materials and Methods

2.1. Patients and Study Design

The study was an analytic, transversal study. The present research analyzed clinical and biological factors that might predict the occurrence of tolerance in patients with cow milk protein allergy.

Seventy-six patients with milk-induced reactions presented for allergological evaluation. The patients were evaluated at the Allergology Department of Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor" in Cluj Napoca and at the

Almedo Clinic in Cluj Napoca between January 2013–November 2021. Only patients with an unequivocal positive immediate allergic reaction after contact with cow milk as well as documented evidence of sIgE to cow milk protein by blood tests and/or a skin prick test were included. The exclusion criteria were: non-IgE-mediated hypersensitivity reactions induced by cow milk, patients without a definitive positive diagnosis of CMA, patients that refused to sign the informed consent, and patients for which follow up was not performed. Based on these criteria, six patients were excluded from the final analysis.

Seventy patients with IgE-mediated CMA that had presented for allergological evaluation were included in the study. The mean age was 44.24 $\pm$ 24.16 months when the patients were included in the evaluation, and the sex ratio was M:F = 1.41. Diagnosis of IgE-mediated CMA was established according to international guidelines, based on history, clinical evaluation, skin prick test (SPT) and sIgE to milk and components.

The study protocol was approved by the University Ethics Committee of the University of Medicine and Pharmacy (293/28 July 2013), according to the principles from Declaration of Helsinki. Each patient signed the informed consent before the study began.

2.2. Allergological Evaluation

Clinical evaluation was performed at the beginning, when the patients were included in the study (see Figure 1). From anamnesis, the following demographic and clinical data were recorded: age, gender, living area (urban/rural) and clinical picture of the first allergic reaction, onset of disease, duration until first allergological diagnosis, family history of atopy, and other allergic diseases associated.

Skin prick of milk protein mix was performed. Skin prick tests were positive if the wheal diameter was $\geq$3 mm compared to the negative control. Standardized allergen extracts (Hal Allergy, Netherlands) were used. The value in mm was recorded as a medium diameter wheal size.

Serological tests implied determination of total Ig, specific IgE for cow milk (whole extract) and casein, beta-lactoglobulin and alpha-lactalbumin. Laboratory test results were obtained through electrochemiluminescence immunoassay method (ECLIA).

The atopy diagnosis was established through skin prick test at enrollment, according to international guidelines [21]. The skin prick test included the following panel of allergens: house dust mites (Derm. Pteronyssinus and Derm. Farinae), pollens (grasses, cereals, birch and weeds), animal dander (cat and dog) and molds (Alternaria alternata). After the positive diagnosis was established, a diet without milk or dairy products was recommended for 6 months. After 6 months, the remission of symptoms or an accidental exposure to milk were assessed. An oral challenge test with milk 3.5%, baked for 30 min was performed in 62 of patients. The positivity of OFC was established if the patient had a clinical manifestation and if the quantity of milk that induced the reactivity was noted. Simple-blind OFC was not performed in patients if the parents refused to sign the informed consent. The simple blind OFC protocol included 4 steps:

1. 2 mL rice milk (commercially available) as placebo;
2. 0.25 mL baked cow milk plus 1.75 mL of rice milk;
3. 0.5 mL baked cow milk plus 1.5 mL of rice milk;
4. 1 mL baked cow milk plus 1 mL of rice milk.

All the doses were given at 30 min time intervals. The OFC was considered positive if the patient presented clinical manifestations in the aforementioned 4 steps. The OFC was considered negative if the patients tolerated 1 mL milk. If the patients tolerated 1 mL milk during OFC, they continued with an accelerated reintroduction of baked milk (see Figure 1 and Table 1) to reach, in 48 h, the maintenance dose of milk that was used in the protocol of oral immunotherapy. The rapid reintroduction of baked milk was performed in the allergological department under medical supervision until the maintenance dose of 200 mL was reached.

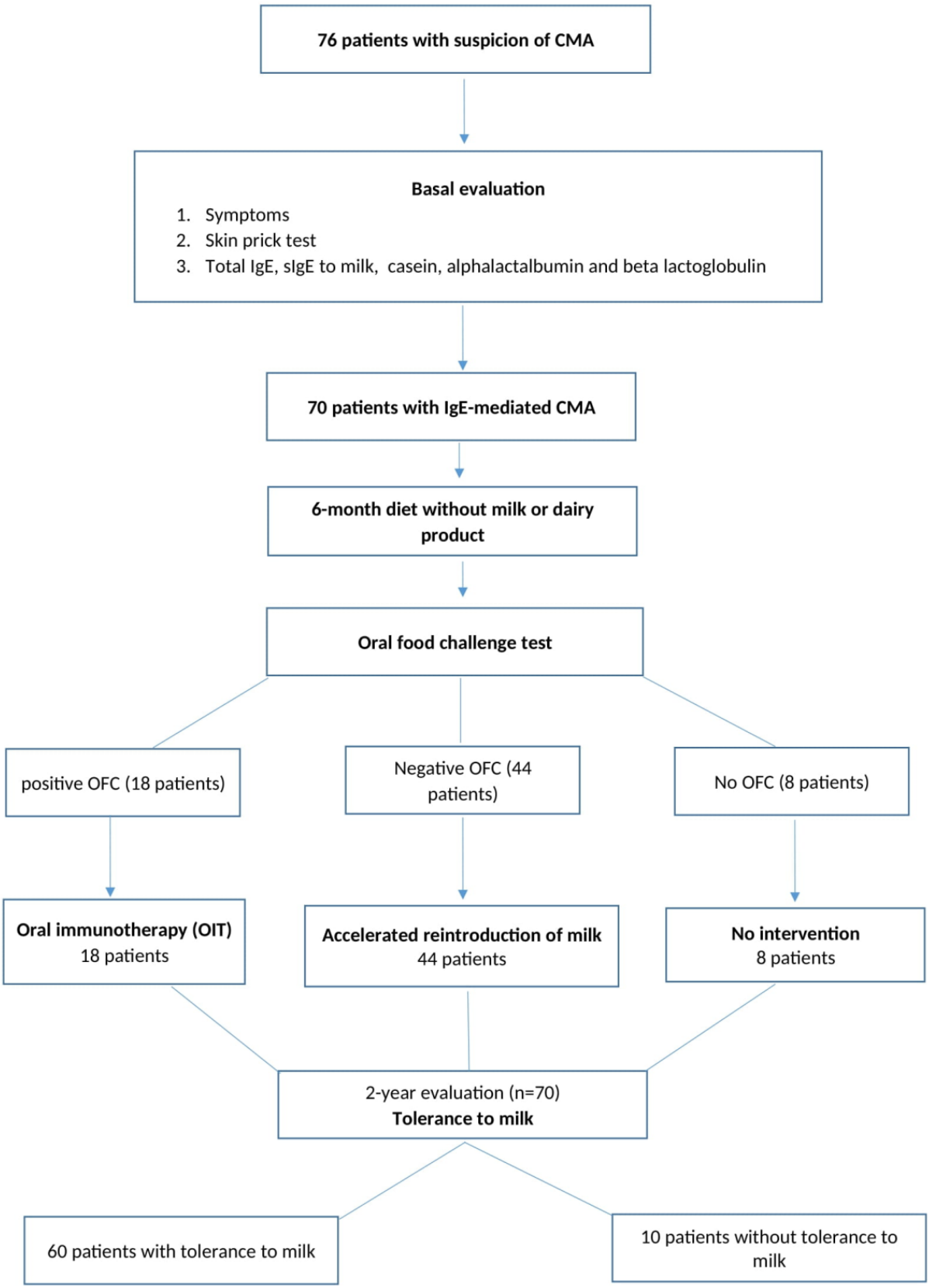

Figure 1. The algorithm of evaluations and therapeutic interventions in patients with cow milk allergy.Abbreviations: CMA, cow's milk allergy; IgE, immunoglobulin E; OFC, oral food challenge.

Table 1. Protocol of rapid reintroduction of milk and oral immunotherapy to milk.

Phases	Rapid Reintroduction of Baked Milk		Oral Immunotherapy	
	Interval of Time between Dose Escalation	**Amount of Baked Milk**	**Interval of Time between Dose Escalation**	**Amount of Baked Milk**
Build up phase	30 min	1 mL	30 min	0.05 mL
	30 min	2 mL	30 min	0.1 mL
	30 min	4 mL	30 min	0.2 mL
	30 min	8 mL	30 min	0.4 mL
	30 min	16 mL	30 min	1 mL
	24 h	25 mL	30 min	2 mL
	48 h	50 mL	24 h	4 mL
			48 h	8 mL
			36 h	16 mL
			1 week	25 mL
			2 weeks	50 mL
Maintenance dose	1 week	100 mL	1 month	100 mL
	1 week	200 mL	3 months	200 mL

2.3. Oral Immunotherapies

A group of patients (18 patients) underwent open oral immunotherapy (see Figure 1). The procedure consisted of the administration of progressively increasing amounts of baked milk 3.5% to induce tolerance and to reduce the allergic symptoms until disappearance. Small amounts of baked milk were administered sublingually initially, with an increasing amount administered orally according to tolerance (build up phase period), to a dose that was given daily (maintenance period) continuously. The initiation of immunotherapy was performed in a specialized allergology unit with existing facilities for emergency assistance if the patients developed adverse reactions. The protocol started with 0.05 mL baked milk, and the maintenance dose of 50 mL was supposed to be reached in 3 weeks. In some patients, the induction phase lasted more than 6 months until the maintenance dose was reached. When the patients were in the maintenance phase, they were allowed to introduce milk substitutes such as yoghurt, cream or ice cream. The protocol of updosing is presented in Table 1. The acquisition of tolerance was established after 2 years follow up.

2.4. Statistical Analysis

Statistical analysis was carried out using the MedCalc Statistical Software version 18.10 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; (accessed on 20 November 2021). Quantitative data were evaluated for normality of distribution using the Kolmogorov–Smirnov test. They were characterized by median and 25–75 percentiles. Qualitative data were expressed as frequency and percentages. Comparisons between groups were performed using the Mann–Whitney or chi-square tests whenever appropriate. The correlation between variables was established using Spearman's correlation. ROC curves were used in order to find cut-off values for quantitative variables that could discriminate between patients with a tolerance to milk and those without. A p value of <0.05 was considered statistically significant.

3. Results

Seventy patients with IgE-mediated CMA were evaluated (Table 2). Most of the patients (62 patients, 88.6%) followed a rapid reintroduction of milk or OIT for milk.

Ten patients (14.28%) did not obtain tolerance to milk within 2 years after the first evaluation and positive diagnosis of cow milk allergy. Only two patients (11.1%) from the group that followed OIT did not gain oral tolerance in this interval of time.

Table 2. Demographic data of patients with cow milk allergy.

Parameter		CMA (*n* = 70)	Patients without Tolerance (*n* = 10)	Patients with Tolerance (*n* = 60)	*p*
Gender	M	41 (58.6%)	8 (80%)	33 (55%)	0.178
	F	29 (49.4%)	2 (20%)	27 (45%)	
Family history of atopy		29 (41.4%)	2 (6.9%)	27 (83.1%)	0.166
Personal history of respiratory allergy		21 (30%)	3 (30%)	18 (30%)	0.918
Personal history of food allergy		20 (28.6%)	3 (30%)	17 (28.6%)	1
Living area	Urban	62 (88.6%)	10 (100%)	52 (83.9%)	0.591
	Non-urban	8 (11.4%)	0	8 (13.3%)	

Demographic data are presented in Table 2.

CMA was noted more frequently in boys than in girls, and more females obtained tolerance after OIT than males (93.1% vs. 80.5%), but the difference was not statistically significant. Twenty-nine patients (41.4%) had a positive family history of atopy, but this did not influence the induction of tolerance compared to patients without a family history of allergy. Personal history of respiratory and/or food allergy were noted in almost one-third of the patients, without any influence in obtaining tolerance. Twelve patients (60%) with other food allergies tested positive to egg, followed by peanuts and other nuts.

The average duration of disease from the onset of the symptoms until the positive diagnosis of CMA was 20 (6.5–40.75) months, and a longer time was noted in patients with persistent allergies compared to those with a tolerance to milk (43.5 (21.5–112.5) vs. 18 (4.5–36), $p = 0.027$). The family or personal history of allergy or the severity of first clinical presentation did not accelerate the presentation to a specialist for evaluation of CMA.

3.1. Clinical Manifestations

The analysis of clinical manifestations revealed that the symptoms occurred, on average, at the age of 9 months (9.72 ± 4.66 years, minimum 1 month, maximum 24 months). The age of onset was higher in patients with persistence of CMA.

Thirty percent of the patients (21 pts) presented anaphylaxis of different degrees of severity. Most of the patients (65.7%) presented cutaneous manifestation such as acute urticaria or aggravation of atopic dermatitis or both (Table 3). The clinical manifestation at the onset of the allergy did not predict the occurrence of tolerance to milk.

Table 3. Primary clinical manifestation of CMA.

Parameter		Patients without Tolerance (*n* = 10)	Patients with Tolerance (*n* = 60)	*p*
Age of symptoms onset (months) *		9 (6–18)	9 (6–11.5)	0.060
Manifestations	Anaphylaxis	3 (14.3%)	18 (85.7%)	0.916
	Acute urticaria	2 (12.5%)	14 (87.5%)	
	Atopic dermatitis	3 (13%)	20 (87%)	
	Digestive symptoms	1 (33.3%)	2 (66.7%)	
	Urticaria + atopic dermatitis	1 (14.3%)	6 (85.7%)	

* Data are expressed as median and percentile.

3.2. Skin Prick Test and sIgE

Skin prick test and specific IgE to milk and major proteins were performed in all cases. The size of the wheal was higher in patients with persistent allergy, but the difference did not reach the level of statistical significance. Basal median values of specific IgE to milk and to casein were significantly higher in patients without oral tolerance (Table 4).

Table 4. Basal results of skin test and laboratory values in patients with cow milk allergy.

Parameter.	Patients without Tolerance (n = 10)	Patients with Tolerance (n = 60)	p
Size of wheal (SPT)	8 (4.75–15.75)	5 (4–8)	0.08
sIgE to milk	12.77 (4.10–86.88)	3.2 (0.6–13.5)	0.039
sIgE to casein	14.3 (1.5–45.72)	0.96 (0.35–5.45)	0.01
sIgE to alpha-lactalbumin	2.3 (0.35–28.02)	2.1 (0.48–7.88)	0.926
sIgE to beta-lactoglobulin	2.42 (0.35–14.9)	1.5 (0.35–5.14)	0.755

Data are expressed as median and 25–27 percentiles.

The basal results of skin prick test and laboratory values were also analyzed in relation to clinical reactivity after OFC. The oral food challenge test was performed after a period of 6 months of eviction diet in order to establish the opportunity of oral immunotherapy. OFC was performed in 62 patients (88.57%), and it was positive for 18 of them (Table 5). The clinical reactivity during OFC was more frequently noted in patients with persistent CMA (p = 0.003).

Table 5. Results of oral food challenge test and correlation with acquired tolerance.

Parameter		Patients without Tolerance (n = 10)	Patients with Tolerance (n = 60)	p
OFC	Negative	1 (10%)	43 (71.67%)	0.003
	Positive	2 (20%)	16 (26.66%)	
	Not done	7 (70%)	1 (1.66%)	

The patients with positive OFC had significantly higher values of specific IgE to milk (p = 0.017), casein (p = 0.006), and beta lactoglobulin (p = 0.011), but not to alpha-lactalbumin (p = 0.083) compared to patients with negative OFC. The size of the wheal at skin prick test was also significantly higher in those patients (p = 0.002).

3.3. Analysis of Patients with Anaphylaxis Induced by Cow Milk Proteins

Twenty-one patients with CMA presented anaphylaxis grade 2 to 4 of severity, from which three patients (14.28%) had a persistent allergy to cow milk. The anaphylaxis as a primary manifestation of CMA was not correlated with a personal history of allergy to other foods or respiratory allergens (p = 1, respectively p = 0.74) and to a familial history of atopy (p = 1). Oral immunotherapy was performed in 18 patients, and all of them obtained tolerance compared to those patients that had a persistent form of CMA and did not follow OIT (p = 0.001). The severity of initial anaphylactic reactions did not predict de-occurrence of oral tolerance (p = 0.792) after OIT.

Specific IgE to milk and casein wase significantly higher in patients with anaphylaxis and persistent allergy to cow milk compared to those who obtained oral tolerance (Table 6).

The ROC curves for basal values of specific IgE for milk and casein were analyzed, and the cut-off values were calculated for these parameters in relation to the presence of tolerance after 2-year follow up after the onset of OIT. The cut off values, AUC, and sensitivity and specificity are presented in Table 7.

Table 6. Basal results of skin test and laboratory values in patients with anaphylaxis induced by cow milk.

Parameter	Patients without Tolerance (n = 3)	Patients with Tolerance (n = 18)	p
Size of wheal (SPT)	15 (5)	7 (5–9)	0.185
sIgE to milk	91.66 (20)	5.3 (1.12–13.1)	0.019
sIgE to casein	74.3 (22.4)	2.3 (0.45–8.87)	0.017
sIgE to alpha-lactalbumin	78.2 (0.9)	2.1 (0.8–8.12)	0.221
sIgE to beta-lactoglobulin	21.8 (0.2)	0.58 (0.35–6.7)	0.534

Data are expressed as median and 25–27 percentiles.

Table 7. ROC curve analysis for oral tolerance at 2-year follow up.

Parameter	AUC	Cut-Off Value	Sensitivity	Specificity	p
sIgE to milk	0.705 (95% CI 0.550−0.860)	2.5 kU/l	45.7% (95% CI 32.7−59.2)	100% (95% CI 69.2−100.0)	0.012
sIgE to casein	0.755 (95% CI 0.581−0.93)	11.73 kU/l	93% (95% CI 83.8−98.2)	60% (95% CI 26.2−87.8)	0.005

During OIT, no severe reactions were noted. Few patients presented mild skin eruptions or perioral contact dermatitis with spontaneous remission or after administration of H1 antihistamines. None of the patients presented bronchospasm, diarrhea or anaphylactic reactions that needed administration of epinephrine.

4. Discussion

The present study assessed the clinical and biological changes in patients with IgE-mediated CMA, showing that both sIgE to milk and casein basal levels could predict the occurrence of oral tolerance after OIT or after rapid reintroduction of milk. The study also demonstrated the efficacy of a modified protocol for oral immunotherapy in inducing oral tolerance to milk.

Cow milk allergy is a common allergy in the pediatric population, being the first food allergy described in the allergic march [2,22]. It may be over- or underdiagnosed, depending on the type of evaluation. Some health care professionals, but especially parents, confuse CMA with lactose intolerance, leading to inappropriate diets. Even if true CMA is diagnosed, the type of elimination diet, substitutive products and the duration of such elimination are not always logical. Complete elimination of cow milk without an appropriate substitution can lead to growth impairment, malnutrition, and deficiencies in nutrients with long term consequences [22]. Food allergies negatively affect quality of life for children and their parents, with a significant disruption in family life and social interactions [23–25]. Both physicians and parents should understand the multifaceted clinical and biological aspects of CMA to know how to manage further diets.

In the present study, the onset of CMA was noted in the first year of life in few patients, with the first symptoms being described afterward, but no later than the age of 2 years. CMA is mostly a disease of infancy and early childhood. Most of the studies reported that affected children presented symptoms within the first 6 months of life and sometimes earlier, usually before 1 month of age and often within 1 week after the introduction of cow milk proteins to their diet [15,22,26]. In the present study, the average onset of CMA was 9 months, later than in the previous studies [27,28], but all of the patients had clinical manifestations within 2 years of life. Boys were more affected by CMA than girls, similar to the EuroPrevall study [29]. The family history of atopy was reported in more than 40% of the patients, as in the EuroPrevall study [29], but the percentage reflects global atopy in mothers and fathers and is not separated by gender.

Eight patients from the countryside are not enough to make proper conclusions about a difference in CMA between patients living in the cities and those living in the countryside. For future studies, an overall online database should be created for doctors from different

departments in order to introduce patients with CMA, especially when small sample sizes are present. Nevertheless, a long period from the first symptoms until the first allergological consultation occurs (median 20 months) is unacceptable. It shows that neither doctors nor the parents are aware of food-induced allergies or comorbidities commonly associated with CMA (e.g., acute urticaria, atopic dermatitis, anaphylaxis, and GERD), and further efforts are needed in order to improve the situation in Romania. Mainly, pediatricians and family doctors must be aware of this topic and should refer probable cases to an allergology department for further evaluation. It is especially important for severe cases presenting clinically with anaphylactic reaction to have proper management and to prevent further acute episodes. Patients with anaphylaxis had earlier presentation to an allergologist (median 10 months), showing that a severe reaction may increase the anxiety of both children and parents and may make them aware of a potential risk. The specialist visit should occur as soon as possible in order to reduce the sequelae, an improper diet, and in order to provide a proper treatment regimen as well as possible oral immunotherapy for patients.

The majority of children with CMA had one or more symptoms that involved one or more organs, mainly the gastrointestinal tract and/or skin. More than half of the patients had skin manifestations (acute urticaria, aggravation of atopic dermatitis, contact dermatitis) as the first manifestation of CMA. Digestive symptoms alone were described only in three patients (4.2%), which is less frequent than in other studies, but digestive symptoms are more common in non-IgE-mediated reactions to milk [29]. Anaphylaxis as the first manifestation was present more frequently in the present analysis (30% of children) compared to previous data [1,8]. An anaphylactic reaction might increase anxiety in the family, allowing parents to be more aware of the risk of a severe reaction. Patients with milder skin reactions probably skip evaluation in the allergology department and are thus treated by a generalist, pediatrician or dermatologist, which may also explain the lower rate of cutaneous manifestations described in this cohort compared to previous data [8,22,29].

Following the ESPGHAN algorithm [30] for the evaluation of children with suspicion of CMA, a simple-blind OFC test was performed in 88.57% of the patients to establish if the patients obtained a tolerance to milk and to assess the opportunity of OIT. OFC should be a part of the routine workup [2,30] along with detailed anamnesis, diagnostic elimination diets, skin prick tests, and sIgE. Lack of OFC in all patients is explained by patients' refusal to partake in it. When cow milk is the only suspected allergen and the only food in the diet, the diagnosis is simpler than in cases where they are already ingesting a variety of foods and OFC could not be a standard procedure. An oral food challenge test was performed in children with more than one food in their diet to confirm a positive diagnosis directly before initiation of OIT. Patients with negative OFC were actually patients with mild CMA that followed a rapid reintroduction of baked milk with an accelerated induction of tolerance.

Oral immunotherapy is a therapeutic method that permits the induction of tolerance and a normal diet after completion of it. OIT to milk is similar to peanut OIT regarding effectiveness in inducing clinical desensitization to the culprit allergen, but with a lower risk of allergic reactions during OIT. Clinical trial data are more limited, and there are no approved formulations for OIT. A significant challenge in determining the efficacy of several therapies for milk and egg allergies is that the natural rate of resolution of these allergies is much higher than for peanuts. In a 2012 meta-analysis of five trials that analyzed milk OIT (including 218 children), milk OIT increased the likelihood of developing full tolerance to milk by 10-fold compared to children without interventions. [17].

The quality of the allergen is critical for both OFC and OIT and may vary in commercial products; thus, it is hard to standardize this method [31]. In the present study, 88.6% of patients followed this procedure with a good response (only 11.1% of them had persistent CMA after 2-year follow up). More patients with an eviction diet who did not follow OIT presented persistent CMA at the end of the follow up period. Garcia-Ara et al. [32] also reported a high successful rate of desensitization after 1-year follow up (88–100%), depending on basal sIgE to milk. In the present study, the patients were not stratified

according to basal evaluation of SPT and sIgE. A lower rate response was also mentioned by Kuitunen et al. (72%) after 6 months of OIT [33]. In another study from Denmark and Australia, patients achieved tolerance in a variable rate (28–77%) at the age of 2 for cow milk, without any interventions [34,35]. This difference could be explained by different inclusion criteria, duration of OIT, or a non-interventional attitude. The present analysis included all the patients with a positive diagnosis of CMA who presented for allergological evaluation. Increasing the duration of OIT and follow up may increase the success rate of it. We did not find associations between tolerance to milk and gender or other food allergies, in accordance with other published results [36,37]. This study sustained the role of OIT in obtaining tolerance, which may permit a normal diet independent from personal or familial history of allergy or from patient gender.

The reported rate of success and the less adverse events of OIT could be explained through a modified protocol of OIT, which used baked milk instead of raw milk until tolerance was induced. The children that obtained tolerance to baked milk after 6–9 months of OIT also tolerated dairy products as a component of the normal diet, or raw milk without any reactions after they switched from heated to unheated milk. Similar results were also reported by Esmaeilzadeh H et al. [38], who demonstrated that introducing baked milk products into the diet of patients with a milk allergy can accelerate the tolerance of unheated milk, but basal sIgE could not predict the success of OIT. Many concerns are raised regarding milk OIT because, unlike most other allergenic foods, milk is typically consumed in diverse forms several times per day, and a total daily dose that could be high may be not tolerated, especially in the presence of anaphylaxis co-factors [39]. Cow milk tolerance can spontaneously occur in the first years of life; thus, the faster tolerance we observed in most of the patients could be a consequence of both immune modulations via OIT with baked milk or may be due to a milder phenotype of CMA. However, this strategy induced a good rate of response to OIT in patients with anaphylaxis as a primary manifestation in the present study; thus, we may suppose that baked milk may accelerate, in a safe manner, the induction of tolerance to milk.

A double-blind placebo0controlled food challenge (DBPCFC) remains the gold standard for a positive diagnosis of CMA, but it is time consuming, expensive, can only be performed under medical guidance, only in specialized clinics, and it has a high risk of inducing severe anaphylactic reactions [9,30]. In addition, the quality of life of patients is affected when they experience a positive challenge test, and for this reason, may refuse to follow an OFC or oral immunotherapy [40]. Development of molecular biology in the last 10 years has permitted an increase in the accuracy of the diagnosis without referring the patient to a DBPCFC. Measurement of sIgE to different allergenic proteins from milk permits an identification of patterns of sensitization in complex polysensitized patients and is useful in identifying different phenotypes of CMA [41].

The present study showed that high levels of sIgE to milk and casein may predict the persistence of CMA despite oral immunotherapy. Previous data showed that patients with persistent CMA have higher values of sIgE to milk than those that can respond to oral immunotherapy [33,42,43], and it may predict the long-term outcome of milk OIT [20,42]. Kuitunen et al. also [33] demonstrated that high basal levels of sIgE to casein, alpha-lactalbumin and betalactoglobulin before the start of OIT were associated with a lower maintenance dose reached at the end of OIT. In addition, Savilahti EM et al. [44] reported that a high level of sIgE to milk and casein could predict a failure to achieve desensitization in milk OIT. It is also important to identify a value of sIge that might predict the resolution of CMA. We calculated a cut off value of 2.5 kU/L for sIgE to milk with 45.76% sensitivity and 100% specificity, but the size of the wheal in the skin prick test was not a predictive marker for OIT outcome. Yavuz ST et al. [45] reported that children with sIgE to milk below 6 kU/L outgrew CMA earlier than those with higher levels. In our cohort, the cut off value for sIgE was lower, but the outcome was to predict the resolution and not the interval of time after which we obtained it.

Component-resolved diagnostics before OIT can help to identify children with a lower probability of a successful OIT outcome. sIgE to casein over 11.73 kU/L predicted a failure of achieving tolerance after OIT, with a sensitivity of 93% and a specificity of 60% in the present study. Kuitunen et al. [33] also reported that sIgE to milk allergens might have a better role in predicting resolution of CMA after OIT compared to other markers. It is essential to establish the role of these markers in order to identify candidates for OIT with a good resolution rate. Patient data derived from modern technology, in combination with a classical approach through the patient's history, can be translated into patient-tailored interventions.

The main strength of the paper is that it presents a clinical and biological analysis of a cohort of patients with IgE-mediated CMA in Romania. The present study offers information from basal evaluation of patients with CMA that might predict the success of a medical intervention in those patients, allowing them to have a better quality of life. The study identifies some aspects that could be improved in the management of CMA. There are also some limitations of the study. First, OFC was not performed in all the patients to measure the exact amount of milk that produces clinical reactivity, and because of this reason, the OIT started in the same way in all included patients. Second, the evaluation of the children was performed at different ages not immediately after the onset of CMA. Most of the parents postponed the evaluation of their children until the moment of entrance in kindergarten or in school to see if they had a risk for severe reactions if an accidental exposure to milk might occur. Third, there was no control group in the present study. It would be interesting to have the possibility to evaluate the patients with mild forms of CMA and to compare the natural resolution of CMA with the active intervention (oral immunotherapy or rapid reintroduction of baked milk). Patients with mild forms of CMA were under pediatrician surveillance, and they followed an eviction diet, which is sometimes a long-term attitude, and they do not benefit from an active intervention.

5. Conclusions

Anaphylaxis with only skin and mucosal involvement represents one of the most frequent manifestations in children with IgE-mediated CMA, although severe anaphylaxis may be present as an initial manifestation of CMA. Basal values of sIgE for milk and casein predict the occurrence of tolerance to milk after 2-year follow up in patients with CMA, including those with anaphylaxis as the first manifestation. OIT, or a rapid reintroduction with baked milk, may be used as an approach for CMA with IgE-mediated mechanisms, and it may result in the induction of tolerance faster and in a higher percentage of patients, allowing for a normal diet without any restrictions.

Author Contributions: Conceptualization, I.A.M., E.D., I.C.B., and D.D.; methodology, I.A.M., I.C.B., L.K.W., A.D.B., and D.D.; validation, A.D.B. and D.D.; formal analysis, I.A.M., I.C.B., I.P., L.K.W., C.T.D., and C.U.; investigation, I.A.M., I.C.B., I.P., C.T.D., E.D., L.K.W., and C.U.; resources, I.A.M., I.C.B., and D.D.; data curation, I.A.M., I.C.B., I.P., C.T.D., and C.U.; writing—original draft preparation, I.A.M., I.C.B., L.K.W., and C.U.; writing—review and editing, I.A.M., I.C.B., E.D., D.D., and A.D.B.; supervision, D.D. and A.D.B.; project administration, I.A.M., L.K.W., I.C.B., and D.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study protocol was approved by the University Ethics Committee of the University of Medicine and Pharmacy (293/28 July 2013).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Flom, J.D.; Sicherer, S.H. Epidemiology of Cow's Milk Allergy. *Nutrients* **2019**, *11*, 1051. [CrossRef] [PubMed]
2. Fiocchi, A.; Schunemann, H.J.; Brozek, J.; Restani, P.; Beyer, K.; Troncone, R.; Martellini, A.; Terracciano, L.; Bahna, S.L.; Rance, F.; et al. Diagnosis and Rationale for Action Against Cow's Milk Allergy (DRACMA): A summary report. *J. Allergy Clin. Immunol.* **2010**, *126*, 1119–1128, e1112. [CrossRef] [PubMed]
3. Rona, R.J.; Keil, T.; Summers, C.; Gislason, D.; Zuidmeer, L.; Sodergren, E.; Sigurdardottir, S.T.; Lindner, T.; Goldhahn, K.; Dahlstorm, J.; et al. The prevalence of food allergy: A me-ta-analysis. *J. Allergy Clin. Immunol.* **2007**, *120*, 638–646. [CrossRef] [PubMed]
4. Burney, P.G.J.; Potts, J.; Kummeling, I.; Mills, E.N.C.; Clausen, M.; Dubakiene, R.; Barreales, L.; Pérez, C.F.; Fernandez-Rivas, M.; Le, T.-M.; et al. The prevalence and distribution of food sensitization in European adults. *Allergy* **2013**, *69*, 365–371. [CrossRef]
5. Lyons, S.A.; Clausen, M.; Knulst, A.C.; Ballmer-Weber, B.K.; Fernandez-Rivas, M.; Barreales, L.; Bieli, C.; Dubakiene, R.; Fernandez-Perez, C.; Jedrzejczak-Czechowicz, M.; et al. Prevalence of Food Sensitization and Food Allergy in Children Across Europe. *J. Allergy Clin. Immunol.* **2020**, *8*, 2736–2746.e9. [CrossRef]
6. Taniuchi, S.; Takahashi, M.; Soejima, K.; Hatano, Y.; Minami, H. Immunotherapy for cow's milk allergy. *Hum. Vaccin. Im-munother.* **2017**, *13*, 2443–2451. [CrossRef]
7. Winberg, A.; West, C.E.; Strinnholm, A.; Nordström, L.; Hedman, L.; Rönmark, E. Assessment of allergy to milk, egg, cod, and wheat in Swedish schoolchildren: A Population Based Cohort Study. *PLoS ONE* **2015**, *10*, e0131804. [CrossRef]
8. Kattan, J.D.; Cocco, R.R.; Jarvinen, K.M. Milk and soy allergy. *Pediatr. Clin. N. Am.* **2011**, *58*, 407–426. [CrossRef]
9. Hochwallner, H.; Schulmeister, U.; Swoboda, I.; Spitzauer, S.; Valenta, R. Cow's milk allergy: From allergens to new forms of diagnosis, therapy and prevention. *Methods* **2014**, *66*, 22–33. [CrossRef]
10. Kim, Y.H.; Kim, Y.S.; Park, Y.; Kim, S.Y.; Kim, K.W.; Kim, H.S.; Sohn, M.H. Investigation of Basophil Activation Test for Diagnosing Milk and Egg Allergy in Younger Children. *J. Clin. Med.* **2020**, *9*, 3942. [CrossRef]
11. Sampson, H.A.; Van Wijk, R.G.; Bindslev-Jensen, C.; Sicherer, S.; Teuber, S.S.; Burks, A.W.; Duboi, A.E.J.; Beyer, K.; Eigenmann, P.A.; Spergel, J.M.; et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. *J. Allergy Clin. Immunol.* **2012**, *130*, 1260–1274. [PubMed]
12. Komata, T.; Soderstrom, L.; Borres, M.P.; Tachimoto, H.; Ebisawa, M.J. The predictive relationship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. *J. Allergy Clin. Immunol.* **2007**, *119*, 1272–1274. [CrossRef] [PubMed]
13. Skripak, J.M.; Matsui, E.C.; Mudd, K.; Wood, R.A. The natural history of IgE-mediated cow's milk allergy. *J. Allergy Clin. Immunol.* **2007**, *120*, 1172–1177. [CrossRef] [PubMed]
14. Spergel, J.M. Natural history of cow's milk allergy. *J. Allergy Clin. Immunol.* **2013**, *131*, 813–814. [CrossRef] [PubMed]
15. Santos, A.; Dias, A.; Pinheiro, J.A. Predictive factors for the persistence of cow's milk allergy. *Pediatr. Allergy Immunol.* **2010**, *21*, 1127–1134. [CrossRef]
16. Shu, S.-A.; Yuen, A.W.T.; Woo, E.; Chu, K.-H.; Kwan, H.-S.; Yang, G.-X.; Yang, Y.; Leung, P.S.C. Microbiota and Food Allergy. *Clin. Rev. Allergy Immunol.* **2019**, *57*, 83–97. [CrossRef]
17. Brożek, J.L.; Terracciano, L.; Hsu, J.; Kreis, J.; Compalati, E.; Santesso, N.; Fiocchi, A.; Schunemann, H.J. Oral immunotherapy for IgE-mediated cow's milk allergy: A systematic review and meta-analysis. *Clin. Exp. Allergy* **2012**, *42*, 363–374. [CrossRef]
18. Nowak-Węgrzyn, A.; Sampson, H.A. Future therapies for food allergies. *J. Allergy Clin. Immunol.* **2011**, *127*, 558–573. [CrossRef]
19. Muntean, I.A.; Bocsan, I.C.; Deleanu, D. Oral Immunotherapy to Wheat in Allergic Asthmatic Female: A Case Report. *Iran. J. Allergy Asthma Immunol.* **2018**, *17*, 291–294.
20. Schoos, A.M.; Bullens, D.; Chawes, B.L.; Costa, J.; De Vlieger, L.; DunnGalvin, A.; Epstein, M.M.; Garssen, J.; Hilger, C.; Knipping, K.; et al. Immunological Outcomes of Al-lergen-Specific Immunotherapy in Food Allergy. *Front. Immunol.* **2020**, *11*, 568598. [CrossRef]
21. Heinzerling, L.; Mari, A.; Bergmann, K.C.; Bresciani, M.; Burbach, G.; Darsow, U.; Durham, S.; Fokkens, W.; Gjomarkaj, M.; Haahtela, T.; et al. The skin prick test—European standards. *Clin. Transl. Allergy* **2013**, *3*, 3. [CrossRef] [PubMed]
22. Lifschitz, C.; Szajewska, H. Cow's milk allergy: Evidence-based diagnosis and management for the practitioner. *Eur. J. Pediatr.* **2015**, *174*, 141–150. [CrossRef] [PubMed]
23. Cummings, A.J.; Knibb, R.; King, R.M.; Lucas, J.S. The psychosocial impact of food allergy and food hypersensitivity in children, adolescents and their families: A review. *Allergy* **2010**, *65*, 933–945. [CrossRef] [PubMed]
24. Carraro, S.; Frigo, A.; Perin, M.; Stefani, S.; Cardarelli, C.; Bozzetto, S.; Baraldi, E.; Zanconato, S. Impact and oral immunotherapy on quality of life in children with cow milk allergy: A pilot study. *Int. J. Immunopathol. Pharmacol.* **2012**, *25*, 793–798. [CrossRef]
25. Efthymiou, D.; Panayi, P.; Feketea, G.; Pitsios, C.; Muntean, I.A.; Vassilopoulou, E. Alliance with the School Personnel Is Crucial for the Management of Food Allergy and Anaphylaxis in School Children. *Foods* **2021**, *10*, 2083. [CrossRef]
26. Luyt, D.; Ball, H.; Makwana, N.; Green, M.R.; Bravin, K.; Nasser, S.M.; Clark, A.T. BSACI guideline for the diagnosis and management of cow's milk allergy. *Clin. Exp. Allergy* **2014**, *44*, 642–672. [CrossRef]
27. Karaman, S.; Erdem, S.B.; Nacaroğlu, H.T.; Karkiner, C.; Can, D. The quantity of unheated milk tolerated as a predictor of tolerance to baked milk. *Asian Pac. J. Allergy Immunol.* **2019**. [CrossRef]

28. Nowak-Wegrzyn, A.; Lawson, K.; Masilamani, M.; Kattan, J.; Bahnson, H.T.; Sampson, H.A. Increased Tolerance to Less Extensively Heat-Denatured (Baked) Milk Products in Milk-Allergic Children. *J. Allergy Clin. Immunol.* **2017**, *6*, 486–495.e5. [CrossRef]
29. Schoemaker, A.A.; Sprikkelman, A.B.; Grimshaw, K.E.; Roberts, G.; Grabenhenrich, L.; Rosenfeld, L.; Siger, S.; Dubakiene, R.; Rudzeviciene, O.; Reche, M.; et al. Incidence and natural history of challenge-proven cow's milk allergy in European children—EuroPrevall birth cohort. *Allergy* **2015**, *70*, 963–972. [CrossRef]
30. Koletzko, S.; Niggemann, B.; Arato, A.; Dias, J.A.; Heuschkel, R.; Husby, S.; Mearin, M.L.; Papadopoulou, A.; Ruemelle, F.M.; Staiano, A.; et al. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. *J. Pediatr. Gastroenterol. Nutr.* **2012**, *55*, 221–229. [CrossRef]
31. Pajno, G.B.; Fernandez-Rivas, M.; Arasi, S.; Roberts, G.; Akdis, C.A.; Alvaro-Lozano, M.; Beyer, K.; Binslev-Jensen, C.; Burks, W.; Ebisawa, M.; et al. EAACI Guidelines on al-lergen immunotherapy: IgE-mediated food allergy. *Allergy* **2018**, *73*, 799–815. [CrossRef] [PubMed]
32. García-Ara, C.; Pedrosa, M.; Belver, M.T.; Martín-Muñoz, M.F.; Quirce, S.; Boyano-Martínez, T. Efficacy and safety of oral desensitization in children with cow's milk allergy according to their serum specific IgE level. *Ann. Allergy Asthma Immunol.* **2013**, *110*, 290–294. [CrossRef] [PubMed]
33. Kuitunen, M.; Englund, H.; Remes, S.; Movérare, R.; Pelkonen, A.; Borres, M.P.; Mäkelä, M.J. High IgE levels to α-lactalbumin, β-lactoglobulin and casein predict less successful cow's milk oral immunotherapy. *Allergy* **2015**, *70*, 955–962. [CrossRef] [PubMed]
34. Høst, A.; Halken, S.; Hp, J.; Ae, C.; Am, H. Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. *Pediatr. Allergy Immunol.* **2002**, *13*, 23–28. [CrossRef] [PubMed]
35. Bishop, J.; Hill, D.; Hosking, C. Natural history of cow milk allergy: Clinical outcome. *J. Pediatr.* **1990**, *116*, 862–867. [CrossRef]
36. Nowak-Wegrzyn, A.; Bloom, K.A.; Sicherer, S.H.; Shreffler, W.G.; Noone, S.; Wanich, N.; Sampson, H.A. Tolerance to extensively heated milk in children with cow's milk allergy. *J. Allergy Clin. Immunol.* **2008**, *122*, 342–347.e2. [CrossRef] [PubMed]
37. Barbosa, C.P.G.; Castro, A.P.M.; Yonamine, G.H.; Gushken, A.K.F.; Beck, C.M.L.; Macedo, P.R.C.; Dorna, M.B.; Santos, C.J.N.; Pastorina, A.C.; Jacob, C.N.A. Baked milk tolerant patient: Is there any special feature? *Allergol. Immunopathol.* **2017**, *45*, 283–289. [CrossRef]
38. Esmaeilzadeh, H.; Alyasin, S.; Haghighat, M.; Nabavizadeh, H.; Esmaeilzadeh, E.; Mosavat, F. The effect of baked milk on accelerating unheated cow's milk tolerance: A control randomized clinical trial. *Pediatr. Allergy Immunol.* **2018**, *29*, 747–753. [CrossRef]
39. Keet, C.A.; Seopaul, S.; Knorr, S.; Narisety, S.; Skripak, J.; Wood, R.A. Long-term follow-up of oral immunotherapy for cow's milk allergy. *J. Allergy Clin. Immunol.* **2013**, *132*, 737–739.e6. [CrossRef]
40. Franxman, T.J.; Howe, L.; Teich, E.; Greenhawt, M.J. Oral Food Challenge and Food Allergy Quality of Life in Caregivers of Children with Food Allergy. *J. Allergy Clin. Immunol.* **2014**, *3*, 50–56. [CrossRef]
41. Linhart, B.; Freidl, R.; Elisyutina, O.; Khaitov, M.; Karaulov, A.; Valenta, R. Molecular Approaches for Diagnosis, Therapy and Prevention of Cow´s Milk Allergy. *Nutrients* **2019**, *11*, 1492. [CrossRef] [PubMed]
42. Kauppila, T.K.; Paassilta, M.; Kukkonen, A.K.; Kuitunen, M.; Pelkonen, A.S.; Mäkelä, M. Outcome of oral immunotherapy for persistent cow's milk allergy from 11 years of experience in Finland. *Pediatr. Allergy Immunol.* **2019**, *30*, 356–362. [CrossRef] [PubMed]
43. Cohen, C.G.; Zhao, W.W.; Ke, D.; Beaudette, L.; Lejtenyi, D.; McCusker, C.; Zhang, X.; Chan, E.S.; Upton, J.E.; Grunebaum, E.; et al. Elevated Cow's Milk–Specific IgE Levels Prior to Oral Immunotherapy Decrease the Likelihood of Reaching the Maintenance Dose. *J. Allergy Clin. Immunol.* **2021**, *10*, 215–221.e2. [CrossRef] [PubMed]
44. Savilahti, E.M.; Savilahti, E. Development of natural tolerance and induced desensitization in cow's milk allergy. *Pediatr. Allergy Immunol.* **2013**, *24*, 114–121. [CrossRef]
45. Yavuz, S.T.; Buyuktiryaki, B.; Sahiner, U.M.; Birben, E.; Tuncer, A.; Yakarisik, S.; Karabulut, E.; Kalayci, O.; Sackesen, C. Factors that predict the clinical re-activity and tolerance in children with cow's milk allergy. *Ann. Allergy Asthma Immunol.* **2013**, *110*, 284–289. [CrossRef]

BMC Pulmonary Medicine

RESEARCH

Open Access

A retrospective study regarding the influence of COVID-19 disease on asthma

Ioana Adriana Muntean[1], Polliana Mihaela Leru[2*], Irena Pintea[1], Ioana Corina Bocsan[3], Carmen Teodora Dobrican[1] and Diana Deleanu[1]

Abstract

Background During the Covid-19 pandemic patients suffering from asthma raised many concerns regarding the outcome ofthe impact of COVID-19 disease on their preexisting condition. The 2021 GINA report indicates that people with asthma do not appear to be at increased risk of a severe form of COVID-19.

Method This study is a retrospective study of patients (n = 163) median age = 27.8 years, M:F = 1:1.26, with asthma evaluated using ACT (asthma control test) and VAS (visual analog scale) before and after COVID-19 disease. An ACT score over 20 points placed patients in the controlled asthma group.

Results The overall evaluation for COVID-19 in our asthma patients revealed that 22.7% of the studied group had the COVID-19 disease (21.5% in the controlled asthma group and 24.5% in uncontrolled asthma group). Asthma disease history was longer in the uncontroled asthma group (128 ± 96.8 months vs. 296 ± 59.7 months, $p = 0.05$). Asthma treatment was conducted according to the GINA guideline, and 18.4% (30 pts) of the patients were on allergen immunotherapy treatment. Significantly more uncontrolled patients were significantly more in Step 1 and 5 of treatment ($p = 0.05$ and $p = 0.03$). During the COVID-19 pandemic, patients in the GINA step 5 of treatment experienced a worsening of asthma, often twice as severe as compared to patients with asthma in GINA step 1–4. In these patients, even mild COVID-19 disease led to worsened asthma symptoms, while severe COVID-19 led to a severe asthma impairment measured by ACT score ($p = 0.03$) and VAS scale ($p = 0.02$), with increased oral corticosteroids consumption.

Conclusion Maintaining optimal asthma control should be able to reduce risk of severe outcomes after COVID-19 disease. Communication via phone with the specialist involved in their asthma care was very comforting for patients, thus confirming the necessity to include phone calls, smart phone's application or online evaluations and counseling in long-term care of chronic diseases.

Keywords Asthma, COVID-19 pandemic, Chronic diseases, Long-term care, SARS-CoV-2 infection

*Correspondence:
Polliana Mihaela Leru
polianaleru@yahoo.com
[1]Allergology Department, Department of Allergology and Immunology and "Octavian Fodor" Institute of Gastroenterology and Hepatology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400347 Cluj-Napoca, Romania.
[2]Clinical Department 5, "Carol Davila" University of Medicine and Pharmacy, Bulevardul Eroii Sanitari 8, 050474 Bucharest, Romania.
[3]Department of Pharmacology, Toxicology and Clinical Pharmacology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400347 Cluj-Napoca, Romania.

Background

Asthma is a heterogenous disease, usually characterized by chronic inflammation of the airways. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, cough that may vary over time and in intensity, together with the variable airflow limitation [1]. During the Covid-19 pandemic, patients suffering from asthma raised concernes and raised many questions regarding their increased risk in

case of SARS-CoV2 infection [2]. The 2021 GINA report and the recent metanalysis indicate that people with asthma do not appear to be at increased risk of acquiring the SARS-CoV2 infection or having a severe form of disease or an increased death rate due to the COVID-19 [1, 2]. The risk of death is related to the recent course of oral corticosteroids administered for uncontrolled asthma [3].

The COVID-19 disease is an acute respiratory syndrome that emerged in the city of Wuhan and rapidly spread throughout the world causing a global pandemic. The Center for Disease Control and Prevention as well as the American Academy of Allergy, Asthma & Immunology consider asthma a risk factor for severe COVID-19 [4]. Coronaviruses are among the top 5 viruses isolated during acute asthma exacerbations, with a higher prevalence in adults [5]. The COVID-19 disease presents respiratory symptoms, from mild to severe, and a significant percentage of patients develop acute respiratory distress syndrome (ARDS) with the Delta variant of the virus and less with Omicron. Severe symptoms are associated with a true cytokine storm, in particular elevation of IL-6, having death as one of the outcomes [6]. Old age and underlying morbidities, such as cardio-vascular diseases, in particular hypertension and metabolic disorders (obesity and diabetes), have been identified as significant risk factors for COVID-19 morbidity and mortality [7, 8]. Unlike asthma, which is not included in the common comorbidities for COVID-19, COPD is considered a risk factor for worse outcomes when infected with SARS-CoV-2. Therefore, COPD is one of the most common comorbidities worldwide, besides other important comorbidities such as chronic kidney diseases (CKD) under conservative treatment or renal replacement therapy (RRT) [9, 10]. The real impact of SARS-CoV-2 on asthma control is still unclear and may be variable in different countries and subgroups of patients.

The current understanding of the interactions between SARS-CoV-2 and asthma is still in the early stages, while observational and experimental data are still awaited to elucidate the relationship between COVID-19 and asthma [11]. Considering the relatively high prevalence of asthma, it is reasonable to hypothesize that asthmatic individuals are relatively resistant to COVID-19 because of the disease characteristics and/or the conventional treatment for asthma. Those patients also protected themselves more using social distancing and facial mask, thus experienced less asthma exacerbations due to distinct viruses [1–4]. The aim of this paper is to assess how asthma patients managed their disease during the pandemic, being monitored by their specialist through based on phone calls or emails and to evaluate the impact of the SARS-CoV-2 virus infection on asthma outcome.

Methods

Study design

This study is a retrospective study of patients (n = 163) with asthma diagnosis treated in a public institution: Allergy Department of the Professor Doctor Octavian Fodor Regional Institute of Gastroenterology and Hepatology, (Cluj-Napoca, Romania), between February 2017 and January 2022. The study protocol was approved by the "Octavian Fodor" Institute of Gastroenterology and Hepatology Ethics Committee (Approval no. 364/11.01.2021), and all the patients signed the informed consent before any assessments were done. Inclusion criteria was the diagnosis of asthma according to the GINA guideline, for at least 1 year. The exclusion criteria were as follows: COPD, systemic autoimmune diseases, chronic infections (e.g., viral B hepatitis, viral C hepatitis which continue to be widespread in Romania) [12].

The total number of patients in our Allergy clinic exceeds 2500 patients/year (outpatient and hospitalized) in the Allergy Department. In 2020 due to the pandemic restrictions there were only 1700 patients. The prevalence of asthma patients among our patients, during the 5 years of evaluation was 1.4%. In Romania the prevalence of asthma according to the ISAAC study was over 15% in school age children, with no data available in adults [13].

Patients and clinical evaluation

Asthma was diagnosed according to the GINA guideline based on, history of typical symptoms: wheeze, shortness of breath, chest tightness, cough, and spirometry with a positive bronchodilator test of 12% and 200 ml increased of FEV1. All the asthma patients over 5 years old were included in this retrospective study. The studied group included 1% of allergic asthmatic children, but the main target patients were adults and adolescents over 12. Patients were from urban areas, but the social-economic status or the environmental exposure to allergen or tobacco smoke was not evaluated in the present study.

The asthma diagnosis was made at least 1 year before the date of the inclusion in the present study. The diagnosis included a previous spirometry with positive bronchodilator test when FEV1 was under 85% from the predicted value. Since spirometry was considered a nebulization method which may increase viral particle spreading, in asthmatic patients who were monitored during COVID-19 pandemic, performing spirometry was not in accordance with international recommendations. Age, sex, and residence (rural/urban), sensitization for inhaled allergen and asthma related symptoms were recorded. Patients were assessed for asthma control monthly using Visual Analog Scale (VAS) for QoL (quality of life) assessment, which was a simple and easy to understand method. Interviews with the patients were by phone or

online as well as face to face duringclinical examination in severe symptomatic patients. In all patients with asthma the stepwise treatment used was according to the GINA guideline [14].

The Asthma Control Test (ACT) is a friendly and accurate asthma control evaluation tool for adults and children. Based on their ACT results patients were included in 2 groups: controlled (ACT ≥ 20 points) and uncontrolled (ACT < 20 points) [15]. Also, VAS scale was used to assess the QoL, in Allergic rhinitis, though it can be used in various chronic diseases [16].

Patients evaluation during the pandemic

All the patients were called or e-mailed, and asked tocomplete VAS and ACT forms, monthly, at home as showed in Table 1. They later sent the results by text message or e-mail. Some of the patients required a face-to-face consultation following the COVID-19 disease, due to asthma severity, to step-up asthma treatment according to GINA recommendations. The asthma outcomes used in the present study were based on subjective methods like the ACT questionnaire and the VAS scale. 'Improvement' was defined by a 3-points ACT increase, 'unchanged' was defined as a ± 2-point ACTchange, and 'worsening' or 'exacerbation' corresponded to a 3 points ACT decrease or in case when hospitalization for respiratory symptoms was required.

Skin prick tests (SPT)

The diagnosis of allergy was established through a skin prick test, according to international guidelines [17]. The allergen panel included international recommendation and particularities of exposure to the following allergens in Romania: Dermatophagoides pteronyssinus, Dermatophagoides farinae, grass pollens, cereals pollen, birch pollen, hazel pollen, cat and dog dander, Alternaria Alternata, Artemisia vulgaris and Ambrosia elatior. SPT was performed at the beginning of the study using standardized allergen extracts (Hal Allergy, Netherlands).

The COVID-19 disease evaluation

COVID-19 disease severity was defined by using the scale provided by the World Health Organization (SpO2 < 94% on room air at sea level, PaO2/FiO2 < 300 mm Hg, a respiratory rate > 30 breaths/min, or lung infiltrates > 50%) [18]. In our clinic we just evaluated the asthma after the infection, the Covid-19 severity diagnosis was taken from medical fies. All the patients with asthma who had Covid-19 disease symptoms were diagnosed using PCR for COVID-19, laboratory tests and CT-scans in a COVID-19 Department at the Infectious Disease Hospital from Cluj-Napoca, not in our Department of Allergology. We analyzed the data from patient's files only retrospectively, calling or emailing the patients to obtain data and the medical documents they received after the evaluation in the COVID-19 Department at the Infectious Disease Hospital. Laboratory tests (complete blood count, CRP, e.g.) were evaluated in our department after 14 days of quarantine, though it was not possible to get tests for all the patients because some with mild forms did not go to hospital, as only GPs evaluated and diagnosed them. All the patients were called and were advised to inform our office if they suspected SARS-CoV-2 infection.

Biological evaluation

The blood tests results obtained à jeun in all patients were: complete blood count including eosinophils by using the SYSMEX-XN-1000 analyzer and CRP, LDH, Troponin tests by using the COBAS PRO C 503/E 801analyzer. The lab test values were obtained from patient's files between day 10 and day 15 after the positive SARS-CoV-2 RT-PCR was confirmed or by the direct evaluation of 30 patients with mild forms of COVID-19 disease on the 15th day.

Table 1 Questions asked during online or phone consultation

Question	Response
ACT	1–5 points
1. In the last 4 weeks, how much of your time did your asthma keep you from getting as much done at work, at school or at work?	
2. In the last 4 weeks, how often have you had shortness of breath?	
3. In the last 4 weeks, how often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness) wake you up at night or earlier than usual in the morning?	
4. In the last 4 weeks, how often have you used your rescue inhaler or nebulizer medication (salbutamol/formoterol + ICS)?	
5.How did you rate your asthma control during the past 4 weeks?	
VAS	0–10 points
How much has your asthma had bothered you (0- no bothersome to 10- extremely bothersome)	
Presence of COVID-19 disease in the last month	Yes/No

Statistical analysis

The statistical analysis was performed using the SPSS version 21 (Chicago, IL, USA) and Microsoft Excel. Data was labeled as nominal, expressed as percentages, and continuous variables. The differences were assessed within groups by the Wilcoxon Signed Rank test and between groups by the Student t test. The Spearman' coefficient of correlation was calculated to highlight differences between continuous variables. The level of statistical significance was set at $p < 0.05$.

Results

Patients' demographic data are presented in Table 2. The treatment data of the studied group of asthma patients during SARS-CoV-2 pandemic (February 2020–April 2021) are presented in Fig. 1. All the asthmatic patients aged over 5 years were included in this study. The Median age was 27.8 (5–85) years, and the sex ratio M:F was 1:1.26. The 73 patients who had ACT score equal or greater than 20 were included in the controlled asthma group and 90 patients (55.2%) were included in uncontrolled asthma group. All patients had undergone SPT for inhaled allergens during their initial evaluation, according to the international guidelines. Of the total number of patients, there were only 9.8% non-atopic, lower than the reported 40% non-atopic ones in the Step-5 GINA guideline.

Asthma treatment was administered according to GINA guideline, and 18.4% (30 pts) of the patients were on allergen immunotherapy treatment (AIT) of which 80% for house dust mites and 20% for other allergens (cat, pollens). In Romania, AIT is not reimbursed by the National Insurance System, so just few of the allergic patients afforded to pay for the treatment, due to socio-economic status. No impairment in asthma symptoms was registered in patients with AIT who had COVID-19 disease.

Table 2 Demographic data of the patients

Parameter	Controlled asthma (n = 73)	Uncontrolled asthma (n = 90)	*p*
Age	21.05 ± 10.2	29.59 ± 19.7	0.6
Sex			
Male	61.6% (45)	30% (27)	0.01
Female	38.4% (28)	70% (63)	0.01
Living area			
Urban	78.2% (62)	68.4% (69)	0.7
Rural	21.8% (11)	31.6% (21)	0.8
Asthma duration (months)	128 ± 96.8	296 ± 59.7	0.05
Rhinitis present	80.8% (59)	77.7% (70)	0.9
Asthma scores			
ACT	22 ± 2	13 ± 5	0.01
VAS	8 ± 2	3 ± 4	0.01
Asthma GINA steps treatment			
Step 1	9.6% (7)	36.6% (33)	0.05
Step 2	19.2% (14)	12.2% (11)	0.8
Step 3	34.2% (25)	6.8% (6)	0.04
Step 4	27.4% (20)	21.1% (19)	0.9
Step 5	5.6% (4)	23.3% (21)	0.03
COVID 19 infection			
No present	79.5% (58)	75.5% (68)	0.9
Present	21.5% (15)	24.5% (22)	0.6

Statistical significance is at $p \leq 0.05$

The COVID-19 disease in Asthma Patients

The overall evaluation for COVID-19 disease in our asthma patients showed that only 22.7% of the studied group were infected (21.5% in the controlled asthma group and 24.5% in uncontrolled asthma group). Of the COVID-19 cases, 80% were mild. No asthma exacerbation was observed in allergen immunotherapy group was observed.

The inflammatory markers in COVID-19 of asthma patients are described in Table 3, showing significant statistical differences between mild and moderate/severe forms of disease.

Three patients in omalizumab group and one patient in benralizumab group presented SARS CoV-2 infection, with a mild to moderate form of disease and one hospital admission (non-vaccinated patient) in omalizumab group. The non-vaccinated 80 years old patient died 6 months later due to stroke, and this was the only death reported in our asthmatic patients group. All the patients experienced a worsening of asthma symptoms and received a 2–3-week course of oral corticosteroids or a step up if they were in step 1–4.

We use phone consultation to evaluate the patients and all our patients had an emergency kit for asthma exacerbation at home containing oral corticosteroids, and, in hospitalized patients a phone contact with the doctor involved in the case was maintained.

Patients in the GINA step 5 of treatment experienced a worsening of asthma, often twice as severe as compared to patients with asthma in GINA step 1–4. In patients with mild COVID-19 the worsening of asthma was treated with step-up treatment according to GINA. Moderate/severe COVID-19 cases were treated according to WHO recommendation in infectious clinic diseases, the treatment including oral corticosteroids, antibiotics, and oxygen. Asthma course developments during COVID-19 disease is described in Tables 4 and 5. Due to similar symptoms of asthma exacerbation and COVID-19, such as dyspnea, cough and chest tightness, worsening

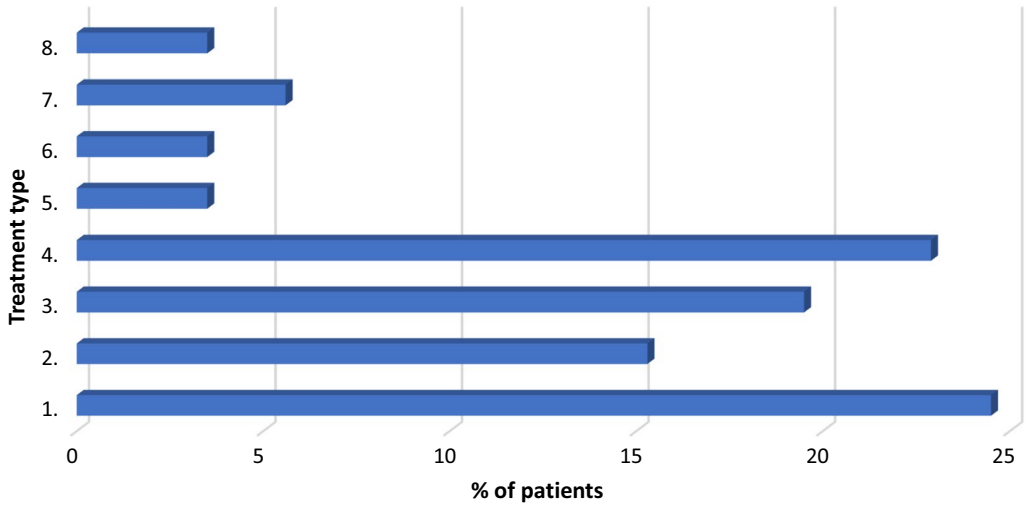

Fig. 1 Pharmacological treatment use for asthmatic patients according to GINA guideline. x-axis (%): number of patients on mentioned type of medication; y-axis (type of medication): (1) as needed ICS and LABA/SABA, (2) ICS low-dose or montelukast, (3) ICS low-dose and LABA, (4) ICS medium-dose and LABA, (5) ICS high-dose and LABA, (6) ICS high-dose and LABA and LAMA, (7) ICS high-dose and omalizumab, (8) ICS high-dose and benralizumab

Table 3 Covid-19 disease in asthma patients' group

Parameter	Mild COVID-19 infection n = 30	Moderate/severe COVID-19 infection n = 7	*p*
Leucocytes 10^3/μL	10.54 (± 9.4)	4.59 (± 2.1)	0.03
CRP (mg/dl)	4.2 (± 5.1)	68.7 (± 42.6)	0.01
Troponin (μg/L)	0.1 (± 0.2)	2.3 (± 1.8)	0.04
LDH (U/l)	538 (± 175.6)	757 (± 195.8)	0.04
Eosinophils 10^3/μL	0.35 (± 0.2)	0.26 (± 0.21)	0.06
ACT	19 (± 5.2)	12 (± 3.8)	0.03
VAS	9 (± 0.8)	7 (± 1.4)	0.02

Data are expressed as mean ± SD. Statistical significance is at $p \leq 0.05$

Table 4 Asthma course influenced by COVID-19 disease

Asthma course	Mild COVID-19 disease (n = 30)	moderate/severe COVID-19 disease (n = 7)
Improvement	1	0
No change	13	0
Uncontrolled/Exacerbation	16	7

Table 5 Asthma in step 5 GINA treatment course influenced by COVID-19 disease

Asthma in step 5 GINA course	mild COVID-19 disease (n = 19)	moderate/severe COVID-19 disease (n = 5)
Improvement	0	0
No change	6	0
Uncontrolled/Exacerbation	13	5

of asthma symptoms was hard to evaluate. No asthma related deaths were reported in this group. Patients in GINA step 5 of treatment (high dose ICS with/without biologicals) were 80% vaccinated, and 20% non-vaccinated. In the non-vaccinated group there was a non-asthma related death 6 months after COVID-19 infection and none in vaccinated group.

Discussion

Asthma is a heterogenous disease with an important social impact and increased costs. Many factors, mainly viral infections, can lead to asthma exacerbations. [1, 19]. The main goals of asthma management are to optimize control of asthma symptoms and to reduce the risk of asthma exacerbation and hospitalization, while minimizing medication adverse effects, especially referred to oral corticosteroids [20]. Asthma is not included in the risk factors for severe COVID-19, but the impact of SARS-CoV-2 virus and its variants on asthma is still being studied, some of the available research showing that asthma may complicate the COVID-19 and conversely [21].

Coronaviruses respiratory infection may lead to asthma exacerbation. It is still unclear how and if SARS-CoV-2 influences the outcome of asthma patients. [10, 20, 21]. Currently, the patients with underlying moderate to severe asthma are a risk group for severe COVID-19 and/or asthma exacerbation [22] mainly evaluated in Delta

variant of the virus and less in Omicron. For patients with asthma there was an overlap with the symptoms of COVID-19, including cough, shortness of breath and chest tightness, consequently it was difficult to distinguish these from those of severe asthma exacerbation [23].

The role of mast cells (MCs) in coronavirus-induced disease have been discussed since the beginning of the Toll-like receptor 3 detection of viral double-stranded ribonucleic acid (RNA), viral sphingosine-1-phosphate (S1P) binding to S1P receptors, and retinoic acid-induced gene I (RIG-I) recognition of uncapped viral RNA). Mast cells express angiotensin converting enzyme 2 receptor (ACE2), now known as the principal receptor for SARS-CoV-2, thus defining a route by which mast cells could also become one of the hosts for this virus, and exacerbate mast-cell related diseases [21–23]. Some researchers claim that allergic diseases could be protective in terms of infection severity from COVID-19, which could be explained by the evidence that ACE-2 receptor is downregulated in allergic patients, including allergic asthmatics [24, 25]. On the other hand, some epidemiological studies indicate that asthma and allergies are comorbidities for severe COVID-19 forms [10]. Another question raised in asthmatic patients was the possible protective role of eosinophils in terms of SARS-COV-2 infection. Patients with asthma may be at a reduced risk of poor outcomes from COVID-19 infection. Eosinophilia, both in those with and without asthma, may be associated with reduced mortality risk, as Ho et al. showed in their study. But in severe asthmatics, defined by oral corticosteroid use in the previous year, it has been observed a slightly increased hazard ratio of mortality despite eosinophilia. [26] In our asthma patients' group there were 6 patients treated with add-on benralizumab, which is an eosinophil depleting biologic treatment. Only one patient in this group had a mild form of COVID-19 disease, with no need of oral corticosteroids course, similar to another case described in the literature [27].

From our group of asthma patients only 22.4% presented COVID-19 disease which is similar with other studies [26]. In the group with uncontrolled asthma, 24.5% of the patients had COVID-19, and there were no statistical differences in terms of getting the infection between controlled and uncontrolled asthmatics. However, it is still important to maintain good asthma control, as poorly controlled asthma may lead to a more complicated COVID-19 course, and some studies have found a higher rate of intubation and prolonged mechanical ventilation in adults with asthma [21]. All the asthma drugs should be available, including inhaled glucocorticoids, long-acting bronchodilators, oral glucocorticoids, and biological agents approved for asthma, and should be continued to be administered during the COVID-19 pandemic [3, 20–23]. Maintaining good asthma control helps minimize the risk of asthma exacerbation as other studies have shown [23, 28]. The group of uncontrolled asthma patients in our study representing 55.2%, was probably due to persistent inflammation in the lower airways even before symptoms appeared [29]. Preexisting airway inflammation may lead to exacerbation during a viral respiratory infection or in presence of other triggers, as other studies showed inflammatory diseases exacerbations during and after COVID-19 [1, 30]. This could be due to failure to acknowledge the asthma symptoms or secondary to minimizing them. Another problem was the high cost of medication without reimbursement, due to logistical problems in long-term care of chronic diseases during the pandemic.

Despite respecting the recommendations according to GINA guidelines, only 44.8% patients from our group were controlled, with ACT score over 20 points. There were more controlled asthma patients in GINA Step 2 and Step 4 and less in GINA Step 1, 3 and 5 there were less controlled. We may hypothesize that in Step 1 of treatment with only as needed medication (low dose ICS/formoterol or salbutamol followed by low ICS dose) patients tend to reduce medication use due to underestimation of their symptoms. Poor asthma control is a risk factor for greater severity of viral-induced exacerbation [1]. Asthma control is difficult to achieve in Step 5 which includes a high ICS dose and different add-on therapies, including biologicals. In their study Racine et al. showed that uncontrolled asthma, smoking, and psychological distress are risk factors for asthma exacerbation [31].

The influence of COVID-19 on asthma in our group showed a worsening of asthma course in 62.1% of infected asthmatics, because even mild COVID-19 could lead to asthma exacerbation, needing a step-up treatment according to GINA guidelines. In our group of patients with severe COVID-19, we observed that there was a statistically significant increase of inflammatory markers correlated with decrease of control evaluated by ACT score and decrease in QoL evaluated by VAS scale. Therefore, maintaining optimal asthma control should be able to reduce the risk of severe outcomes in COVID-19, like Jackson et al. showed in their study since 2015 [32]. Even though over half of the patients included in our study had uncontrolled asthma, no COVID-19 related deaths were reported.

The clinical evaluation and spirometry were difficult to maintain in our country during the pandemic, only patients that underwent a biological treatment or subcutaneous AIT were evaluated monthly (omalizumab) or at 2 months (benralizumab after the first 3 months). The possibility of maintaining phone contact with the

specialist involved in their asthma care was very comforting for patients with asthma. This may lead to the necessity to include phone-calls, smart phone's application or online evaluations and counseling in asthma patient's care [33]. In our study all asthma patients had the possibility to reach their physician by phone or e-mail. The patients were educated to recognize and to self-manage their asthma exacerbation using ICS/formoterol treatments, salbutamol and ICS or OCS as stated in the GINA guideline recommendations are [1].

Another problem raised during pandemic was the influence of inhaled cortico-steroids on COVID-19 disease. There is no solid evidence that inhaled glucocorticoids, or the biological agents used for asthma, which do not have a systemic immunosuppressive effect, have an adverse effect on the course of COVID-19 [33–35]. In our study, we noticed that patients in GINA Step 1 with as needed treatment experienced more often asthma exacerbations than patients with daily intake of ICS, which shows a protective effect for asthma exacerbation. The results from two studies indicate that individuals with nonallergic asthma have a higher risk for severe outcome of COVID-19 than those with allergic asthma [21, 25]. In our study we mainly included patients with allergic asthma, thus probably influencing the good outcome (no death reported after COVID-19 disease) could be influenced by atopic status or genetic polymorphisms, which are not studied in our population.

In the allergen immunotherapy (AIT) group, no asthma exacerbation was noted, since AIT was not discontinued according to international recommendation [36]. Therefore, asthma was controlled in this patients' group, raising no concerns, as was the case in similar groups of asthma phenotypes [37]. Patients with severe asthma, uncontrolled in step 5 GINA treatment are at higher risk for severe outcome, those patients experienced a worsening of asthma symptoms twice as often compared to asthma patients in GINA step 1–4. From our infected patients' group, 64.8% were in Step 5 GINA treatment, which support other study findings proving that severe asthma is influenced by COVID-19 [38]. No death was reported in the severe asthma patients' group even when they had a severe impairment of the lung function. In the omalizumab group which also included also an unvaccinated old patient, there was a protection despite the severe COVID-19, as other studies showed regarding the use of omalizumab in allergic diseases showed [39, 40]. Biological treatment may act protectively via several pathways. Omalizumab prevents IgE from binding to its receptor on plasmacytoid dendritic cells, leading to lower IFN-1 production by cross-linking of IgE [41]. In conjunction with asthma and COVID-19 severity, it was suggested in other studies that those with more severe asthma who require high dose of inhaled corticosteroids (Step 5 GINA) to maintain asthma control may be at risk for worse prognosis from COVID-19 [1, 22, 37].

As a first limitation of this study we should mention that the laboratory tests, SpO2 values and imaging of asthma patients before COVID-19 were not available, so it was not possible to evaluate those parameters before and after COVID-19. The second limitation was the difficulty to discriminate respiratory symptoms such as dyspnea, cough, and chest tightness due to worsening of asthma from those caused by COVID-19. Another limitation of our study is the subjectivity of the outcome measures, given that during the pandemic spirometry was not recommended, being considered a nebulization method which might increase the viral particle spreading. Another limitation is that we had a retrospective study, which is also less powerful than other types of studies, such as prospective studies. Strength of our study consists in a large group of asthma patients diagnosed and monitored before the COVID-19 disease who were evaluated with asthma control test questions during the pandemic. Although, the COVID-19 immunization through vaccination was not our study's topic, patients with asthma are recommended to receive the COVID-19 vaccination [42]. In our group of asthmatics only one death was reported in a non-vaccinated patient, that was a non-asthma related death 6 months after COVID-19 infection and none in the vaccinated group, but no conclusion can be drawn due to the small sample size. According to the current information we cannot estimate if there was a selection bias for survival among this asthma patient population especially because the study is a retrospective one.

Conclusion

We conclude in our study that the influence of COVID-19 on asthma may lead to the worsening of symptoms mainly in moderately severe COVID-19 cases and in uncontrolled asthma. Therefore, in asthmatic patients, besides every effort that should be made to avoid exposure to the SARS-CoV-2 virus, all regular medications necessary to maintain asthma control should be available and be used. Maintaining optimal asthma control should be able to reduce risk of severe outcomes in COVID-19 disease, therefore asthma even uncontrolled is not a risk factor for COVID-19 related fatalities in allergic asthma patients. The possibility of phone contact with the specialist involved in their asthma care was very comforting for patients, thus confirming the necessity to include phone calls, smart phone's applications or online evaluations and counseling in long-term care of chronic diseases.

Acknowledgements
Not applicable.

Author contributions
Conceptualization, IAM and DD methodology, IAM, ICB and PML; software, IAM; validation, IAM, ICB and DD; formal analysis, PML, IP and CTD; investigation, IAM, IP and CTD; resources, IAM, PML, IP and DD; data curation, IAM, ICB and PML; writing—original draft preparation, IAM, IP, CTD and PML writing—review and editing, IAM and DD; visualization IAM; supervision, DD; project ad-ministration IAM, PML, IP and DD; funding acquisition—not applicable. All authors have read and agreed to the published version of the manuscript.

Funding
Not applicable.

Availability of data and materials
Data are available at Allergology Department, Octavian Fodor" Institute of Gastroenterology and Hepatology, Cluj-Napoca.

Declarations

Declarations
All the patients or parents for patients under 16 years old signed the informed consent before any medical data were collected for the study according to the Declaration of Helsinki.

Ethics approval and consent to participate
The study protocol was approved by the "Octavian Fodor" Institute of Gastroenterology and Hepatology Ethics Committee (Approval no. 364/11.01.2021).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 3 September 2022 Accepted: 3 January 2023
Published online: 17 January 2023

References

1. Ginasthma.org. (2022). https://ginasthma.org/wp-content/uploads/2022/05/GINA-Main-Report-2022-FINAL-22-05-03-WMS.pdf. Accessed 22 June 2022
2. Liu S, Cao Y, Du T, Zhi Y. Prevalence of Comorbid Asthma and related outcomes in COVID-19: a systematic review and Meta-analysis. J Allergy Clin Immunol Pract. 2021;9(2):693–701. https://doi.org/10.1016/j.jaip.2020.11.054.
3. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, Curtis HJ, Mehrkar A, Evans D, Inglesby P, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature. 2020;584(7821):430–6. https://doi.org/10.1038/s41586-020-2521-4.
4. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497–506. https://doi.org/10.1016/S0140-6736(20)30183-5. Erratum in: Lancet. 2020 Jan 30; PMID: 31986264; PMCID: PMC7159299.
5. Lovinsky-Desir S, Deshpande DR, De A, Murray L, Stingone JA, Chan A, Patel N, Rai N, DiMango E, Milner J, et al. Asthma among hospitalized patients with COVID-19 and related outcomes. J Allergy Clin Immunol. 2020;146(5):1027-1034e4.
6. Morais-Almeida M, Pité H, Aguiar R, Ansotegui I, Bousquet J. Asthma and the Coronavirus disease 2019 pandemic: a literature review. Int Arch Allergy Immunol. 2020;181(9):680–8. https://doi.org/10.1159/000509057.
7. Halpin DMG, Faner R, Sibila O, Badia JR, Agusti A. Do chronic respiratory diseases or their treatment affect the risk of SARS-CoV-2 infection. Lancet Respir Med. 2020;8(5):436–8. https://doi.org/10.1016/S2213-2600(20)30167-3.
8. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Lancet Respir Med. 2020;8(4):e21. https://doi.org/10.1016/S2213-2600(20)30116-8. Erratum in: Lancet Respir Med. 2020 Jun;8(6):e54. PMID: 32171062; PMCID: PMC7118626.
9. Shi C, et al. Predictors of mortality in patients with coronavirus disease 2019: a systematic review and meta-analysis. BMC Infect Dis. 2021;21(1):663.
10. Gao YD, et al. Risk factors for severe and critically ill COVID-19 patients: a review. Allergy. 2021;76(2):428–55.
11. Liu S, Zhi Y, Ying S. COVID-19 and asthma: reflection during the pandemic. Clin Rev Allergy Immunol. 2020;59(1):78–88. https://doi.org/10.1007/s12016-020-08797-3.
12. Paquet C, Babes VT, Drucker J, Sénémaud B, Dobrescu A. Viral hepatitis in Bucharest. Bull World Health Organ. 1993;71(6):781–6.
13. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet. 2006;368(9537):733–43. https://doi.org/10.1016/S0140-6736(06)69283-0. Erratum in: Lancet. 2007 Sep 29;370(9593):1128. PMID: 16935684.
14. Ginasthma.org. (2020). https://ginasthma.org/wp-content/uploads/2020/04/Main-pocket-guide_2020_04_03-final-wms.pdf. Accessed 22 June 2022
15. Jia CE, Zhang HP, Lv Y, Liang R, Jiang YQ, Powell H, Fu JJ, Wang L, Gibson PG, Wang G. The Asthma control test and Asthma control questionnaire for assessing asthma control: systematic review and meta-analysis. J Allergy Clin Immunol. 2013;131(3):695–703.
16. Bousquet J, Bedbrook A, Czarlewski W, Onorato GL, Arnavielhe S, Laune D, Mathieu-Dupas E, Fonseca J, Costa E, The Lourenço O, MASK study group. Guidance to, et al. good practice: ARIA digitally-enabled, integrated, person-centred care for rhinitis and asthma. Clin Transl Allergy. 2018;2019(9):16. https://doi.org/10.1186/s13601-019-0252-0. Erratum in: Clin Transl Allergy. 2019 Oct 9;9:52. PMID: 30911372; PMCID: PMC6413444.
17. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, Durham S, Fokkens W, Gjomarkaj M, Haahtela T, et al. The skin prick test: European standards. Clin Transl Allergy. 2013;3(1):3. https://doi.org/10.1186/2045-7022-3-3.
18. https://www.who.int/publications/i/item/covid-19-therapeutic-trial-synopsis
19. Expert Panel Working Group of the National Heart, Lung, and Blood Institute (NHLBI) administered and coordinated National Asthma Education and Prevention Program Coordinating Committee (NAEPPCC), Cloutier MM, Baptist AP, Blake KV, Brooks EG, Bryant-Stephens T, DiMango E, Dixon AE, Elward KS, Hartert T, Krishnan JA et al. 2020 Focused Updates to the Asthma Management Guidelines: A Report from the National Asthma Education and Prevention Program Coordinating Committee Expert Panel Working Group. J Allergy Clin Immunol. 2020;146(6):1217–1270. 10.1016/j.jaci.2020.10.003. Erratum in: J Allergy Clin Immunol. 2021 Apr;147(4):1528–1530. PMID: 33280709; PMCID: PMC7924476.
20. Lupia T, Scabini S, Mornese Pinna S, Di Perri G, De Rosa FG, Corcione S. 2019 novel coronavirus (2019-nCoV) outbreak: a new challenge. J Glob Antimicrob Resist. 2020;21:22–7. https://doi.org/10.1016/j.jgar.2020.02.021.
21. Mahdavinia M, Foster KJ, Jauregui E, Moore D, Adnan D, Andy-Nweye AB, Khan S, Bishehsari F. Asthma prolongs intubation in COVID-19. J Allergy Clin Immunol Pract. 2020;8(7):2388–91. https://doi.org/10.1016/j.jaip.2020.05.006.
22. Chhiba KD, Patel GB, Vu THT, Chen MM, Guo A, Kudlaty E, Mai Q, Yeh C, Muhammad LN, Harris KE, et al. Prevalence and characterization of asthma in hospitalized and nonhospitalized patients with COVID-19. J Allergy Clin Immunol. 2020;146(2):307-314e4.
23. Zhu Z, Hasegawa K, Ma B, Fujiogi M, Camargo C, Liang L. Association of asthma and its genetic predisposition with the risk of severe COVID-19. J Allergy Clin Immunol. 2020;146(2):327-329e4.
24. Jackson DJ, et al. Association of respiratory allergy, asthma, and expression of the SARS-CoV-2 receptor ACE2. J Allergy Clin Immunol. 2020;146(1):203–206e3.

25. Scala E, Abeni D, Tedeschi A, Manzotti G, Yang B, Borrelli P, Marra A, Giani M, Sgadari A, Saltalamacchia F, Asero R. Atopic status protects from severe complications of COVID-19. Allergy. 2021;76(3):899–902. https://doi.org/10.1111/all.14551.
26. Ho KS, et al. The relationship between asthma, eosinophilia, and outcomes in coronavirus disease 2019 infection. Ann Allergy Asthma Immunol. 2021;127(1):42–8.
27. Renner A, Marth K, Patocka K, Pohl W. COVID-19 in a severe eosinophilic asthmatic receiving benralizumab: a case study. J Asthma. 2021;58(9):1270–2.
28. Yang JM, Koh HY, Moon SY, Yoo IK, Ha EK, You S, Kim SY, Yon DK, Lee SW. Allergic disorders and susceptibility to and severity of COVID-19: a nationwide cohort study. J Allergy Clin Immunol. 2020;146(4):790–8.
29. Muntean IA, Bocsan IC, Vesa S, Miron N, Nedelea I, Buzoianu AD, Deleanu D. Could FeNO predict asthma in patients with house dust mites allergic rhinitis? Medicina (Kaunas). 2020;56(5):235. https://doi.org/10.3390/medicina56050235.
30. Muntean IA, Pintea I, Bocsan IC, Dobrican CT, Deleanu D. COVID-19 disease leading to chronic spontaneous urticaria exacerbation: a Romanian retrospective study. Healthcare (Basel). 2021;9(9):1144. https://doi.org/10.3390/healthcare9091144.
31. Racine G, Forget A, Moullec G, Jiao T, Blais L, Lemiere C. Predictors of asthma control and exacerbations: a real-world study. J Allergy Clin Immunol Pract. 2021;9(7):2802-2811.e2. https://doi.org/10.1016/j.jaip.2021.04.049.
32. Jackson DJ, Trujillo-Torralbo MB, del-Rosario J, Bartlett NW, Edwards MR, Mallia P, Walton RP, Johnston SL,. The influence of asthma control on the severity of virus-induced asthma exacerbations. J Allergy Clin Immunol. 2015;136(2):497-500.e3.
33. Akenroye AT, Wood R, Keet C. Asthma, biologics, corticosteroids, and coronavirus disease 2019. Ann Allergy Asthma Immunol. 2020;125(1):12–3. https://doi.org/10.1016/j.anai.2020.05.001.
34. Saeed H, Abdelrahim ME, Rabea H, Salem HF. Impact of advanced patient counseling using a training device and smartphone application on asthma control. Respir Care. 2020;65(3):326–32. https://doi.org/10.4187/respcare.06903.
35. Choi JC, Jung SY, Yoon UA, You SH, Kim MS, Baek MS, Jung JW, Kim WY. Inhaled corticosteroids and COVID-19 risk and mortality: a nationwide cohort study. J Clin Med. 2020;9(11):3406. https://doi.org/10.3390/jcm9113406.
36. Pfaar O, Agache I, Bonini M, et al. COVID-19 pandemic and allergen immunotherapy-an EAACI survey. Allergy. 2021;76(11):3504–16. https://doi.org/10.1111/all.14793.
37. Myers LC, Parodi SM, Escobar GJ, Liu VX. Characteristics of hospitalized adults with COVID-19 in an integrated health care system in California. JAMA. 2020;323(21):2195–8. https://doi.org/10.1001/jama.2020.7202.
38. Kow CS, Capstick T, Hasan SS. Are severe asthma patients at higher risk of developing severe outcomes from COVID-19? Allergy. 2021;76(3):959–60. https://doi.org/10.1111/all.14589.
39. Poddighe D, Kovzel E. Impact of anti-type 2 Inflammation biologic therapy on COVID-19 clinical course and outcome. J Inflamm Res. 2021;14:6845–53. https://doi.org/10.2147/JIR.S345665.
40. Leru PM, Anton VF. Real-life benefit of omalizumab in improving control of bronchial asthma during COVID-19 pandemic. Cureus. 2021;13(8):e17268. https://doi.org/10.7759/cureus.17268.
41. Bakakos A, Bakakos P, Rovina N. Unraveling the relationship of asthma and COVID-19. J Pers Med. 2021;16(12):1374. https://doi.org/10.3390/jpm11121374.
42. https://www.asthma.org.uk/advice/triggers/coronavirus-covid-19/what-should-people-with-asthma-do-now/

Publisher's Note

Journal of
Clinical Medicine

MDPI

Article

IL-31—Pruritus Interleukin: Serum Values and Clinical Impact in Chronic Spontaneous Urticaria—A Romanian Retrospective Study

Carmen-Teodora Dobrican-Băruța [1], Diana Mihaela Deleanu [1], Ioana Adriana Muntean [1,*], Irena Pintea [1,*], Cristian Marius Florea [2] and Gabriela Adriana Filip [3]

[1] "Iuliu Hatieganu" University of Medicine and Pharmacy, Department of Allergology and Clinical Immunology, 400012 Cluj-Napoca, Romania; dobrican.carmen@umfcluj.ro (C.-T.D.-B.); diana.deleanu@umfcluj.ro (D.M.D.)
[2] "Iuliu Hatieganu" University of Medicine and Pharmacy, Department of Cardiology, 400012 Cluj-Napoca, Romania; florea.cristian@umfcluj.ro
[3] "Iuliu Hatieganu" University of Medicine and Pharmacy, Department of Physiology, 400012 Cluj-Napoca, Romania; gabriela.filip@umfcluj.ro
* Correspondence: adriana.muntean@umfcluj.ro (I.A.M.); irena.nedelea@umfcluj.ro (I.P.)

Abstract: (1) Background: This study aimed to evaluate the implications of interleukin-31 (IL-31) in the pathogenesis of chronic spontaneous urticaria (CSU) and to assess the differences that occur between its serum values compared to controls. Additionally, the serum IL-31 levels were measured alongside other clinical and paraclinical parameters that were identified in the patients to understand its immunological importance in this skin disease and to determine if it could potentially serve as a therapeutic target in CSU in the future. (2) Methods: The serum levels of IL-31 were estimated in 50 patients diagnosed with CSU according to the accepted international guidelines. Additionally, 38 controls who had not experienced any episodes of urticaria during their lifetime were included. (3) Results: Significantly elevated serum IL-31 levels were observed in CSU patients compared to the controls ($p < 0.0001$). Although no direct correlations were found between IL-31 and inflammatory markers (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)), eosinophils, or total immunoglobulins E (IgE), significant differences in IL-31 levels were identified based on CSU severity, quality of life impact, itch intensity, and response to histamine H1 receptor antagonists (H1 antihistamines) ($p < 0.05$ for all). (4) Conclusions: Our findings underscore that IL-31 is not directly associated with general inflammation, eosinophilic response, or atopy in CSU. Nevertheless, its expression is influenced by key disease characteristics: severity, pruritus, and H1 antihistamine response. This investigation provides essential insights into CSU pathogenesis, potentially leading to novel therapeutic interventions. An enhanced understanding of these mechanisms is crucial due to the limitations of current treatment modalities in terms of fully managing CSU symptoms.

Keywords: IL-31; CSU; UAS-7; pruritus; inflammation; atopy; IgE; eosinophils

Citation: Dobrican-Băruța, C.-T.; Deleanu, D.M.; Muntean, I.A.; Pintea, I.; Florea, C.M.; Filip, G.A. IL-31—Pruritus Interleukin: Serum Values and Clinical Impact in Chronic Spontaneous Urticaria—A Romanian Retrospective Study. *J. Clin. Med.* **2023**, *12*, 5957. https://doi.org/10.3390/jcm12185957

Academic Editors: Carlo Pincelli and Marco Manfredini

Received: 9 August 2023
Revised: 8 September 2023
Accepted: 10 September 2023
Published: 13 September 2023

1. Introduction

Chronic urticaria, characterized by a persistent rash and intense pruritus lasting over six weeks, involves complex pathogenetic mechanisms that remain incompletely understood [1,2]. The roles of mast cells, basophils, and eosinophils in the disease's pathogenesis are well-known, leading to the release of vasoactive mediators, including histamine, proinflammatory compounds, and newly synthesized cytokines [1,2]. Cytokine-mediated inflammation, particularly alarmins such as interleukin-33 (IL-33), interleukin-25 (IL-25), and thymic stromal lymphopoietin (TSLP), originating from mast cells, play significant roles in inflammation [3–5]. Additionally, interleukin-31 (IL-31), which is produced by various immune cells including T helper 2 (Th2) cells, mast cells, macrophages, and dendritic cells, has some importance in pruritus and inflammatory responses [6–8].

Current therapies for chronic spontaneous urticaria (CSU) include second-generation H1 antihistamines, omalizumab, ciclosporin, and corticotherapy [1]. However, many patients continue to suffer from uncontrolled symptoms, necessitating the exploration of novel biological treatments [6]. Notably, CSU shares intense pruritus in common with other autoimmune and allergic skin diseases, such as bullous pemphigoid (BP), psoriasis, and atopic dermatitis (AD) [7–9]. While histamine and neuropeptides have traditionally been linked to pruritus, recent evidence highlights IL-31, known as the pruritogenic cytokine, as a pivotal factor in the itch mechanism [7–9]. Initially attributed to activated T helper cells, recent studies reveal eosinophils as significant IL-31 sources, implying their role in mediating itch severity [2,10–12]. IL-31, besides inducing itching, exerts potential immunomodulatory functions in supporting Th2-type immunity, contributing to the autoimmune diseases associated with IgE, including chronic urticaria [9,10,13–15]. Recognizing IL-31's implication in pruritus and its relevance in AD and other allergic conditions has fostered its consideration as a therapeutic target [7,8,10]. However, the precise implications of IL-31 in CSU, encompassing disease severity, itch intensity, and quality of life impact, remain incompletely elucidated. To address this gap, our study aims to unravel the correlations between serum IL-31 levels and diverse clinical and paraclinical parameters in CSU patients. By unveiling these associations, our investigation seeks to provide novel insights into CSU's underlying mechanisms and potentially establish IL-31 as a promising therapeutic focus.

Distinguishing itself from prior research centered on IL-31's role in pruritus, inflammation, and immune modulation, our study specifically delves into IL-31's involvement within the context of CSU [6–8]. Through these intricate associations, our research offers a fresh perspective on IL-31's significance in CSU and sets the stage for future research directions.

Within this manuscript, we present our findings, highlighting the elevated IL-31 levels found in CSU patients compared to controls. We delve into the correlations between IL-31 and disease severity, itch intensity, and treatment responsiveness. Furthermore, we investigate how IL-31 levels relate to the impact on patients' quality of life. By illuminating these multifaceted connections, our study enriches our understanding of IL-31's role in CSU and establishes a foundation for potential therapeutic advancements. The prospect of blocking IL-31 receptors through therapeutic approaches, exemplified by Nemolizumab, emerges as a potential strategy for treating disorders involving IL-31, including CSU [16–18].

In summary, this study endeavors to uncover IL-31's distinctive contribution to CSU, highlighting the novel connections between this cytokine and disease manifestations. With IL-31 as our focal point, we aspire to pave the way for therapeutic interventions that alleviate the burden of CSU for affected individuals.

2. Materials and Methods

This research undertakes a retrospective, analytical approach to a study conducted at the Allergology Department of the Regional Institute of Gastroenterology and Hepatology in Cluj-Napoca (IRGH), Romania. The study encompassed 50 patients rigorously diagnosed with CSU in accordance with international consensus guidelines [1]. These guidelines define CSU as the recurrence of a specific maculopapular rash, optionally accompanied by angioedema, manifesting at least twice a week for a period exceeding six weeks [1,2]. To draw a meaningful comparison, a control group was assembled from 38 Institute staff members. Therefore, the inclusion criteria for patients required a diagnosis of CSU in accordance with the referenced guidelines [1]. Notably, patients were required to lack systemic illnesses concurrent with urticaria, such as systemic mastocytosis, Schnitzler syndrome, and urticarial vasculitis, which were considered exclusion criteria for this study. The exclusion criteria for patients also included renal, hepatic, psychiatric, and infectious diseases that may be accompanied by cutaneous eruptions or itching. Conversely, the inclusion criteria for control subjects mandated a complete absence of urticaria episodes throughout their lifetimes. The exclusion criteria for the control group incorporated individuals with a history of acute or chronic urticaria episodes from any etiology, as well as those with systemic conditions characterized by urticaria, wheals, or other cutaneous exanthems,

along with pruritus, either individually or in combination, as concurrent symptoms. These systemic conditions encompass renal and hepatic disorders, psychogenic pruritus, other psychiatric conditions, infectious diseases, and malignancies. This study was approved by the Ethics Committee of the "Iuliu Hatieganu" University of Medicine and Pharmacy (UMF), Cluj-Napoca, Romania (AVZ270/10.10.2022) and by the IRGH (12637/11.10.2022). Informed consent was obtained from all participants.

Demographic data and the baseline characteristics of all participants were recorded, including age, sex, and serum IL-31 levels. In patients with CSU, additional data were collected, such as the duration of the disease, the severity of the disease, and the presence or absence of atopy, proven by a positive skin-prick test to environmental allergens. Complementary paraclinical tests were also performed, including a complete blood count, an analysis of inflammatory parameters such as ERS and CRP, coproparasitological examination, and total serum IgE. All laboratory analyses were performed at the hospital's central laboratory.

Venous blood samples were taken from all these participants and the samples were centrifuged. The serum was later frozen and kept at −80 degrees Celsius. IL-31 concentrations in the serum were assessed using a commercially available enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions (Human IL-31 ELISA Kit, BioLegend, 8999 BioLegend Way, San Diego, CA 92121, United States).

The urticaria activity score over 7 days (UAS7) is an internationally authorized tool used to assess the severity of CSU. It combines the scores for hives and itch severity over a continuous period of 7 days, with scores ranging from 0 to 42. The hives score ranges from 0 to 3, indicating the level of hives: 0 represents no hives, 1 indicates mild hives (fewer than 20 hives in 24 h), 2 corresponds to moderate hives (20–50 hives in 24 h), and 3 signifies severe hives (more than 50 hives in 24 h or a large confluent area of hives). Likewise, the itch severity score ranges from 0 to 3, reflecting the intensity of itchiness: 0 signifies no itch, 1 indicates a mild itch (present but not bothersome), 2 represents a moderate itch (troublesome but not disrupting normal daily activities or sleep), and 3 indicates a severe itch (interfering with normal daily activities or sleep). Based on the UAS7 scores, the patients' chronic spontaneous urticaria severity is categorized into three disease activity states: scores from 0 to 15 are classified as mild, scores from 16 to 27 are categorized as moderate, and scores from 28 to 42 are considered severe [19,20].

Additionally, the weekly itch severity is also grouped into three levels: scores from 0 to 7 are considered mild, scores from 8 to 14 are classified as moderate, and scores from 15 to 21 are categorized as severe.

The dermatology life quality index (DLQI) is a widely used tool to assess how CSU affects a person's quality of life. It consists of ten questions, each scored from 0 to 3, with higher scores indicating a more significant impact. The total DLQI score can range from 0 to 30, and the scoring breakdown is as follows: 0 to 1—no impact on quality of life, 2 to 5—small impact, 6 to 10—moderate impact, 11 to 20—very large impact, and 21 to 30—extremely large impact [21,22].

Statistical Analysis

The data were reported as the median, along with the interquartile range, unless specifically mentioned otherwise. To assess the normality of the data distribution, the Shapiro–Wilk test was conducted. For comparisons between the study groups, the Mann–Whitney U test or the Kruskal–Wallis test was utilized, depending on the nature of the data. The Mann–Whitney U test was chosen when comparing two independent groups, such as the serum IL-31 levels between healthy controls and urticaria patients. This test is suitable for non-normally distributed continuous data and allows us to assess whether there are significant differences in the central tendencies of the two groups. To provide a comprehensive understanding of the practical significance of these findings, we calculated the effect size measures. Effect size measures help elucidate the magnitude of observed differences beyond statistical significance. Thus, in our analysis, we included

effect size measures using the Rosenthal coefficient (R) for the Mann–Whitney test, which quantifies the practical significance of the differences observed between these two groups. We calculated this coefficient manually, based on the U statistic and sample sizes. To avoid confusion between the traditional notation "r" for the Rosenthal coefficient and the "r" notation for the Pearson correlation coefficient, we have chosen to represent the effect size coefficient, Rosenthal's, as "R" in our study. Conversely, the Kruskal–Wallis test was employed when comparing IL-31 levels among more than two independent groups or when dealing with ordinal data. This test extends the Mann–Whitney U test to multiple groups and examines whether there are significant differences in the medians of these groups. In our study, it was used to compare IL-31 levels among patients with different disease severities. To assess the practical significance of the Kruskal–Wallis test results, we calculated the effect size using the epsilon-squared (ε^2) method. This approach provides a measure of the proportion of variability in the dependent variable that can be attributed to group differences, helping us interpret the practical significance of the findings.

Categorical data were compared using Pearson's chi-squared test, which is appropriate for analyzing the associations between categorical variables. This test helped us to determine whether there were significant differences in the categorical variables, such as atopic status, between groups. The relationships between different parameters were determined using Spearman's correlation, a non-parametric measure suitable for assessing non-linear associations between variables. This analysis enabled us to examine the strength and direction of correlations between IL-31 levels and other clinical parameters. To investigate whether IL-31 could discriminate between healthy controls and urticaria patients, the receiver operating characteristics (ROC) curve for IL-31 was analyzed, and the area under the curve (AUC) was calculated. This analysis allowed us to assess the diagnostic performance of IL-31 in differentiating between the two groups. GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, CA, USA) was used for conducting the statistical analyses and generating graphs. Statistical significance was set at a p-value of <0.05, indicating that results with p-values lower than this threshold were considered statistically significant. The choice of these specific tests and methods aimed to comprehensively analyze the relationships and differences in IL-31 levels and the associated clinical parameters within the context of CSU.

3. Results

3.1. Clinical Data

We meticulously collected the foundational clinical characteristics of the study subjects. A comprehensive synthesis of these characteristics is presented in Table 1 for detailed review [Table 1].

Table 1. Characteristics of the study participants. Note: F, female; M, male. Age is presented as mean ± standard deviation (SD).

Characteristic	CSU Patients	Controls
Number, n	50	38
Sex (F/M)	36/14	26/12
Age, yrs.	50.14 ± 16.10	44.32 ± 9.23
Atopy (Atopic/Non-atopic)	14/36	10/28

3.2. Serum IL-31 Levels in UCS Patients and Controls

The statistical analysis showed a highly significant difference in serum IL-31 levels between patients and controls, with IL-31 being significantly higher in patients ($p < 0.0001$). The median IL-31 level in patients was 121.5, while in the controls, it was 29.21 (Figure 1). We utilized the Rosenthal coefficient (R) as an effect size measure in conjunction with the Mann–Whitney U test to evaluate the clinical significance of the IL-31 level differences. The computed Cohen's r value was approximately 0.485, indicating a moderate degree of practical relevance. In essence, this suggests that the observed increase in IL-31 levels

among patients compared to controls transcends statistical significance, underscoring its substantial clinical implications.

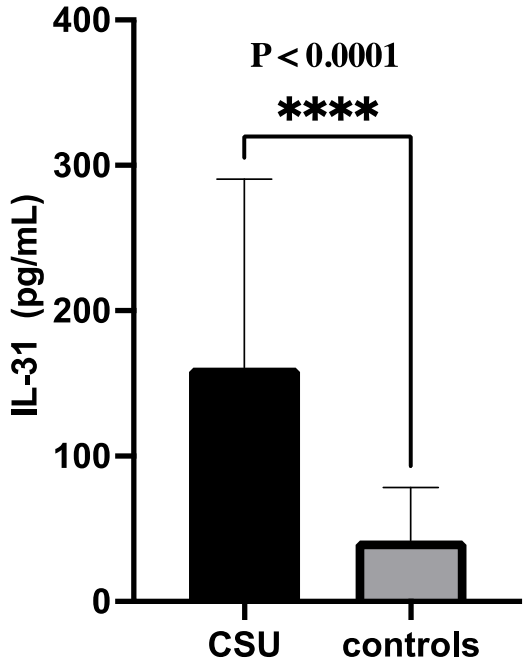

Figure 1. IL-31 serum levels in CSU patients versus (vs.) controls; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, and **** = $p < 0.0001$.

In our study, ROC analysis was also performed to evaluate the levels of IL-31 between patients with CSU and the control subjects. The AUC was calculated to be 0.9039, with a standard error (SE) of 0.03220 and a 95% confidence interval ranging from 0.8408 to 0.9671 (Figure 2).

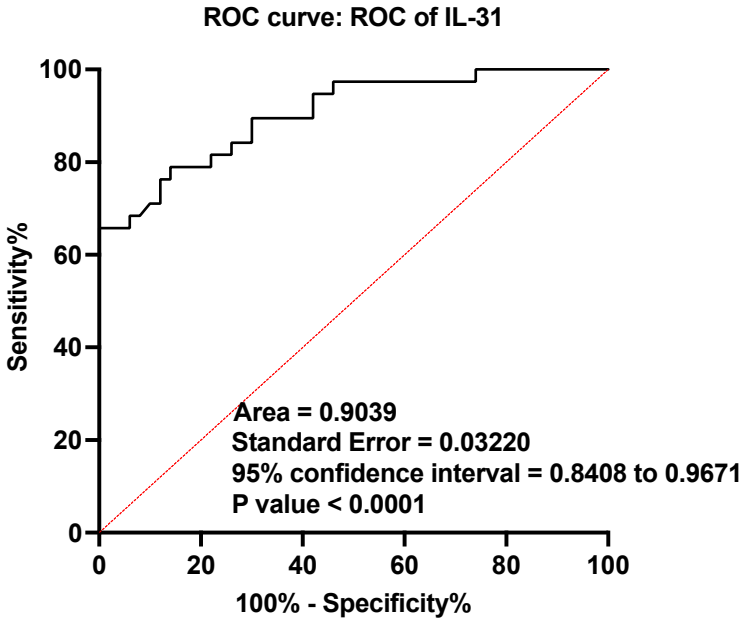

Figure 2. Receiver operating characteristics (ROC) curve of IL-31 in CSU compared with healthy controls; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, $p < 0.0001$, AUC—area under the curve = 0.9039, and SE—standard error = 0.03220.

3.3. Serum IL-31 Levels in Relation to Other Paraclinical Parameters: Eosinophils, Inflammation Markers, and Total IgE Serum Level

There were no significant correlations between IL-31 levels and other paraclinical parameters, such as serum eosinophils, or inflammatory markers, such as ESR or CRP, as well as total IgE levels. The Pearson correlation coefficient (r) for the serum level of IL-31 and serum eosinophils is −0.06714, indicating a weak negative correlation between the two variables. However, this correlation was not statistically significant (p = 0.6432) as the p-value was greater than 0.05. The analysis was based on 50 XY pairs, and the results suggested that there was no significant relationship between serum interleukin-31 levels and serum eosinophil levels in patients (Figure 3). Using the same statistical analyses, correlations between IL-31 and ESR, CRP, and total IgE levels were investigated, but no statistically significant associations were found between them.

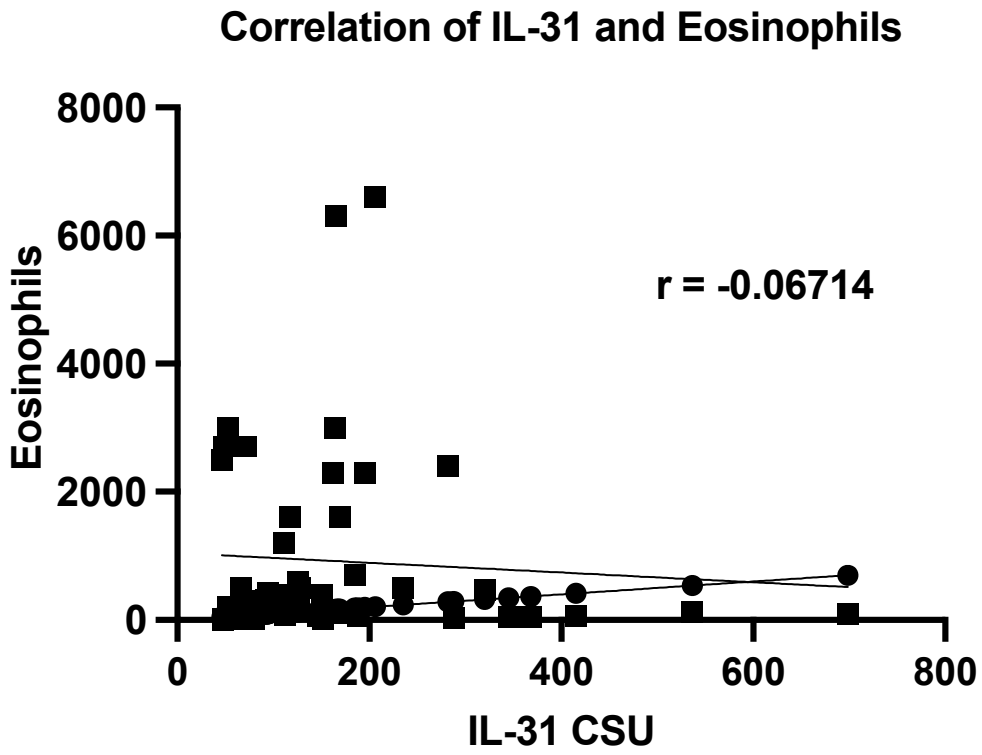

Figure 3. Correlation of serum IL-31 and eosinophils. IL-31—interleukin 31, CSU—chronic spontaneous urticaria, p = 0.6432; r—the Pearson correlation coefficient for the serum level of IL-31 and serum eosinophils is −0.06714 (r = −0.06714).

3.4. Serum IL-31 Levels in Relation to Atopy

Another objective of our study was to examine potential variations in serum IL-31 levels in relation to atopic status, specifically contrasting these levels between atopic and non-atopic CSU patients. Statistical analysis conducted using the Mann–Whitney test yielded a non-significant p-value of 0.2491, thus indicating that there was no statistically significant difference in serum IL-31 levels between these subgroups (Figure 4). The Rosenthal coefficient (R) for the comparison between non-atopic and atopic groups regarding IL-31 levels is approximately −0.214. This result suggests a small or negligible effect. In other words, the observed difference between the two groups in terms of IL-31 levels is not clinically or practically significant since the coefficient is close to zero.

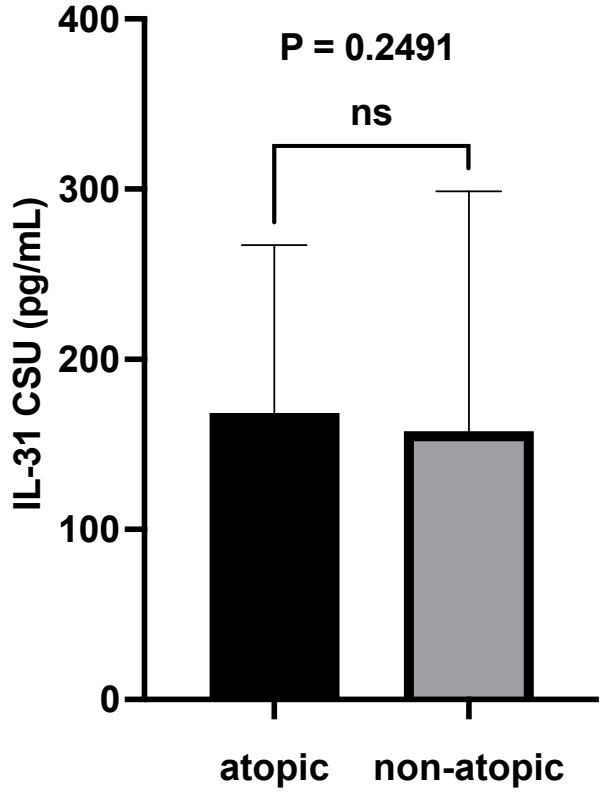

Figure 4. IL-31 serum levels in atopic vs. non-atopic patients; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, ns—non-significant, and $p = 0.2491$.

3.5. Serum IL-31 Levels in Relation to Clinical Tools

3.5.1. Serum IL-31 Levels in Relation to UAS7

According to UAS7, which, as explained in materials and methods, divides patients into three categories of disease severity, our patients fell into the moderate and severe forms, with no patient having the mild form of the disease. Thus, we divided the patients into two categories: moderate and severe, and evaluated whether there are differences in IL-31 levels between them. The examination of statistical relationships revealed a significant positive correlation (r = 0.5673, $p < 0.0001$) between IL-31 serum levels in CSU patients and UAS7 scores, underscoring the finding that individuals with higher UAS7 scores exhibited elevated IL-31 levels (Figure 5). Furthermore, the investigation encompassed a comparison of IL-31 levels within distinct disease severity groups using the Mann–Whitney U test. For patients with moderate disease, the mean IL-31 level stood at 106 (standard deviation 42), with a median of 94 (range: 80 to 127). Conversely, patients with severe disease exhibited a mean IL-31 level of 200 (standard deviation 156), accompanied by a median of 163 (range: 77 to 282). The utilization of the Mann–Whitney U test demonstrated a significant differentiation between these two groups ($p = 0.0026$). Specifically, IL-31 levels were notably higher in patients grappling with severe disease compared to those with moderate disease presentation (Figure 6). The Rosenthal coefficient (R) for the comparison between the "moderate form" and "severe form" groups in terms of IL-31 levels was approximately 0.327. This result suggests a moderate effect size. It indicates that there is a moderate practical significance associated with the difference in IL-31 levels between these two groups. In other words, the observed difference in IL-31 levels is not only statistically significant but also carries meaningful clinical implications. Notably, the analysis incorporated data from 21 patients with a moderate disease form and 29 patients with a severe manifestation of the condition.

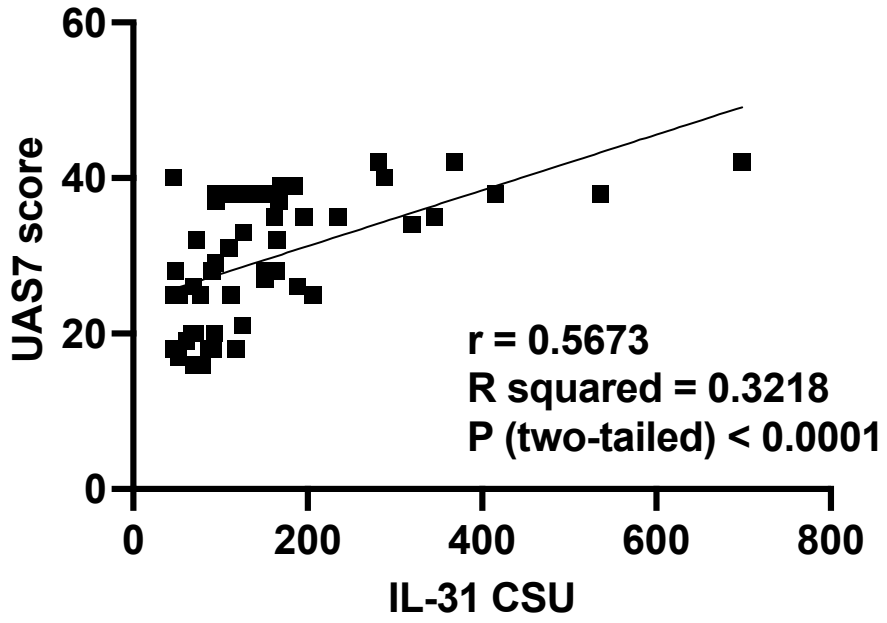

Figure 5. Serum IL-31 levels in patients with moderate vs. severe forms of CSU, according to UAS7; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, UAS7—urticaria activity score per 7 days, - the Pearson correlation coefficient—r = 0.5673, and $p < 0.0001$.

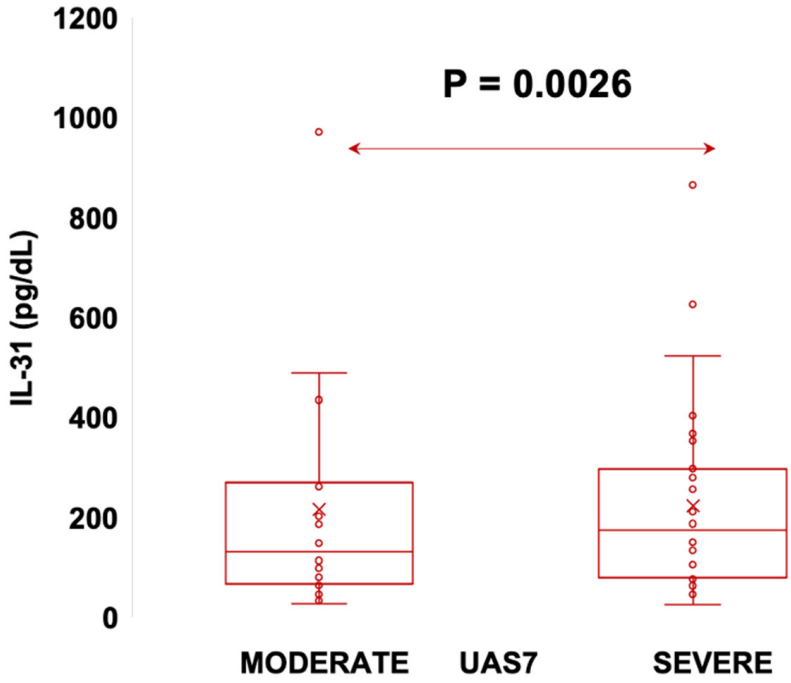

Figure 6. The Mann–Whitney U test, showing the level of serum IgE in moderate and severe forms of CSU, where $p = 0.0026$; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, UAS7—urticaria activity score per 7 days, and $p = 0.0026$.

3.5.2. Serum IL-31 Levels in Relation to the Severity of the Pruritus

Additionally, as shown in the second subtitle of this paper, the severity of itch was also quantified separately by summing the scores over 7 days, and patients were divided into three categories, based on the intensity of the pruritus. Scores from 0 to 7 were considered mild, scores from 8 to 14 were classified as moderate, and scores from 15 to 21 were categorized as severe (Figure 7).

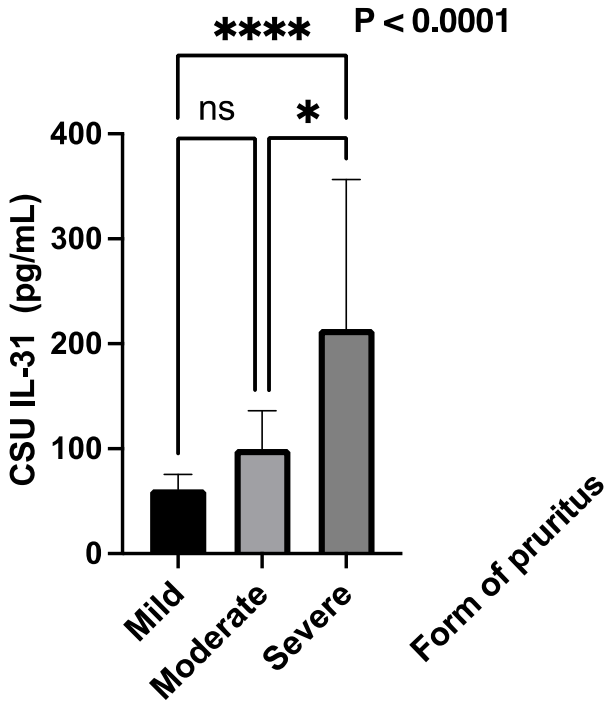

Figure 7. Serum IL-31 levels and the forms of pruritus quantified by the intensity of itch; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, ns—non-significant, and **** = $p < 0.0001$, * = $p < 0.05$.

The statistical analysis used the Kruskal–Wallis test to examine the association between different forms of pruritus. The test resulted in a highly significant p-value of less than 0.0001, indicating that there were statistically significant differences between the groups with different forms of pruritus. The p-value summary is denoted by ****, confirming the high significance of the result. The test also revealed that the medians of the pruritus scores vary significantly ($p < 0.05$) among the three groups. The Kruskal–Wallis statistic value is 26.79, which further supports the evidence of significant differences in pruritus scores among the groups (Figure 8). The effect-size epsilon-squared (ε^2) value for the Kruskal–Wallis test using the epsilon-squared method is approximately 0.149. This suggests a moderate effect size, indicating that around 14.9% of the variability in the dependent variable can be attributed to the differences between the groups. In other words, there are meaningful differences among the groups in our study.

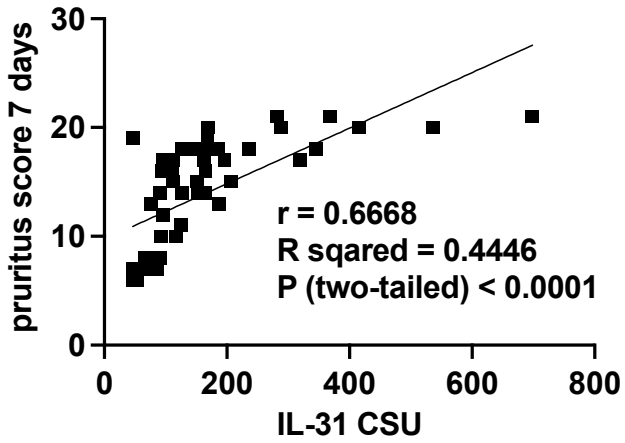

Figure 8. Correlation of serum IL-31 levels in CSU patients and pruritus score per 7 days; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, $p < 0.0001$, and the Pearson correlation coefficient—r = 0.6668.

3.5.3. Serum IL-31 Levels in Relation to DLQI

The impact of CSU on patients' quality of life was assessed using the dermatology life quality index (DLQI), as explained in the Materials and Methods (Section 2). The results revealed that patients in the "very important impact" group (n = 33) had a mean IL-31 level of 104 (SD = 47), with a median of 93, ranging from 70 to 127 pg/mL. On the other hand, the "extremely important impact" group (n = 16) exhibited a significantly higher mean IL-31 level of 276 (SD = 170) with a median of 244, ranging from 160 to 351 pg/mL. Additionally, it is worth noting that there was one patient with a "moderate" impact on quality of life, as per the DLQI, who had an IL-31 level of 79.635 pg/mL. These findings suggest that higher IL-31 levels may be associated with a more pronounced impact on the quality of life in patients with the disease (Figures 9 and 10).

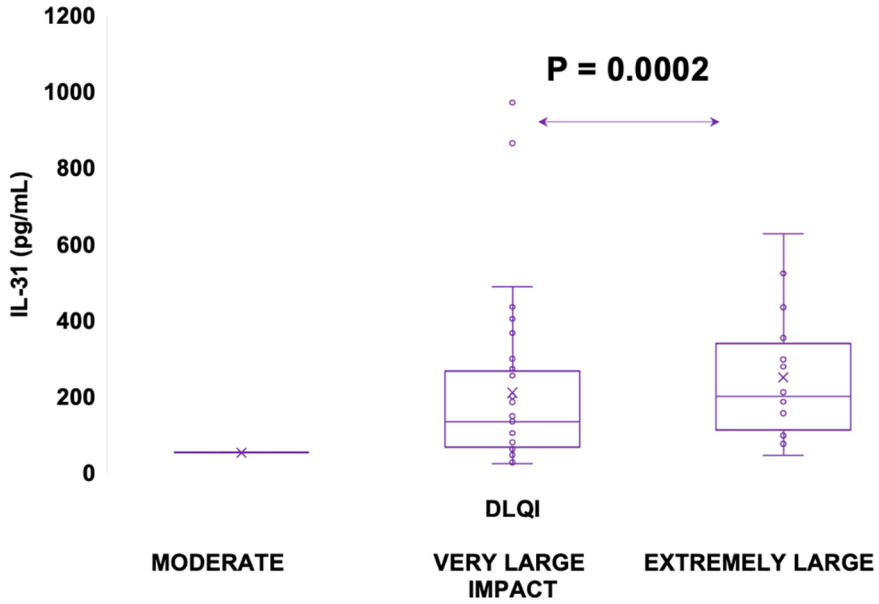

Figure 9. Serum IL-31 levels in CSU patients experiencing a moderate impact, very large impact, and extremely large impact on quality of life, according to the DLQI; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, DLQI—dermatology life quality index, and $p = 0.0002$.

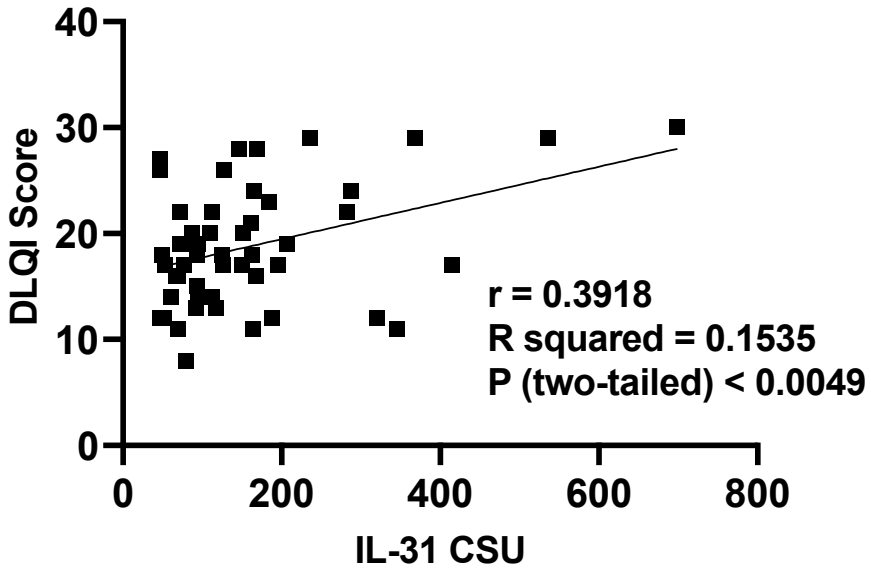

Figure 10. Correlation of serum IL-31 levels in CSU patients and DLQI score; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, DLQI—dermatology life quality index, $p < 0.0049$, and the Pearson correlation coefficient—r = 0.3918.

3.6. Serum IL-31 Levels in Relation to Response to Antihistamines AH1

The IL-31 levels in CSU patients and their response to the maximum recommended dose of second-generation h1 antihistamines [1] were compared, dividing the patients into two groups, as follows: those who responded to AH1—"YES"; those who did not respond to AH1 requiring the following therapeutic steps—"NO". The Mann–Whitney test resulted in a *p*-value of 0.0290, indicating a statistically significant difference in the treatment response between the two groups. The "*p*-value summary" denoted by one asterisk (*) confirms a significance at the alpha level of 0.05. The test used a two-tailed *p*-value, considering both higher and lower values. The sum of ranks in the "YES" and "NO" groups was 256.5 and 1019, respectively, with a Mann–Whitney U value of 151.5. The difference between the medians of the two groups was 46.06 (actual difference) or 43.56 (the Hodges–Lehmann estimate). This suggests that the group with a negative response to the treatment ("NO") had a higher median value of serum IL-31 levels (136.9) compared to the group with a positive response ("YES") with a median of 90.86 (Figure 11).

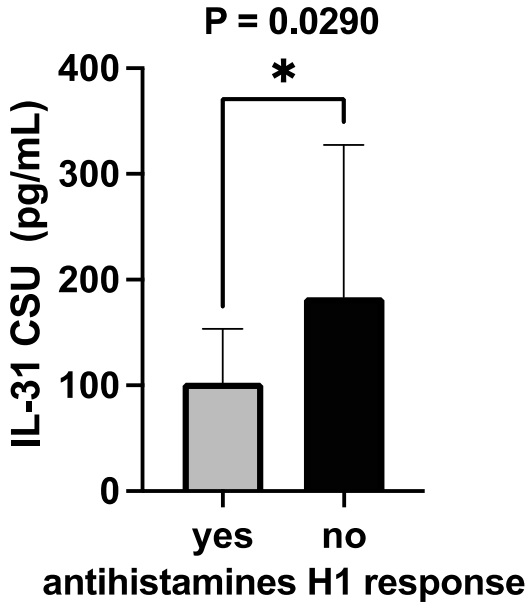

Figure 11. CSU patients' serum IL-31 levels and the response to antihistamines H1; IL-31—interleukin 31, CSU—chronic spontaneous urticaria, antihistamines H1—histamine H1 receptor antagonists, and * $p = 0.0290$.

We obtained an effect size coefficient, Rosenthal's coefficient (R) of approximately 0.295. This value indicates a moderate level of practical significance. In essence, it signifies that the difference in response to AH between the "YES" and "NO" groups is not only statistically significant but also carries meaningful clinical implications. This means that beyond the statistical significance observed in our Mann–Whitney test, the practical importance of the difference in AH responses between these two groups is underscored by the calculated effect size.

4. Discussion

CSU is a debilitating skin disease that has a considerable impact on the daily life of affected patients, not only through the characteristic lesions but especially through itching. It affects approximately 1% of the global population and represents a substantial socioeconomic burden, with absenteeism from the workplace and high costs for the necessary treatments [23–26]. Thus, there is an urgent need to better understand the pathogenesis of CSU to develop improved diagnostic and treatment approaches [2,6,27,28]. Despite this need, there has been less focus on pruritus in the specialized literature that studies chronic urticaria, the symptom being more pathognomonic for atopic dermatitis, which

is why IL-31 was also studied further in the context of this condition [7,9,29]. Based on these considerations, our study aimed to investigate the correlation between the cytokine attributed to pruritus and the severity scores of the urticaria, the intensity of the itch, and the impact on the quality of life of affected patients. Our findings revealed a significant association between IL-31 levels in CSU patients and pruritus intensity, disease severity, and quality of life. Additionally, we observed that patients who responded to the first steps of treatment, up to the maximum dose of H1 antihistamines, had lower serum levels of IL-31 than those who did not respond to these drugs and required other therapeutic steps. Among these therapeutic options, anti-IL-31 therapy could also be considered, as has been considered for other itchy skin conditions [7,9,29].

IL-31 plays a significant role in cutaneous inflammation and has been extensively studied for its involvement in tissue homeostasis, inflammation, immune defense, neuroimmune circuits, and pruritus [7,29–33]. It belongs to the IL-6-derived cytokine family and is associated with proinflammatory characteristics. While its physiological function is not fully understood, it has been implicated in various inflammatory disorders in humans, such as AD, inflammatory bowel disease, and asthma [9,16,17,29–32]. IL-31 is produced by TH2 cells and immature dendritic cells, activating neurons and keratinocytes through its receptors, IL31RA/OSMRβ. The intradermal application of IL-31 induces itching, further illustrating its ability to activate target neurons [34,35]. In our study, we found significantly increased levels of IL-31 in patients with CSU compared to healthy controls, which is consistent with observations from other studies [4,5,30]. We also computed the effect size coefficients, which revealed a moderate to large level of significance. In essence, the statistical analysis demonstrated not only a highly significant difference in serum IL-31 levels between patients and controls but also conveyed that this difference holds practical importance in the context of our study. It highlights the potential clinical relevance of elevated IL-31 levels in patients, which may warrant further investigation and consideration in the management of CSU.

We also conducted an ROC analysis to assess the discriminatory ability of IL-31 in distinguishing between individuals with CSU and those without the condition (control subjects). The AUC value that we obtained was 0.9039. This value indicates a high degree of accuracy in distinguishing between the two groups using the IL-31 parameter. The analysis showed that IL-31 is quite effective in making this distinction, with a high degree of certainty. The numbers we provided give us confidence in the accuracy and reliability of our results. This finding supports the inclusion of urticaria in the category of pruritogenic inflammatory skin diseases, along with the others mentioned above.

During the course of our study, we meticulously scrutinized the foundational clinical characteristics of both the CSU patient group and the carefully matched control cohort. Strikingly, no statistically significant disparities emerged in these pivotal attributes, such as age and sex, underscoring the meticulous selection process that aimed to eliminate potential confounders arising from these fundamental demographics. Surprisingly, we also noticed the absence of correlations between the serum levels of IL-31 and eosinophils in our patients, despite prior theoretical assumptions suggesting an association between IL-31 and eosinophils [2,10–12]. This discrepancy between IL-31 and eosinophils suggests that while IL-31 may contribute to certain aspects of allergic inflammation, its role might not be as straightforward as previously assumed. Intriguingly, our findings regarding serum IL-31 levels in relation to atopic status present a complex picture that challenges our initial expectations. While previous research has suggested a potential link between IL-31 and atopic conditions [9,16,17,29–32,35,36] such as AD, the non-significant difference in IL-31 levels between atopic and non-atopic CSU patients, as indicated by the Mann–Whitney test (p-value = 0.2491), adds a layer of complexity to our understanding. Furthermore, no significant correlations were identified between non-specific inflammatory markers, such as ESR and CRP, and the serum levels of IL-31. These findings strengthen the notion that IL-31 may not primarily function as a non-specific pro-inflammatory cytokine. Rather, it appears to exhibit distinct characteristics that are intricately connected to the pruritogenic

mechanism, as indicated by the observed positive correlations. These correlations, as detailed in the results section and elaborated upon in the subsequent paragraphs of this discussion, underscore the connections we identified between serum IL-31 levels and the specific clinical impacts of pruritus, assessed through the presented scoring systems.

We observed significant correlations between the level of IL-31 and the severity of CSU, as quantified through the UAS7 assessment. These findings align with previous investigations that have also reported associations between IL-31 serum levels and disease severity in patients suffering from CSU [4], atopic dermatitis [36], and uremic pruritus [37]. Notably, a relatively recent study from 2020 demonstrated contrasting results since it found no correlations between IL-31 levels in CSU and psoriasis patients with varying degrees of disease severity, as evaluated by UAS7 and psoriasis area and severity index (PASI) scores, respectively [5]. The correlations observed in our study, corroborating previous research, bolster the prominence of IL-31 in the realm of pruritic skin ailments, particularly CSU. The robust associations between IL-31 and clinical severity scores underscore the significance of this cytokine in the complex tapestry of pruritic pathophysiology. The noteworthy discordant findings in the context of psoriasis and CSU severity further emphasize the need for comprehensive investigations to unravel the multifaceted role of IL-31 in diverse pruritic conditions. It is evident that our findings, along with other pertinent research, lay the groundwork for a deeper understanding of IL-31's involvement in pruritic skin disorders, particularly CSU. The substantial correlations uncovered in this study contribute to the growing body of evidence highlighting IL-31's potential as a key player in pruritus etiology and severity. Nonetheless, further exploration and nuanced studies are essential to elucidate the exact mechanisms that underlie the observed correlations, thereby paving the way for potential therapeutic interventions targeting IL-31 to ameliorate pruritus and enhance the quality of life for affected individuals.

In line with contemporary investigations, other studies have emphasized the significance of this cytokine in the pathogenesis of various conditions, including autoimmune skin diseases, atopic dermatitis, and other atopic skin inflammation, as well as in its impact on the quality of life of patients afflicted by these conditions [7,9,29]. Following the lead of these studies, we aimed to investigate whether there exists a correlation between serum IL-31 levels and the impact on the lives of patients with CSU through the utilization of the DLQI score. Thus, we have demonstrated that IL-31 levels in CSU exhibit a direct proportionality with the impact on patients' quality of life. This finding aligns with the outcomes reported by authors in studies referenced earlier [7,9,29], which similarly encompass conditions marked by pruritus, elevated serum IL-31 levels, and a substantial impact on quality of life. Collectively, these observations underscore a salient association between serum IL-31 levels and their implications for individuals grappling with pruritic disorders. Nevertheless, to solidify and enhance the comprehensiveness of the relationship between IL-31 levels and their impact on quality of life, further investigations involving a larger cohort are imperative.

Past investigations have unveiled alterations in IL-31 levels following targeted therapeutic interventions. For instance, in individuals afflicted by psoriasis, levels of IL-31 in serum exhibited a significant reduction following exposure to narrowband ultraviolet radiation [38]. Furthermore, the efficacious administration of omalizumab to patients grappling with CSU led to noteworthy declines in serum IL-31 concentrations [39]. Additionally, a study involving CSU patients who displayed positive responses to omalizumab elucidated a substantial correlation linking ameliorated clinical symptoms with diminished IL-31-secreting T cells [40]. Although the present study did not explicitly assay IL-31 levels after specific interventions, it was observed that patients who were unresponsive to antihistamine treatment during the initial treatment stages exhibited elevated IL-31 values. In other words, those who solely responded to H1 antihistamines, the primary treatment tier in CSU, demonstrated lower serum IL-31 levels compared to those requiring more intricate therapeutic regimens. These findings, strengthened by effect size coefficients, emphasize the potential clinical relevance of the observed disparities in antihistamine

responses and call for further exploration and consideration in clinical practice. Subsequent investigations aimed at quantifying IL-31 levels contingent on diverse treatment modalities and correlating them with cytokine profiles warrant consideration. Such endeavors hold the potential to unravel the nuanced implications of IL-31 in the disease's pathogenesis, progression, and therapeutic responses.

Strengths and Limitations of the Study

The present investigation encompasses a range of strengths that enhance its scientific significance within the realm of CSU research. By comprehensively exploring the intricate relationships between serum IL-31 levels and various clinical parameters, as well as the nuanced impact on quality-of-life indicators, this study contributes to a deeper understanding of the potential implications of IL-31 in the context of CSU. Moreover, the meticulous examination of multifaceted aspects such as disease severity, itch intensity, and therapeutic responsiveness bolsters the study's comprehensive nature.

The strategic inclusion of a control group within the study design further substantiates the validity of the findings by allowing for meaningful comparisons against a reference baseline. Employing well-established diagnostic criteria and employing standardized methodologies, the study demonstrates methodological rigor, ensuring the reliability and reproducibility of results. Additionally, the judicious application of diverse statistical analyses adds a layer of robustness to the interpretation of observed associations.

Notwithstanding these strengths, the study does bear certain limitations that warrant acknowledgment. The relatively constrained sample size could potentially limit the extrapolation of findings to broader populations, necessitating circumspection when considering the broader implications. The inherent cross-sectional nature of the study design precludes the establishment of causal relationships between IL-31 levels and the clinical parameters explored. To unveil the temporal dynamics and elucidate potential causal mechanisms, prospective longitudinal investigations are imperative.

The single-center setting, while providing a controlled environment, introduces the possibility of selection bias and may restrict the generalizability of the findings to more heterogeneous populations. Collaborative endeavors encompassing multiple centers could mitigate this limitation and enhance the external validity of the study outcomes.

In conclusion, while this study substantively enriches our understanding of the intricate interplay between IL-31 and CSU, it is important to underscore that the strengths and limitations outlined herein underscore the necessity for continued research. Ongoing endeavors with larger and more diverse cohorts, encompassing various settings and employing longitudinal designs, are poised to refine and substantiate the current findings, culminating in a more nuanced comprehension of the intricate interrelationships governing IL-31's role in the clinical context of CSU.

5. Conclusions

In conclusion, our study offers valuable insights into the multifaceted interactions between IL-31 and CSU, shedding light on the complex role of this cytokine in pruritic skin disorders. The significant associations that we observed between serum IL-31 levels and pruritus intensity, disease severity (UAS7), and quality of life (DLQI) underscore IL-31's potential as a key player in the pathophysiology of CSU. Moreover, the correlation between lower IL-31 levels and a positive response to initial H1 antihistamine treatment highlights the potential relevance of IL-31 as a therapeutic target.

The intricate web of relationships that we have unveiled underscores the intricate nature of pruritus etiology and severity and supports the need for further investigation into the underlying mechanisms. As IL-31 emerges as a potential biomarker for disease severity and therapeutic response, future studies with larger, more diverse cohorts and prospective longitudinal designs will provide a deeper understanding of IL-31's temporal dynamics and causal relationships.

While our study advances our understanding of IL-31's involvement in CSU, it is essential to acknowledge the study's limitations, such as the sample size and single-center setting, which call for continued research in more diverse settings. Collectively, the findings presented here contribute to the ongoing pursuit of improved diagnostic tools and therapeutic approaches for individuals grappling with the burden of CSU.

Author Contributions: Conceptualization, C.-T.D.-B.; data curation, C.-T.D.-B. and I.A.M.; formal analysis, C.M.F.; investigation, C.-T.D.-B.; methodology, C.-T.D.-B., I.A.M. and G.A.F.; resources, D.M.D.; software, I.A.M.; supervision, G.A.F.; validation, C.-T.D.-B. and G.A.F.; writing—original draft, C.-T.D.-B.; writing—review and editing, C.-T.D.-B., I.P. and G.A.F. All authors have read and agreed to the published version of the manuscript.

Funding: This work received support from the "Iuliu Hatieganu" University of Medicine and Pharmacyof Cluj-Napoca, Romania through a grant—Proiect Cercetare Doctorala (PCD 2018-2019), contract number 1529/19/18.01.2019.

Institutional Review Board Statement: This study was approved by the Ethics Committee of the UMF "Iuliu Hatieganu" (AVZ270/10.10.2022) and by the Octavian Fodor Institute of Gastroenterology and Hepatology (12637/11.10.2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to especially thank the chemist Nicoleta Decea for her support and coordination in performing the IL-31 ELISA determinations. his work was granted by project PDI-PFE-CDI 2021, entitled Increasing the Performance of Scientific Research, Supporting Excellence in Medical Research and Innovation, PROGRES, no. 40PFE/30.12.2021.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zuberbier, T.; Latiff, A.H.A.; Abuzakouk, M.; Aquilina, S.; Asero, R.; Baker, D.; Ballmer-Weber, B.; Bangert, C.; Ben-Shoshan, M.; Bernstein, J.A.; et al. The international EAACI/GA^{2}LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. *Allergy* **2021**, *77*, 734–766. [CrossRef] [PubMed]
2. Dobrican, C.-T.; Muntean, I.A.; Pintea, I.; Petricău, C.; Deleanu, D.-M.; Filip, G.A. Immunological signature of chronic spontaneous urticaria (Review). *Exp. Ther. Med.* **2022**, *23*, 381. [CrossRef] [PubMed]
3. Kay, A.B.; Clark, P.; Maurer, M.; Ying, S. Elevations in T-helper-2-initiating cytokines (interleukin-33, interleukin-25 and thymic stromal lymphopoietin) in lesional skin from chronic spontaneous ('idiopathic') urticaria. *Br. J. Dermatol.* **2015**, *172*, 1294–1302. [CrossRef] [PubMed]
4. Lin, W.; Zhou, Q.; Liu, C.; Ying, M.; Xu, S. Increased plasma IL-17, IL-31, and IL-33 levels in chronic spontaneous urticaria. *Sci. Rep.* **2017**, *7*, 17797. [CrossRef] [PubMed]
5. Chaowattanapanit, S.; Choonhakarn, C.; Salao, K.; Winaikosol, K.; Julanon, N.; Wongjirattikarn, R.; Foocharoen, C.; Sompornrattanaphan, M. Increased serum IL-31 levels in chronic spontaneous urticaria and psoriasis with pruritic symptoms. *Heliyon* **2020**, *6*, e05621. [CrossRef]
6. Kaplan, A.; Lebwohl, M.; Giménez-Arnau, A.M.; Hide, M.; Armstrong, A.W.; Maurer, M. Chronic spontaneous urticaria: Focus on pathophysiology to unlock treatment advances. *Allergy* **2022**, *78*, 389–401. [CrossRef]
7. Sonkoly, E.; Muller, A.; Lauerma, A.I.; Pivarcsi, A.; Soto, H.; Kemeny, L.; Alenius, H.; Dieu-Nosjean, M.-C.; Meller, S.; Rieker, J.; et al. IL-31: A new link between T cells and pruritus in atopic skin inflammation. *J. Allergy Clin. Immunol.* **2006**, *117*, 411–417. [CrossRef]
8. Gutzmer, R.; Gschwandtner, M.; Rossbach, K.; Mommert, S.; Werfel, T.; Kietzmann, M.; Baeumer, W. Pathogenetic and therapeutic implications of the histamine H4 receptor in inflammatory skin diseases and pruritus. *Front. Biosci.* **2011**, *3*, 985–994. [CrossRef]
9. Gibbs, B.F.; Patsinakidis, N.; Raap, U. Role of the Pruritic Cytokine IL-31 in Autoimmune Skin Diseases. *Front. Immunol.* **2019**, *10*, 1383. [CrossRef]
10. Church, M.K.; Kolkhir, P.; Metz, M.; Maurer, M. The role and relevance of mast cells in urticaria. *Immunol. Rev.* **2018**, *282*, 232–247. [CrossRef]
11. Zhang, Q.; Putheti, P.; Zhou, Q.; Liu, Q.; Gao, W. Structures and biological functions of IL-31 and IL-31 receptors. *Cytokine Growth Factor Rev.* **2008**, *19*, 347–356. [CrossRef]

12. Kasraie, S.; Niebuhr, M.; Werfel, T. Interleukin (IL)-31 induces pro-inflammatory cytokines in human monocytes and macrophages following stimulation with staphylococcal exotoxins. *Allergy* **2009**, *65*, 712–721. [CrossRef] [PubMed]
13. Rauber, M.M.; Pickert, J.; Holiangu, L.; Möbs, C.; Pfützner, W. Functional and phenotypic analysis of basophils allows determining distinct subtypes in patients with chronic urticaria. *Allergy* **2017**, *72*, 1904–1911. [CrossRef] [PubMed]
14. Raap, U.; Gehring, M.; Kleiner, S.; Rüdrich, U.; Eiz-Vesper, B.; Haas, H.; Kapp, A.; Gibbs, B.F. Human basophils are a source of—And are differentially activated by—IL-31. *Clin. Exp. Allergy* **2017**, *47*, 499–508. [CrossRef]
15. Ying, S.; Kikuchi, Y.; Meng, Q.; Kay, A.; Kaplan, A.P. TH1/TH2 cytokines and inflammatory cells in skin biopsy specimens from patients with chronic idiopathic urticaria: Comparison with the allergen-induced late-phase cutaneous reaction. *J. Allergy Clin. Immunol.* **2002**, *109*, 694–700. [CrossRef] [PubMed]
16. Fioranelli, M.; Roccia, M.G.; Lotti, T. Therapy with anti-interleukin-31 receptor A antibody for atopic dermatitis. *Dermatol. Ther.* **2017**, *30*, e12490. [CrossRef] [PubMed]
17. Kabashima, K.; Furue, M.; Hanifin, J.M.; Pulka, G.; Wollenberg, A.; Galus, R.; Etoh, T.; Mihara, R.; Nakano, M.; Ruzicka, T. Nemolizumab in patients with moderate-to-severe atopic dermatitis: Randomized, phase II, long-term extension study. *J. Allergy Clin. Immunol.* **2018**, *142*, 1121–1130.e7. [CrossRef]
18. Oyama, S.; Kitamura, H.; Kuramochi, T.; Higuchi, Y.; Matsushita, H.; Suzuki, T.; Goto, M.; Adachi, H.; Kasutani, K.; Sakamoto, A.; et al. Cynomolgus monkey model of interleukin-31-induced scratching depicts blockade of human interleukin-31 receptor A by a humanized monoclonal antibody. *Exp. Dermatol.* **2016**, *27*, 14–21. [CrossRef]
19. Mathias, S.D.; Crosby, R.D.; Zazzali, J.L.; Maurer, M.; Saini, S.S. Evaluating the minimally important difference of the urticaria activity score and other measures of disease activity in patients with chronic idiopathic urticaria. *Ann. Allergy Asthma Immunol.* **2012**, *108*, 20–24. [CrossRef]
20. Hawro, T.; Ohanyan, T.; Schoepke, N.; Metz, M.; Peveling-Oberhag, A.; Staubach, P.; Maurer, M.; Weller, K. The urticaria activity score-validity, reliability, and responsiveness. *J. Allergy Clin. Immunol. Pract.* **2018**, *6*, 1185–1190.e1. [CrossRef]
21. Baiardini, I.; Braido, F.; Bindslev-Jensen, C.; Bousquet, P.J.; Brzoza, Z.; Canonica, G.W.; Compalati, E.; Fiocchi, A.; Fokkens, W.; Gerth van Wijk, R.; et al. Recommendations for assessing patient-reported outcomes and health-related quality of life in patients with ur-ticaria: A GA(2)LEN taskforce position paper. *Allergy* **2011**, *66*, 840–844. [CrossRef] [PubMed]
22. Weller, K.; Groffik, A.; Magerl, M.; Tohme, N.; Martus, P.; Krause, K.; Metz, M.; Staubach, P.; Maurer, M. Development and construct validation of the angioedema quality of life questionnaire. *Allergy* **2012**, *67*, 1289–1298. [CrossRef] [PubMed]
23. O'Donnell, B.F.; Lawlor, F.; Simpson, J.; Morgan, M.; Greaves, M.W. The impact of chronic urticaria on the quality of life. *Br. J. Dermatol.* **1997**, *136*, 197–201. [CrossRef] [PubMed]
24. Parisi, C.A.; Ritchie, C.; Petriz, N.; Morelo Torres, C. Direct medical costs of chronic urticaria in a private health organization of Buenos Aires, Argentina. *Value Health Reg. Issues* **2016**, *11*, 57–59. [CrossRef]
25. Broder, M.S.; Raimundo, K.; Antonova, E.; Chang, E. Resource use and costs in an insured population of patients with chronic idiopathic/spontaneous urticaria. *Am. J. Clin. Dermatol.* **2015**, *16*, 313–321. [CrossRef]
26. Graham, J.; McBride, D.; Stull, D.; Halliday, A.; Alexopoulos, S.T.; Balp, M.M.; Griffiths, M.; Agirrezabal, I.; Zuberbier, T.; Brennan, A. Cost utility of omalizumab compared with standard of care for the treatment of chronic spontaneous urticaria. *Pharm. Econ.* **2016**, *34*, 815–827. [CrossRef]
27. Zhou, B.; Li, J.; Liu, R.; Zhu, L.; Peng, C. The Role of Crosstalk of Immune Cells in Pathogenesis of Chronic Spontaneous Urticaria. *Front. Immunol.* **2022**, *13*, 879754. [CrossRef] [PubMed]
28. Yang, X.; Chen, L.; Wang, S.; Wu, Y.; Zhou, X.; Meng, Z. The correlation between Th17/Treg immune dysregulation and the disease severity in chronic spontaneous urticaria patients. *Immun. Inflamm. Dis.* **2023**, *11*, e920. [CrossRef]
29. Saleem, M.D.; Oussedik, E.; D'amber, V.; Feldman, S.R. Interleukin-31 pathway and its role in atopic dermatitis: A systematic review. *J. Dermatol. Treat.* **2017**, *28*, 591–599. [CrossRef]
30. Bağci, I.S.; Ruzicka, T. IL-31: A new key player in dermatology and beyond. *J. Allergy Clin. Immunol.* **2018**, *141*, 858–866. [CrossRef]
31. Lai, T.; Wu, D.; Li, W.; Chen, M.; Yi, Z.; Huang, D.; Jing, Z.; Lü, Y.; Lv, Q.; Li, D.; et al. Interleukin-31 expression and relation to disease severity in human asthma. *Sci. Rep.* **2016**, *6*, 22835. [CrossRef] [PubMed]
32. Lei, Z.; Liu, G.; Huang, Q.; Lv, M.; Zu, R.; Zhang, G.-M.; Feng, Z.-H.; Huang, B. Original article: SCF and IL-31 rather than IL-17 and BAFF are potential indicators in patients with allergic asthma. *Allergy* **2007**, *63*, 327–332. [CrossRef] [PubMed]
33. West, N.R.; Hegazy, A.N.; Owens, B.M.J.; Bullers, S.J.; Linggi, B.; Buonocore, S.; Coccia, M.; Görtz, D.; This, S.; Stockenhuber, K.; et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor–neutralizing therapy in patients with inflammatory bowel disease. *Nat. Med.* **2017**, *23*, 579–589. [CrossRef] [PubMed]
34. Roosterman, D.; Goerge, T.; Schneider, S.W.; Bunnett, N.W.; Steinhoff, M. Neuronal control of skin function: The skin as a neuro-immuno-endocrine organ. *Physiol. Rev.* **2006**, *86*, 1309–1379. [CrossRef]
35. Datsi, A.; Steinhoff, M.; Ahmad, F.; Alam, M.; Buddenkotte, J. Interleukin-31: The "itchy" cytokine in inflammation and therapy. *Allergy* **2021**, *76*, 2982–2997. [CrossRef]
36. Raap, U.; Wichmann, K.; Bruder, M.; Ständer, S.; Wedi, B.; Kapp, A.; Werfel, T. Correlation of IL-31 serum levels with severity of atopic dermatitis. *J. Allergy Clin. Immunol.* **2008**, *122*, 421–423. [CrossRef]
37. Ko, M.-J.; Peng, Y.-S.; Chen, H.-Y.; Hsu, S.-P.; Pai, M.-F.; Yang, J.-Y.; Wen, S.-Y.; Jee, S.-H.; Wu, H.-Y.; Chiu, H.-C. Interleukin-31 is associated with uremic pruritus in patients receiving hemodialysis. *J. Am. Acad. Dermatol.* **2014**, *71*, 1151–1159.e1. [CrossRef]

38. Narbutt, J.; Olejniczak, I.; Sobolewska-Sztychny, D.; Sysa-Jedrzejowska, A.; Słowik-Kwiatkowska, I.; Hawro, T.; Lesiak, A. Narrow band ultraviolet B irradiations cause alteration in interleukin-31 serum level in psoriatic patients. *Arch. Dermatol. Res.* **2012**, *305*, 191–195. [CrossRef]
39. Altrichter, S.; Hawro, T.; Hänel, K.; Czaja, K.; Lüscher, B.; Maurer, M.; Church, M.; Baron, J. Successful omalizumab treatment in chronic spontaneous urticaria is associated with lowering of serum IL-31 levels. *J. Eur. Acad. Dermatol. Venereol.* **2014**, *30*, 454–455. [CrossRef]
40. Rauber, M.; Pickert, J.; Holiangu, L.; Möbs, C.; Pfützner, W. Omalizumab response correlates with reduced IFN-γ-, IL-10- and IL-31-secreting cells in chronic spontaneous urticaria. *J. Eur. Acad. Dermatol. Venereol.* **2020**, *34*, 2078–2085. [CrossRef]

biomedicines

MDPI

Communication

Managing Severe Adverse Reactions to Biologicals in Severe Asthma

Radu-Gheorghe Balan [1,2], Diana Mihaela Deleanu [1,3,4], Irena Pintea [1,3,*], Carmen Teodora Dobrican Baruta [1,3,*], Milena Adina Man [5,6], Ioana Corina Bocsan [1,7,8] and Ioana Adriana Muntean [1,3]

1 Department of Allergology and Immunology, "Iuliu Haţieganu" University of Medicine and Pharmacy, Str. Croitorilor 19–21, 400058 Cluj-Napoca, Romania; balan.radu.gheorghe@elearn.umfcluj.ro (R.-G.B.); diana.deleanu@umfcluj.ro (D.M.D.); bocsan.corina@umfcluj.ro (I.C.B.); adriana.muntean@umfcluj.ro (I.A.M.)
2 Department of Science and Technology, George Emil Palade University of Medicine, Pharmacy, Science and Technology, 540067 Târgu Mureş, Romania
3 Department of Allergology, "Professor Doctor Octavian Fodor" Regional Institute of Gastroenterology and Hepatology, 400162 Cluj-Napoca, Romania
4 Department of Internal Medicine, "Professor Doctor Octavian Fodor" Regional Institute of Gastroenterology and Hepatology, 400162 Cluj-Napoca, Romania
5 Department of Medical Sciences, "Iuliu Haţieganu" University of Medicine and Pharmacy, 400337 Cluj-Napoca, Romania; manmilena50@yahoo.com
6 Department of Pneumology, "Leon Daniello" Clinical Hospital of Pulmonology, 400332 Cluj-Napoca, Romania
7 Department of Pharmacology, Toxicology and Clinical Pharmacology, "Iuliu Haţieganu" University of Medicine and Pharmacy, 400337 Cluj-Napoca, Romania
8 Almedo—Allergology and Clinical Immunology Outpatient Clinic, 400394 Cluj-Napoca, Romania
* Correspondence: irena.nedelea@umfcluj.ro (I.P.); dobrican.carmen@umfcluj.ro (C.T.D.B.)

Abstract: Background: The use of biological agents in the treatment of various inflammatory and malignancy conditions has expanded rapidly. However, these agents can induce hypersensitivity reactions, posing significant clinical challenges. Methods: We conducted a retrospective study that included nine patients with severe asthma who experienced hypersensitivity reactions to biological agents (omalizumab, benralizumab and dupilumab). Results: Hypersensitivity reactions to biologicals in severe asthma were observed in 9 of 68 patients treated. In five cases, treatment was stopped or changed to another available biological, and for four patients administered under close surveillance, titrated provocation or desensitization was applied. Successful desensitization was achieved in three of the patients, allowing them to continue therapy without adverse reactions. Improvements in asthma control were observed post-desensitization, leading to the reduced need for systemic steroid treatments and an increase in quality of life. Conclusions: This study highlights the importance of recognizing hypersensitivity reactions to biologicals to have an appropriate approach for patients with severe asthma. As an effective approach for patients experiencing hypersensitivity reactions to biological agents, desensitization allows treatment continuation.

Keywords: asthma; monoclonal antibodies; biologic therapy; hypersensitivity; desensitization

Citation: Balan, R.-G.; Deleanu, D.M.; Pintea, I.; Dobrican Baruta, C.T.; Man, M.A.; Bocsan, I.C.; Muntean, I.A. Managing Severe Adverse Reactions to Biologicals in Severe Asthma. *Biomedicines* **2023**, *11*, 3108. https://doi.org/10.3390/biomedicines11123108

Academic Editors: Hiroshi Wakao and Diego Bagnasco

Received: 11 October 2023
Revised: 16 November 2023
Accepted: 17 November 2023
Published: 21 November 2023

1. Introduction

In recent years, there has been a rapid increase in the number of biological agents approved by pharmaceutical agencies across the globe for treating various inflammatory and malignancy conditions. As the use of these agents continues to expand, despite their therapeutic potential, their mechanism of action exposes them to the risk of immune-mediated effects, which can have significant implications [1,2].

To gain a deeper understanding of adverse reactions related to biological agents, it is essential to consider key differences between them and conventional drugs. Unlike

most drugs, which are generally small molecules with molecular weights below 1 kDa (kilodaltons), biological agents are large-sized proteins designed to structurally resemble autologous proteins and have much larger molecular weights exceeding 1 kDa. Furthermore, drugs are chemically synthesized, while biological agents are produced using molecular genetic techniques and purified from genetically modified cells. Most biological agents require parenteral administration to avoid gastrointestinal degradation, whereas drugs can often be administered orally or parenterally and undergo metabolism. While drug metabolism can sometimes generate immunogenic intermediaries, biological agents are subject to processing but not metabolism. Ultimately, biological agents exhibit intrinsic immune-mediated effects because they originate from non-self proteins, a characteristic usually associated with smaller synthetic compounds in drugs [1].

Due to these differences, efforts have been made to categorize adverse reactions to biological agents using classification systems that emphasize their immune-target effects. In 2006, Pichler proposed such a classification scheme, which was later elaborated by Haussman and his colleagues [1–3]. This classification assigns Greek letters (alpha, beta, gamma, delta and epsilon) to various types of reactions (Table 1).

Table 1. Adverse reactions to biological agents [1,2].

Type	Example Reaction (Causative Drug)
α: Overstimulation	Cytokine release syndrome (cytokine storm) (muromunab, TGN1412)
β: Hypersensitivity	Common acute infusion reactions (rituximab), delayed infusion reactions (etanercept, adalimumab), anaphylaxis (muromunab, cetuximab, omalizumab)
γ: Cytokine of immune imbalance	
Immunodeficiency	Increased risk of tuberculosis—anti-TNF (tumor necrosis factor) agents Hypogammaglobulinemia (rituximab)
Autoimmunity	Systemic lupus erythematosus or vasculitis: IFN-γ(interferon gamma)
Atopic disorders	Atopic dermatitis (anti-TNF agents)
δ: Cross-reactivity	Acne from anti-EGFR (epidermal growth factor receptor): cetuximab, panitumumab
ε: Nonimmunological side effects	Neuropsychiatric side effects including confusion or depression (IFN-α)

Beta-type reactions to biological agents involve hypersensitivity reactions, which can manifest as immediate or delayed responses. Factors that influenced these reactions include the type of immune response triggered: IgE (immunoglobulin E) mediated complement activation or immune complexes disease. Also, in the immune response towards biologicals, the degree of humanization of the monoclonal antibody and the presence of adjuvants or excipients should also be considered in the assessment of the reaction. Immediate reactions may involve the production of IgE antibodies targeting non-self peptide sequences, although immediate IgE-mediated hypersensitivity reactions are not commonly the primary cause of these reactions. Many patients can tolerate the same agent when administered at a slower rate or with premedication, such as antihistamines and steroids. However, IgE-mediated anaphylactic reactions have been reported with several biological agents, including infliximab, omalizumab and cetuximab, which were available early on the market, but also in new agents such as benralizumab and checkpoint inhibitors (nivolumab and ipilimumab). Studies have shown that IgE-mediated anaphylactic reactions to cetuximab are associated with pre-existing IgE antibodies against galactose-α-1,3-galactose. Common acute infusion reactions, largely comprising predictable and mild symptoms, represent most reactions to monoclonal antibodies. The underlying mechanism is not fully understood but may involve the release of proinflammatory cytokines [4–9].

Complement activation is also considered to play a role in immediate hypersensitivity reactions, as C3a (complement component 3 activated) and C5a (complement component 5 activated) cleavage products can directly stimulate mast cells and trigger mast cell ac-

tivation independent of IgE. The degree of humanization of monoclonal antibodies has evolved, with fully humanized and human monoclonal antibodies having reduced immunogenicity compared to antibodies derived from mice. However, even humanized monoclonal antibodies contain non-self peptide sequences that can lead to the formation of anti-human antibodies. The consequences of these antibodies are usually delayed and involve the production of IgG antibodies, which can lead to drug inactivation but not necessarily severe symptoms. Complement is also believed to play a role in delayed reactions through the formation of immune complexes and serum sickness-like reactions. Some case reports have suggested T-cell-mediated hypersensitivity causing delayed maculopapular exanthems [3–5].

In severe asthma, there are different biological therapies available, as detailed in Table 2, such as anti-immunoglobulin E (anti-IgE) therapy (omalizumab), anti-interleukin-5 (anti-IL-5) (mepolizumab, reslizumab), anti-alpha subunit-IL-5 receptor (benralizumab), an anti-alpha subunit of the IL-4 receptor/IL-13 receptor (dupilumab) or anti-thymic stromal lymphopoietin (TSLP) (tezepelumab). The recommendation for a specific biological action depends on several factors such as their age, serum total IgE level, eosinophils level and the presence of type-2 asthma phenotype. The selection of treatment is also performed according to national protocols and availability of them in each country [10,11].

Table 2. Biologicals available for severe asthma in Europe in 2023 (alphabetical order) [11].

Generic Name	Trade Name	Target
Benralizumab	Fasenra®	alpha subunit of the IL5R
Dupilumab	Dupixent®	alpha subunit of the IL-4R and the IL-13R
Mepolizumab	Nucala®	IL5
Omalizumab	Xolair®	IgE
Reslizumab	Cinqair®	IL5
Tezepelumab	Tezspire®	TSLP

It is important to note that hypersensitivity reactions, including IgE-mediated ones, can present symptoms that overlap with common infusion reactions (such as gastrointestinal symptoms, dyspnea, transient skin redness, back pain) and symptoms of the underlying disease. Symptoms suggestive of hypersensitivity reactions may include urticaria with/without angioedema, wheezing, frequent cough, severe rash or anaphylactic symptoms. Hypersensitivity reactions to biological agents appear to be less common and have been reported for various agents, including omalizumab, natalizumab, rituximab, dupilumab and cetuximab [4,12–18].

Desensitization, as a management strategy for hypersensitivity reactions to biological agents, has emerged as an option, mainly when other biologicals are not available, but has yielded varying results [3,4].

In this study, we evaluated the hypersensitivity reactions to biological recommended for severe asthma that were available in our country in 2023; reactions were evaluated in three clinical settings specialized in severe asthma treatment.

2. Materials and Methods

We present a case series of adverse reactions to monoclonal antibodies (MoAb) used in severe asthma. From a group of 68 patients with severe asthma (treated in the Pneumology Department, the Allergology Department, Almedo Clinic), we evaluated 9 patients with adverse reactions requiring hospitalization or emergency room visits.

Out of the nine patients, three underwent successful desensitization to biologicals used in the treatment of severe asthma: two to omalizumab and another to dupilumab. For data collection, we reviewed the electronic and physical medical records.

Our study received approval from the Institutional Review Board at our facility (no. 10577/09.12.2021), and we obtained informed consent from all patients before proceeding with any investigation. The desensitization procedures took place between 2019 and 2022

at the Allergology Department of IRGH Cluj-Napoca, a specialized unit for treating allergic drug reactions.

3. Results

Of 68 patients with severe asthma under biologicals (from 2016 to 2023 in all three centers), 14 patients (20.5%) are treated with omalizumab, 16 patients with dupilumab (23.5%) and 38 patients (56%) with benralizumab. Hypersensitivity reactions are described below for each patient and summarized in Table 3.

Table 3. Summary of investigations in 9 cases of MoAb (monoclonal antibodies) adverse reactions.

Patient	Age (y)/Sex	Index Reaction to MoAb	Drug Cause	SPT	IDT	Follow-Up Attitude
1	60 F	AFX/UNK	Dupilumab	UD negative	1:1000 negative 1:100 negative 1:10 negative	Desensitization/itrated provocation with successful continuation of treatment
2	31 F	AFX	Omalizumab	UD positive	ND	Desensitization with successful continuation of treatment
3	40 M	AFX/UNK	Omalizumab	UD negative	1:10 negative	Titrated provocation with successful continuation of treatment
4	53 M	AR	Benralizumab	ND	ND	Successful continuation of treatment
5	48 M	AFX	Omalizumab	ND	ND	Treatment cessation
6	30 F	SS	Omalizumab	ND	ND	Treatment cessation
7	50 F	Severe adverse reaction	Omalizumab	ND	ND	Treatment cessation
8	47 F	AFX/UNK	Benralizumab	UD negative / 1:10 negative	1:10 negative	Treatment cessation
9	68 F	Delayed hypersensitivity (maculopapular rash with arthralgias and abdominal pain)	Dupilumab	UD negative	1/10 negative	Treatment cessation

AFX, anaphylaxis; F, female; M, male; UD, undiluted; ND, not done; SS, serum sickness; UNK, unknown; AR, adverse reaction.

3.1. Patient 1

A 60-year-old female with a history of severe persistent asthma since childhood had a moderate obstructive pattern in baseline spirometry with significant reversibility (FEV1 (Forced Expiratory Volume in the 1st second) = 57%, with an 18% bronchodilator response). After experiencing multiple ICU admissions and exacerbations necessitating oral steroids, she was started dupilumab 200 mg, resulting in significant improvement in asthma control after the first month of treatment. However, 2 h after the third dose of dupilumab, she developed a systemic hypersensitivity reaction to dupilumab. This reaction included facial edema and paresthesia with an extended local reaction at the site of administration in the absence of significant lung function impairment (FEV1 = 55%). While under the care of the Leon Daniello Pneumology Hospital, she was treated with hydrocortisone, salmeterol

and diphenhydramine, with slow remission of symptoms during the following three days. Dupilumab was stopped, and OCS (oral corticosteroids) were added.

Two months later, she was referred to our center for testing and possible desensitization to dupilumab. Skin prick testing with undiluted dupilumab (200 mg/mL) yielded a weak irritation reaction (wheal of 2–3 mm in the absence of erythema or pruritus), and intradermal testing with concentrations starting from 0.2 mg/mL to 20 mg/mL (1:1000, 1:100, 1:10) were all negative (Table 3). Desensitizing was attempted with progressive administration of dupilumab diluted in saline solution every 30 min, according to Table 4. However, after receiving the fifth dose (40 mg, total dose of 70 mg), she developed undereye edema; desensitization was paused, and the patient was monitored for further developments. The next day, we continued desensitization according to Table 4 up to a cumulated dose of 200 mg dupilumab, which she tolerated. The protocol was completed without further complications, and she has since continued receiving 200 mg dupilumab every two weeks without any hypersensitivity reactions. She was able to taper off systemic steroids, and her asthma was well controlled.

Table 4. Proposed dupilumab desensitization protocol/titrated provocation.

Day	Dose	Solution Concentration (mg/mL)	Dosage (mg)
1	1	2	0.2
	2	20	0.4
	3	200	10
	4	200	20
	5	200	40
		Cumulative dose (mg)	70.00
2	6	200	20
	7	200	40
	8	200	80
	9	200	40
		Cumulative dose (mg)	200

3.2. Patient 2

A 31-year-old female had severe, persistent asthma and a history of two episodes of severe anaphylaxis to tree nuts, with baseline spirometry revealing a moderate obstructive pattern with significant reversibility (FEV1 = 63%, with a 21% bronchodilator response). She exhibited sensitivity to house dust mite allergens, various pollens and molds with a total IgE level of 487 IU/mL. Her asthma required daily prednisone in addition to high-dose inhaled corticosteroids combined with long-acting beta-agonists for control. After starting omalizumab, she was able to discontinue daily oral corticosteroids. However, after 3 months of omalizumab therapy, she presented an episode of grade II anaphylaxis [19]: generalized urticaria, facial edema, shortness of breath and extended local reaction at the site of administration immediately after injection.

She was referred to our center for testing and possible desensitization to omalizumab. Our target dose for desensitization was 300 mg out of her regular dose of 450 mg per month. Skin prick testing with undiluted omalizumab (150 mg/mL) was positive (Table 3). The desensitization was performed according to the protocol detailed in Table 5 over the course of two days. She completed the protocol without complications and has continued monthly treatments without further reactions. Her asthma has improved, allowing her to discontinue administration of oral corticosteroids (OCS).

3.3. Patient 3

A 40-year-old man with severe uncontrolled persistent asthma had baseline spirometry revealing mild restriction and moderate obstructive pattern without significant reversibility (FEV1 = 68%, with a 9% bronchodilator response). He also presented NSAID (non-steroidal anti-inflammatory drugs) exacerbated respiratory disease and chronic rhinosinusitis with

nasal polyposis. He exhibited sensitivity to house dust mite allergens and grass pollen and had a history of grade II food-related anaphylaxis (peanuts, shrimp and pumpkin seeds). The total IgE level was 1434 IU/mL. His asthma required high-dose inhaled corticosteroids combined with long-acting beta-agonists and leukotriene receptor antagonists, and it was still uncontrolled. After the first dose of omalizumab, within 20 min, he experienced dyspnea, wheezing, angioedema and conjunctivitis; omalizumab was stopped.

Table 5. Omalizumab desensitization protocol.

Day	Dose	Solution Concentration (mg/mL)	Dosage (mg)
1	1	1.5	0.15
	2	15	1.5
	3	150	4.5
	4	150	22.5
	5	150	37.5
		Cumulative dose (mg)	66.15
2	6	150	15
	7	150	22.5
	8	150	37.5
	9	150	75
		Cumulative dose (mg)	150

He was referred to our center for testing and possible desensitization to omalizumab. Our target dose was 300 mg out of his regular dose of 450 mg per month. Skin prick testing with undiluted omalizumab (150 mg/mL) and intradermal testing with diluted omalizumab in saline (15 mg/mL, 1:10) were all negative (Table 3). Since skin prick tests were negative, we began the titrated provocation protocol detailed in Table 6. He completed the protocol without complications, and he has continued monthly treatments with the indicated dose without further reactions. His asthma has improved, allowing him to discontinue administration of oral corticosteroids (OCS).

Table 6. Omalizumab titrated provocation (minimal risk patients, negative skin tests).

Day	Dose	Solution Concentration (mg/mL)	Dosage (mg)
1	1	150	15
	2	150	30
	3	150	45
	4	150	60
	5	150	150
		Cumulative dose (mg)	300

3.4. Patient 4

A 53-year-old man with severe, persistent asthma: baseline spirometry revealed mild restriction and moderate obstructive pattern without significant reversibility (FEV1 = 61%, with a 7.5% bronchodilator response) and chronic rhinosinusitis with nasal polyposis. His asthma required high-dose inhaled corticosteroids combined with long-acting beta-agonists for control. Treatment was initiated with benralizumab 30 mg/month. One hour after the first dose, the patient experienced severe headache, hypertension (200/120 mmHg), tachycardia (120 bpm) and, later that evening, associated arthralgia and myalgia. Following emergency cardiological investigations, the patient started treatment with antihypertensive medication. Biological treatment with benralizumab was continued a month later without any further adverse effects.

3.5. Patient 5

A 48-year-old male with severe, persistent asthma with baseline spirometry revealing moderate–severe obstructive pattern with significant reversibility (FEV1 = 54%, with a 20% bronchodilator response) had presented grade III anaphylaxis to a bee sting and grade IV anaphylaxis to penicillin and ampicillin [19]. His asthma remains uncontrolled despite treatment with high-dose inhaled corticosteroids combined with long-acting beta-agonists and leukotriene receptor antagonists, as well as frequent courses of high-dose oral steroids. Treatment was initiated with omalizumab (300 mg q2wk). Five years of omalizumab treatment was tolerated. In the 5th year of treatment, 20 min after administration, the patient exhibited severe headache, vertigo, severe arterial hypotension and wheezing, interpreted as a grade III/IV anaphylactic reaction. Symptoms remitted after epinephrine administration, and he was hospitalized (for a total of 1000 mcg, epinephrine i.m.). Omalizumab was stopped, and OCS were added again for 6 months until dupilumab treatment was initiated when it was approved in Romania. The patient received dupilumab and tolerated the treatment with cessation of OCS.

3.6. Patient 6

A 30-year-old female with severe, persistent asthma presented baseline spirometry, revealing a moderate obstructive pattern with significant reversibility (FEV1 = 64%, with a 13% bronchodilator response) during asthma initial evaluation. She exhibited sensitivity to house dust mites and dog dander. Her asthma was still uncontrolled (FEV1 = 68%, ACT (asthma control test) = 10 maximum points) despite treatment with high-dose inhaled corticosteroids combined with long-acting beta-agonists and leukotriene receptor antagonists, as well as frequent courses of high-dose oral steroids. Treatment was initiated with omalizumab (300 mg q2wk). Three days after the first injection of omalizumab, the patient exhibited flu-like symptoms: myalgia, arthralgia and painful laterocervical adenopathy with generalized urticarial lesions, which needed a course of 4 weeks of OCS until remission. Subsequent attempts at continuing biological treatment with half of the dose led to the same outcome. The pattern of symptoms was interpreted as serum sickness due to omalizumab; thus, cessation of treatment was chosen.

3.7. Patient 7

A 50-year-old female with severe, persistent asthma and baseline spirometry revealing a moderate obstructive pattern with significant reversibility (FEV1 = 68%, with an 18% bronchodilator response) exhibited sensitivity to bees and yellow jacket venom. Her asthma required high-dose inhaled corticosteroids combined with long-acting beta-agonists and leukotriene receptor antagonists for control, as well as frequent courses of high-dose oral steroids. Treatment was initiated with omalizumab (300 mg q2wk). Two days after the second injection of omalizumab, the patient exhibited intense asthenia, nausea and dyspnea, which persisted for 3 weeks and made her unable to attend work; an OCS course for 2 weeks was helpful for her asthma and asthenia. The pattern of symptoms was interpreted as an adverse reaction to omalizumab. Other neurologic, rheumatologic or infectious diseases were excluded. No other biologic agent was initiated, despite the aggravation of her asthma, and several courses of OCS were needed.

3.8. Patient 8

A 47-year-old female with severe, persistent asthma and baseline spirometry revealing a moderate obstructive pattern with significant reversibility (FEV1 = 56%, with a 20% bronchodilator response) exhibited sensitivity to grass pollen. Her asthma required high-dose inhaled corticosteroids combined with long-acting beta-agonists and leukotriene receptor antagonists, as well as frequent courses of high-dose oral steroids, and still was uncontrolled. Treatment was initiated with benralizumab (30 mg q8wk). Five minutes after the eighth dose of benralizumab, the patient exhibited loss of consciousness (30 s), same-level fall, snoring and diaphoresis. After 24 h, she also develops mild urticaria. She was

admitted to the ER, and the neurological assessment excluded an underlying neurological pathology. Symptoms remitted after systemic corticosteroids and supportive treatment.

Skin prick testing with dilute and undiluted benralizumab (30 mg/mL, 1:1; 3 mg/mL, 1:10) as well as intradermal testing with a concentration of dilute benralizumab in saline (3 mg/mL, 1:10), were performed, and all were negative (Table 3). Twenty-four hours after skin prick and intradermal tests, she developed generalized urticaria that ceased in twenty-four hours without treatment. Benralizumab was stopped.

3.9. Patient 9

A 68-year-old female with severe allergic asthma has had multiple allergen sensitizations since she was 20 years old (house dust mites, cat, grass pollen, birch pollen, cockroaches), with also two episodes of anaphylaxis in the last 5 years from nuts and banana. At 40 years old, baseline spirometry revealed a moderate obstructive pattern with significant reversibility (FEV1 = 62% from predicted, with a 14% bronchodilator response). Her asthma required high-dose inhaled corticosteroids combined with long-acting beta-agonists and leukotriene receptor antagonists, as well as frequent courses of high-dose oral steroids in the last year, and despite this treatment, asthma was still uncontrolled (FEV1= 67% from predicted, ACT test under 15 points 6 months before biological treatment initiation). Treatment was initiated with dupilumab (200 mg q2wk) with improvement in asthma control and lung function after the first doses and continuing 1 year after. After the 11th month of treatment, the patient developed large local reactions at the injection site (erythema, itch, edema around 5 mm). Forty-eight hours after the dose of dupilumab in the 15th month, the patient exhibited maculopapular rash (as shown in Figure 1), arthralgias, bloating, shivers and abdominal pain. She was presented in the ER; lab tests were performed for infectious disease screening, and inflammatory status showed no abnormalities. The dermatological evaluation was performed to exclude other dermatological diseases, but drug-induced maculopapular rash was confirmed. Symptoms remitted after systemic corticosteroids, antihistamines and supportive treatment. Dupilumab was stopped, and an allergy work-up was made after 4 weeks.

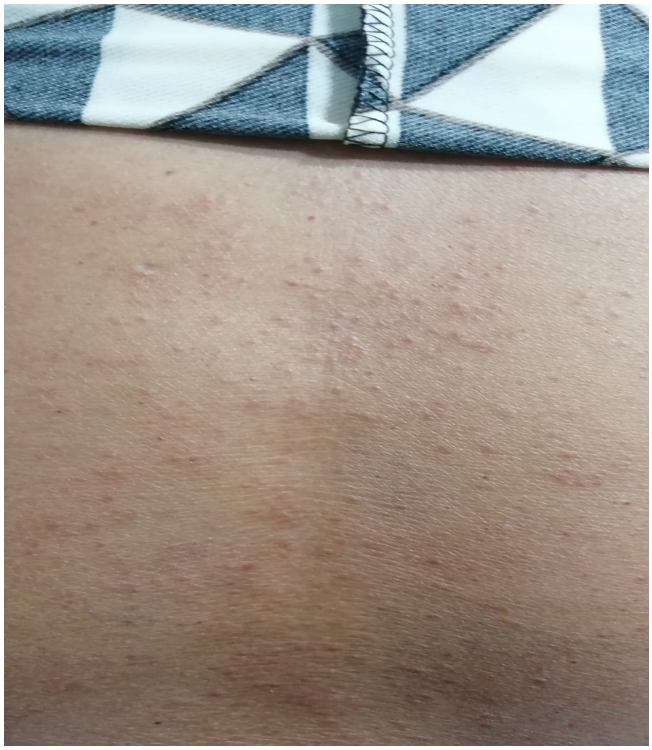

Figure 1. Skin aspect on the back, extended to all skin.

Skin prick testing with undiluted dupilumab after 4 weeks (200 mg/mL, 1:1) and intradermal testing with a concentration of dilute dupilumab in saline (20 mg/mL, 1:10) were performed and were all negative (Table 3). The patient tolerated several courses with vitamins from group B with Polysorbate 80 as an excipient. It is necessary to evaluate the risk/benefit ratio in order to purchase the titrated provocation; because severe asthma was controlled after dupilumab, we obtained informed consent and began the provocation test. This type of delayed hypersensitivity does not always present with a positive skin test, and provocation is the gold standard diagnosis method. The patient followed step 1 (day 1) from Table 3 at a cumulative dose of 20 mg dupilumab; after 2 h, the patient presented with shivers, lingual edema, metallic taste and a decrease in arterial tension (30 mmHg difference from baseline value); thus, the provocation test was stopped. Symptoms remitted after epinephrine, antihistamines, saline solution and systemic i.v. corticosteroids (8 mg dexamethasone).

4. Discussion

Biologicals in the treatment of severe asthma were introduced in Romania in 2016 (omalizumab), 2019 (benralizumab) and 2022 (dupilumab), with an increase in quality of life for those patients. This treatment is "life changing" for severe asthma patients, so the appearance of adverse reactions as hypersensitivity reactions could lead to important problems.

Although monoclonal antibodies have demonstrated their efficacy in clinical studies for severe asthma, several factors must be considered when initiating and monitoring biological treatment in severe asthma. In this clinical decision process, the risks/benefits of biologic therapy need to be understood to adequately counsel patients and appropriately monitor for potential adverse events. Also, because biologicals in severe asthma improve the level of control and quality of life, cessation should be considered as a last resort, especially when no other biological therapy is available, and desensitization protocols should be carefully implemented [20–26].

Omalizumab has been associated with an increased risk of anaphylaxis, and the product label includes a black box warning from the FDA for anaphylaxis, so an emergency kit with epinephrine is mandatory for users. The diagnosis of anaphylaxis is often difficult in severe asthma patients. Also, asthma increases the risk of severe anaphylaxis. On the other hand, omalizumab is useful in the treatment of severe IgE-mediated anaphylaxis, and it is used in desensitization protocols for other drugs or pre-treatment in allergen immunotherapy [20–27].

Risk factors for developing anaphylaxis to omalizumab include a prior history of anaphylaxis, which was present in the clinical history of our three patients with omalizumab anaphylaxis [28,29].

Diagnostic hypersensitivity reactions to monoclonal antibodies are difficult; testing for IgE-mediated hypersensitivity reactions may involve skin and intradermal tests, but it is crucial to determine non-irritating concentrations for drug testing. Omalizumab is the only biological agent for which non-irritating concentrations have been systematically established, but there are unmet needs to establish non-irritating concentrations for other biologicals. Various dilutions of omalizumab for skin and intradermal tests were studied in 2010 to ensure safety and interpretable results for immediate IgE-mediated hypersensitivity reactions. It was found that dilutions in sterile water caused irritant reactions, leading to the subsequent use of saline dilutions. An established non-irritant concentration for omalizumab testing with saline dilution is 1:100,000 (equivalent to a concentration of 1.25 mg/mL). However, the utility of omalizumab skin testing and additional data on positive and negative predictive values remain unknown [30,31].

Desensitization is a procedure performed only by specialized centers, where patients receive increasing doses of a drug to which they had previously experienced a hypersensitivity reaction until the target therapeutic dosage is achieved. The exact mechanism by which desensitization occurs is not entirely understood but involves temporary tolerance of mast cells and basophils to the drug involved in the hypersensitivity reaction. Successful

desensitization protocols have been published for other monoclonal antibodies, especially for omalizumab, which has a high rate of anaphylaxis [29–31]. In patients with a negative skin test or minimal risk, we chose a titrated provocation, which leads to a rapid increase in the amount of drug up to the therapeutical dose.

Several case reports describe the onset of adverse events like arthralgia after use of omalizumab. Specifically, serum sickness-like reactions have been described, as we also reported in our communication [26,32,33].

Benralizumab is mainly indicated in eosinophilic severe asthma; hypersensitivity reactions (urticaria, polyangiitis, and even anaphylaxis in one case) were reported in placebo-controlled trials. In our patient in whom we suspected anaphylaxis to benralizumab, serum tryptase was not evaluated immediately after the reaction, which could be helpful, but it is not a common attitude in ER (emergency room) because it is not available. In this case, due to the increased severity of the reaction, benralizumab was ceased. Several case reports describe the development of inflammatory disorders in patients receiving benralizumab, including cytokine-release reaction, which we also described in one patient, but with the disappearance of the symptoms as we continued the treatment. Also, we described possible anaphylaxis to benralizumab; only another case was reported, so we considered that anaphylaxis is rare [26,29,34].

Dupilumab is newly introduced in our country, but hypersensitivity reactions occur in less than 1% of patients, including generalized urticaria, serum sickness, rash, erythema nodosum and anaphylaxis. Generally, dupilumab is well tolerated, but eosinophilia and conjunctivitis are reported more commonly [26,29,35]. Diagnosis of hypersensitivity reactions is difficult. Non-irritating concentrations for dupilumab skin tests have not yet been established, although some case series with a limited number of patients determined concentrations of undiluted dupilumab (150 mg/mL) for skin prick test and a dilution of 1:10 in saline for intradermal testing [32]. Since, for many patients, treatment continuation is particularly important for severe asthma management, in low-risk patients such as our case, we can safely readministered the drug.

Excipients are used as drug preservatives in many products, like polysorbate 80 from omalizumab and dupilumab, which may induce allergic reactions, which is a problem that needs to be discussed. Of course, patients with multiple hypersensitivity reactions to drugs containing polysorbate 80 are the candidates for this type of allergy work-up, but there is still no standard evaluation or commercial test substance for skin tests. Drug provocation with two different drugs containing polysorbate 80 should be considered in these patients, but it is time consuming and has a high cost and risks for patients [35,36].

In general, biologicals are well tolerated in patients with severe asthma, but real-life experiences are still published. New studies showed that there is an increased risk of adverse events related to the drugs for patients with biologicals in severe asthma [10,36,37]. Omalizumab presents an increased risk for anaphylaxis, as we observed among our patients. We need a good surveillance system in adverse events reporting that is voluntary and easy to manage, which is not accurately described in the literature.

We share our experience with successful desensitization or titrated provocation in three patients with severe, previously steroid-dependent asthma who experienced hypersensitivity reactions to biologicals. They have continued therapy for at least 12 months post-desensitization without experiencing adverse reactions. Following successful desensitization, all patients showed significant improvements in asthma control and were able to discontinue or reduce oral steroids.

One limitation of our study is that none of the patients had their serum tryptase levels checked after their reactions, which could have supplied additional evidence of mast cell degranulation in immediate-type hypersensitivity reactions. Moreover, we did not perform skin testing for excipients, for example, polysorbate 80, an excipient in both omalizumab and dupilumab, known to cause hypersensitivity reactions, as they are not commercially available for testing. There are studies that reveal these problems and the necessity to make registries for harmonized reports of hypersensitivity reactions to biological therapies [37,38].

Another limitation of our study is the small sample size since the treatments are still recently introduced in clinical practice, and severe asthma is observed in a small percentage of asthmatic patients [10].

5. Conclusions

In conclusion, it is important to report adverse events and follow up with patients with severe asthma under biological treatment to make a correct decision. Desensitization or titrated provocation emerges as a crucial therapeutic choice for managing patients who exhibit hypersensitivity reactions to biologicals because, in many cases, biologicals are "a life-changing" treatment inducing asthma remission. This approach can lead to substantial enhancements in asthma control and quality of life while reducing the necessity for systemic steroid treatments.

Author Contributions: R.-G.B., D.M.D., I.P., C.T.D.B., M.A.M., I.C.B. and I.A.M. participated in the analysis of current published data, as well as in the planning and writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was granted by project PDI-PFE-CDI 2021, entitled Increasing the Performance of Scientific Research, Supporting Excellence in Medical Research and Innovation, PROGRES, no. 40PFE/30.12.2021.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of IRGH Cluj-Napoca (protocol code no. 10577/09.12.2021) for studies involving humans.

Informed Consent Statement: Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The datasets used during the present study are available from the corresponding author upon reasonable request.

Acknowledgments: We would like to acknowledge Mlești Mihai Adrian, the Manager of the Octavian Fodor Regional Institute of Gastroenterology and Hepatology (Cluj-Napoca, Romania).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Patel, S.V.; Khan, D.A. Adverse Reactions to Biologic Therapy. *Immunol. Allergy Clin. N. Am.* **2017**, *37*, 397–412. [CrossRef]
2. Pichler, W.J. Adverse side-effects to biological agents. *Allergy* **2006**, *61*, 912–920. [CrossRef]
3. Pintea, I.; Petricau, C.; Dumitrascu, D.; Muntean, A.; Branisteanu, D.C.; Branisteanu, D.E.; Deleanu, D. Hypersensitivity reactions to monoclonal antibodies: Classification and treatment approach (Review). *Exp. Ther. Med.* **2021**, *22*, 949. [CrossRef]
4. Brennan, P.J.; Bouza, T.R.; Hsu, F.I.; Sloane, D.E.; Castells, M.C. Hypersensitivity reactions to mAbs: 105 desensitizations in 23 patients, from evaluation to treatment. *J. Allergy Clin. Immunol.* **2009**, *124*, 1259–1266. [CrossRef]
5. Burks, A.W.; O'Hehir, R.E.; Broide, D.H.; Holgate, S.T.; Bacharier, L.B.; Hershey, G.K.K.; Peebles, R.S. (Eds.) Chapter 5: Cytokines in allergic inflammation. In *Middleton's Allergy: Principles and Practice*; Elsevier Sanders: Philadelphia, PA, USA, 2019.
6. Aubin, F.; Carbonnel, F.; Wendling, D. The complexity of adverse side-effects to biological agents. *J. Crohn's Colitis* **2013**, *7*, 257–262. [CrossRef]
7. Suntharalingam, G.; Perry, M.R.; Ward, S.; Brett, S.J.; Castello-Cortes, A.; Brunner, M.D.; Panoskaltsis, N. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N. Engl. J. Med.* **2006**, *355*, 1018–1028. [CrossRef]
8. Bugelski, P.J.; Achuthanandam, R.; Capocasale, R.J.; Treacy, G.; Bouman-Thio, E. Monoclonal antibody induced cytokine-release syndrome. *Expert Rev. Clin. Immunol.* **2009**, *5*, 499–521. [CrossRef]
9. Abramowicz, D.; Crusiaux, A.; Goldman, M. Anaphylactic shock after retreatment with OKT3 monoclonal antibody. *N. Engl. J. Med.* **1992**, *327*, 736.
10. Available online: https://ginasthma.org/wp-content/uploads/2023/07/GINA-2023-Full-report-23_07_06-WMS.pdf (accessed on 10 October 2023).
11. Agache, I.; Beltran, J.; Akdis, C.; Akdis, M.; Canelo-Aybar, C.; Canonica, G.W.; Casale, T.; Chivato, T.; Corren, J.; Del Giacco, S.; et al. Efficacy and safety of treatment with biologicals (benralizumab, dupilumab, mepolizumab, omalizumab and reslizumab) for severe eosinophilic asthma. A systematic review for the EAACI Guidelines—Recommendations on the use of biologicals in severe asthma. *Allergy* **2020**, *75*, 1023–1042. [CrossRef]

12. Chung, C.H.; Mirakhur, B.; Chan, E.; Le, Q.T.; Berlin, J.; Morse, M.; Murphy, B.A.; Satinover, S.M.; Hosen, J.; Mauro, D.; et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N. Engl. J. Med.* **2008**, *358*, 1109–1117. [CrossRef]
13. Makatsori, M.; Kiani-Alikhan, S.; Manson, A.L.; Verma, N.; Leandro, M.; Gurugama, N.P.; Longhurst, H.J.; Grigoriadou, S.; Buckland, M.; Kanfer, E.; et al. Hypogammaglobulinaemia after rituximab treatment-incidence and outcomes. *QJM Int. J. Med.* **2014**, *107*, 821–828. [CrossRef]
14. Devos, S.A.; Van den Bossche, N.; De Vos, M.; Naeyaert, J.M. Adverse skin reactions to anti- TNF-alpha monoclonal antibody therapy. *Dermatology* **2003**, *206*, 388–390. [CrossRef]
15. Chan, J.L.; Davis-Reed, L.; Kimball, A.B. Counter-regulatory balance: Atopic dermatitis in patients undergoing infliximab infusion therapy. *J. Drugs Dermatol.* **2004**, *3*, 315–318.
16. Perez-Soler, R.; Saltz, L. Cutaneous adverse effects with HER1/EGFR-targeted agents: Is there a silver lining? *J. Clin. Oncol.* **2005**, *23*, 5235–5246. [CrossRef]
17. Cheifetz, A.; Mayer, L. Monoclonal antibodies, immunogenicity, and associated infusion reactions. *Mt. Sinai J. Med.* **2005**, *72*, 250–256.
18. Cheifetz, A.; Smedley, M.; Martin, S.; Reiter, M.; Leone, G.; Mayer, L.; Plevy, S. The incidence and management of infusion reactions to infliximab: A large center experience. *Am. J. Gastroenterol.* **2003**, *98*, 1315–1324. [CrossRef]
19. Brown, S.G. Clinical features and severity grading of anaphylaxis. *J. Allergy Clin. Immunol.* **2004**, *114*, 371–376. [CrossRef]
20. Busse, W.; Corren, J.; Lanier, B.Q.; McAlary, M.; Fowler-Taylor, A.; Della Cioppa, G.; van As, A.; Gupta, N. Omalizumab, Anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J. Allergy Clin. Immunol.* **2001**, *108*, 184–190. [CrossRef]
21. Soler, M.; Matz, J.; Townley, R.; Buhl, R.; O'brien, J.; Fox, H.; Thirlwell, J.; Gupta, N.; Della Cioppa, G. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur. Respir. J.* **2001**, *18*, 254–261. [CrossRef]
22. Cox, L.; Platts-Mills, T.A.; Finegold, I.; Schwartz, L.B.; Simons, F.E.; Wallace, D.V. American Academy of Allergy, Asthma&Immunology/American College of Allergy, Asthma and Immunology Joint Task Force Report on omalizumab-associated anaphylaxis. *J. Allergy Clin. Immunol.* **2007**, *120*, 1373–1377.
23. Dreyfus, D.H.; Randolph, C.C. Characterization of an anaphylactoid reaction to omalizumab. *Ann. Allergy Asthma Immunol.* **2006**, *96*, 624–627. [CrossRef]
24. Price, K.S.; Hamilton, R.G. Anaphylactoid reactions in two patients after omalizumab administration after successful long-term therapy. *Allergy Asthma Proc.* **2007**, *28*, 313–319. [CrossRef]
25. Wenzel, S.; Castro, M.; Corren, J.; Maspero, J.; Wang, L.; Zhang, B.; Pirozzi, G.; Sutherland, E.R.; Evans, R.R.; Joish, V.N.; et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting beta2 agonist: A randomized double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet* **2016**, *388*, 31–44. [CrossRef]
26. Sitek, A.N.; Li, J.T.; Pongdee, T. Risks and safety of biologics: A practical guide for allergists. *World Allergy Organ. J.* **2023**, *16*, 100737. [CrossRef]
27. Fernandez, J.; Ruano-Zaragoza, M.; Blanca-Lopez, N. Omalizumab and other biologics in drug desensitization. *Curr. Opin. Allergy Clin. Immunol.* **2020**, *20*, 333–337. [CrossRef]
28. Lieberman, P.L.; Jones, I.; Rajwanshi, R.; Rosén, K.; Umetsu, D.T. Anaphylaxis associated with omalizumab administration: Risk factors and patient characteristics. *J. Allergy Clin. Immunol.* **2017**, *140*, 1734.e4–1736.e4. [CrossRef]
29. Li, L.; Wang, Z.; Cui, L.; Xu, Y.; Guan, K.; Zhao, B. Anaphylactic risk related to omalizumab, benralizumab, reslizumab, mepolizumab, and dupilumab. *Clin. Transl. Allergy* **2021**, *11*, e12038. [CrossRef]
30. Owens, G.; Petrov, A. Successful desensitization of three patients with hypersensitivity reactions to omalizumab. *Curr. Drug Saf.* **2011**, *6*, 339–342. [CrossRef]
31. Bernaola, M.; Hamadi, S.A.; Lynch, D.M.; Marquis, K.A.; Silver, J.N.; Castells, M.C.; Hsu, J.T. Successful administration of omalizumab by desensitization protocol following systemic reactions in 12 patients. *J. Allergy Clin. Immunol. Pract.* **2021**, *9*, e1–e2508. [CrossRef]
32. Sitek, A.; Chiarella, S.E.; Pongdee, T. Hypersensitivity reactions to biologics used in the treatment of allergic diseases: Clinical features, diagnosis and management. *Front. Allergy* **2023**, *4*, 1219735. [CrossRef]
33. Weiss, S.L.; Smith, D.M. A Case of Serum Sickness-Like Reaction in an Adult Treated with Omalizumab. *Mil. Med.* **2020**, *185*, e912–e913. [CrossRef]
34. Morgenstern-Kaplan, D.; Vasquez-Echeverri, E.; Carrillo-Martin, I.; Chiarella, S.E.; Gonzalez-Estrada, A. Cytokine-release hypersensitivity reaction after the first dose of benralizumab for severe eosinophilic asthma. *Ann. Allergy Asthma Immunol.* **2021**, *127*, 701–702. [CrossRef]
35. Available online: https://www.regeneron.com/downloads/dupixent_fpi.pdf (accessed on 10 October 2023).
36. Stone, C.A.J.; Liu, Y.; Relling, M.V.; Krantz, M.S.; Pratt, A.L.; Abreo, A.; Hemler, J.A.; Phillips, E.J. Immediate Hypersensitivity to Polyethylene Glycols and Polysorbates: More Common Than We Have Recognized. *J. Allergy Clin. Immunol. Pract.* **2019**, *7*, 1533.e8–1540.e8. [CrossRef]

37. Akarsu, A.; Soyer, O.; Sekerel, B.E. Hypersensitivity Reactions to Biologicals: From Bench to Bedside. *Curr. Treat. Options Allergy* **2020**, *7*, 71–83. [CrossRef]
38. Jappe, U.; Beckert, H.; Bergmann, K.C.; Gülsen, A.; Klimek, L.; Philipp, S.; Pickert, J.; Rauber-Ellinghaus, M.M.; Renz, H.; Taube, C.; et al. Biologics for atopic diseases: Indication, side effect management, and new developments. *Allergol. Select* **2021**, *5*, 1–25. [CrossRef]

International Journal of
Molecular Sciences

MDPI

Article

The Alarmin Triad—IL-25, IL-33, and TSLP—Serum Levels and Their Clinical Implications in Chronic Spontaneous Urticaria

Carmen-Teodora Dobrican-Băruța [1,2], Diana Mihaela Deleanu [1,2], Ioana Adriana Muntean [1,2,*], Irena Nedelea [1,2], Radu-Gheorghe Bălan [1], Gabriela Adriana Filip [3,*] and Lucia Maria Procopciuc [4]

1 Department of Allergology and Immunology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400012 Cluj Napoca, Romania; dobrican.carmen@umfcluj.ro (C.-T.D.-B.)
2 Allergology Department, "Octavian Fodor" Institute of Gastroenterology and Hepatology, 400162 Cluj Napoca, Romania
3 Department of Anatomy, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400006 Cluj-Napoca, Romania
4 Department of Biochemistry, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400349 Cluj-Napoca, Romania; luciamariaprocopciuc@yahoo.com
* Correspondence: adriana.muntean@umfcluj.ro (I.A.M.); gabriela.filip@umfcluj.ro or adrianafilip33@yahoo.com (G.A.F.)

Abstract: This study delves into the critical role of alarmins in chronic spontaneous urticaria (CSU), focusing on their impact on disease severity and the quality of life (QoL) of patients. We investigated the alterations in alarmin levels in CSU patients and their correlations with the Urticaria Activity Score (UAS7) and the Dermatology Life Quality Index (DLQI). We analyzed serum levels of interleukin-25 (IL-25), interleukin-33 (IL-33), and thymic stromal lymphopoietin (TSLP) in 50 CSU patients, comparing these to 38 healthy controls. The study examined the relationship between alarmin levels and clinical outcomes, including disease severity and QoL. Elevated levels of IL-33 and TSLP in CSU patients ($p < 0.0001$) highlight their potential role in CSU pathogenesis. Although IL-25 showed higher levels in CSU patients, this did not reach statistical significance ($p = 0.0823$). Crucially, IL-33's correlation with both UAS7 and DLQI scores underscores its potential as a biomarker for CSU diagnosis and severity assessment. Of the alarmins analyzed, IL-33 emerges as particularly significant for further exploration as a diagnostic and prognostic biomarker in CSU. Its substantial correlation with disease severity and impact on QoL makes it a compelling candidate for future research, potentially serving as a target for therapeutic interventions. Given these findings, IL-33 deserves additional investigation to confirm its role and effectiveness as a biomarker and therapeutic target in CSU.

Keywords: alarmins; IL-33; IL-25; TSLP; CSU; urticaria; skin; mast cells; UAS7; DLQI

Citation: Dobrican-Băruța, C.-T.; Deleanu, D.M.; Muntean, I.A.; Nedelea, I.; Bălan, R.-G.; Filip, G.A.; Procopciuc, L.M. The Alarmin Triad—IL-25, IL-33, and TSLP—Serum Levels and Their Clinical Implications in Chronic Spontaneous Urticaria. *Int. J. Mol. Sci.* **2024**, *25*, 2026. https://doi.org/10.3390/ijms25042026

Academic Editors: Filomena Napolitano and Valentina Giudice

Received: 31 December 2023
Revised: 1 February 2024
Accepted: 4 February 2024
Published: 7 February 2024

1. Introduction

Chronic spontaneous urticaria (CSU), historically known also as chronic idiopathic urticaria (CIU), is a condition that has garnered increased attention in the realm of dermatological research due to its rising global prevalence. The current medical discourse, guided by the EAACI/GA2LEN/EDF/WAO guidelines, predominantly utilizes the term CSU to describe this disorder characterized by unprovoked wheals, angioedema, or both, persisting for more than six weeks. The distinctiveness of CSU lies in its extended duration and spontaneous symptom onset, setting it apart from other types of urticaria and highlighting the complexity of its pathogenesis [1,2].

The escalation in the global incidence of CSU has coincided with an evolving understanding of its etiological factors [2]. This condition intricately intertwines immunological and non-immunological elements, such as stress, pseudoallergens, autoimmunity, infection, and inflammation [3,4]. The recent COVID-19 pandemic has further spurred investigations

into the potential links between viral infections, including SARS-CoV-2, and the onset or aggravation of CSU [5]. Over the years, the exploration of CSU has progressively unveiled the involvement of a broad spectrum of immune cells in its pathophysiology. Initially, significant attention was directed towards the roles of eosinophils and basophils [6–8] in the development and perpetuation of the disease. However, recent advancements in immunological research have shifted this focus, highlighting mast cells as the central figures in the initiation and propagation of CSU's pathogenic mechanisms. This paradigm shift underscores the complexity of immune cell interactions in CSU, particularly emphasizing the critical role of mast cells in orchestrating the inflammatory responses [3,9–11]. Concurrent with the intensified scrutiny of mast cells in CSU, a broad array of cytokines associated with these cells has come under the research spotlight. This includes pro-inflammatory cytokines like IL-1 beta and IL-6, which are instrumental in driving the inflammatory cascade [12–14]. Additionally, IL-17, often implicated in autoimmunity, and IL-31, commonly referred to as the" pruritus cytokine", have been identified as having significant implications in the pathogenesis and clinical manifestation of CSU [15,16]. These findings have expanded our understanding of the complex interactions between various cytokines and mast cells in the pathogenesis of CSU. Ultimately, this has led to a heightened interest in alarmins—cytokines originating from mast cells that initiate Th2 responses. Assessing the dynamics of these alarmins, including IL-25, IL-33, and TSLP, is now recognized as a critical step in understanding their role in CSU [17]. The role of mast cells extends beyond CSU, serving as essential mediators in various skin diseases through their interaction with cytokines such as alarmins [3,9–11]. These interactions are pivotal in the development of skin inflammation, as demonstrated by studies indicating that IL-33 can induce skin inflammation with mast cell and neutrophil activation, suggesting a broad immunological role of IL-33 in conditions like psoriasis and atopic dermatitis [18]. Similarly, the neurobeachin-like 2 protein has been identified as a regulator of mast cell homeostasis, affecting their differentiation, proliferation, and cytokine production, which is crucial for understanding mast cell functions in allergic reactions and skin diseases [19]. Furthermore, the IL-33 and IL-37 axis elucidates the balancing act between pro-inflammatory and anti-inflammatory responses in skin and allergic diseases, highlighting the therapeutic potential of targeting these pathways [20]. Moreover, the initiation of vascular responses to contact allergens by mast cells, as mediated by cell stress signals, emphasizes the significance of mast cells and alarmins in immediate and chronic inflammatory responses [21]. Despite the growing interest in alarmins, their exact roles in the pathogenesis of CSU remain underexplored. Our study aims to fill this knowledge gap by investigating the dynamics of serum alarmin levels in CSU patients and their correlations with disease severity and impact on QoL. By assessing the levels of IL-25, IL-33, and TSLP in CSU patients and comparing them with those in healthy controls, we seek to elucidate the contribution of these cytokines to the pathophysiology of CSU. Furthermore, a deep understanding of the correlation between alarmins and the clinical impact of CSU, as quantified by assessment tools like the UAS7 and the DLQI, is crucial in refining therapeutic strategies. These assessment tools are indispensable in gauging the severity and the QoL implications of CSU, thus guiding treatment approaches. This line of investigation is particularly relevant in the context of the current focus on biologic therapies and monoclonal antibodies aimed at cytokines involved in allergic and autoimmune diseases. Currently, omalizumab is the only monoclonal antibody approved for CSU, yet there exists a subset of patients who do not respond adequately to this treatment [1,2,22]. This scenario underscores the pressing need for continued research into the mechanisms of treatment resistance and factors influencing treatment response in CSU [23–25]. The exploration of alternative therapeutic targets and the development of novel monoclonal antibodies are pivotal in addressing these challenges and improving patient outcomes.

2. Results

2.1. Collection and Presentation of Clinical and Paraclinical Data

In this study, we meticulously gathered and analyzed essential clinical data from all participants, including demographic information and significant clinical characteristics. Additionally, we evaluated a comprehensive set of laboratory parameters and paraclinical metrics for both control subjects and CSU patients. Table 1 provides a comparative analysis of the characteristics between the two groups, presenting key demographics such as the total number of participants, gender distribution, average age (with standard deviation), and atopy status. Furthermore, the table includes critical paraclinical parameters, including IL-25, TSLP, IL-33, total IgE, Eos, RF, ESR, and CRP. This integrated approach allows for a comprehensive exploration of both clinical and laboratory aspects, shedding light on the nuanced differences between control and CSU groups.

Table 1. Overview of participant characteristics.

Characteristics	Controls	CSU Patients	*p*-Values (Mann–Whitney U)
Total Participants (n)	33	50	N/A
Average Age (yrs. ± SD)	44.32 ± 9.23	50.14 ± 16.10	N/A
Gender Distribution (F/M)	26/12	36/14	N/A
Atopy Status (Atopic/Non-atopic)	10/28	14/36	N/A
IL-25 (pg/mL ± SD)	105.03 ± 89.21	140.27 ± 100.16	0.0823
TSLP (pg/mL ± SD)	434.57 ± 169.43	1200.42 ± 1348.36	<0.0001
IL-33 (pg/mL ± SD)	21.70 ± 22.68	220.67 ± 201.17	<0.0001
Total IgE (IU/L ± SD)	41.23 ± 27.08	168.63 ± 178.99	<0.0001
Eos (×1000 cells/μL ± SD)	0.163 ± 0.102	0.495 ± 0.704	0.1015
RF (IU/mL ± SD)	10.75 ± 2.82	14.02 ± 1.58	<0.0001
ESR (mm/h ± SD)	12 ± 6.71	14.29 ± 9.89	0.8074
CRP (mg/dL ± SD)	0.36 ± 0.33	0.36 ± 0.27	0.7408

Note: CSU, chronic spontaneous urticaria; n, number; yrs, years; F, female; M, male; IL-25, interleukin-25; TSLP, thymic stromal lymphopoietin; IL-33, interleukin-33; pg/mL, picograms per milliliter; total IgE, total immunoglobulin E; IU/L, international units per liter; Eos, absolute eosinophil count; cells/μL, cells per microliter of blood; RF, rheumatoid factor; IU/mL, international units per milliliter; ESR, erythrocyte sedimentation rate; mm/h, millimeters per hour; CRP, C-reactive protein; mg/dL, milligrams per deciliter. Age, IL-25, TSLP, IL-33, total IgE, Eos, RF, ESR, and CRP are presented as mean ± standard deviation (SD). *p*-values indicate the statistical significance of differences between CSU patients and controls, with $p < 0.05$ considered significant. N/A, not applicable, is used where statistical comparison was not relevant or was not conducted, such as for total participant count, average age, atopy status, and gender distribution.

2.2. Serum Alarmin Levels and ROC Analysis in CSU Patients versus Controls

Our analysis revealed a markedly significant disparity in the serum levels of alarmins (IL-25, IL-33, and TSLP) between CSU patients and control subjects.

2.2.1. Analysis of IL-25, IL-33, and TSLP Serum Levels in CSU Patients and Healthy Controls

In our detailed assessment of serum alarmin levels comparing CSU patients to controls, the Mann–Whitney U test revealed distinct patterns for IL-25, IL-33, and TSLP. Specifically for IL-25, CSU patients exhibited median levels of 125.9 pg/mL against 61.94 pg/mL in controls, a difference that is not statistically significant ($p = 0.0823$) (Figure 1a). In contrast, the levels of IL-33 and TSLP in CSU patients significantly surpassed those in controls, with median levels of 160.8 pg/mL for IL-33 and 551.4 pg/mL for TSLP, sharply deviating from the control medians of 10.98 pg/mL and 396.2 pg/mL, respectively, both with *p*-values of <0.0001 (Figure 1b,c). These visual summaries present clearly different profiles for alarmin levels, providing valuable insights into their diagnostic relevance in CSU.

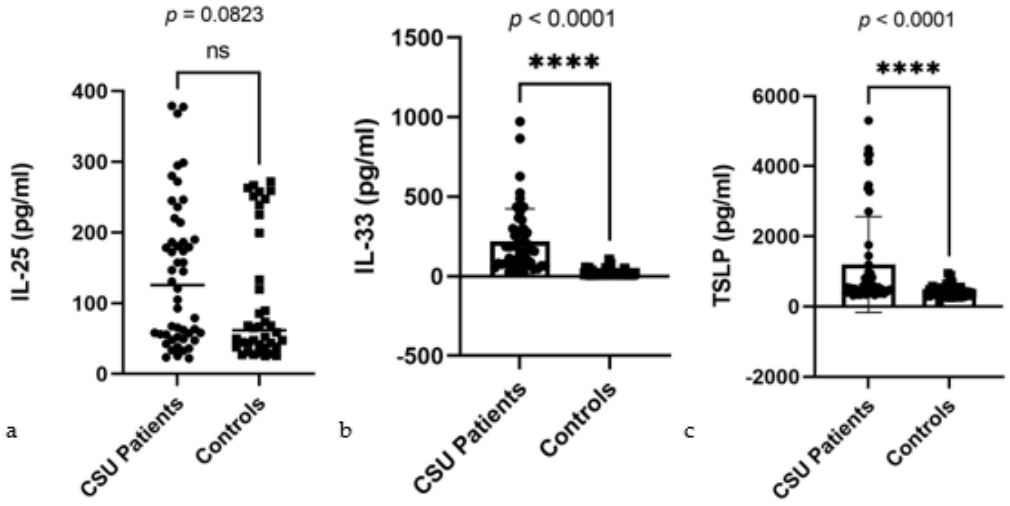

Figure 1. Serum alarmin levels in CSU patients and controls: (**a**) IL-25—interleukin 25, CSU—chronic spontaneous urticaria, ns—non-significant, $p = 0.0823$; (**b**) IL-33—interleukin 33, CSU—chronic spontaneous urticaria, **** = $p < 0.0001$; (**c**) TSLP—thymic stromal lymphopoietin, CSU—chronic spontaneous urticaria, **** = $p < 0.0001$.

2.2.2. Diagnostic Performance of Serum Alarmins in CSU: ROC Curve Assessment

Receiver operating characteristic (ROC) analysis was conducted to evaluate the diagnostic performance of serum IL-25 levels in differentiating CSU patients from controls. The area under the ROC curve (AUC) was calculated to be 0.6511, with a standard error of 0.06018. The 95% confidence interval for the AUC ranged from 0.5331 to 0.7690. The analysis yielded a statistically significant *p*-value of 0.0156, indicating a discriminative capacity of serum IL-25 levels between the two groups (Figure 2).

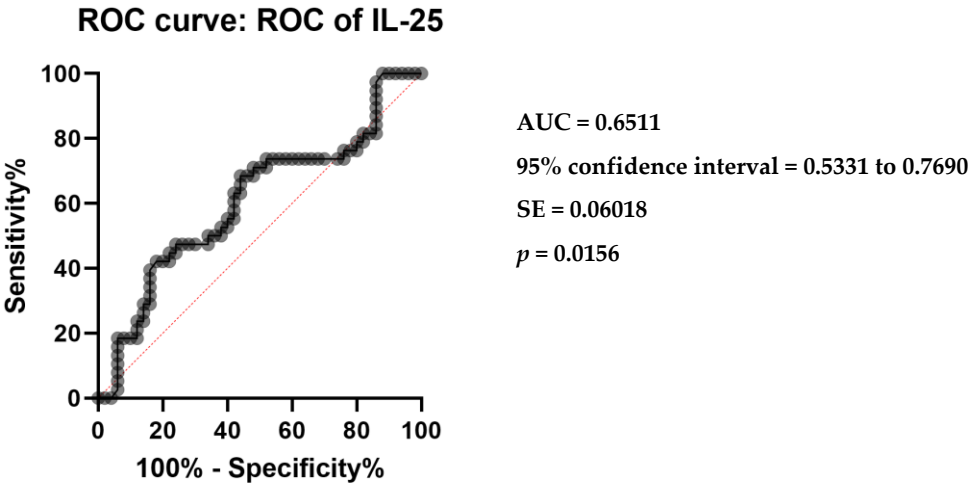

Figure 2. Receiver operating characteristics (ROC) curve of IL-25 in CSU compared with healthy controls; IL-25—interleukin 25, CSU—chronic spontaneous urticaria, $p = 0.0156$, AUC—area under the curve = 0.6511, and SE—standard error = 0.06018.

In parallel assessments, the diagnostic capacities of serum IL-33 and TSLP levels were examined through ROC curve analyses. For IL-33, the AUC was 0.9658, indicative of a high discriminative ability to distinguish CSU patients from controls, supported by a significant *p*-value of less than 0.0001 (Figure 3a). The analysis denotes IL-33 as a robust biomarker, with a narrowly defined standard error of 0.01623 and a 95% confidence interval between 0.9340 to 0.9976. Similarly, TSLP demonstrated a notable diagnostic accuracy, with an AUC of 0.7600, a standard error of 0.05048, and a 95% confidence interval from 0.6611 to 0.8589,

also yielding a highly significant *p*-value of less than 0.0001 (Figure 3b). These analyses collectively underscore the potential of serum IL-33 and TSLP levels in the diagnostic stratification of CSU patients.

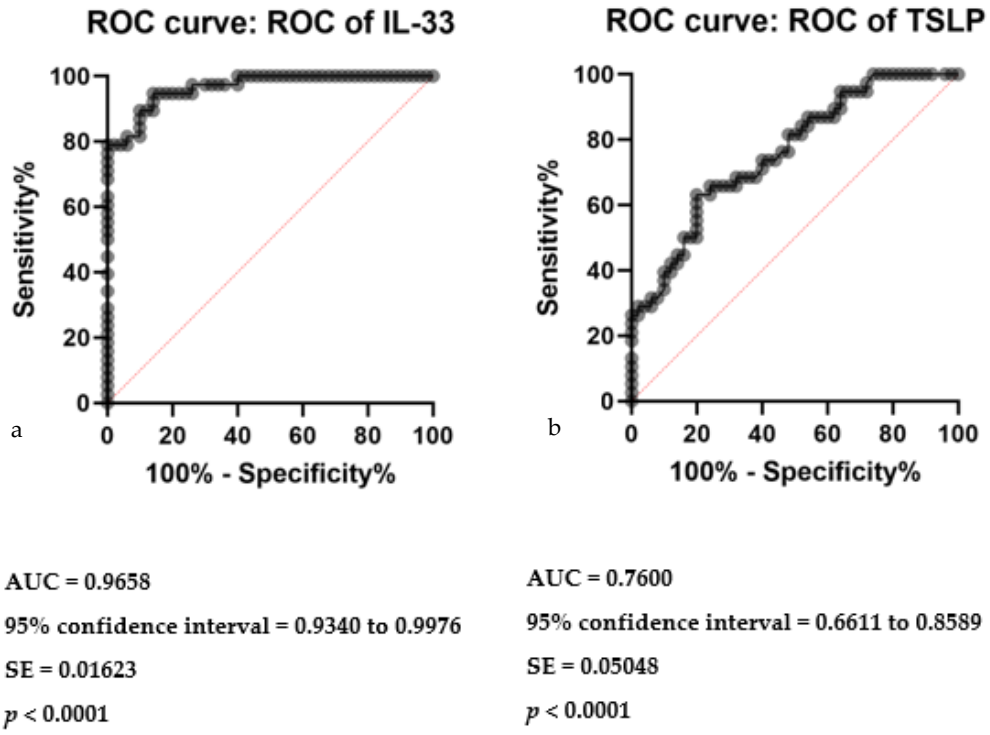

Figure 3. ROC curve analysis of serum IL-33 and TSLP in CSU: (**a**) Receiver operating characteristics (ROC) curve of IL-33 in CSU compared with healthy controls; IL-33—interleukin 33, CSU—chronic spontaneous urticaria, $p < 0.0001$, AUC—area under the curve = 0.9658, SE—standard error = 0.01623. (**b**) Receiver operating characteristics (ROC) curve of TSLP in CSU compared with healthy controls; TSLP—thymic stromal lymphopoietin, CSU—chronic spontaneous urticaria, $p < 0.0001$, AUC—area under the curve = 0.7600, SE—standard error = 0.05048.

2.3. Inter-Alarmin Correlations in Serum

Within the scope of our analysis, a detailed examination of potential correlations between the serum levels of the alarmins—IL-25, IL-33, and TSLP—was conducted. This investigation included pairwise comparisons among these cytokines to identify any interdependencies or associative patterns. However, our findings revealed that none of these inter-alarmin correlations reached statistical significance. All of the observed relationships yielded *p*-values exceeding the threshold of 0.05, indicating a lack of meaningful association. Furthermore, the correlation coefficients for these comparisons also presented as statistically non-significant, further substantiating the absence of notable interrelations among these alarmin levels in our study cohort.

2.4. Correlation Analysis of Serum Alarmin Levels with Inflammatory Markers in CSU

Subsequently, our investigation extended to assess correlations between each of these alarmins and various inflammatory markers, including ESR, CRP, RF, and serum eosinophil counts. Additionally, potential associations between these alarmins and clinical markers of atopy, as well as serum IgE levels in CSU patients, were explored. In all of these instances, the correlation analyses did not reveal any statistically significant relationships. The Pearson correlation coefficients obtained in these analyses were not indicative of significant correlations, affirming the absence of meaningful associations between alarmin levels and the aforementioned inflammatory and atopic markers (Figure 4).

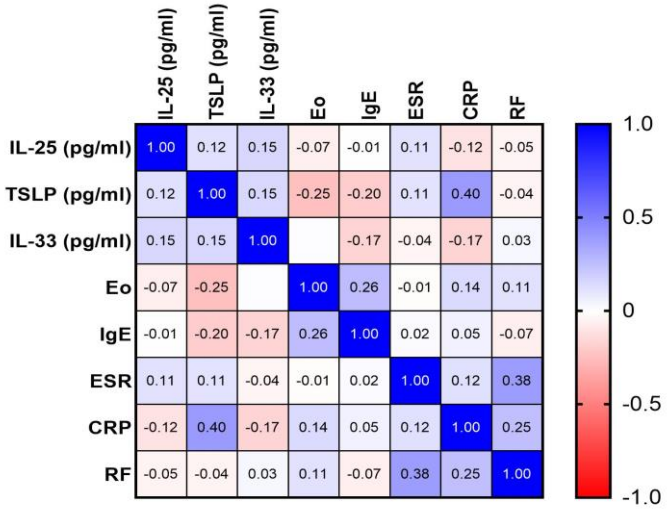

Figure 4. Correlation matrix of alarmin and inflammatory markers in CSU; CSU—chronic spontaneous urticaria, IL-25—interleukin-25, TSLP—thymic stromal lymphopoietin, IL-33—interleukin-33, Eo—eosinophil count, IgE—immunoglobulin E, ESR—erythrocyte sedimentation rate, CRP—C-reactive protein, RF—rheumatoid factor.

An exception to these findings was observed in the case of TSLP. A clinically significant direct correlation between serum TSLP levels and CRP was identified, as indicated by a *p*-value of 0.0045 and a Pearson correlation coefficient (r) of 0.3954. The details of this correlation are graphically represented in Figure 5, providing a visual interpretation of the relationship between TSLP and CRP in the context of CSU.

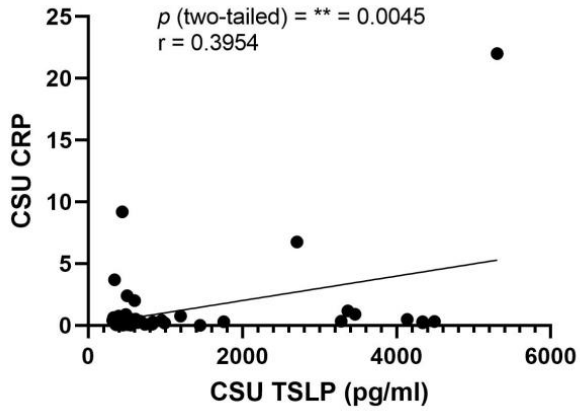

Figure 5. Correlation of serum TSLP and CRP in CSU; TSLP—thymic stromal lymphopoietin, CRP—C-reactive protein, CSU—chronic spontaneous urticaria, *p* = ** = 0.0045; r—the Pearson correlation coefficient for the serum level of TSLP and CRP is 0.3954 (r = 0.3954).

2.5. Correlation between Clinical Tools (UAS7 and DLQI) in CSU

In evaluating the relationship between the urticaria activity score over 7 days (UAS7) and the dermatology life quality index (DLQI), a Pearson correlation analysis was conducted among patients with CSU. The analysis yielded a Pearson correlation coefficient (r) of 0.8176, indicating a strong positive correlation between the two clinical instruments. This suggests that as the severity of urticaria symptoms increases, as measured with the UAS7, there is a concomitant and proportional impact on the patients' QoL, as reflected by the DLQI scores. The strength of this association is further underscored by a highly significant *p*-value of less than 0.0001. The 95% confidence interval for the correlation coefficient extends from 0.6981 to 0.8928, reinforcing the reliability of this strong correlation. Additionally, the R squared value of 0.6685 implies that approximately 66.85% of the

variability in the DLQI scores can be explained by the variability in the UAS7 scores among the CSU patient cohort. These findings highlight the interdependence of symptom severity and QoL in patients with CSU, and validate the use of UAS7 and DLQI as complementary tools in clinical assessments (Figure 6).

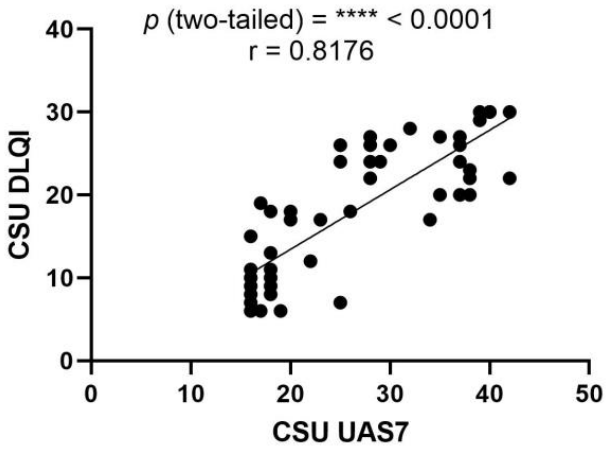

Figure 6. Correlation between UAS7 and DLQI in CSU; UAS7—Urticaria Activity Score over 7 days, DLQI—Dermatology Life Quality Index, CSU—chronic spontaneous urticaria, p = **** < 0.0001; r—the Pearson correlation coefficient for the UAS7 and DLQI is 0.8176 (r = 0.8176).

2.6. Correlation between Serum Alarmin Levels and UAS7 in CSU

2.6.1. Analysis of Serum IL-25 and TSLP Levels and Their Correlation with UAS7

Pearson correlation analyses for serum IL-25 and TSLP levels against UAS7 scores in CSU patients were performed. The correlation for IL-25 levels (Figure 7a) yielded an r value of 0.1629, with a 95% confidence interval from −0.1210 to 0.4221, and a p-value of 0.2584, indicating a minimal and non-significant correlation. Similarly, serum TSLP levels (Figure 7b) showed an r value of −0.01836 with a 95% confidence interval between −0.2952 to 0.2613, and a p-value of 0.8993, also suggesting no significant association. The R squared values for IL-25 and TSLP were 0.02653 and 0.0003369, respectively, underscoring the lack of predictive value for UAS7 scores in this CSU patient cohort.

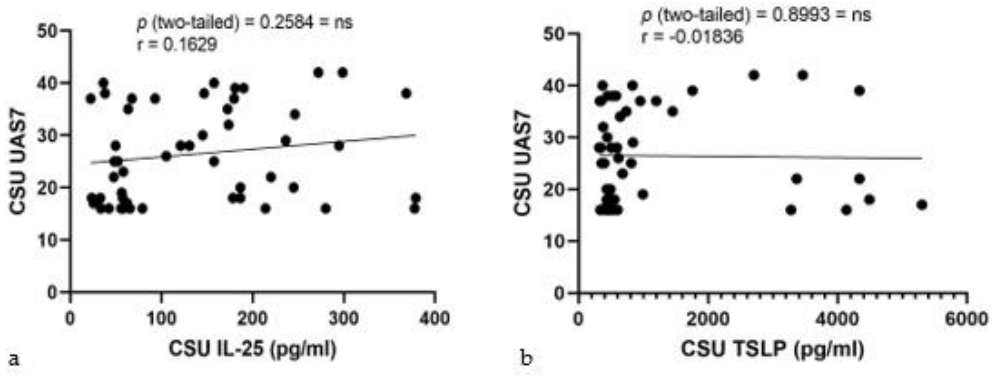

Figure 7. Correlation of serum IL-25 and TSLP levels with UAS7 in CSU: (**a**) Serum IL-25 levels and UAS7 in CSU; IL-25—interleukin-25, UAS7—Urticaria Activity Score over 7 days, CSU—chronic spontaneous urticaria, p = ns (not significant) = 0.2584; r—the Pearson correlation coefficient for serum IL-25 levels and UAS7 scores is 0.1629 (r = 0.1629). (**b**) Serum TSLP levels and UAS7 in CSU; TSLP—thymic stromal lymphopoietin, UAS7—Urticaria Activity Score over 7 days, CSU—chronic spontaneous urticaria, p = ns (not significant) = 0.8993; r—the Pearson correlation coefficient for serum TSLP levels and UAS7 scores is −0.01836 (r = −0.01836).

2.6.2. Analysis of Serum IL-33 Levels and Their Correlation with UAS7

This investigation probed for a correlation between serum IL-33 levels and UAS7 scores in CSU. The Pearson correlation coefficient (r) was determined to be 0.7510, denoting a strong positive correlation, with a highly significant *p*-value of less than 0.0001. The 95% confidence interval for this correlation extends from 0.5976 to 0.8514, indicating a high degree of precision in the relationship between these variables. Additionally, an R squared value of 0.5640 suggests that approximately 56.40% of the variability in UAS7 can be explained by the variability in serum IL-33 levels (Figure 8).

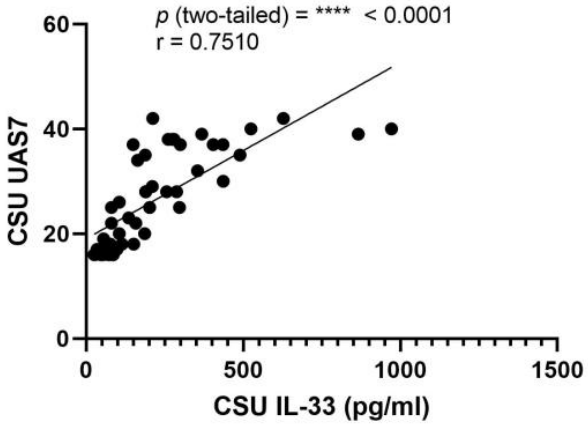

Figure 8. Correlation between serum IL-33 levels and UAS7 in CSU; IL-33—interleukin-33, UAS7—Urticaria Activity Score over 7 days, CSU—chronic spontaneous urticaria, *p* = **** < 0.0001; r—the Pearson correlation coefficient for serum IL-33 levels and UAS7 scores is 0.7510 (r = 0.7510).

2.7. Correlation between Serum Alarmin Levels and DLQI in CSU

2.7.1. Analysis of Serum IL-25 and TSLP Levels and Their Correlation with DLQI

In the correlation studies of serum alarmin levels and DLQI in CSU, IL-25 levels displayed a low positive but non-significant correlation with DLQI, yielding an r value of 0.2595, a *p*-value of 0.0688, and explaining minimal variance in DLQI (R^2 = 0.06734) (Figure 9a). Concurrently, serum TSLP levels showed a very weak and negative correlation with DLQI, denoted by a Pearson r of −0.1457 and a non-significant *p*-value of 0.3128, indicating no substantial correlation with the QoL in the study population (R^2 = 0.02122) (Figure 9b).

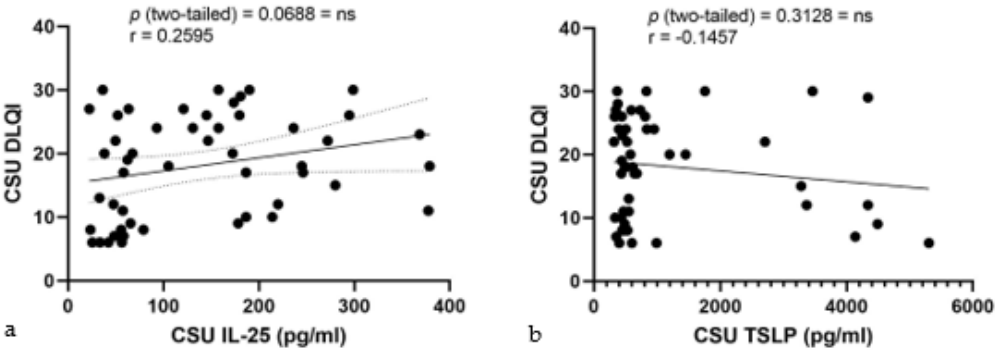

Figure 9. Correlation of serum IL-25 and TSLP levels with DLQI in CSU: (**a**) Correlation between serum IL-25 levels and DLQI in CSU; IL-25—interleukin-25, DLQI—Dermatology Life Quality Index, CSU—chronic spontaneous urticaria, *p* = ns (not significant) = 0.0688; r—the Pearson correlation coefficient for serum IL-25 levels and DLQI scores is 0.2595 (r = 0.2595). (**b**) Correlation between serum TSLP levels and DLQI in CSU; TSLP—thymic stromal lymphopoietin, DLQI—Dermatology Life Quality Index, CSU—chronic spontaneous urticaria, *p* = ns (not significant) = 0.3128; r—the Pearson correlation coefficient for serum TSLP levels and DLQI scores is −0.1457 (r = −0.1457).

2.7.2. Analysis of Serum IL-33 Levels and Their Correlation with DLQI

A Pearson correlation assessment revealed a significant positive relationship between serum IL-33 and DLQI scores (r = 0.7981, $p < 0.0001$), indicating a substantial association in CSU patients. The 95% confidence interval for the correlation coefficient spans from 0.6683 to 0.8808, with an R squared value of 0.6370, reflecting that over 63% of the variability in DLQI is accounted for by changes in IL-33 serum levels (Figure 10).

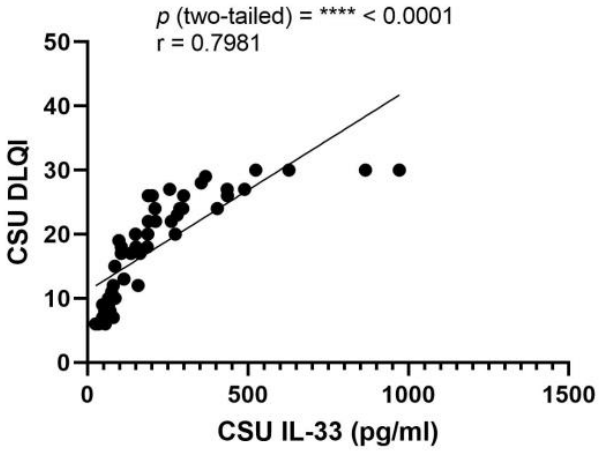

Figure 10. Correlation between serum IL-33 Levels and DLQI in CSU; IL-33—interleukin-33, DLQI—Dermatology Life Quality Index, CSU—chronic spontaneous urticaria, p = **** < 0.0001; r—the Pearson correlation coefficient for serum IL-33 levels and DLQI scores is 0.7981 (r = 0.7981).

Given the robust correlation between serum IL-33 levels and DLQI impact, further statistical analysis was warranted. Subsequently, the Kruskal–Wallis test, a non-parametric method suitable for comparing medians across multiple groups, was employed. This test yielded a p-value of 0.1216, indicating no significant median differences among the groups (Figure 11a). This suggests that categorization into these specific groups does not reveal significant disparities in how serum IL-33 levels affect the DLQI scores. While the Pearson correlation indicated a significant individual-level predictor of QoL, the Kruskal–Wallis test did not reflect these differences across the grouped categories. This juxtaposition of findings highlights that IL-33 levels correlate with QoL on a continuous scale, but such associations may not be as apparent when examining group medians, possibly due to the loss of nuanced data in group categorization.

Expanding upon the correlations identified through the Pearson correlation and the Kruskal–Wallis test, further analysis was conducted using the Mann–Whitney U test for more focused pairwise comparisons between DLQI impact categories. Although no significant difference in serum IL-33 levels was found between the moderate and important categories ($p = 0.1876$), a significant distinction was observed when comparing the moderate and very important categories ($p = 0.0299$) (Figure 11b,d). This outcome elucidates a significant disparity in IL-33 levels that aligns with the varying impact on QoL, reinforcing the association identified by the Pearson correlation. This granular approach through pairwise comparisons reveals nuances of the data, demonstrating that significant differences in IL-33 levels emerge when considering specific pairs of DLQI impact categories, even though such differences may not be apparent across a broader comparison of all categories together. Slight elevations in IL-33 serum levels were observed in the important category compared to the very important category. These differences are particularly pronounced due to the presence of outliers, as indicated in Figure 11a,c, rather than differences in group medians. This pattern reinforces the presence of significant statistical differences just between the groups at the extremes. Patients with a very important impact of the disease on their QoL exhibit significantly higher serum IL-33 levels than those with a moderate impact, as clearly

demonstrated in Figure 11d. These findings further substantiate the role of IL-33 levels as a marker reflective of the disease's impact severity on patient QoL.

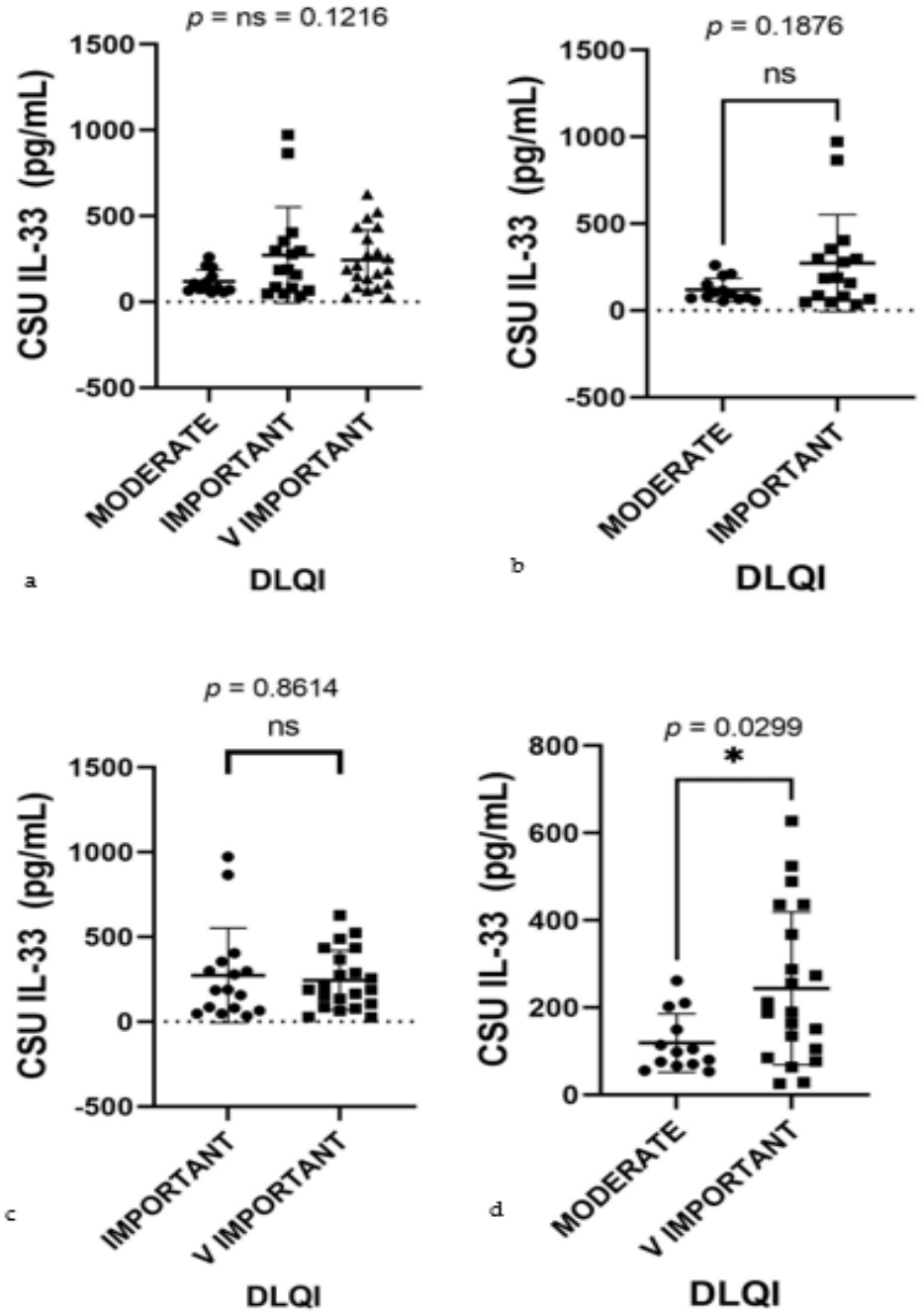

Figure 11. Serum IL-33 levels by DLQI classes in CSU patients; IL-33—interleukin-33, DLQI—Dermatology Life Quality Index (the DLQI classes are demarcated as moderate, important, and very important, illustrating the gradation of urticaria's impact on patients' lives), CSU—chronic spontaneous urticaria. (**a**) Moderate, important, and very important DLQI classes, p = ns (not significant) = 0.1216. (**b**) Moderate and important DLQI classes, showing no significant difference in serum IL-33 levels, with a p value of ns (not significant) = 0.1876. (**c**) Important and very important DLQI classes, p = ns (not significant) = 0.8614. (**d**) Moderate and very important DLQI classes, revealing a significant difference in serum IL-33 levels, with a p value of 0.0299 (* indicates statistical significance).

3. Discussion

The pivotal role of mast cells in the pathogenesis CSU is well-established and underscored by numerous recent studies [3,9–11,15,17]. This recognition justifies and heightens the scientific interest in focusing our attention on the cytokines that activate these cells, namely alarmins (IL-25, IL-33, and TSLP). The involvement of such cytokines in mast cell activation and their potential to exacerbate CSU has been increasingly illuminated by research [3,9–11,15,17]. This divergence emphasizes the critical contribution of alarmins to the mechanism of the disease.

The potential for targeting these cytokines to mitigate the symptoms of CSU represents an intriguing and scientifically promising avenue for exploration. While the effectiveness of such therapeutic interventions has yet to be definitively proven, the development of biologic therapies aimed at these pathways, including tezepelumab (anti-TSLP), etokimab, and itepekimab (both targeting IL-33), as well as astegolimab and GSK3772847 (targeting the ST2 subunit of the IL-33 receptor), are currently undergoing clinical evaluation [26,27]. This innovative therapeutic strategy marks a significant shift in the approach to managing CSU, offering the potential to transform patient outcomes by addressing the underlying mechanisms of the disease directly. The exploration of these biologics in the clinical setting is poised to advance our understanding and treatment of CSU, reflecting a novel paradigm in our therapeutic arsenal against this challenging condition.

This study examined the serum levels of alarmins to elucidate their association with the clinical severity of CSU and its impact on patient QoL. Our analysis indicates that IL-33 levels are notably higher in CSU patients, correlating with increased disease severity and diminished QoL as reflected by the UAS7 and DLQI scores. These observations suggest that IL-33's influence in CSU extends beyond that of a biomarker, acting as a pivotal mediator in the disease's pathogenesis and affecting both clinical outcomes and patient well-being. The significant correlation between IL-33 with the UAS7 and DLQI scores underlines the necessity for developing IL-33-targeted therapeutic approaches, especially for patient subgroups inadequately managed by current treatments, such as omalizumab [26,27]. The findings by Manti et al. [28] support this approach, emphasizing the potential of monoclonal antibodies in enhancing CSU management.

Consistent with the insights provided by Puxeddu et al. [29] and further elaborated in reviews by Cayrol and Girard [30], our results confirm IL-33's essential role in the immunological cascade of CSU, highlighting its extensive biological relevance across various immunological conditions. Trier et al. [31] have identified IL-33's capacity to aggravate histaminergic itch, a prevalent CSU symptom, suggesting a potential interaction with sensory pathways that exacerbates itch severity. This underscores IL-33's dual functionality in both immunological responses and neurosensory processes, expanding its potential as a therapeutic target in CSU and similar pruritic conditions [32].

Furthermore, the review by Murdaca et al. [33] on the IL-33/IL-31 axis sheds light on the multifaceted nature of IL-33, proposing its dual role as a novel biomarker and therapeutic target for Th2-driven diseases. These perspectives are particularly relevant considering our data, which corroborates IL-33's critical involvement in CSU. By integrating these findings, our research not only supports but also expands upon the existing literature, reinforcing the argument for IL-33-targeted therapies as a promising avenue to transform CSU management and patient outcomes.

In our investigation, IL-25's elevated presence in the serum of CSU patients, while notable, did not translate into a significant statistical impact, presenting an intriguing facet in the complex tapestry of CSU pathogenesis. This contrast in our findings could indicate a nuanced role for IL-25 in CSU, potentially contributing to the condition in a less direct or potent manner than the pronounced effects seen with IL-33. The interplay of IL-25 in the context of CSU may involve intricate immune interactions that attenuate its measurable impact on disease severity and patient-reported QoL.

The nuanced role of IL-25 within the immune system reflects the complex intercellular signaling regulated by alarmin cytokines, pivotal for skin homeostasis and implicated

in allergic inflammation processes. The analysis by Hasegawa et al. [34] positions IL-25 as crucial in both promoting allergic skin inflammation and facilitating wound healing, underscoring its intricate function in skin disorders. Additionally, Stanbery et al. [35] expand the scope of IL-25's impact, illustrating its involvement in a broader spectrum of immune responses, including those against viral infections and in cancer, thus highlighting IL-25's versatility as a cytokine affecting diverse immune mechanisms.

This broader understanding suggests that the serum IL-25 levels observed in our CSU study, while not showing a direct statistical correlation, contribute to a more comprehensive narrative. Documented increased IL-25 expression in lesional skin by Kay et al. [17], in conjunction with the regulatory roles of type 2 alarmin cytokines in skin immunity, as outlined by Hasegawa et al. [34] and the varied functions of IL-25 in tissue immunity highlighted by Stanbery et al. [35], suggest IL-25's nuanced involvement in CSU. This prompts further investigation into IL-25's specific contributions to CSU pathophysiology and symptomatology.

Moreover, our research adds to the ongoing dialogue on the identification of effective biomarkers for CSU, with IL-33 demonstrating significant potential as indicated by ROC curve analysis. Conversely, IL-25's weaker performance, despite its statistical relevance, hints at a more limited role when considered in isolation, emphasizing the need for a multifaceted approach in biomarker development and the utility of these cytokines in clinical assessments.

In our study, we observed elevated levels of TSLP in CSU, aligning with findings from other studies such as those reported by Hoy et al. [36]. This observation underscored TSLP's role in the inflammation associated with CSU, although its direct correlation with disease severity and patient QoL, as measured by DLQI and UAS7 indices, remained minimal, as discussed by Wang and Zuo [37]. The association between TSLP and CRP highlighted its involvement in generalized inflammatory responses, without a clear link to specific CSU severity or QoL outcomes.

The development and approval of tezepelumab, an anti-TSLP monoclonal antibody, underscored by Damask et al. [38], marked a pivotal advancement in targeting this cytokine for CSU treatment, offering new hope for patients who are unresponsive to standard treatments. Further research by Hashimoto et al. [39] suggested TSLP's involvement in CSU's pruritic symptoms through basophil activation, extending its impact to sensory mechanisms within the disease. Observations by Treudler and Simon [40] on emerging biologics targeting TSLP indicated potential for broader therapeutic applications in addressing allergic and immunological facets of CSU.

However, contrasting evidence by Metz et al. [41] regarding TSLP levels in CSU necessitated additional research to resolve discrepancies in cytokine profiles and their implications for clinical manifestations and QoL in CSU patients. Collectively, these insights [36–41] called for an integrated approach to CSU therapy that considered TSLP's multifaceted role in the immune response, advocating for refined strategies in patient care and management.

The absence of inter-alarmin correlations suggests independent pathways of action for these cytokines in CSU pathogenesis. This is particularly notable, as it challenges the notion of a synergistic alarmin network and prompts further inquiry into these cytokines' discrete roles. Alarmin cytokines, such as IL-25, IL-33, and TSLP, are secreted by various immune and epithelial cells in response to tissue damage, infection, or inflammation. While they are known to play critical roles in immune responses, the exact order and mechanisms of their secretion within the immune cascade remain a subject of ongoing investigation.

The correlation between clinical tools—UAS7 and DLQI—demonstrates a robust link between symptom severity and QoL, affirming the interdependence of these measures in CSU. This strong correlation is especially significant, as it validates the clinical relevance of these tools in both research and practice.

While CSU and atopic dermatitis (AD) can be clinically distinguished by specialist clinicians, they share significant similarities in their inflammatory mechanisms, partic-

ularly in the role of pro-inflammatory cytokines like IL-4, IL-5, IL-13, and IL-31. This intersection underscores the necessity of mentioning this second condition here, allowing for a comparative and parallel overview to enhance our understanding of their shared pathophysiological features in an academic context. These cytokines, implicated in the onset of pruritus—a common symptom in both conditions—underscore the potential for a unified approach to therapy. Biologics such as dupilumab, targeting the IL-4 receptor to block type-2 inflammation, mepolizumab against IL-5, and tezepelumab and etokimab targeting TSLP and IL-33, respectively, alongside nemolizumab, which focuses on the IL-31 receptor crucial for mediating itch, represent pivotal advancements. These developments offer targeted relief from the inflammatory and pruritic aspects of AD and CSU [42].

By engaging with these existing contributions to the field, our study not only corroborates the established narratives, but also expands upon them, suggesting new avenues for research and potential therapeutic interventions that leverage the systemic nature of alarmin activity. It is within this expanded, complex framework that our study positions itself, aiming to bridge the gaps in current knowledge and inspire a forward momentum in both research and clinical practice.

Strengths and Limitations

This study's exploration into the serum levels of IL-25, IL-33, and TSLP offers valuable insights into CSU pathophysiology. One of the main strengths is the detailed examination of the correlation between these cytokines and both CSU severity and QoL outcomes, which enhances our understanding of the disease's underlying mechanisms. The inclusion of a control group adds robustness to our findings, and the rigorous statistical analyses employed substantiate the strength of the observed associations.

However, there are limitations to consider. The cross-sectional nature of the study precludes the establishment of causality, and does not capture the longitudinal dynamics of cytokine levels. While IL-33's elevation in CSU patients suggests a significant role, the absence of a corresponding increase in TSLP levels in relation to QoL measures points to the complex interplay of cytokines in CSU.

The study's sample size, though sufficient for our analysis, may not reflect the full spectrum of the CSU population, and the single-center design limits the generalizability of the results. This highlights the necessity for larger, multi-center studies to confirm these findings across a more diverse patient cohort.

In conclusion, our findings provide a solid foundation for the potential targeting of IL-33 in CSU management, given its strong correlation with disease severity and QoL. Yet, the intricate web of cytokine interactions and their impact on CSU remains an area ripe for further investigation. Future research, ideally involving multi-center longitudinal studies with larger participant pools, is essential to advance our comprehension of cytokine activity in CSU and to guide therapeutic interventions.

4. Materials and Methods

This retrospective, analytical study was carried out at the Allergology Department of the Regional Institute of Gastroenterology and Hepatology, Cluj-Napoca, Romania. It involved a cohort of 50 CSU patients, diagnosed according to the latest international guidelines [1]. These guidelines describe CSU as a recurrent, maculopapular rash that may include angioedema, appearing at least bi-weekly for over six weeks [1,2]. For comparative analysis, 38 healthy staff members from the institute were selected as a control group. The CSU patients met diagnostic criteria as per the guidelines [1], and were free from concurrent systemic illnesses such as systemic mastocytosis, Schnitzler syndrome, and urticarial vasculitis. Additional exclusion criteria included patients with renal, hepatic, psychiatric, or infectious conditions presenting with cutaneous manifestations or itching. The control group, with same distribution by age and gender, was strictly composed of individuals with no history of urticaria and excluded those with systemic diseases causing urticaria or pruritus. This study received ethical approval from the "Iuliu Hatieganu" University

of Medicine and Pharmacy, Cluj-Napoca, Romania (AVZ270/10.10.2022), and the IRGH (12637/11.10.2022). Informed consent was obtained from all participants. Demographic information and baseline characteristics such as age, sex, and serum levels of the alarmins IL-33, IL-25, and TSLP were recorded for all participants. For CSU patients, additional data including disease duration, severity, and presence or absence of atopy were collected. This was defined by a positive skin-prick (SPT) test to environmental allergens. Complementary tests included complete blood counts, ERS and CRP analysis, coproparasitological exams, and total serum IgE measurements, all conducted at the hospital's central laboratory. Venous blood was collected from the participants and centrifuged, and the serum was stored at −80 °C. The levels of IL-33, IL-25, and TSLP in the serum were determined using specific ELISA kits, following the manufacturer's instructions (Elabscience, 14780 Memorial Drive, Suite 108, Houston, TX, 77079, USA).

The Urticaria Activity Score over 7 days (UAS7) is a comprehensive, globally recognized metric for evaluating the severity of CSU [43,44]. This tool captures the extent and impact of urticarial symptoms over a week, quantifying both hive formation and itch intensity. The UAS7 incorporates two distinct subscores, one for hives and the other for itch severity, each evaluated daily. Hive Score: This is assessed on a scale from 0 to 3, where 0 denotes the absence of hives, 1 signifies mild hives (less than 20 hives in a 24-hour period), 2 indicates moderate hives (between 20 and 50 hives), and 3 reflects severe hives (over 50 hives or extensive confluent areas). Itch Severity Score: Similar to the hive score, itch severity is measured on a scale from 0 to 3. A score of 0 indicates no itching, 1 represents mild itching (noticeable but not bothersome), 2 is for moderate itching (disturbing but not interfering significantly with daily activities or sleep), and 3 signifies severe itching (severely disruptive to daily activities and sleep). The total UAS7 score is the sum of these daily assessments, with a maximum potential score of 42. The cumulative score categorizes CSU severity into three levels: mild (0–15), moderate (16–27), and severe (28–42), enabling a nuanced understanding of the disease's impact on the patient.

The Dermatology Life Quality Index (DLQI) is a widely employed instrument to gauge the impact of CSU on a patient's QoL [45,46]. This index consists of ten questions, addressing various aspects of daily life that may be affected by the condition. Each question is scored from 0 to 3, based on the degree of impact. The questions cover a broad spectrum of life domains, including symptoms and feelings, daily activities, leisure, work or school performance, personal relationships, and treatment-related issues. The cumulative score from these questions provides a total DLQI score, ranging from 0 (no impact) to 30 (extremely large impact). This total score is further broken down into five categories: 0–1 (no impact), 2–5 (small impact), 6–10 (moderate impact), 11–20 (very large impact), and 21–30 (extremely large impact). Thus, the DLQI offers an insightful, patient-centered perspective on how CSU affects various facets of an individual's life, complementing the clinical severity assessed using the UAS7.

Statistical Analysis

Our data are presented predominantly as medians and interquartile ranges. We employed the Shapiro–Wilk test to evaluate the normal distribution of the data. For group comparisons, the study utilized the Mann–Whitney U test or the Kruskal–Wallis test, based on the data characteristics. Specifically, the Mann–Whitney U test was applied for comparing two distinct groups, such as the serum levels of IL-25, IL-33, and TSLP between healthy controls and CSU patients. This test is particularly suited for non-normally distributed continuous data and facilitates the assessment of significant differences in the groups' central tendencies.

For comparisons involving more than two independent groups or ordinal data, the Kruskal–Wallis test was the method of choice. This test extends the Mann–Whitney U test to multiple groups, and assesses if there are significant differences in their medians. In our research, it was particularly useful for analyzing IL-25, IL-33, and TSLP levels across various disease severity groups.

Categorical data comparisons were conducted using Pearson's chi-squared test, suitable for examining associations between categorical variables. Correlations between various parameters were examined using Spearman's correlation, a non-parametric measure ideal for assessing non-linear relationships.

Furthermore, we explored the diagnostic potential of IL-25, IL-33, and TSLP using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) for these cytokines was calculated to determine their effectiveness in distinguishing CSU patients from healthy controls.

All statistical analyses and graph generation were conducted using GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, CA, USA). A p-value of <0.05 was set as the threshold for statistical significance. This comprehensive selection of tests and methods was aimed at thoroughly analyzing the interrelationships and disparities in IL-25, IL-33, and TSLP levels and their clinical correlates within the context of CSU.

5. Conclusions

Our research into the alarmin cytokines IL-25, IL-33, and TSLP has identified a notable elevation of IL-33 in CSU patients, correlating significantly with both UAS7 and DLQI scores, thereby substantiating its role in disease severity and patient QoL impact. In contrast, the elevated serum levels of IL-25 did not correspond with significant clinical correlations, suggesting a more intricate role within the cytokine interplay of CSU. Elevated TSLP levels, while indicative of its involvement in allergic and immunological pathways, did not show a direct correlation with symptom severity or patient QoL in CSU. The study acknowledges the recent approval of tezepelumab, an anti-TSLP therapy, for other allergic and immunological disorders, which may inform future therapeutic strategies in CSU management. The discrepancies in cytokine profiles, particularly concerning TSLP, underscore the imperative for continued research to elucidate their roles in CSU. Overall, these findings advocate for further exploration into cytokine-modulating treatments, with IL-33 presenting a viable target. Future research, ideally through larger, multi-center longitudinal studies, is essential to deepen our understanding of cytokine dynamics in CSU and optimize therapeutic interventions.

Author Contributions: Conceptualization, C.-T.D.-B., I.A.M. and G.A.F.; data curation, C.-T.D.-B., D.M.D. and I.A.M.; formal analysis, R.-G.B.; investigation, C.-T.D.-B.; methodology, C.-T.D.-B., I.A.M. and G.A.F.; resources, D.M.D.; software, I.A.M.; supervision, G.A.F. and L.M.P.; validation, C.-T.D.-B. and G.A.F.; writing—original draft, C.-T.D.-B.; writing—review and editing, C.-T.D.-B., I.N. and G.A.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received support from the "Iuliu Hatieganu" University of Medicine and Pharmacy of Cluj-Napoca, Romania, through a grant—Proiect Cercetare Doctorala (PCD), contract number 2462/18/17.01.2020.

Institutional Review Board Statement: This study was approved by the ethics committee of the UMF "Iuliu Hatieganu" [AVZ270/10.10.2022] and by the "Octavian Fodor" Institute of Gastroenterology and Hepatology [12637/11.10.2022].

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to especially thank the chemist Nicoleta Decea for her support and coordination in performing the IL-25, IL-33 and TSLP ELISA determinations.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zuberbier, T.; Latiff, A.H.A.; Abuzakouk, M.; Aquilina, S.; Asero, R.; Baker, D.; Ballmer-Weber, B.; Bangert, C.; Ben-Shoshan, M.; Bernstein, J.A.; et al. The international EAACI/GA2LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. *Allergy* **2021**, *77*, 734–766. [CrossRef] [PubMed]
2. Maurer, M.; Eyerich, K.; Eyerich, S.; Ferrer, M.; Gutermuth, J.; Hartmann, K.; Jakob, T.; Kapp, A.; Kolkhir, P.; Larenas-Linnemann, D.; et al. Urticaria: Collegium Internationale Allergologicum (CIA) Update 2020. *Int. Arch. Allergy Immunol.* **2020**, *181*, 321–333. [CrossRef]
3. Dobrican, C.-T.; Muntean, I.A.; Pintea, I.; Petricău, C.; Deleanu, D.-M.; Filip, G.A. Immunological signature of chronic spontaneous urticaria (Review). *Exp. Ther. Med.* **2022**, *23*, 381. [CrossRef] [PubMed]
4. Bansal, C.J.; Bansal, A.S. Stress, Pseudoallergens, Autoimmunity, Infection and Inflammation in Chronic Spontaneous Urticaria. *Allergy Asthma Clin. Immunol.* **2019**, *15*, 56. [CrossRef] [PubMed]
5. Muntean, I.A.; Pintea, I.; Bocsan, I.C.; Dobrican, C.T.; Deleanu, D. COVID-19 Disease Leading to Chronic Spontaneous Urticaria Exacerbation: A Romanian Retrospective Study. *Healthcare* **2021**, *9*, 1144. [CrossRef]
6. MacGlashan, D. Autoantibodies to IgE and FcεRI and the Natural Variability of Spleen Tyrosine Kinase Expression in Basophils. *J. Allergy Clin. Immunol.* **2019**, *143*, 1100–1107.e11. [CrossRef]
7. Rauber, M.M.; Pickert, J.; Holiangu, L.; Möbs, C.; Pfützner, W. Functional and Phenotypic Analysis of Basophils Allows Determining Distinct Subtypes in Patients with Chronic Urticaria. *Allergy* **2017**, *72*, 1904–1911. [CrossRef]
8. Zhou, B.; Li, J.; Liu, R.; Zhu, L.; Peng, C. The Role of Crosstalk of Immune Cells in Pathogenesis of Chronic Spontaneous Urticaria. *Front. Immunol.* **2022**, *13*, 879754. [CrossRef]
9. Church, M.K.; Kolkhir, P.; Metz, M.; Maurer, M. The role and relevance of mast cells in urticaria. *Immunol. Rev.* **2018**, *282*, 232–247. [CrossRef]
10. Yanase, Y.; Takahagi, S.; Ozawa, K.; Hide, M. The Role of Coagulation and Complement Factors for Mast Cell Activation in the Pathogenesis of Chronic Spontaneous Urticaria. *Cells* **2021**, *10*, 1759. [CrossRef]
11. Saini, S.S.; Paterniti, M.; Vasagar, K.; Gibbons, S.P., Jr.; Sterba, P.M.; Vonakis, B.M. Cultured Peripheral Blood Mast Cells From Chronic Idiopathic Urticaria Patients Spontaneously Degranulate Upon IgE Sensitization: Relationship to Expression of Syk and SHIP-2. *Clin. Immunol.* **2009**, *132*, 342–348. [CrossRef] [PubMed]
12. dos Santos, J.C.; Azor, M.H.; Nojima, V.Y.; Lourenço, F.D.; Prearo, E.; Maruta, C.W.; Rivitti, E.A.; da Silva Duarte, A.J.; Sato, M.N. Increased Circulating Pro-inflammatory Cytokines and Imbalanced Regulatory T-cell Cytokines Production in Chronic Idiopathic Urticaria. *Int. Immunopharmacol.* **2008**, *8*, 1433–1440. [CrossRef]
13. Bonnekoh, H.; Maurer, M.; Krause, K. Interleukin-1-related cytokines as potential biomarkers in autoinflammatory skin diseases. *Pediatr. Rheumatol.* **2015**, *13* (Suppl. 1), P197. [CrossRef]
14. Kuna, M.; Štefanović, M.; Ladika Davidović, B.; Mandušić, N.; Birkić Belanović, I.; Lugović-Mihić, L. Chronic Urticaria Biomarkers IL-6, ESR and CRP in Correlation with Disease Severity and Patient Quality of Life—A Pilot Study. *Biomedicines* **2023**, *11*, 2232. [CrossRef]
15. Lin, W.; Zhou, Q.; Liu, C.; Ying, M.; Xu, S. Increased plasma IL-17, IL-31, and IL-33 levels in chronic spontaneous urticaria. *Sci. Rep.* **2017**, *7*, 17797. [CrossRef]
16. Dobrican-Băruța, C.-T.; Deleanu, D.M.; Muntean, I.A.; Pintea, I.; Florea, C.M.; Filip, G.A. IL-31—Pruritus Interleukin: Serum Values and Clinical Impact in Chronic Spontaneous Urticaria—A Romanian Retrospective Study. *J. Clin. Med.* **2023**, *12*, 5957. [CrossRef]
17. Kay, A.B.; Clark, P.; Maurer, M.; Ying, S. Elevations in T-helper-2-initiating cytokines (interleukin-33, interleukin-25, and thymic stromal lymphopoietin) in lesional skin from chronic spontaneous ('idiopathic') urticaria. *Br. J. Dermatol.* **2015**, *172*, 1294–1302. [CrossRef]
18. Hueber, A.J.; Alves-Filho, J.C.; Asquith, D.L.; Michels, C.; Millar, N.L.; Reilly, J.H.; Graham, G.J.; Liew, F.Y.; Miller, A.M.; McInnes, I.B. IL-33 induces skin inflammation with mast cell and neutrophil activation. *Eur. J. Immunol.* **2011**, *41*, 2229–2237. [CrossRef]
19. Drube, S.; Grimlowski, R.; Deppermann, C.; Fröbel, J.; Kraft, F.; Andreas, N.; Stegner, D.; Dudeck, J.; Weber, F.; Rödiger, M.; et al. The Neurobeachin-like 2 Protein Regulates Mast Cell Homeostasis. *J. Immunol.* **2017**, *199*, 2948–2957. [CrossRef] [PubMed]
20. Borgia, F.; Custurone, P.; Li Pomi, F.; Vaccaro, M.; Alessandrello, C.; Gangemi, S. IL-33 and IL-37: A Possible Axis in Skin and Allergic Diseases. *Int. J. Mol. Sci.* **2023**, *24*, 372. [CrossRef] [PubMed]
21. Hoppe, A.; Katsoulis-Dimitriou, K.; Edler, H.J.; Dudeck, J.; Drube, S.; Dudeck, A. Mast cells initiate the vascular response to contact allergens by sensing cell stress. *J. Allergy Clin. Immunol.* **2020**, *145*, 1476–1478. [CrossRef]
22. Kaplan, A.; Lebwohl, M.; Giménez-Arnau, A.M.; Hide, M.; Armstrong, A.W.; Maurer, M. Chronic spontaneous urticaria: Focus on pathophysiology to unlock treatment advances. *Allergy* **2022**, *78*, 389–401. [CrossRef]
23. Pedersen, N.H.; Sørensen, J.A.; Ghazanfar, M.N.; Zhang, D.G.; Vestergaard, C.; Thomsen, S.F. Biomarkers for Monitoring Treatment Response of Omalizumab in Patients with Chronic Urticaria. *Int. J. Mol. Sci.* **2023**, *24*, 11328. [CrossRef]
24. Asero, R.; Ferrucci, S.M.; Calzari, P.; Consonni, D.; Cugno, M. Thyroid Autoimmunity in CSU: A Potential Marker of Omalizumab Response? *Int. J. Mol. Sci.* **2023**, *24*, 7491. [CrossRef]
25. Bostan, O.C.; Damadoglu, E.; Sarac, B.E.; Kilic, B.; Sahiner, M.; Karaaslan, C.; Karakaya, G.; Kalyoncu, A.F. Cytokine Profiles of Chronic Urticaria Patients and The Effect of Omalizumab Treatment. *Dermatol. Pract. Concept.* **2023**, *13*, e2023272. [CrossRef]

26. Metz, M.; Maurer, M. Use of Biologics in Chronic Spontaneous Urticaria—Beyond Omalizumab Therapy? *Allergol. Select* **2021**, *5*, 89–95. [CrossRef]
27. Metz, M.; Kolkhir, P.; Altrichter, S.; Siebenhaar, F.; Levi-Schaffer, F.; Youngblood, B.A.; Church, M.K.; Maurer, M. Mast cell silencing: A novel therapeutic approach for urticaria and other mast cell-mediated diseases. *Allergy* **2023**, *79*, 37–51. [CrossRef]
28. Manti, S.; Giallongo, A.; Papale, M.; Parisi, G.F.; Leonardi, S. Monoclonal antibodies in treating chronic spontaneous urticaria: New drugs for an old disease. *J. Clin. Med.* **2022**, *11*, 4453. [CrossRef]
29. Puxeddu, I.; Italiani, P.; Giungato, P.; Pratesi, F.; Panza, F.; Bartaloni, D.; Rocchi, V.; Del Corso, I.; Boraschi, D. Free IL-18 and IL-33 cytokines in chronic spontaneous urticaria. *Cytokine* **2013**, *61*, 741–743. [CrossRef]
30. Cayrol, C.; Girard, J.P. Interleukin-33 (IL-33): A critical review of its biology and the mechanisms involved in its release as a potent extracellular cytokine. *Cytokine* **2022**, *156*, 155891. [CrossRef]
31. Trier, A.M.; Ver Heul, A.M.; Fredman, A.; Kropski, J.A.; Dai, R.; Mitchell, D.; Serezani, A.P.; Guttman-Yassky, E.; Richmond, J.; Gilfillan, A.M.; et al. IL-33 potentiates histaminergic itch. *J. Allergy Clin. Immunol.* **2023**. [CrossRef]
32. Garcovich, S.; Maurelli, M.; Gisondi, P.; Peris, K.; Yosipovitch, G.; Girolomoni, G. Pruritus as a Distinctive Feature of Type 2 Inflammation. *Vaccines* **2021**, *9*, 303. [CrossRef]
33. Murdaca, G.; Greco, M.; Tonacci, A.; Negrini, S.; Borro, M.; Puppo, F.; Gangemi, S. IL-33/IL-31 Axis in Immune-Mediated and Allergic Diseases. *Int. J. Mol. Sci.* **2019**, *20*, 5856. [CrossRef]
34. Hasegawa, T.; Oka, T.; Demehri, S. Alarmin Cytokines as Central Regulators of Cutaneous Immunity. *Front. Immunol.* **2022**, *13*, 876515. [CrossRef]
35. Stanbery, A.G.; Smita, S.; von Moltke, J.; Tait Wojno, E.D.; Ziegler, S.F. TSLP, IL-33, and IL-25: Not just for allergy and helminth infection. *J. Allergy Clin. Immunol.* **2022**, *150*, 1302–1313. [CrossRef]
36. Hoy, S.M. Tezepelumab: First Approval. *Drugs* **2022**, *82*, 461–468. [CrossRef]
37. Wang, S.-H.; Zuo, Y.-G. Thymic Stromal Lymphopoietin in Cutaneous Immune-Mediated Diseases. *Front. Immunol.* **2021**, *12*, 698522. [CrossRef]
38. Damask, C.C.; Ryan, M.W.; Casale, T.B.; Castro, M.; Franzese, C.B.; Lee, S.E.; Levy, J.M.; Lin, S.Y.; Lio, P.A.; Peters, A.T.; et al. Targeted Molecular Therapies in Allergy and Rhinology. *Otolaryngol.-Head Neck Surg.* **2021**, *164*, S1–S21. [CrossRef]
39. Hashimoto, T.; Rosen, J.D.; Sanders, K.M.; Yosipovitch, G. Possible roles of basophils in chronic itch. *Exp. Dermatol.* **2019**, *28*, 1373–1379. [CrossRef]
40. Treudler, R.; Simon, J.-C. Developments and perspectives in allergology. *J. Der Dtsch. Dermatol. Ges.* **2023**, *21*, 399–403. [CrossRef]
41. Metz, M.; Krull, C.; Maurer, M. Histamine, TNF, C5a, IL-6, -9, -18, -31, -33, TSLP, neopterin, and VEGF are not elevated in chronic spontaneous urticaria. *J. Dermatol. Sci.* **2013**, *70*, 222–225. [CrossRef] [PubMed]
42. Di Filippo, P.; Russo, D.; Attanasi, M.; Di Pillo, S.; Chiarelli, F. Immunological Targets of Biologic Drugs in Allergic Skin Diseases in Children. *Biomedicines* **2021**, *9*, 1615. [CrossRef] [PubMed]
43. Mathias, S.D.; Crosby, R.D.; Zazzali, J.L.; Maurer, M.; Saini, S.S. Evaluating the minimally important difference of the urticaria activity score and other measures of disease activity in patients with chronic idiopathic urticaria. *Ann. Allergy Asthma Immunol.* **2012**, *108*, 20–24. [CrossRef] [PubMed]
44. Hawro, T.; Ohanyan, T.; Schoepke, N.; Metz, M.; Peveling-Oberhag, A.; Staubach, P.; Maurer, M.; Weller, K. The urticaria activity score-validity, reliability, and responsiveness. *J. Allergy Clin. Immunol. Pract.* **2018**, *6*, 1185–1190.e1. [CrossRef]
45. Baiardini, I.; Braido, F.; Bindslev-Jensen, C.; Bousquet, P.J.; Brzoza, Z.; Canonica, G.W.; Compalati, E.; Fiocchi, A.; Fokkens, W.; Gerth van Wijk, R.; et al. Recommendations for assessing patient-reported outcomes and health-related quality of life in patients with urticaria: A GA^2LEN taskforce position paper. *Allergy* **2011**, *66*, 840–844. [CrossRef]
46. Weller, K.; Groffik, A.; Magerl, M.; Tohme, N.; Martus, P.; Krause, K.; Metz, M.; Staubach, P.; Maurer, M. Development and construct validation of the angioedema quality of life questionnaire. *Allergy* **2012**, *67*, 1289–1298. [CrossRef]