Ocular surface disease in atopic dermatitis and the effect of dupilumab treatment

Clinical characteristics, risk factors, and pathomechanism

Roselie Achten

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Clinical characteristics, risk factors, and pathomechanism

Oogklachten bij patiënten met constitutioneel eczeem en het effect van dupilumab behandeling

Klinische karakteristieken, risicofactoren en pathomechanisme

(met een samenvatting in het Nederlands)

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CONTENTS

PART I

Introduction

CHAPTER 1

General introduction

ATOPIC DERMATITIS AND OCULAR COMORBIDITIES

Atopic dermatitis (AD) is a chronic, inflammatory skin disease that affects up to 10% of the adults.[1] In most cases, AD begins in early childhood but it can develop at any age. AD is characterized by repeated flare ups with pruritus, pain, and sleep disturbance, leading to a reduction in quality of life.[2] Patients with AD often have several other atopic comorbidities, like allergic asthma, food allergy, and allergic rhinitis.[3] AD is also associated with ocular comorbidities, and an overall prevalence of conjunctivitis was found in 31.7% of the AD patients, compared to 13.3% in controls.[4] In patients with moderate-to-severe AD, the reported prevalence of conjunctivitis was even higher, up to 39.6%.[4] Uncontrolled AD around the eyes can lead to chronic scratching and rubbing, transferring for example the S. aureus bacteria into the eyes which may result in blepharoconjunctivits.[5] Other frequently reported ocular diseases in moderate-to-severe AD patients are eczema on the eyelids, superficial punctate keratopathy, cataract, increased risk of retinal detachment, keratoconus, and viral ocular infections.[5,6] Furthermore, patients with AD have an eight-fold higher risk of developing allergic conjunctivitis than adults without AD.[7,8] Allergic conjunctivitis includes seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal keratoconjunctivitis (VKC), and atopic keratoconjunctivitis (AKC), which have different pathomechanisms.[7,9] Initial development of SAC and PAC begins with ocular exposure to an allergen that induces an immune response.[9] The chronic inflammation of VKC is primarily mediated by T helper (Th) 2-lymphocyte derived cytokines, while the pathogenesis of AKC involves both Th1- and Th2-lymphocyte derived cytokines.

The pathogenesis of AD is complex and multifactorial, and is characterized by epithelial barrier dysfunction and a hyperactive adaptive immune system, characterized by a hyperactive Th2 response.[10,11] The majority of AD patients have mild disease and can be adequately treated with emollients, topical corticosteroids, topical calcineurin inhibitors, and/or UV light therapy.[12] The treatment of moderate-to-severe AD can be more challenging. These patients may need treatment with systemic immunosuppressive drugs, including cyclosporine A, methotrexate, azathioprine, and mycophenolate mofil.[13] However, these treatments may have multiple side effects and are sometimes ineffective.[13]

Since 2018, new targeted therapies have become available to treat moderate-to-severe AD.[14,15] Dupilumab is the first biologic agent available for the treatment of moderate-to-severe AD patients. It is a fully human monoclonal antibody that targets the interleukin (IL)-4 receptor alpha (IL-4Rα), resulting in inhibition of the Th2 pathway. Dupilumab has proven its effectiveness and safety in both clinical trials and daily practice studies.[16-18]

OCULAR SIDE EFFECTS DURING DUPILUMAB TREATMENT

Ocular surface disease (OSD) can be used as an umbrella term for various ocular diseases such as conjunctivitis, blepharitis, and dry eyes. In both clinical trials and daily practice studies, dupilumab-associated ocular surface disease (DAOSD) is the most commonly described side effect and has been reported in up to 34% of the dupilumab-treated AD patients.[16,17,19] Remarkably, no increased incidence of DAOSD was found in dupilumab trails for other type 2 diseases, like asthma, suggesting that AD patients may have a predisposition to develop DAOSD during dupilumab treatment. In addition, increased rates of OSD during treatment with other Th2 blocking agents, like tralokinumab treatment, are also reported.[20] Due to the increased incidence of OSD during dupilumab and tralokinumab treatment, the importance of a better understanding of OSD in patients with moderate-to-severe AD has been emphasized.[19,20]

Patients with DAOSD have reported symptoms of itch, burning sensation, foreign body sensation, redness, dryness, photophobia, and watery eyes.[21-24] A few studies investigated the ophthalmological characteristics of DAOSD, and reported blepharitis, Meibomian gland dysfunction, blepharoconjunctivitis, keratitis, conjunctival injection, limbal vascularization, and signs of limbitis (e.g. hyperemia and/or nodular swelling of the limbus).[21-24] Safety analysis of dupilumab up to 4 years of treatment showed that most cases of ocular adverse events resolved during the study treatment period (n=775/888, 87%).[25] In severe and chronic DAOSD, limbal stem cell deficiency may develop due to chronic inflammation with long-term irreversible consequences, including cellular invasion of the conjunctival epithelium onto the cornea, leading to impaired epithelial wound healing, which may require long-term care and management.[26,27] Most studies on DAOSD are based on patient-reported diagnosis, and do not include a standardized ophthalmic examination. Bortoluzzi et al. described that patient-reported diagnoses are not always reliable, as there was a lack of correlation between the ophthalmic evaluation of the ocular surface and an OSD questionnaire.[28] This highlights the importance of ophthalmological examination in dupilumab-treated AD patients. Since most of the dupilumab-treated AD patients that develop DAOSD will not notice and thus not actively report this side effect, knowledge about clinical risk factors, which can potentially predict which patients will develop DAOSD, is important.

Several studies have investigated risk factors for the development of OSD during treatment with dupilumab in AD patients. These studies suggested that the increased rates of DAOSD in AD patients are associated with more severe AD at baseline, prior history of (allergic) conjunctivitis, prior history of asthma, eyelid involvement of

AD, presence of other atopic comorbidities, a family history of AD, high levels of serum IgE, high levels of thymus and activation-regulated chemokine (TARC), and peripheral blood eosinophilia.[17,19,24,29-32] However, most of these studies are based on retrospective and/or small cohorts, so larger prospective studies that examine risk factors for DAOSD are needed.

Treatment of DAOSD can be challenging and several therapies have been described in small case-series or case reports, including corticosteroid eye drops, antihistamine eye drops, and artificial tears.[33] Tacrolimus skin ointment (0.03% or 0.1%) applied on the skin of the eyelids was effective in 4 patients with blepharoconjunctivitis during dupilumab treatment.[34] Currently, no official guidelines are available for the management of DAOSD.

THE HYPOTHEZISED PATHOMECHANISM OF DAOSD

The exact pathomechanism of DAOSD remains unclear. Several pathomechanisms have been suggested to be responsible for the development of DAOSD. Bakker et al. examined conjunctival biopsies of 6 dupilumab-treated AD patients with DAOSD, and found a scarcity of intraepithelial goblet cells (GCs).[35] Voorberg et al. reported an increasing amount of GCs after discontinuation of dupilumab in a patient with DAOSD (n=1).[36] Both studies suggest that GCs may play a role in the development of DAOSD. Conjunctival GCs are specialized in the secretion of mucins that are important in the maintenance of the tear film stability. They also play an important role in the mucosal immune system by producing immune regulatory factors.[37-39] IL-13 is the predominant cytokine promoting GC proliferation. The blocking effect of dupilumab on IL-13 signalling might lead to reduction of GCs in dupilumab-treated AD patients.[35,40] A second study of Bakker et al. investigated conjunctival biopsies of AD patients with DAOSD (n=6) and found increased local T-helper 1 (Th1) related cytokine production. The reduced GC production due to IL-13 blocking could lead to a decreased expression of immune-regulatory factors and protective mucus, thereby enhancing the conjunctival inflammation. The blocking effect on the Th2 immune response by dupilumab could lead to skewing towards the Th1 and Th17 pathways, leading to aggravation of conjunctival inflammation. Taken together, the high incidence of DAOSD in dupilumab-treated moderate-to-severe AD patients might be partly explained by loss of conjunctival GCs due to blocking of IL-13 and partly by the skewing towards the Th1 and Th17 pathways (Figure 1). Several other hypotheses have been proposed for the mechanisms inducing DAOSD, including an local under-treatment of dupilumab in the eyes.[19,41]

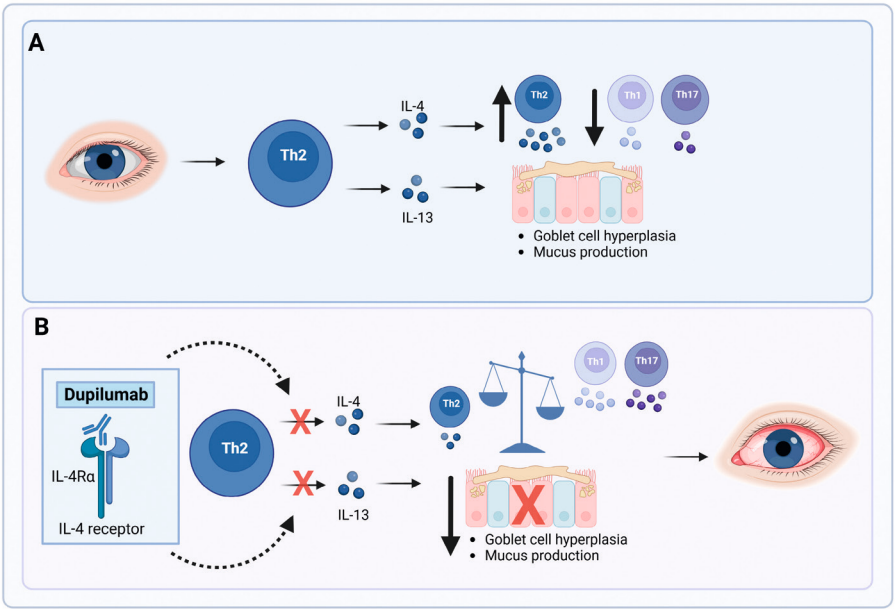

FIGURE 1. Hypothesis on the pathomechanism of dupilumab-associated ocular surface disease.
A. Normally, T helper (Th)-2 cells produce cytokines like interleukin (IL)-4 and IL-13. IL-4 plays a role in the differentiation of Th2 cells and the inhibition of differentiation of Th1 and Th17 cells. IL-13 stimulates goblet cell (GC) hyperplasia and mucus production. **B.** Dupilumab blocks IL-4 and IL-13 signalling. Blocking of IL-4 leads to less proliferation of Th2 cells, and to less inhibition of the proliferation of Th1 and Th17 cells. This might lead to a disbalance between these two, leading to an irritative conjunctivitis and a Th-1 inflammatory response. The blocking effect of dupilumab on IL-13 might lead to less GC hyperplasia, leading to less GCs and less mucus production. This figure is created in Biorender.

OUTLINE OF THIS THESIS

The aim of this thesis is to investigate OSD in moderate-to-severe AD patients, before (**chapter 2**) and during dupilumab treatment (**chapter 3-10**). We want to evaluate clinical risk factors that are contributing to the development of DAOSD, and learn more about the (clinical) characteristics of DAOSD. In addition, we aim to evaluate the pathomechanism of DAOSD.

In **chapter 2**, we describe ocular surface characteristics, both clinical and immunological, in moderate-to-severe AD patients before the start of dupilumab treatment. Risk factors for the development of DAOSD are evaluated in **chapter 3**. The course and development of DAOSD and the effect of dupilumab on conjunctival GCs in dupilumab-treated AD patients are described in **chapter 4**. The long-term follow-up and treatment outcomes of 33 AD patients with DAOSD are described in

chapter 5. In **chapter 6** we describe the effect on the ocular surface after switching from dupilumab treatment to tralokinumab treatment in 4 AD patients. A case series on dupilumab-associated uveitis, is reported in **chapter 7**.

The last part of this thesis examines the pathomechanism of DAOSD in AD patients. We have investigated tear fluid of dupilumab-treated AD patients and looked for both dupilumab tear fluid levels (**chapter 8**) and tear fluid biomarkers before and during dupilumab treatment (**chapter 9**). Lastly, by means of flow cytometry analysis of conjunctival impression cytology the immune cells and their possible role in the development of DAOSD are investigated (**chapter 10**). Implications and future prospects of our outcomes are outlined in the discussion (**chapter 11**).

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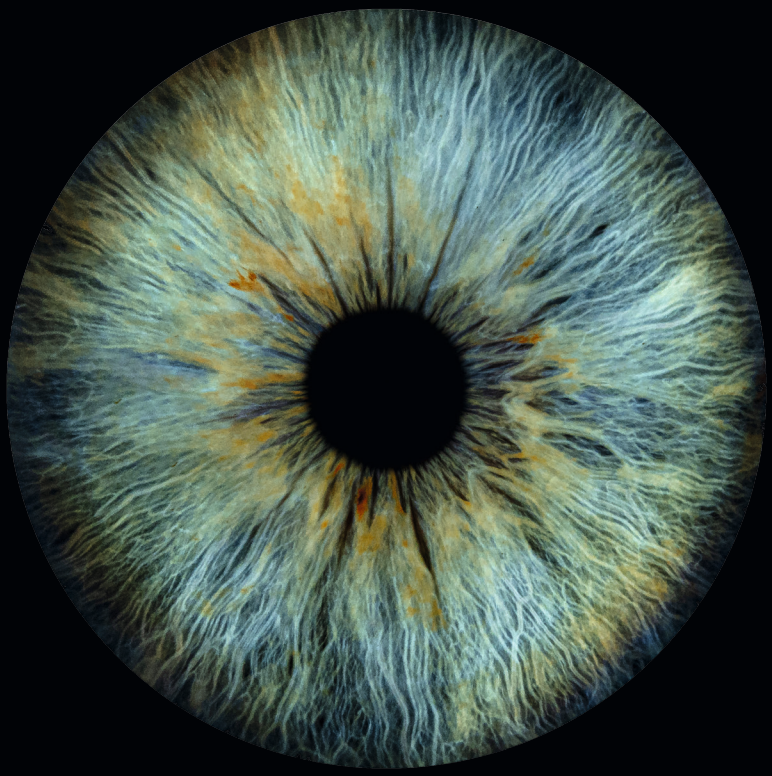
PART II

Ocular surface disease in moderate-to-severe atopic dermatitis patients and its pathomechanism



CHAPTER 2

Ocular surface disease is common in moderate-to-severe atopic dermatitis patients

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Clinical and Experimental Allergy: 2022 June; 52(6):801-805.

KEY MESSAGES

- In a single-center study, we assessed ocular surface disease prevalence in moderate-to-severe atopic dermatitis.
- Before starting dupilumab, 60/70 (90%) of patients already had ocular surface disease.
- Ocular surface disease was associated with lower conjunctival goblet cell density and more severe AD.

To the Editor,

High rates of ocular surface disease (OSD) in atopic dermatitis (AD) patients have been reported during dupilumab treatment. One of the hypothesis about the pathomechanism to be responsible for its development is focal scarcity of intraepithelial goblet cells.[1] An association between moderate-to-severe AD patients and low goblet cell density (GCD) has also been reported previously.[2] Despite the association between AD and OSD reported in previous literature, moderate-to-severe AD patients do not commonly undergo ophthalmological evaluation.[2] To better understand the pathomechanism of dupilumab-associated OSD (DAOSD), more insight in the occurrence of OSD in moderate-to-severe AD population is needed. Therefore, we investigated the frequency, severity, and pathogenesis of OSD in moderate-to-severe AD patients before the start of dupilumab.

This prospective study included adult moderate-to-severe AD patients treated with topical corticosteroids on the skin, between February 2020 and September 2021 from the University Medical Centre Utrecht, the Netherlands. The patients provided written informed consent and were registered in the BioDay registry, which is funded by the manufacturer of dupilumab.[3] Ethics approval was obtained by the local Medical Research Ethics Committee (METC 18/239).

All patients were examined by a dermatologist and an ophthalmologist before starting dupilumab treatment. AD severity was assessed by the Eczema Area and Severity Index (EASI). Clinical ophthalmological characteristics and symptoms of OSD were reported. Patients were divided into having no, mild, moderate, or severe OSD based on the Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score, and the severity classification of the most severely affected eye was used.[3] Conjunctival impression cytology (CIC) by using the Eyeprim device was performed to investigate the number of conjunctival goblet cells (GCs) and its major secretory mucin, which is Mucin5AC (MUC5AC). The CIC from the left eye was stained with Periodic Acid-Schiff and hematoxylin following the Eyeprim protocol.[4] Afterwards, the GCD per sample was calculated. Flow cytometry analyses of CIC of the right eye were performed in a representative subgroup of the included patients. CIC is further explained in the online access repository following: https://zenodo.org/record/6275350.

Differences between no or mild OSD and moderate-to-severe OSD were calculated with the Chi-square test and with the Mann-Whitney U test. CIC results were reported per severity category of OSD. Statistical analyses were conducted with SPSS Statistics version 25.0.0.2 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows). Figures were created by using Prism (version 9 GraphPad Software).

A total of 70 moderate-to-severe AD patients (median EASI 15.0 (IQR 10.8-20.9)) were included (Table 1). Mild, moderate, and severe OSD were reported in 32/70 (45.7%), 24/70 (34.3%), and 7/70 (10.0%) patients respectively. Only 7/70 (10.0%) patients showed no signs of OSD. Significantly higher EASI scores were found in patients with moderate-to-severe OSD compared to patients with no or mild OSD (17.7 (IQR 13.7-24.9) vs. 11.8 (IQR 9.0-16.7), p=<0.001). Additionally, occurrence of both AD eyelid involvement and AD facial involvement in the past year was significantly higher in patients with moderate-to-severe OSD compared to patients with no or mild OSD (n=28 (90.3%) vs. n=18 (46.2%), p=<0.001 and n=31 (100.0%) vs. n=33 (84.6%), p=0.030, respectively). Of all patients with moderate-to-severe OSD, 23/31 patients (74.2%) experienced OSD symptoms.

TABLE 1. Patient, dermatological, and ophthalmological characteristics

	Total cohort (n=70)	Severity of OSD		P-value
		No or mild OSD (n=39)	Moderate-to-severe OSD (n=31)	
Age (years), median (IQR)†	38.5 (27.0-53.3)	41.0 (27.0-59.0)	32.0 (24.0-46.0)	0.090
Men, n (%)	35 (50.0)	15 (38.5)	20 (64.5)	0.030
Age of onset of AD, n (%)				0.135
Childhood	62 (88.6)	32 (82.1)	30 (96.8)	n/a
Adolescence	5 (7.1)	4 (10.3)	1 (3.2)	n/a
Adult	3 (4.3)	3 (7.7)	0 (0.0)	n/a
History of self-reported episodic acute allergic conjunctivitis, n (%)	55 (78.6)	28 (71.8)	27 (87.1)	0.121
Allergic asthma, n (%)	35 (50.0)	18 (46.2)	17 (54.8)	0.470
Allergic rhinitis, n (%)	51 (72.9)	28 (71.8)	23 (74.2)	0.823
Food allergy, n (%)	34 (48.6)	21 (53.8)	13 (41.9)	0.322
History of rosacea, n (%)	2 (2.9)	1 (2.6)	1 (3.2)	1.000
EASI score, median (IQR)†	15.0 (10.8 – 20.9)	11.8 (9.0-16.7)	17.7 (13.7-24.9)	0.001
IGA score, median (IQR)†	3 (3-3)	3 (2-3)	3 (3-4)	0.002
AD eyelid involvement in the past year, n (%)	46 (65.7)	18 (46.2)	28 (90.3)	<0.001
AD facial involvement in the past year, n (%)	64 (91.4)	33 (84.6)	31 (100.0)	0.030
TARC (pg./ml), median (IQR)†	1564 (811-2716)	1411 (787-1975)	1919 (1348-3154)	0.026
Missing, n (%)	1 (1.4)	0 (0)	1 (3.2)	n/a
Peripheral blood eosinophils ($\times 10^9$/L), median (IQR)†	0.29 (0.16-0.51)	0.29 (0.14-0.50)	0.31 (0.20-0.52)	0.624
Eosinophilia (≥0.45$\times 10^9$/L), n (%)	21 (30.4)	12 (30.8)	9 (30.0)	0.945
Missing, n (%)	1 (1.4)	0 (0)	1 (3.2)	n/a
Visited an ophthalmologist before, n (%)	36 (51.4)	19 (48.7)	17 (54.8)	0.611
Previous use of ophthalmic medication, n (%)	45 (64.3)	24 (61.5)	21 (67.7)	0.591
Lubricant eye drops	23 (32.9)	8 (20.5)	15 (48.4)	0.014
Antihistamine eye drops	22 (31.4)	13 (33.3)	9 (29.0)	0.700
Anti-inflammatory ointment for the external eyelids	5 (7.1)	2 (5.1)	3 (9.7)	0.649

TABLE 1. (Continued)

	Total cohort (n=70)	Severity of OSD		P-value
		No or mild OSD (n=39)	Moderate-to-severe OSD (n=31)	
Anti-inflammatory therapy (eye drops or eye ointment)	14 (20.0)	4 (10.3)	10 (32.3)	0.022
Other	15 (21.4)	6 (15.4)	9 (29.0)	0.167
Wearing contact lenses, n (%)	6 (8.6)	4 (10.3)	2 (6.5)	0.687
Current use of ophthalmic medication, n (%)				
Lubricant eye drops	3 (4.3)	0 (0.0)	3 (9.7)	0.082
Antihistamine eye drops	6 (8.6)	4 (10.3)	2 (6.5)	0.687
Anti-inflammatory ointment for the external eyelids	2 (2.9)	1 (2.6)	1 (3.2)	1.000
Anti-inflammatory therapy (eye drops or eye ointment)	2 (2.9)	1 (2.6)	1 (3.2)	1.000
Medical history of any eye disease, n (%)	22 (31.4)	9 (23.1)	13 (41.9)	0.091
Medical history of allergic eye disease ‡, n (%)	3 (4.3)	1 (2.6)	2 (6.5)	0.580
Medical history of non-allergic eye disease §, n (%)	12 (17.1)	5 (12.8)	7 (22.6)	0.282
Medical history of other eye disease, n (%)	8 (11.4)	3 (7.7)	5 (16.1)	0.452
Presence of symptoms of OSD, n (%)	40 (57.1)	17 (43.6)	23 (74.2)	0.010
Redness	20 (28.6)	5 (12.8)	15 (48.4)	0.001
Pruritus	35 (50.0)	14 (35.9)	21 (67.7)	0.008
Watery eyes	20 (28.6)	10 (25.6)	10 (32.3)	0.542
Burning sense	12 (17.1)	4 (10.3)	8 (25.8)	0.086
Pain	6 (8.6)	1 (2.6)	5 (16.1)	0.081
Photophobia	6 (8.6)	3 (7.7)	3 (9.7)	1.000
Presence of clinical characteristics of OSD, n (%)				
Blepharitis	50 (71.4)	19 (48.7)	31 (100.0)	<0.001
Meibomian gland dysfunction	45 (64.3)	15 (38.5)	30 (96.8)	<0.001
Tarsal conjunctivitis	57 (81.4)	26 (66.7)	31 (100.0)	<0.001
Bulbar conjunctivitis	38 (54.3)	11 (28.2)	27 (87.1)	<0.001
Limbitis	4 (5.7)	0 (0.0)	4 (12.9)	0.034
Limbal vascularization	42 (60.0)	13 (33.3)	29 (93.5)	<0.001
Punctate corneal lesions	20 (29.0)	6 (15.4)	14 (46.7)	0.005
Hurricane fluorescein staining	0 (0.0)	0 (0.0)	0 (0.0)	n/a

Severity of OSD is based on eye with the highest severity within a patient. P-values were calculated with Chi-square test.

† indicates p-values were calculated with Mann-Whitney U tests.

‡ Atopic keratoconjunctivitis; vernal keratoconjunctivitis; giant papillary conjunctivitis

§ Keratoconus; pellucid marginal degeneration; keratitis; uveitis; herpetic keratitis; blepharitis; glaucoma; cataract; macular edema; amblyopia; Meibomian gland dysfunction; retinal detachment.

Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IGA scale, Investigator's Global Assessment Scale; IQR, interquartile range; OD, oculus dexter (right eye); OS, oculus sinister (left eye); OSD, Ocular surface disease; SD, standard deviation; TARC, thymus and activation-regulated chemokine.

A lower conjunctival GCD median was found in patients with OSD compared to patients without OSD (Figure 1a). Flow cytometry analyses of CIC showed a trend of higher median fluorescence intensity (MFI) of MUC5AC within MUC5AC+ GCs in patients with more severe OSD (Figure 1b). This indicates that patients with more severe OSD had more MUC5AC production by GCs.

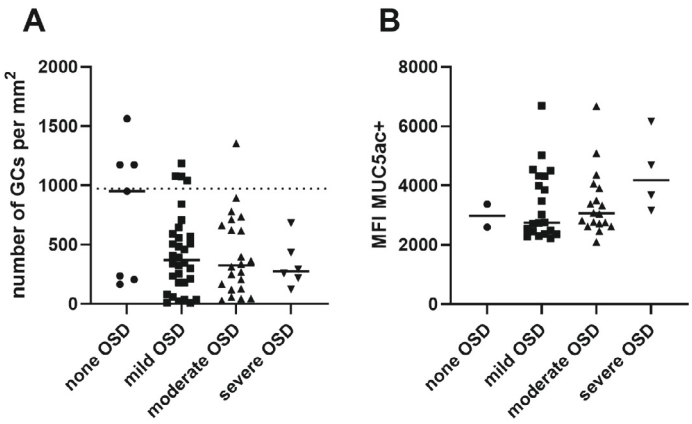

FIGURE 1. Results of conjunctival impression cytology of moderate-to-severe atopic dermatitis patients.

Groups are based on the UTOPIA categories.[3] The bold lines display the median. **A.** Number of goblet cells (GCs) per mm^2 in 67 patients. The dotted line displays the mean GC density from normal covered conjunctiva sites (973 cells/mm^2), based on Doughty et al.[5] The median GC density of patients without ocular surface disease (OSD) was 950 cells/mm^2 (IQR 205-1174). The median GC density of patients with mild, moderate, and severe OSD were 370 cells/mm^2 (IQR 182-580), 325 cells/mm^2 (IQR 129-664), and 276 cells/mm^2 (IQR 219-434), respectively. **B.** Flow cytometry data displaying the median fluorescence intensity (MFI) of MUC5AC within MUC5AC+ GCs in 45 patients.

This prospective study demonstrates that OSD is very frequent in adult patients with moderate-to-severe AD (90%), and is associated with lower conjunctival GCD compared to GCD of healthy controls reported in literature.[5] Moderate-to-severe OSD was found in 44.3% of the AD patients and was associated with more severe AD.

Our results show higher rates of OSD in AD patients (90%) than previous studies, reporting an incidence of 32.4-55.8% of OSD in severe AD patients.[1] All of our patients were examined by an ophthalmologist following a standardized protocol, which is more reliable than patient-reported diagnosis and explain the higher rates of OSD. In our study, 25% of the patients with moderate-to-severe OSD did not report OSD symptoms. Bortoluzzi et al.[6] reported low Ocular Surface Disease Index, which focusses partly on symptoms of OSD, in patients with severe ocular surface involvement. These findings are comparable to our results and explain the underreporting of ocular

comorbidity in AD based on patient reported diagnosis. Furthermore, significantly more of our included patients with moderate-to-severe OSD reported presence of eyelid and facial eczema in the past year compared to patients with no or mild OSD. Dogru et al.[2] reported also that OSD in AD patients was associated with facial and eyelid eczema and that patients with facial atopy had higher grades of conjunctival squamous metaplasia. Additionally, patients from our study with moderate-to-severe OSD had more severe AD based on EASI and serum Thymus and Activation-Regulated Chemokine (TARC) levels, shown in Table 1, suggesting that more severe AD is associated with moderate-to-severe OSD. Abovementioned points underline the importance of ophthalmological examination in patients with moderate-to-severe AD, especially in patients with presence of eyelid eczema or severe AD including the face, in which low-threshold referral to an ophthalmologist is recommended. Diagnosing OSD is important since it may be associated with chronic limbitis, possibly leading to irreversible limbal stem cell deficiency and subsequently to irreversible long-term visual loss.[3]

In our study, lower conjunctival GCD was found in patients with OSD, compared to patients without OSD. Since there were only seven patients without OSD, having a large variation in GCD, no significant differences in GCD could be found between these groups. However, median GCD of patients with OSD was much lower compared to mean GCD from normal covered conjunctiva sites (973 cells/mm^2) described in literature, assuming that lower GCD is associated with OSD.[5] In contrast, GC hyperplasia and mucin hypersecretion are reported in allergic conjunctivitis.[7] In our cohort, OSD was accompanied by low conjunctival GCD, which makes it different from (episodic) allergic conjunctivitis.

In addition to the lower GCD in patients with OSD, higher MFI of MUC5AC was found in patients with more severe OSD. MUC5AC is the major GC secretory mucin and protects and lubricates the ocular surface.[2] Dogru et al.[2] investigated OSD in atopic patients and suggested that the increased expression of MUC5AC might be a defence response of the ocular surface to compensate for the ailing ocular surface condition, with eventually decreased expression of MUC5AC as a result of progression of atopic OSD. This higher expression of MUC5AC protein as a defence response might explain the higher expression of MUC5AC protein found in our patients with more severe OSD.

The development of ocular side effects during dupilumab treatment in AD patients emphasises the importance of gaining more insight into the ocular comorbidities in patients with moderate-to-severe AD.[1] This current study shows that 90% of the moderate-to-severe AD patients have OSD before the initiation of dupilumab, at

least in our center, which leads to the question of what effect dupilumab will have on pre-existing OSD. Previously, we have shown scarcity of conjunctival GCD with increased local Th1 related cytokine production in a case-series of patients with DAOSD.[8] Inhibition of Interleukin (IL)-4/IL-13 signaling by dupilumab, combined with increased local Th1 related cytokine production may be the basis for the loss of GCs and their important immunomodulary function in the conjunctiva. Additionally, a small observational study by Barnett et al.[9] reported a relative deficiency of MUC5AC in tear levels in AD patients with DAOSD. The abovementioned hypothesis and conclusions will be studied in future research, in which we will evaluate the included patients of this current study longitudinally during dupilumab treatment.

This study has some limitations. First, severity of only one eye was included, which might have led to loss of information. However, a preliminary analysis showed a very strong association (Spearman correlation) of 0.953 between severity of both eyes, assuming that this has not influenced our results. Second, due to the small number of patients without OSD having a large variation in GCD, no significant differences were found in GCD between patients with- and without OSD. However, by comparing GCD of patients with OSD to GCD of healthy controls described in the literature, we can conclude that moderate-to-severe AD patients with OSD have lower GC counts. Third, since this is an explorative study in which two small subgroups were compared, we did not correct for multiple testing. Larger cohorts or other comparable studies are needed to support our results.

In conclusion, this prospective, single-center cohort study shows that OSD is a common finding in adult patients with moderate-to-severe AD and is associated with low conjunctival GCD, more severe AD, and the presence of facial AD and/or eyelid eczema. As many patients with OSD did not report OSD symptoms, low-threshold referral to an ophthalmologist is recommended in patients with the mentioned risk factors. The results of this study provide an important basis for unravelling the pathomechanism of ocular side effects associated with IL-4/IL-13 blocking treatment in future studies.

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SUPPLEMENTARY

Methods

Processing of conjunctival impression cytology (CIC) samples – Periodic Acid-Schiff staining

The CIC sample obtained of the left eye was stained with Periodic Acid-Schiff (PAS) and haematoxylin following the Eyeprim protocol.[4] The sample was subsequently placed in the periodic acid solution for 15 minutes, in Schiff's reagent for 30 minutes, and in the Haematoxylin solution for 1 minute. During this procedure, the sample was washed several times with distilled water. Afterwards, the sample was air-dried and placed on a microscope slide with a drop of VectaMount mounting medium. The sample was sealed using the coverslip sealant CoverGrip.

Within 24 hours of the staining of the CIC, the sample was photographed with an Olympus BX41 light microscope attached to a microphotography system (Olympus DP25). All photographs were taken using the CellSense Entry software for Windows (Version 1.3, 2010). Two overview pictures were taken with a 10x and 20x objective (100x and 200x magnification, respectively). Based on the study of Doughty et al.[10], which recommended taking photographs of five to seven fields, six non-overlapping areas were photographed using a 40x objective (400x magnification). A standardized protocol to select these locations was used. The goblet cells (GCs) in these six images were counted by two independent researchers using ImageJ for Windows (ImageJ 1.47v; Java 1.6.0_20). The average GC number per sample was calculated. Afterwards, this number was converted into GC per millimetre squared (mm^2).

Processing of CIC samples – Flow cytometry

Flow cytometry analyses were performed in a representative subgroup of the included patients. CIC samples obtained of the right eye were collected in PBS (Sigma) containing 0.05% (w/v) paraformaldehyde (Alfa Aesar) and stored at 4°C for flow cytometry analysis. Within 3 weeks of sample collection, cells were extracted by gentle manual agitation using a 0.70µM easystrainer (Greiner Bio-One) for 2 minutes. To include only living cells in further analysis, cells were initially stained with Fixable Viability Dye eF506 (Invitrogen) in PBS for 25 min at 4°C. For intracellular staining, cells were fixed and permeabilized using eBioscience Fixation and Permeabilization buffers (Invitrogen) for 30 minutes and stained with Cytokeratin-19 AF647 (BD Biosciences) and MUC5AC AF594 (Santa Cruz Biotechnology) containing 10% FcR blocking reagent (Miltenyi Biotec) for another 30 minutes at 4°C. Data acquisition was performed on a FACS Fortessa flow cytometer (BD Biosciences) and data was analyzed using FlowJo Software (Tree Star Inc.)

TABLE S1. Patient, dermatologic, and ophthalmologic characteristics per subgroup

	Total cohort (n=70)	Cohort GC density (n=67)	Cohort MFI MUC5AC (n=45)
Age (years), median (IQR)	38.5 (27.0-53.3)	39.0 (27.0-54.0)	38.0 (27.0-53.0)
Men, n (%)	35 (50.0)	34 (50.7)	24 (53.3)
Age of onset of AD, n(%)			
Childhood	62 (88.6)	59 (88.1)	40 (88.9)
Adolescence	5 (7.1)	5 (7.5)	4 (8.9)
Adult	3 (4.3)	3 (4.5)	1 (2.2)
History of self-reported episodic acute allergic conjunctivitis, n (%)	55 (78.6)	52 (77.6)	34 (75.6)
Allergic asthma, n (%)	35 (50.0)	34 (50.7)	24 (53.3)
Allergic rhinitis, n (%)	51 (72.9)	49 (73.1)	33 (73.3)
Food allergy, n (%)	34 (48.6)	33 (49.3)	25 (55.6)
History of rosacea, n (%)	2 (2.9)	2 (3.0)	2 (4.4)
EASI score, median (IQR)	15.0 (10.8 – 20.9)	14.9 (10.8-20.1)	14.0 (10.7-18.4)
IGA score, median (IQR)	3 (3-3)	3 (3-3)	3 (2.5-3)
AD eyelid involvement in the past year, n (%)	46 (65.7)	43 (64.2)	34 (75.6)
AD facial involvement in the past year, n (%)	64 (91.4)	61 (91.0)	44 (97.8)
TARC (pg./ml), median (IQR)	1564 (811-2716)	1566 (812-2661)	1432 (789-2135)
Missing, n (%)	1 (1.4)	1 (1.5)	1 (2.2)
Peripheral blood eosinophils ($\times 10^9$/L), median (IQR)	0.29 (0.16-0.51)	0.29 (0.15-0.50)	0.29 (0.16-0.49)
Eosinophilia ($\geq 0.45 \times 10^9$/L), n (%)	21 (30.4)	19 (28.8)	12 (27.3)
Missing, n (%)	1 (1.4)	1 (1.5)	1 (2.2)
Severity of OSD, n(%)			
No OSD	7 (10.0)	7 (10.4)	2 (4.4)
Mild OSD	32 (45.7)	32 (47.8)	21 (46.7)
Moderate OSD	24 (34.3)	22 (32.8)	18 (40.0)
Severe OSD	7 (10.0)	6 (9.0)	4 (8.9)
Visited an ophthalmologist before, n (%)	36 (51.4)	34 (50.7)	25 (55.6)
Previous use of ophthalmic medication, n (%)	45 (64.3)	44 (65.7)	30 (66.7)
Lubricant eye drops	23 (32.9)	22 (32.8)	16 (35.6)
Antihistamine eye drops	22 (31.4)	21 (31.3)	16 (35.6)
Anti-inflammatory ointment for the external eyelids	5 (7.1)	5 (7.5)	4 (8.9)
Anti-inflammatory therapy (eye drops or eye ointment)	14 (20.0)	13 (19.4)	8 (17.8)
Other	15 (21.4)	15 (22.4)	12 (26.7)
Medical history of any eye disease, n (%)	22 (31.4)	20 (29.9)	15 (33.3)
Medical history of allergic eye disease ‡, n(%)	3 (4.3)	3 (4.5)	2 (4.4)
Medical history of non-allergic eye disease §, n(%)	12 (17.1)	12 (17.9)	9 (20.0)
Medical history of other eye disease, n(%)	8 (11.4)	6 (9.0)	5 (11.1)

TABLE S1. (Continued)

	Total cohort (n=70)	Cohort GC density (n=67)	Cohort MFI MUC5AC (n=45)
Wearing contact lenses, n (%)	6 (8.6)	6 (9.0)	6 (13.3)
Current use of ophthalmic medication, n (%)			
Lubricant eye drops	3 (4.3)	2 (3.0)	2 (4.4)
Antihistamine eye drops	6 (8.6)	5 (7.5)	4 (8.9)
Anti-inflammatory ointment for the external eyelids	2 (2.9)	2 (3.0)	2 (4.4)
Anti-inflammatory therapy (eye drops or eye ointment)	2 (2.9)	2 (3.0)	2 (4.4)
Presence of symptoms of OSD, n (%)	40 (57.1)	37 (55.2)	27 (60.0)
Redness	20 (28.6)	19 (28.4)	12 (26.7)
Pruritus	35 (50.0)	32 (47.8)	23 (51.1)
Watery eyes	20 (28.6)	20 (29.9)	15 (33.3)
Burning sense	12 (17.1)	12 (17.9)	9 (20.0)
Pain	6 (8.6)	5 (7.5)	4 (8.9)
Photophobia	6 (8.6)	4 (6.0)	4 (8.9)
Presence of clinical characteristics of OSD, n(%)			
Blepharitis	50 (71.4)	47 (70.1)	36 (80.0)
Meibomian gland dysfunction	45 (64.3)	42 (62.7)	30 (66.7)
Tarsal conjunctivitis	57 (81.4)	54 (80.6)	39 (86.7)
Bulbar conjunctivitis	38 (54.3)	35 (52.2)	25 (55.6)
Limbitis	4 (5.7)	4 (6.0)	3 (6.7)
Limbal vascularization	42 (60.0)	40 (59.7)	33 (73.3)
Punctate corneal lesions	20 (29.0)	18 (26.9)	12 (26.7)
Hurricane fluorescein staining	0 (0.0)	0 (0.0)	0 (0.0)

‡ Atopic keratoconjunctivitis; vernal keratoconjunctivitis; giant papillary conjunctivitis
§ Keratoconus; pellucid marginal degeneration; keratitis; uveitis; herpetic keratitis; blepharitis; glaucoma; cataract; macular edema; amblyopia; Meibomian gland dysfunction; retinal ablation.
Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; GC, goblet cell; IGA scale, Investigator's Global Assessment Scale; IQR, interquartile range; MFI, Median Fluorescence Intensity; MUC5AC, Mucin 5AC; OD, oculus dexter (right eye); OS, oculus sinister (left eye); OSD, Ocular surface disease; SD, standard deviation; TARC, thymus and activation-regulated chemokine.

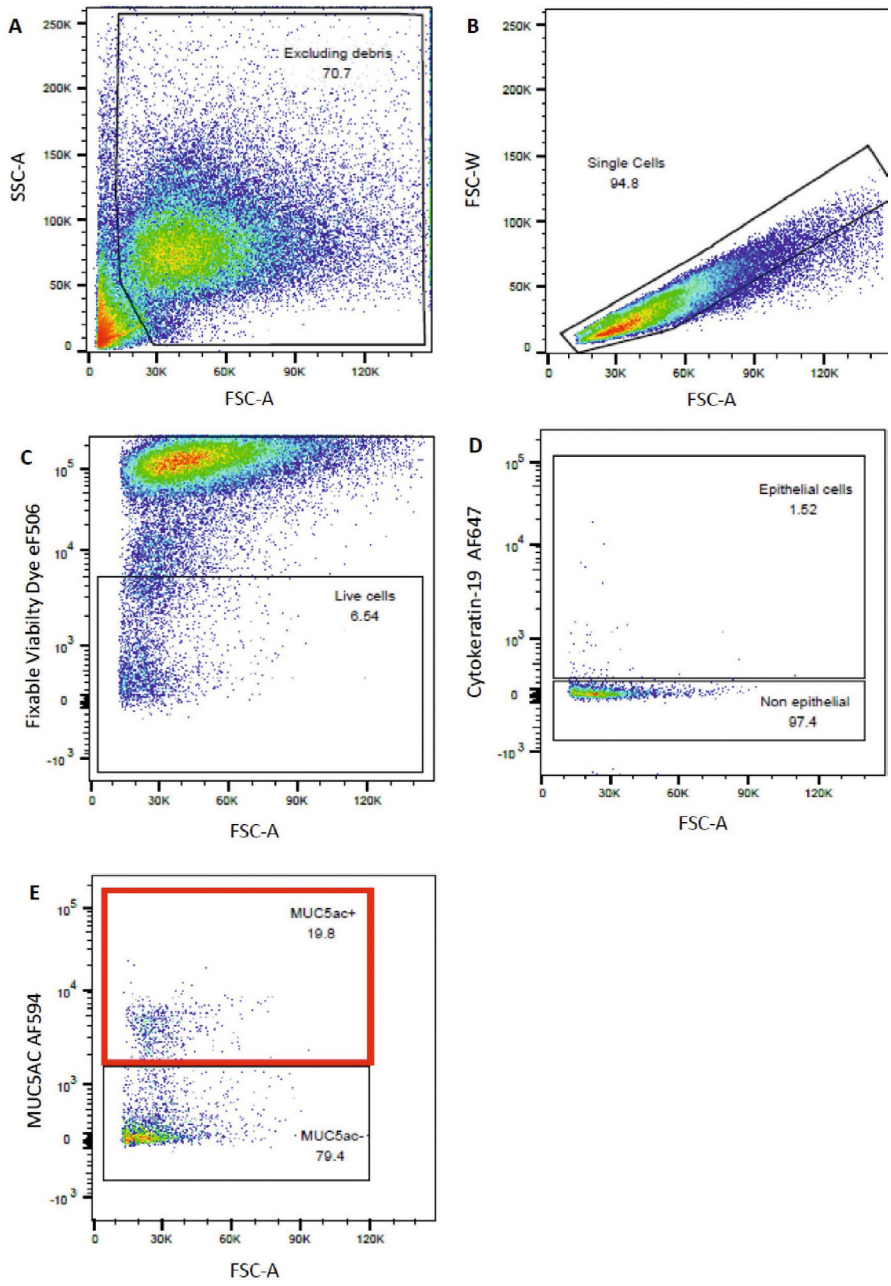

FIGURE S1. The different steps to gate Mucin 5AC+ (MUC5AC) cells are depicted.
Debris was excluded (A), single cells were gated (B), auto fluorescence and dead cells were excluded (C), exclusion of epithelial cells (cytokeratin-19+) (D) and finally MUC5AC+ cells were gated (E). Median Fluorescence Intensity was determined within MUC5AC+ population.

PART III

Ocular surface disease as adverse event in moderate-to-severe atopic dermatitis patients treated with dupilumab

CHAPTER 3

Identification of risk factors for dupilumab-associated ocular surface disease in patients with atopic dermatitis

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ABSTRACT

This study identified risk factors for the development of dupilumab-associated ocular surface disease (DAOSD) in moderate-to-severe atopic dermatitis (AD) patients in a large prospective daily practice cohort. Data from the Dutch BioDay Registry were used to assess the risk of developing DAOSD by performing univariate and multivariate logistic regression analyses. A total of 469 patients were included, of which 152/469 (32.4%) developed DAOSD. Multivariate analysis showed a statistically significant association of the development of DAOSD with a history of any eye disease (history of self-reported episodic acute allergic conjunctivitis excluded) combined with the use of ophthalmic medication at the start of dupilumab (odds ratio 5.16, 95% confidence interval 2.30-11.56, $p<0.001$). In conclusion, a history of any eye disease (history of self-reported episodic acute allergic conjunctivitis excluded) combined with the use of ophthalmic medication at baseline was associated with the development of DAOSD in AD patients.

SIGNIFICANCE

Dupilumab-associated ocular surface disease (DAOSD) is the most frequently reported side effect during dupilumab treatment in atopic dermatitis patients. Although risk factors have been studied, data of large prospective daily practice studies are lacking. In this study, we found a significant association between the development of DAOSD and a history of any eye disease (history of self-reported episodic acute allergic conjunctivitis excluded) combined with the use of ophthalmic medication at the start of dupilumab treatment. Information about previous eye diseases and current ophthalmic medication can be helpful to assess the risk of the development of DAOSD.

INTRODUCTION

Dupilumab, a monoclonal antibody directed against the interleukin (IL)-4-receptor alpha subunit inhibiting both IL-4 and IL-13 signalling, is the first biologic agent for the treatment of patients with moderate-to-severe atopic dermatitis (AD). In multiple clinical trials and daily practice studies, dupilumab has proven to be effective with limited side effects.[1-6] Dupilumab significantly improves signs and symptoms of AD and increases the quality of life in difficult-to-treat AD patients. Nevertheless, dupilumab-associated ocular surface disease (DAOSD), including conjunctivitis, eye pruritus, limbal nodules, and blepharoconjunctivitis developing during dupilumab treatment[7,8], is the most frequently reported side effect in dupilumab-treated AD patients, and is reported in 9-34% in both clinical trials and daily practice studies.[5,9,10] Remarkably, increased rates of DAOSD were not reported in dupilumab trials in other type-2 inflammatory diseases, like asthma and chronic rhino sinusitis with nasal polyposis, suggesting that AD patients may have a predisposition to develop DAOSD.[9]

Currently, the pathomechanism of the development of DAOSD in AD patients remains unclear. DAOSD and associated risk factors have only been studied in small cohorts and data from clinical trials.[5,9,11-14] These studies suggested that the increased rates of DAOSD in AD patients are associated with more severe AD at baseline, prior history of conjunctivitis, presence of other atopic comorbidities, eyelid involvement in AD, high levels of thymus and activation-regulated chemokine (TARC), high levels of serum IgE, and peripheral blood eosinophilia. However, prospective studies on risk factors for the development of DAOSD in AD patients in daily practice are scarce.

Therefore, we conducted a prospective cohort study to identify risk factors for the development of DAOSD in a large group of AD patients in daily practice.

MATERIALS AND METHODS

Study design and patients

This prospective multi-centre observational cohort study included patients with moderate-to-severe AD treated with dupilumab between October 2017 and February 2020 and registered in the Dutch prospective BioDay registry.[5,10] Patients were included from 8 different hospitals, including 3 academic hospitals. Included patients were aged ≥18 years and had a follow-up period for at least 16 weeks. This time point was chosen since most patients develop DAOSD within the first 16 weeks of dupilumab treatment.[7,9,15] Patients who discontinued dupilumab within the first 16 weeks, but continued their follow-up for at least 16 weeks, were still included in the analyses.

At baseline, all patients received a 600-mg loading dose of dupilumab, followed by 300-mg injections every other week. Physicians diagnosed and reported the development of DAOSD by asking patients if they experienced symptoms of DAOSD like tearing, pruritus, or pain. In cooperation with the ophthalmologist, protocols were developed regarding the diagnosis and treatment of the DAOSD. There was no ophthalmological examination prior to the initiation of dupilumab. Only patients with difficult-to-treat DAOSD that could not be controlled with lubricant eye drops and/or tacrolimus skin ointment (1mg/g) for the external eyelids, which was started after the onset of DAOSD, were referred to an ophthalmologist. The Medical Research Ethics Committee confirmed that this study did not fall under the scope of the Medical Research Involving Human Subjects act (METC 18/239). The study was performed according to the Declaration of Helsinki Principles. All patients provided written informed consent.

Data collection

All data were extracted from the BioDay registry. Data collected at baseline included demographic information, medical history, physical examination, laboratory screening, and drug use (including previous and current ophthalmic drug use). Clinicians reported the medical history of any eye disease by asking patients about their general medical history and checking the electronical patient file. The medical history of any eye disease was divided into a medical history of allergic eye disease, and a medical history of non-allergic eye disease. A medical history of atopic keratoconjunctivitis, vernal keratoconjunctivitis, or giant papillary conjunctivitis was classified as a medical history of allergic eye disease. The medical history of non-allergic eye disease included keratoconus, pellucid marginal degeneration, keratitis, uveitis, herpetic keratitis, blepharitis, glaucoma, cataract, macula oedema, amblyopia, meibomitis, and retinal ablation. A history of self-reported episodic acute allergic conjunctivitis was reported separately, as this is usually not confirmed by an ophthalmologist. The severity of AD was assessed by the Eczema Area and Severity Index (EASI) and the Investigator's Global Assessment (IGA). According to the Harmonising Outcome Measures for Eczema (HOME), EASI scores were classified as mild (0-5.9), moderate (6.0-22.9) and severe AD (23.0-72.0).[16] Patients being treated with oral immunosuppressive drugs at the time of initiation of dupilumab treatment were also classified as severe AD. EASI and laboratory results were collected at baseline, after 4 weeks, and after 16 weeks of treatment.

Statistical analyses

Patient characteristics were described using absolute numbers (N) and percentages for categorical variables, median with interquartile ranges (IQR) for non-normally distributed continuous variables, and mean with standard deviations (SD) for normally distributed continuous variables.

Univariate and multivariate logistic regression analyses were used to assess the contribution of potential risk factors to the development of DAOSD. Potential risk factors were selected based on (1) knowledge from previous literature, and (2) clinical experience. Risk factors based on knowledge from previous literature included severity of AD, eosinophils levels at baseline, presence of eyelid eczema, presence of other atopic conditions, and prior history of conjunctivitis.[5,9,11-14] Since Thyssen[17] hypothesized that the development of DAOSD is related to the Demodex mites, which are elevated in rosacea, a history of rosacea was included as a variable. Another study by Thyssen et al.[18] concluded that the number of prescriptions of ophthalmic medication per person was higher in AD patients compared to control subjects. Additionally, Simonetti et al.[19] suggested that the use of moisturizing eye drops could be considered as a preventive resource for the development of DAOSD. For these reasons, the use of ophthalmic medication at baseline was included as a variable in our analyses.

Univariate analysis was performed on the variables as registered. In the multivariate analysis, some variables were combined, whilst others were either categorized or excluded. History of any eye disease was combined with the use of ophthalmic medication at baseline. AD severity categories based on EASI scores were included in the multivariate analyses, as these are widely used to define AD severity and have validated severity-ranges.[16] Additionally, AD eyelid involvement in the past year was excluded from the multivariate analyses due to a high number of missing values. No selection of risk factors was performed based on p-values from the univariate analyses.

The assumption of a linear association of continuous risk factors and the outcome was assessed with restricted cubic spline.[20] Results were reported as Odds Ratio's (OR) with 95% confidence intervals (95% CI) and p-values. P-values were adjusted for multiple testing using the Benjamini-Hochberg procedure[21], which controls False Discovery Rate (FDR). FDR adjusted p-values <0.05 were considered statistically significant.

To investigate the possible association between clinical effectiveness of dupilumab and the occurrence of DAOSD, changes in EASI scores were calculated between baseline and after 4 weeks of dupilumab treatment, and between baseline and after 16 weeks of dupilumab treatment (Table 2). In addition, association between dupilumab-induced blood eosinophilia and the occurrence of DAOSD was studied. Comparison of the DAOSD group with the non-DAOSD group was conducted using the non-parametric Mann-Whitney-U test. Changes in EASI scores and blood eosinophilia were not included in multivariate analyses since these values reflect effectiveness of the treatment of dupilumab, and this study focussed on factors that may be assessed prior to start of the dupilumab.

Statistical analyses were conducted with SPSS Statistics version 25.0.0.2 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows) and the rms library package in R (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient and baseline characteristics

A total of 469 dupilumab-treated AD patients were included at baseline with a median EASI score of 16.8 (IQR 10.9-26.1) and a median IGA score of 3 (IQR 3-4), shown in Table 1. A medical history of any eye disease (excluding a history of self-reported episodic acute allergic conjunctivitis) was reported in 68/469 (14.5%) patients, and 81/469 (17.3%) patients were using ophthalmic drugs at the initiation of dupilumab treatment. A history of self-reported episodic acute allergic conjunctivitis was found in 274/469 (59.8%) patients. Within the first 16 weeks of dupilumab treatment, 11/469 (2.3%) patients discontinued their treatment. Three of these patients experienced ocular side effects (2 patients with DAOSD and 1 patient with a cornea perforation). The other eight patients stopped with dupilumab due to other reasons. All other baseline characteristics are shown in Table 1.

TABLE 1. Baseline characteristics

	Total cohort (n = 469)	DAOSD diagnosed by physician	
		Yes (n = 152)	No (n=317)
Age at baseline (years), median (IQR)	42.0 (28.0-54.0)	45.5 (29.0-54.0)	41.0 (28.0-54.0)
Age at baseline(square), median (IQR)	1764.0 (784.0-2916.0)	2070.5 (841.0-2916.0)	1681.0 (784.0-2916.0)
Men, n (%)	284 (60.6)	95 (62.5)	189 (59.6)
History of any eye disease, n (%)[a,b]	68 (14.5)	37 (24.3)	31 (9.8)
History of allergic eye disease, n (%)[a]	32 (6.8)	18 (11.8)	14 (4.4)
History of non-allergic eye disease, n (%)[b]	54 (11.5)	30 (19.7)	24 (7.6)
Use of ophthalmic drugs at baseline[c]	81 (17.3)	52 (34.2)	29 (9.1)
No history of any eye disease and no ophthalmic drugs at baseline, n (%) *reference*	401 (85.5)	115 (75.7)	286 (90.2)
History of any eye disease and no ophthalmic drugs at baseline, n (%)	35 (7.5)	14 (9.2)	21 (6.6)
History of any eye disease and ophthalmic drugs at baseline, n (%)	33 (7.0)	23 (15.1)	10 (3.2)
History of self-reported episodic acute allergic conjunctivitis, n (%)			
Yes, n (%)	274 (59.8)	101 (67.8)	173 (56.0)
No, n (%)	184 (40.2)	48 (32.2)	136 (44.0)
Missing, n (%)	11 (2.3)	3 (2.0)	9 (2.8)
Allergic asthma, n (%)			
Yes, n (%)	269 (58.2)	93 (61.6)	176 (56.6)
No, n (%)	193 (41.8)	58 (38.4)	135 (43.4)
Missing, n (%)	7 (1.5)	1 (0.7)	6 (1.9)
Allergic rhinitis, n (%)			
Yes, n (%)	308 (66.7)	107 (71.3)	201 (64.4)
No, n (%)	154 (33.3)	43 (28.7)	111 (35.6)
Missing, n (%)	7 (1.5)	2 (1.3)	5 (1.6)
Food allergy, n (%)			
Yes, n (%)	214 (46.6)	82 (54.7)	132 (42.7)
No, n (%)	245 (53.4)	68 (45.3)	177 (57.3)
Missing, n (%)	10 (2.1)	2 (1.3)	8 (2.5)
History of rosacea, n (%)	5 (1.1)	1 (0.7)	4 (1.3)
EASI score at baseline, median (IQR)	16.8 (10.9-26.1)	19.3 (12.7 - 27.8)	15.2 (10.2 - 24.8)
EASI score at baseline, per severity category			
Mild AD (EASI 0.1 - 5.9) n, (%) *reference*	31 (6.7)	8 (5.3)	23 (7.5)
Moderate AD (EASI 6.0 - 22.9) n, (%)	270 (58.7)	81 (53.3)	189 (61.4)
Severe AD (EASI 23.0 - 72.0) n, (%)	159 (34.6)	63 (41.4)	96 (31.2)

TABLE 1. (Continued)

	Total cohort (n = 469)	DAOSD diagnosed by physician	
		Yes (n = 152)	No (n=317)
Missing, n (%)	9 (1.9)	0 (0)	9 (2.8)
IGA score at baseline, median (IQR)	3 (3-4)	3 (3-4)	3 (3-4)
Missing, n (%)	12 (2.6)	2 (1.3)	10 (3.2)
AD eyelid involvement in the past year, n (%)			
Yes, n (%)	131 (65.5)	47 (83.9)	84 (58.3)
No, n (%)	69 (34.5)	9 (16.1)	60 (41.7)
Missing, n (%)	269 (57.4)	96 (63.2)	173 (54.6)
Eosinophilia (≥0.45×10^9/L) at baseline, n (%)			
Yes, n (%)	168 (37.9)	61 (42.1)	107 (35.9)
No, n (%)	275 (62.1)	84 (57.9)	191 (64.1)
Missing, n (%)	26 (5.5)	7 (4.6)	19 (6.0)

[a] Atopic keratoconjunctivitis; vernal keratoconjunctivitis; giant papillary conjunctivitis
[b] Keratoconus; pellucid marginal degeneration; keratitis; uveitis; herpetic keratitis; blepharitis; glaucoma; cataract; macula oedema; amblyopia; meibomitis; retinal ablation
[c] Ketotifen; cromoglicic acid; levocabastine; dextran/hypromellose; sodium hyaluronate/carbomer; other lubricant eyedrops; oxytetracycline/hydrocortisone; dexamethasone; fluormetholone liquifilm; cyclosporine; tobramycin/dexamethasone; prednisolone; hydrocortisone; tacrolimus eye ointment; pimecrolimus skin ointment; chloramphenicol; prostaglandin f-analogue; beta-blocker eyedrops; other ophthalmic drugs
Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; DAOSD, dupilumab associated ocular surface disease; IGA scale, Investigator's Global Assessment Scale; IQR, interquartile range; SD, standard deviation.

Development of DAOSD and univariate logistic regression odds ratios

Of all included patients, 152/469 (32.4%) developed DAOSD, shown in Table 1. Of these patients, 93/152 (61.2%) patients were referred to an ophthalmologist. Of all patients who developed DAOSD, 37/152 (24.3%) had a history of any eye disease, which was significantly higher compared to the patients that did not develop DAOSD (n=31/317 (9.8%), OR 2.97, 95% CI 1.76-5.01, p<0.001, Figure 1, see Table S1 in the Supplementary). The number of patients who were using ophthalmic medication at baseline was significantly higher in the DAOSD group compared to the non-DAOSD group (OR 5.16, 95% CI 3.11-8.58, p<0.001). Moreover, the number of patients having a history of any eye disease combined with the use of ophthalmic medication at baseline was significantly higher in the DAOSD group compared to the non-DAOSD group (OR of 5.72 (95% CI 2.64-12.40, p<0.001).

Furthermore, a history of self-reported episodic acute allergic conjunctivitis (OR 1.65, 95% CI 1.10-2.49, p=0.039) and food allergy (OR 1.62, 95% CI 1.09-2.39, p=0.039) were found to be significantly associated with the development of DAOSD. Of the patients that developed DAOSD, more had AD on their eyelids in the past year, leading to a statistically significant association between eyelid involvement in AD in the past year and the development of DAOSD (OR 3.73, 95% CI 1.70-8.19, p=0.004). Since this variable contained more than 100 missing values, it was excluded from the multivariate analyses. EASI score at baseline showed no statistically significant association with the development of DAOSD (OR 1.02, 95% CI 1.00-1.03, p=0.079).

Multivariate logistic regression odds ratios

Multivariate analysis showed a statistically significant association between having a history of any eye disease combined with the use of ophthalmic medication at baseline and the development of DAOSD (OR 5.16, 95% CI 2.30-11.56, p<0.001, Figure 1). No significant association was found between a history of self-reported episodic acute allergic conjunctivitis and the development of DAOSD in multivariate analysis (OR 1.33, 95% CI 0.77-2.29, p=0.438).

With the exception of age, continuous risk factors showed a linear association with DAOSD development. Analysis with restricted cubic splines showed an association similar to a quadratic effect. For ease of interpretation, age and age-square were included in univariate and multivariate analysis.

Effectiveness of dupilumab and the development of DAOSD

After 4 weeks of treatment with dupilumab, the decrease in EASI score from baseline was significantly higher in the DAOSD group, compared to the non-DAOSD group (p=0.016, Table 2). The significant difference in decrease continued during 16 weeks of treatment (p=0.007).

Changes in peripheral blood eosinophils during dupilumab treatment

A significantly higher increase of peripheral blood eosinophils was found in the DAOSD group compared to the non-DAOSD group after comparing baseline values with values after 16 weeks of dupilumab treatment (0.16 x 10^9/L (IQR -0.05-0.53) and 0.04 x 10^9/L (IQR -0.08-0.26), respectively, p=0.001, Table 2).

TABLE 2. Peripheral blood eosinophils and EASI at baseline compared to 4 and 16 weeks after dupilumab treatment

	Week 4			Week 16		
	DAOSD (n=152)	**No DAOSD (n=317)**	**P-value**	**DAOSD (n=152)**	**No DAOSD (n=317)**	**P-value**
Δ Eosinophils from baseline, x10^9/L, median (IQR)	0.07 (-0.08 - 0.32)	0.03 (-0.07 – 0.21)	0.125	0.16 (-0.05 - 0.53)	0.04 (-0.08 – 0.26)	**0.001**
Missing, n	11	33	-	15	43	-
Δ EASI from baseline, median (IQR)	-10.2 (-16.3 – -5.05)	-7.6 (-13.6 – -3.6)	**0.016**	-14.4 (-21.8 - 7.7)	-10.8 (-17.6 – -6.1)	**0.007**
Missing, n	1	16	-	6	24	-

Data were analysed by using the Mann-Whitney U test.
Abbreviations: EASI, Eczema Area and Severity Index; DAOSD, dupilumab associated ocular surface disease; IQR, interquartile range.

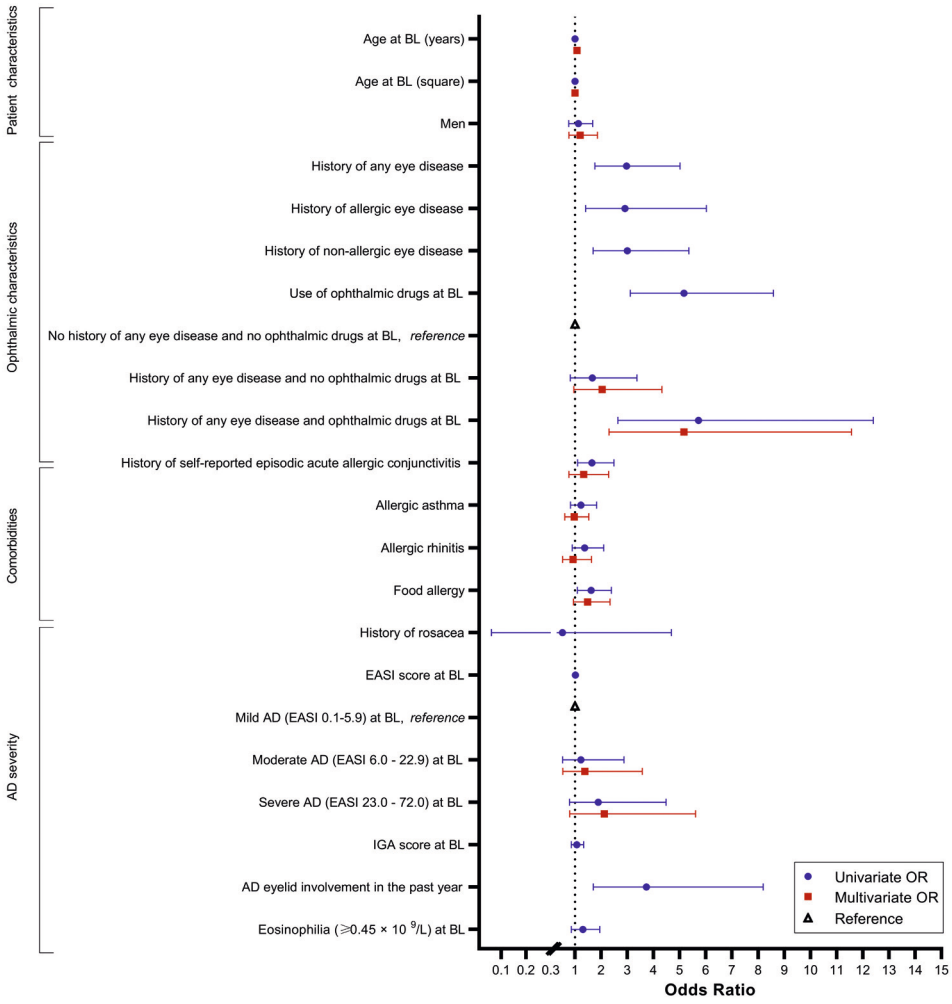

FIGURE 1. Univariate and multivariate odds ratios with 95% confidence interval of risk factors for the development of dupilumab associated ocular surface disease in AD patients.

Abbreviations: AD, atopic dermatitis; BL, baseline; EASI, Eczema Area and Severity Index; IGA scale, Investigator's Global Assessment Scale; OR, odds ratio.

DISCUSSION

In this large prospective cohort study, 32.4% of AD patients developed DAOSD. We found that a history of any eye disease, other than a history of self-reported episodic acute allergic conjunctivitis, combined with the use of ophthalmic medication indicating current disease at baseline, was associated with the development of DAOSD. In addition, the development of DAOSD was associated with higher clinical

effectiveness of dupilumab at 4 and 16 weeks and a stronger increase in blood eosinophils at 16 weeks of treatment compared to the non-DAOSD group.

In both clinical trial populations and data from small daily practice studies, a prior history of allergic conjunctivitis is a frequently reported risk factor for the development of DAOSD.[9,12-14] However, in most studies, the definition of allergic conjunctivitis is very broad. In the current study, we used a more refined classification of allergic conjunctivitis and separated a history of self-reported episodic acute allergic conjunctivitis from a history of more chronic allergic eye disease, such as atopic keratoconjunctivitis and vernal keratoconjunctivitis (mostly confirmed by an ophthalmologist). In our study, only univariate analysis showed a statistically significant association between the development of DAOSD and a history of self-reported episodic acute allergic conjunctivitis. This association was not significant in the multivariate analysis, possibly due to correction for confounding in the multivariate analysis, like other atopic diseases or more severe ophthalmological pathology. Multivariate analyses showed a significant association between the development of DAOSD and a history of any eye disease (history of self-reported episodic acute allergic conjunctivitis excluded) combined with ophthalmic drugs use at baseline. Ongoing ophthalmic treatment for previous eye disease might indicate ongoing ophthalmic pathology, leading to a higher risk of the development of DAOSD. It is unknown as to why patients with allergic and non-allergic eye disease combined with ophthalmic drugs are at risk of DAOSD. However, it is known that preservatives in eye drops cause conjunctival allergy and irritation.[22] Whether this hypersensitivity is related to DAOSD development is unclear since no information is available about the presence of preservatives in eye drops in our series. Additionally, some of the eye diseases classified as non-allergic might indirectly be related to AD or its treatment, like cataract and glaucoma.[23,24]

Another frequently reported associated risk factor with the development of DAOSD in AD patients is AD severity at baseline. Akinlade et al.[9] performed a post-hoc analysis of published clinical trial data on dupilumab treatment in AD regarding the development of DAOSD. The conclusion of this post-hoc analysis was that a higher incidence of DAOSD in AD patients was associated with higher baseline AD severity. However, Akinlade et al.[9] used different and inconsistent banding of the EASI scores, making the comparison of these results with our study difficult. In the current study, EASI scores were classified according to the validated HOME criteria[16], and no relationship was found between the baseline severity of AD and the development of DAOSD. In addition, there is an important difference in patient population between the studies; Akinlade et al.[9] included participants of clinical trials, while our study included

patients in daily practice, representing a more diverse population regarding baseline AD severity. Ariens et al.[5] also found that development of DAOSD was significantly associated with higher EASI scores at baseline. Despite the fact that these patients also participated in the BioDay registry, the results are not comparable with our current study. The selected cohort of Ariens et al.[5] is smaller and the patients were participating in an Early Access program, which allowed the most severe AD patients to start dupilumab ahead of market access. Therefore, these patients had high EASI scores at baseline, comparable with the clinical trial population, while our cohort was more diverse.

Although baseline AD severity was not significantly associated with the development of DAOSD in our study, patients showing a larger decrease in EASI score at 4 weeks and 16 weeks of treatment more often developed DAOSD. This might indicate that higher clinical effectiveness of dupilumab in AD is associated with higher risk of developing DAOSD. We previously investigated the long-term follow-up of DAOSD and found improvement of DAOSD after prolongation of the dosing interval of dupilumab to 300mg every three to five weeks.[15] Since dose reduction improved signs and symptoms of the DAOSD, and EASI reduced significantly more in the group of dupilumab treated patients that developed DAOSD, it might be possible that these patients have higher serum levels of dupilumab, and are thus relatively overdosed.

Another reported potential risk factor to develop DAOSD is the presence of eyelid eczema. Touhouche et al.[14] reported that this was significantly associated with the occurrence of DAOSD, with an OR of 8.7 (95% CI 1.8-40.6). This is in line with findings of our univariate analysis, in which eyelid involvement in AD in the past year was statistically more present in the group of patients that developed DAOSD (OR 3.73; 95% CI 1.70-8.19, P=0.004). Dogru et al.[25] investigated ocular surface disorders in patients with severe AD. They described the presence of eyelid eczema, which was seen in 476/724 eyes (65.7%), as one of the most dominant ocular diseases in severe AD patients. Another study of Dogru et al.[26] investigated conjunctival impression cytology samples in AD patients. They concluded that AD patients with facial eczema showed significantly higher grades of conjunctival squamous metaplasia.

In the current cohort, significantly more patients who developed DAOSD had eyelid eczema in the past year. This might indicate that these patients had (undiagnosed) ocular surface disease, which exacerbated during the use of dupilumab. For this reason, it is important to ask patients about presence of redness, tearing, itch, burning sense, photophobia, and painful eyes before the start of dupilumab. Low-threshold referral to the ophthalmologist is recommended, especially in patients with a history

of any eye disease that are currently using ophthalmic treatment and patients having eyelid eczema in the past year.

To the best of our knowledge, this is the first large prospective daily practice cohort study that investigated risk factors associated with the development of DAOSD in AD patients. Patients were seen by physicians aware of the ophthalmological comorbidity in AD, and standardized procedures were followed, leading to accurate data collection. We included many different variables, and our prospective database provided information about ophthalmic treatment at the start of dupilumab. Our study has some limitations. Firstly, no ophthalmological slit lamp examination was performed before the start of dupilumab. Therefore, only patient-reported information regarding the ophthalmological history was available, and pre-existing ophthalmological pathology, which can only be found during ophthalmic slit-lamp examination, could not be excluded. Secondly, the variable AD eyelid involvement in the past year had many missing values and was therefore not included in the multivariate logistic analyses. Sensitivity analyses evaluated the effect of the inclusion of eyelid involvement in the past year. However, this showed minor differences (see Table S2 in the Supplementary). Thirdly, only the absence or presence of DAOSD was reported during an outpatient visit. As a result, the exact date of onset of DAOSD is missing. However, previous literature reported that most patients developed DAOSD within the first 16 weeks of treatment. [7,9,15] All of our included patients had a follow-up duration of at least 16 weeks, and the absence or presence of DAOSD was reported, making the information about the date of onset of DAOSD less relevant for the primary analyses. Fourthly, both a history of any eye disease and ocular medication are broad definitions. However, the subgroups of individual eye diseases and individual ocular medications were too small to analyse. Therefore, these subgroups were not included in analysis. Lastly, for some variables, like a history of rosacea, no conclusions can be made due to the small number of patients. Larger cohorts or other comparable cohort studies need to be conducted to support our results.

In conclusion, in this large prospective cohort study in which one-third of the AD patients developed DAOSD, we showed that a history of any eye disease, but not a history of self-reported episodic acute allergic conjunctivitis, combined with the use of ophthalmic medication at baseline was associated with the development of DAOSD. In addition, high effectiveness of dupilumab appeared to be associated with development of DAOSD. Future studies are warranted to investigate the association between specific ophthalmological characteristics at baseline and the development of DAOSD and to determine (preventive) treatment strategies.

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SUPPLEMENTARY

TABLE S1. Univariate-and multivariate logistic results

	Univariate logistic analyses results				Multivariate logistic analyses results[d]			
	Odds ratio	**95% CI**	***P*-value**	**Adjusted p-value[e]**	**Odds ratio**	**95% CI**	***P*-value**	**Adjusted p-value[e]**
Age at baseline (years)	1.00	0.99-1.02	0.537	0.614	1.08	1.00- 1.17	0.049	0.166
Age at baseline(square)	1.00	1.00-1.00	0.869	0.869	1.00	1.00 -1.00	0.061	0.166
Men	1.13	0.76-1.68	0.551	0.614	1.20	0.77 -1.86	0.416	0.541
History of any eye disease[a,b]	2.97	1.76-5.01	**0.000**	**0.000**	-	-	-	-
History of allergic eye disease[a]	2.91	1.41-6.02	**0.004**	**0.013**	-	-	-	-
History of non-allergic eye disease[b]	3.00	1.69-5.35	**0.000**	**0.000**	-	-	-	-
Use of ophthalmic drugs at baseline[c]	5.16	3.11-8.58	**0.000**	**0.000**	-	-	-	-
No history of any eye disease and no ophthalmic drugs at baseline, *reference*	-	-	**0.000**	**0.000**	-	-	**0.000**	**0.000**
History of any eye disease and no ophthalmic drugs at baseline	1.66	0.82-3.37	0.163	0.256	2.04	0.96-4.32	0.064	0.166
History of any eye disease and ophthalmic drugs at baseline	5.72	2.64-12.40	**0.000**	**0.000**	5.16	2.30-11.56	**0.000**	**0.000**
History of self-reported episodic acute allergic conjunctivitis	1.65	1.10-2.49	**0.016**	**0.039**	1.33	0.77-2.29	0.303	0.438
Allergic asthma	1.23	0.83-1.83	0.307	0.422	0.97	0.61-1.53	0.879	0.879
Allergic rhinitis	1.37	0.90-2.10	0.141	0.254	0.93	0.53-1.63	0.798	0.865
Food allergy	1.62	1.09-2.39	**0.016**	**0.039**	1.48	0.94-2.34	0.088	0.184
History of rosacea	0.52	0.06-4.68	0.558	0.614	-	-	-	-
EASI score at baseline	1.02	1.00-1.03	**0.036**	0.079	-	-	-	-

TABLE S1. (Continued)

	Univariate logistic analyses results				Multivariate logistic analyses results[d]			
	Odds ratio	**95% CI**	***P*-value**	**Adjusted p-value[e]**	**Odds ratio**	**95% CI**	***P*-value**	**Adjusted p-value[e]**
EASI score at baseline, per severity category			0.085	0.170	-	-	-	-
Mild AD (EASI 0.1 - 5.9), *reference*	-	-	-	-	-	-	0.099	0.184
Moderate AD (EASI 6.0 - 22.9)	1.23	0.53-2.87	0.628	0.658	1.38	0.54-3.57	0.502	0.593
Severe AD (EASI 23.0 - 72.0)	1.89	0.79-4.48	0.150	0.254	2.12	0.80-5.61	0.131	0.213
IGA score at baseline	1.07	0.86-1.33	0.542	0.614	-	-	-	-
AD eyelid involvement in the past year	3.73	1.70-8.19	**0.001**	**0.004**	-	-	-	-
Eosinophilia (≥0.45×10^9/L) at baseline	1.30	0.86-1.95	0.210	0.308	-	-	-	-

Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IGA scale, Investigator's Global Assessment Scale; IQR, interquartile range; SD, standard deviation;

[a] Atopic keratoconjunctivitis; vernal keratoconjunctivitis; giant papillary conjunctivitis

[b] Keratoconus; pellucid marginal degeneration; keratitis; uveitis; herpetic keratitis; blepharitis; glaucoma; cataract; macula oedema; amblyopia; meibomitis; retinal ablation

[c] Ketotifen; cromoglicic acid; levocabastine; dextran/hypromellose; sodium hyaluronate/carbomer; other lubricant eyedrops; oxytetracycline/hydrocortisone; dexamethasone; fluormetholone liquifilm; cyclosporine; tobramycin/dexamethasone; prednisolone; hydrocortisone; tacrolimus eye ointment; pimecrolimus skin ointment; chloramphenicol; prostaglandin f-analogue; beta-blocker eyedrops; other ophthalmic drugs.

[d] In the multivariate analysis, some variables were combined, categorized or excluded. History of any eye disease was combined with the use of ophthalmic medication at baseline. AD severity categories based on EASI scores were included in the multivariate analyses. AD eyelid involvement in the past year was excluded from the multivariate analyses due to a high number of missing values.

[e] P-values were adjusted for multiple testing using the Benjamini-Hochberg procedure[21], which controls False Discovery Rate (FDR). FDR adjusted P values <0.05 were considered statistically significant.

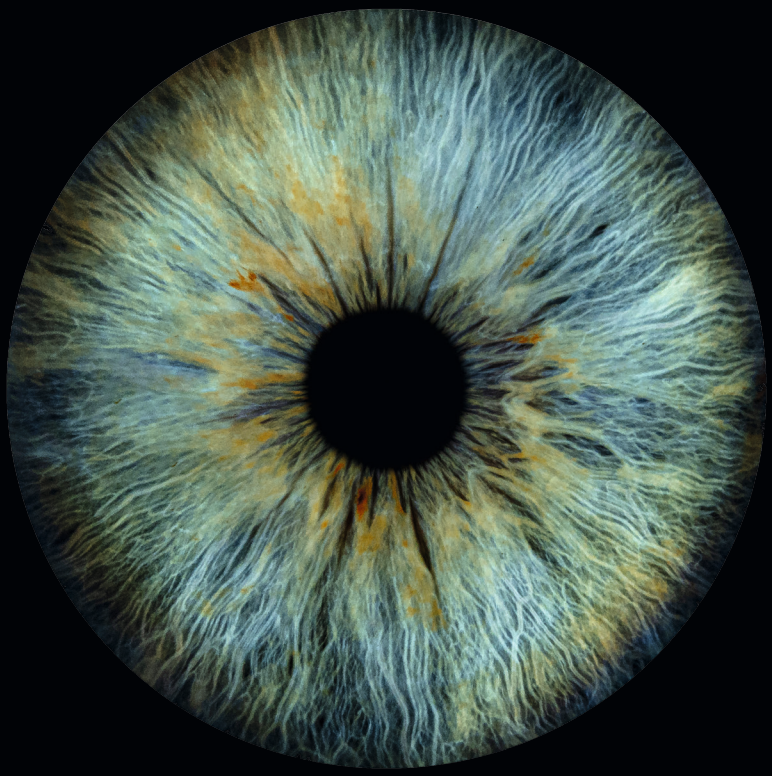
TABLE S2. Sensitivity analyses – Multivariate analyses with and without AD eyelid involvement in the past year

	Multivariate analyses with eyelid involvement (n=193)[a]			Multivariate analyses without eyelid involvement (n=193)[a]		
	Odds ratio	**95% CI**	***P*-value**	**Odds ratio**	**95% CI**	***P*-value**
Age at baseline (years)	1.01	0.89-1.14	0.924	0.99	0.89-1.12	0.921
Age at baseline(square)	1.00	1.00-1.00	0.960	1.00	1.00-1.00	0.950
Men	1.14	0.56-2.32	0.716	1.18	0.59-2.34	0.637
No history of any eye disease and no ophthalmic drugs at baseline, *reference*	-	-	-	-	-	-
History of any eye disease and no ophthalmic drugs at baseline	0.71	0.17-2.97	0.642	0.70	0.17-2.80	0.609
History of any eye disease and ophthalmic drugs at baseline	3.44	0.84-14.03	0.085	3.98	1.00-15-78	**0.050**
Self-reported episodic acute allergic conjunctivitis	1.59	0.64-3.97	0.317	1.86	0.75-4.60	0.178
Allergic asthma	0.72	0.35-1.46	0.361	0.79	0.40-1.58	0.509
Allergic rhinitis	0.78	0.31-1.98	0.595	0.85	0.34-2.13	0.731
Food allergy	1.11	0.55-2.25	0.774	1.07	0.53-2.14	0.854
EASI score at baseline, per severity category[b]	-	-	-	-	-	-
Mild and moderate AD (EASI 0.1 – 22.9) *reference*	-	-	-	-	-	-
Severe AD (EASI 23.0 - 72.0)	0.94	0.45-1.99	0.870	0.859	0.41-1.78	0.684
AD eyelid involvement in the past year	4.00	1.68-9.50	**0.002**	-	-	-

Abbreviations: AD, atopic dermatitis; CI, confidence interval; EASI, Eczema Area and Severity Index

[a] The selected patients for this sensitivity analysis (n=193) were patients in which the presence or absence of AD eyelid involvement in the past year had been reported at baseline. As a result of this smaller sample size, the odds ratio, 95% confidence intervals, and p-values were not comparable with those of the main analyses.

[b] Since there were only few patients with mild AD in which the presence or absence of AD eyelid involvement in the past year was known at baseline, patients with mild AD and moderate AD were combined into one category.



CHAPTER 4

Dupilumab-associated ocular surface disease in atopic dermatitis patients: clinical characteristics, ophthalmic treatment response, and conjunctival goblet cell analysis

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ABSTRACT

Background

Dupilumab-associated ocular surface disease (DAOSD) is frequently reported as side effect in atopic dermatitis (AD) patients. Therefore, the aim of this study was to investigate the frequency and severity of DAOSD, ophthalmic treatment response and to learn more about the effect of dupilumab on conjunctival goblet cells (GC).

Methods

This prospective study included dupilumab-treated AD patients between February 2020 and June 2022 from the University Medical Centre Utrecht. Patients were examined by an ophthalmologist and a dermatologist before start (baseline), and after 4 and 28 weeks of dupilumab treatment. Ophthalmological examination was assessed by the Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score. DAOSD was defined as an increase in UTOPIA score of ≥3 points from baseline. To quantify conjunctival GCs and to investigate the percentage of Cytokeratin 19 (CK19)-CD45-Mucin 5AC (MUC5AC)+ cells, conjunctival impression cytology samples were analysed.

Results

Ocular surface disease (OSD) was present in 91.3% (n=63/69) patients at baseline. DAOSD was observed in 28.9% (n=20/69) patients, in whom GC numbers remained stable and the percentage of CK19-CD45-MUC5AC+ cells decreased at onset of DAOSD compared with baseline. After 28 weeks of dupilumab treatment, DAOSD was seen in 14.5% (n=10/69) patients. Of the 85.5% (n=59/69) patients without DAOSD or with controlled DAOSD at week 28, 40.7% (n=24/59) patients received anti-inflammatory ophthalmic drugs.

Conclusions

OSD is common in moderate-to-severe AD patients before starting dupilumab. During treatment with dupilumab DAOSD severity improves with early ophthalmic treatment. The decrease in percentage of CK19-CD45-MUC5AC+ cells during dupilumab.

INTRODUCTION

Dupilumab, the first biologic treatment for moderate-to-severe atopic dermatitis (AD) patients, is directed against the interleukin (IL)-4 receptor alpha (IL-4Rα), inhibiting the signalling of IL-4 and IL-13.[1] It has proven its effectiveness and safety in both clinical trials and daily practice studies.[1-3] Dupilumab-associated ocular surface disease (DAOSD) is the most frequently reported adverse event in dupilumab-treated AD patients.[2-5] Remarkably, high rates of DAOSD have not been reported in patients treated with dupilumab for other diseases like asthma, suggesting that AD patients may have a predisposition to develop DAOSD.[4] DAOSD in AD patients can be severe and persistent, with potentially irreversible consequences in case of chronic limbal inflammation and may lead to discontinuation of treatment with dupilumab.[5,6] Several treatment options for DAOSD have been described in small case reports and case series, including artificial tears, antihistaminic eye drops, tacrolimus skin ointment and corticosteroid eye drops.[7-9] However, there is no official guideline for the treatment of DAOSD, and knowledge about the effect of ophthalmic treatment is needed.[8,9]

We previously examined conjunctival biopsies of AD patients with DAOSD and found focal scarcity of intraepithelial goblet cells (GCs).[10] However, in a recent study, we found that 90% of the moderate-to-severe AD patients already had signs of ocular surface disease (OSD) accompanied with low conjunctival GCs before the start of dupilumab.[11] In addition, more severe AD and high AD-related severity tear fluid biomarkers were reported in moderate-to-severe AD patients with moderate-to-severe OSD.[11,12] Taken together, this suggests that AD patients indicated for dupilumab have a relative high risk for AD-related OSD prior to the initiation of treatment.

It has been hypothesized that the blocking effect of dupilumab on IL-13 reduces GC proliferation and mucin production, thereby further increasing the risk of ocular surface inflammation.[10] Additionally, Barnett et al. reported a relative deficiency of Mucin 5AC (MUC5AC), which is the main mucin produced by the conjunctival GCs, in tear fluid of dupilumab-treated AD patients with DAOSD.[13] However, the exact patho-mechanism of DAOSD remains unclear.

To better understand DAOSD and its patho-mechanism in AD patients, the aim of this study was to investigate the frequency and severity of OSD in dupilumab-treated AD patients before start and during dupilumab treatment, the effect of ophthalmic treatment on (DA)OSD, and to learn more about the effect of dupilumab on the number of GCs and its function.

MATERIALS AND METHODS

Study design and patients

Between February 2020 and June 2022, adult moderate-to-severe AD patients eligible for treatment with dupilumab were included in this prospective, monocentre, observational cohort study at the University Medical Centre Utrecht in the Netherlands. Included patients were not using systemic immunosuppressive therapies for at least two weeks prior to the start of dupilumab, and dupilumab was dosed according to the label (300 mg every two weeks) for at least 28 weeks. Written informed consent was provided by all patients, and this study was considered as non-interventional by the Medical Research Ethics Committee.

Data collection

Patients were seen by both a dermatologist and an ophthalmologist prior to the start of dupilumab, and after 4 and 28 weeks of treatment with dupilumab. An additional ophthalmic visit was performed if patients reported OSD symptoms and/or if ophthalmic medication was required due to worsening of OSD during dupilumab treatment. The study design is shown in Figure 1.

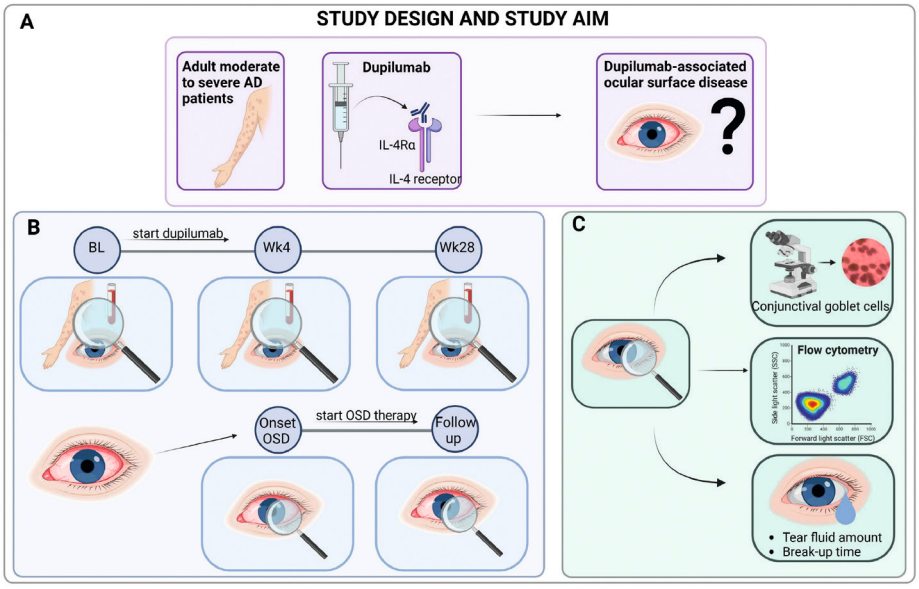

FIGURE 1. Study design of the prospective study.
A. Aim of the study. **B.** Time points of the different visits and the conducted investigations. **C.** Additional analysis to the clinical ophthalmological examination. Abbreviations: atopic dermatitis, AD; BL, baseline; interleukin, IL; ocular surface disease, OSD; week, wk. Figure created in Biorender.

AD severity was assessed by the Eczema Area and Severity Index (EASI) and the Investigator's Global Assessment (IGA). Additionally, data regarding other atopic comorbidities, eyelid eczema or facial eczema in the past year, and laboratory results including peripheral blood eosinophils and thymus and activation-regulated chemokine (TARC) were collected.

The ophthalmological examination was based on the Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score, which assesses the severity of inflammation of the eyelids, conjunctiva (both bulbar and tarsal), and the limbus.[5] The overall severity of each eye was classified as no (UTOPIA score 0), mild (UTOPIA score 1-4), moderate (UTOPIA score 5-8), or severe OSD (UTOPIA score ≥9), following the severity ranges of the UTOPIA score.[5] In case of different severity between the eyes of a single patient, data were analysed based on the classification of the most severe eye. DAOSD was defined as the first visit at which an increase in UTOPIA score of ≥3 points was observed during dupilumab treatment compared to baseline. In addition, patients were asked about the presence or absence of OSD symptoms (red eyes, watery eyes, pruritus, pain, photophobia, and burning sensation), and the past or current use of ophthalmic medication. Tear fluid production from both eyes was measured by the Schirmer's test.[14] Tear film stability was examined by the tear break-up time.[15]

Conjunctival impression cytology (CIC) was performed to investigate the number of conjunctival GCs and its main secretory mucin, MUC5AC. CIC samples of controls (non-atopic, no use of ophthalmic medication, no contact lens wearer) were included as well. The CIC of the left eye was stained with Periodic Acid-Schiff and haematoxylin to visualize the GCs, as described previously.[11] Subsequently, GCs per millimetre squared (mm^2) were calculated. In a random selected subgroup of patients (flow cytometry cohort total n=49, n=48 at baseline, n=30 at week 4, n=47 at week 28), the CIC of the right eye was analysed by flow cytometry to learn more about the function of the GCs, by investigating the percentage of cytokeratin 19-(CK19) CD45- MUC5AC+ cells, as described previously (gating strategy shown in Figure S1).[11]

Treatment of OSD

Ophthalmological treatment was started in patients with signs and symptoms of OSD. Treatment included tacrolimus skin ointment for the eyelids, eye drops (including lubricants, antihistaminic, and steroidal and non-steroidal anti-inflammatory eye drops (e.g. hydrocortisone, cyclosporine, and dexamethasone)), or eye ointment (including lubricants and steroidal and non-steroidal anti-inflammatory eye ointment (e.g. oxytetracycline/hydrocortisone)). OSD treatment depended on the severity of OSD, and could also consist of a combination of different ophthalmic drugs.

Statistical analysis

Differences between time points during treatment with dupilumab were calculated using the Wilcoxon signed-rank test and the McNemar test. Sub analysis including patients with DAOSD (UTOPIA ≥3 points increase from baseline) were analysed by comparing their baseline values with their values at the onset of DAOSD. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were conducted with SPSS Statistics version 26.0.0.1 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows). Figures were created by using Prism (version 9.3.0 GraphPad Software) and Biorender.

RESULTS

Patient and baseline characteristics

A total of 69 moderate-to-severe AD patients with a median age of 38 years (interquartile range (IQR) 27-52 years) were included, of which 47.8% (n=33/69) were men. At the start of dupilumab (i.e. baseline), median EASI was 14.7 (IQR 10.8-18.0). Most patients had facial involvement of AD in the past year (n=63/69, 91.3%) and a history of self-reported episodic acute allergic conjunctivitis was reported in 71.0% (n=49/69) of the patients. All other patient characteristics (from both the total cohort and the flow cytometry cohort) are shown in Table 1.

TABLE 1. Patient characteristics at baseline

	Total cohort (n=69)	Flow cytometry cohort (n=49)
Age (years), median (IQR)	38 (27-52)	38 (27-52)
Men, n (%)	33 (47.8)	23 (46.9)
Age of onset of AD, n (%)		
Childhood	63 (91.3)	45 (91.8)
Adolescence	4 (5.8)	4 (8.2)
Adult	2 (2.9)	0 (0.0)
History of self-reported episodic acute allergic conjunctivitis, n (%)	49 (71.0)	33 (67.3)
Allergic asthma, n (%)	37 (53.6)	29 (59.2)
Allergic rhinitis, n (%)	50 (72.5)	35 (71.4)
Food allergy, n (%)	35 (50.7)	29 (59.2)
History of rosacea, n (%)	2 (2.9)	2 (4.1)
EASI score at baseline, median (IQR)	14.7 (10.8-18.0)	14.0 (10.8-17.0)
IGA score at baseline, median (IQR)	3 (3-3)	3 (2-3)
AD eyelid involvement in the past year, n (%)	47 (68.1)	38 (77.6)

TABLE 1. (Continued)

	Total cohort (n=69)	Flow cytometry cohort (n=49)
AD facial involvement in the past year, n (%)	63 (91.3)	47 (95.9)
TARC (pg./ml), median (IQR)	1456 (810-2160)	1332 (787-1905)
Severity of OSD before the start of dupilumab†, n (%)		
No OSD	6 (8.7)	2 (4.1)
Mild OSD	37 (53.6)	28 (57.1)
Moderate OSD	20 (29.0)	16 (32.7)
Severe OSD	6 (8.7)	3 (6.1)

† Severity of OSD is based on eye with the highest severity within a patient. Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IGA scale, Investigator's Global Assessment Scale; IQR, interquartile range; OSD, Ocular Surface Disease; TARC, thymus and activation-regulated chemokine.

Clinical ophthalmological characteristics

At baseline, 91.3% (n=63/69) of the patients had ophthalmological characteristics of OSD. In 38/64 (59.4%) of the patients, ophthalmic treatment was started at baseline and was still ongoing at week 4 of dupilumab treatment (5 missing cases due to COVID19 at week 4). The median UTOPIA score was 4.0 (IQR 2.0-5.0) at baseline, which decreased non-significantly to 3.0 (IQR 3.0-5.0) at 4 weeks, and stayed stable at 3.0 (IQR 2.0-5.0) at 28 weeks of dupilumab treatment (Figure 2A and Table S1). DAOSD (UTOPIA ≥3 points increase from baseline) was observed in 28.9% (n=20/69) of the patients, which developed after a median of 12.5 weeks (IQR 4.3-30.8 weeks) of dupilumab treatment. These patients showed a significant increase in their UTOPIA score at the onset of DAOSD compared with baseline (6.0 (5.0-9.0) vs. 2.0 (IQR 0.5-3.0), p<0.001, respectively) (Figure 2B and Table S2). The individual items of the UTOPIA score remained similar during treatment with dupilumab in the total cohort (Table S1). However, all individual items of the UTOPIA score were significantly more frequently present in patients with DAOSD at the onset of DAOSD compared with baseline, and limbitis was present in 25.0% (n=5/20) at the onset of DAOSD (Table S2).

Interestingly, only half of the patients did report any ocular symptoms, both at baseline and during dupilumab treatment (n=37/69 (53.6%) at baseline, n=33/64 (51.6%) at week 4, and n=38/69 (55.1%) at week 28, Figure 2C, Table S1). However, all patient with severe OSD reported symptoms both before and during dupilumab treatment (Figure 2C). In addition, 90% (n=18/20) of the patients with DAOSD reported symptoms at the onset of DAOSD (Figure 2D and Table S2).

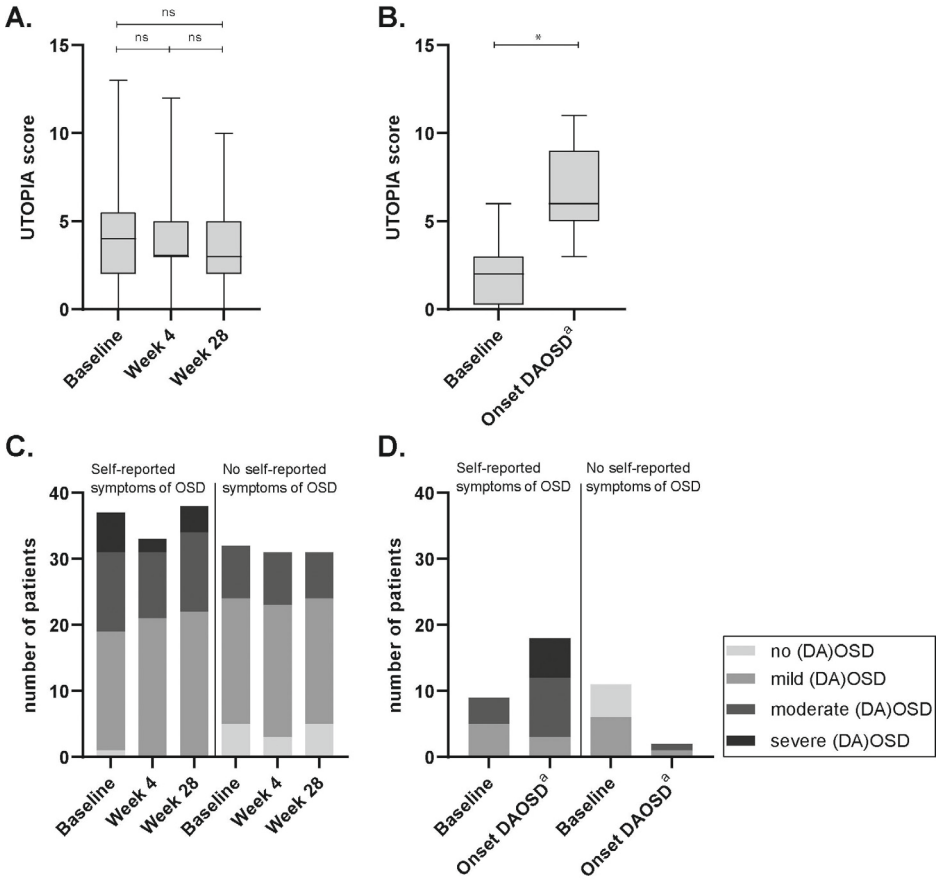

FIGURE 2. Clinical characteristics of ocular surface disease before and during dupilumab treatment.

A. Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score of the total cohort before the start of dupilumab (baseline, n=69), and after 4 (n=64, 5 missing cases due to COVID19) and 28 weeks of dupilumab treatment (n=69). **B.** UTOPIA score of the patients who developed DAOSD (n=20) at baseline and at the onset of DAOSD[a]. **C.** Total group of 69 patients divided by number of patients with self-reported symptoms of OSD or who had no self-reported symptoms of OSD at baseline, and after 4 and 28 weeks of dupilumab treatment. **D.** Patients who developed DAOSD (n=20) divided by number of patients with self-reported symptoms of OSD or who had no self-reported symptoms of OSD at baseline and at the onset of DAOSD[a] Abbreviations: non-significant = ns; ocular surface disease = OSD; Utrecht Ophthalmic Inflammatory and Allergic disease = UTOPIA.

* indicates statistical significance.

[a] Increase in UTOPIA score of ≥3 points compared to baseline.

In the total cohort, a normal tear break-up time was observed at baseline, which decreased slightly during dupilumab treatment (Table S1). In addition, tear production, which was measured by Schirmer's test, was normal at baseline and increased slightly

during treatment with dupilumab (Table S1). Among patients with DAOSD, tear break-up time remained stable, and tear production increased at the onset of DAOSD compared with baseline (Table S2).

Goblet cell numbers and mucin production

CIC was conducted to measure GC numbers per mm^2 and to investigate the percentage of CK19-CD45-MUC5AC+ cells to examine the functionality of the GCs. At baseline, GC numbers were significantly lower in AD patients compared with healthy controls (n=12) (Figure 3A). GC numbers showed a non-significant decrease during the first 4 weeks of dupilumab treatment. After 28 weeks of dupilumab treatment, significantly higher GC numbers were found compared to 4 weeks of treatment but not compared to baseline (Figure 3A). At onset of DAOSD (n=20/69, 28.9%), median GCs remained stable compared to baseline (Figure 3B). Examination of GC numbers by severity category in the total cohort showed lower GC numbers in patients with more severe OSD at baseline and at week 28 (Figure 3C). In addition, GC numbers were lower in patients with more severe DAOSD and at the onset of DAOSD (Figure 3D).

In the flow cytometry cohort (n=49) the percentage of CK19-CD45-MUC5AC+ cells significantly decreased after 4 and 28 weeks of dupilumab treatment compared with baseline (Figure 3E). Additionally, the percentage of CK19-CD45-MUC5AC+ cells at baseline and at the onset of DAOSD was available from 7/20 patients of the flow cytometry cohort, and showed a non-significant decrease during dupilumab treatment (Figure 3F and Table S2).

Together this indicates that the number of GCs is not decreased by dupilumab, but that there is an impaired function of the conjunctival GCs as a result of dupilumab treatment.

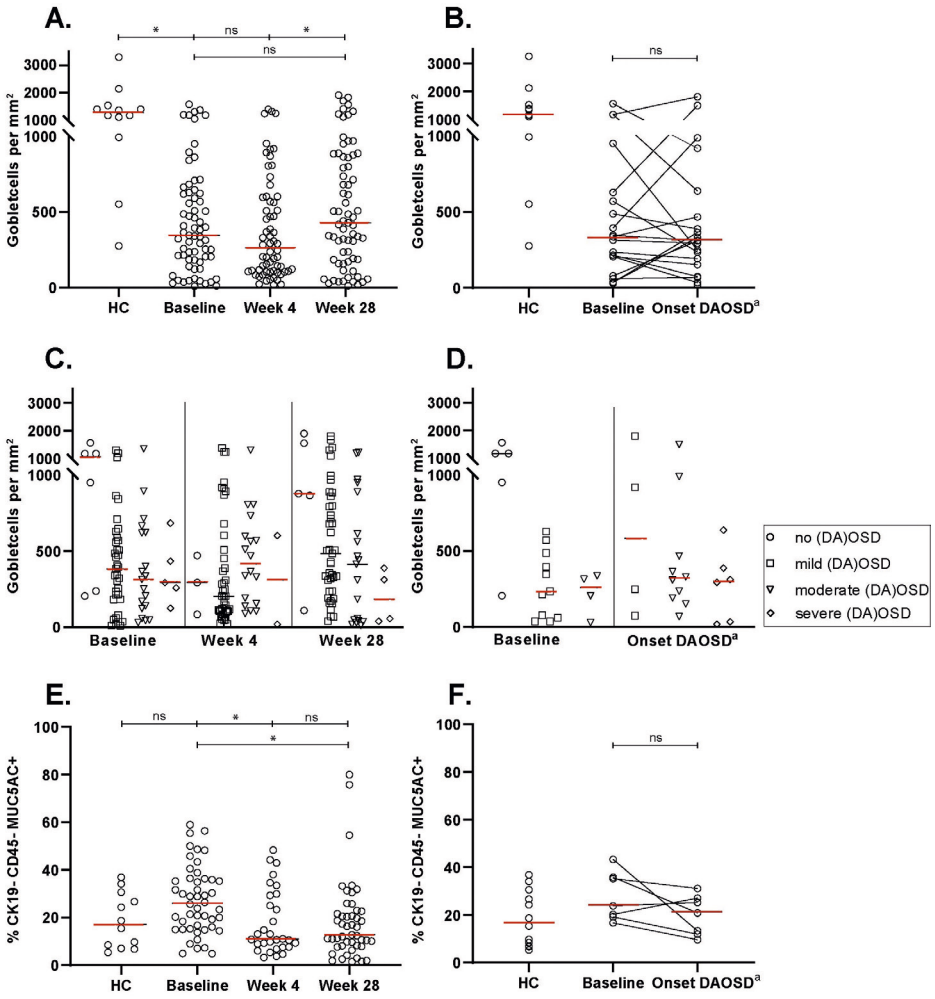

FIGURE 3. Results of conjunctival impression cytology from dupilumab-treated atopic dermatitis patients.

The medians are displayed by red lines. **A.** Goblet cells (GCs) per squared millimetre (mm^2) of the total cohort before the start of dupilumab (baseline, n=67), and after 4 (n=64) and 28 weeks (n=69) of dupilumab treatment compared to healthy controls (HC, n=12). **B.** GCs per mm^2 in patients who developed DAOSD (n=20) before the start of dupilumab (baseline), at the onset of DAOSD[a], and in HCs (n=12). **C.** GCs per mm^2 per severity of OSD of the total cohort before the start of dupilumab (baseline), and after 4 and 28 weeks of dupilumab treatment. Only significant differences are shown ($p<0.05$). **D.** GCs per mm^2 per severity of OSD in patients who developed DAOSD (n=20) before the start of dupilumab (baseline) and at the onset of DAOSD[a]. Only significant differences are shown ($p<0.05$). **E.** Percentage of cytokeratin 19 (CK19)- CD45- Mucin5AC (MUC5AC)+ cells of the flow cytometry cohort before the start of dupilumab (baseline, n=48), after 4 weeks (n=30) and 28 weeks (n=47) of dupilumab treatment, and in HCs (n=12). **F.** Percentage of CK19-CD45-MUC5AC+ cells in patients who developed DAOSD (n=7/20 available samples from the flow cytometry cohort) before the start of dupilumab (baseline), at the onset of DAOSD[a], and in HCs (n=12).
Abbreviations: Cytokeratin19 = CK19; DAOSD = dupilumab-associated ocular surface disease;

MUC5AC = mucin 5 AC; non-significant = ns; ocular surface disease = OSD; Utrecht Ophthalmic Inflammatory and Allergic disease = UTOPIA.
* indicates statistical significance.
[a] Increase in UTOPIA score of ≥3 points compared to baseline.

The effect of ophthalmic treatment

At baseline, only 5.8% (n=4/69) of the patients used ophthalmic medication, which increased during dupilumab treatment to 59.4% (n=38/64) of the patients at week 4 (which was initiated at baseline) and 55.1% (n=38/69) of the patients at week 28 (Figure 4A and Table S3). Tacrolimus skin ointment for the external eyelids was the most frequently used therapy (32.8% (n=21/64) at week 4 and 26.1% (n=18/69) at week 28) (Figure 4B and Table S3). The majority of patients that were treated with anti-inflammatory ophthalmic medication (i.e. tacrolimus skin ointment for the external eyelids or anti-inflammatory eye drops or eye ointment) at week 28 showed a tendency of lower UTOPIA scores compared with patients that were not treated or who received anti-histamine eye drops (Figure 4C and Figure S2).

At the onset of DAOSD, 45.0% (n=9/20) of the patients were already treated with ophthalmic medications, of whom five patients were receiving anti-inflammatory treatment (n=4/5 tacrolimus skin ointment for the external eyelids and n=1/5 non-steroidal and steroidal anti-inflammatory eye drops or eye ointment) (Figure 4A, B, and Table S3). At week 28 of dupilumab treatment, DAOSD was controlled (i.e. patients with a previous increase ≥3 points in UTOPIA score from baseline but no longer at week 28) in 50% (n=10/20) of the patients, of whom six patients were receiving anti-inflammatory ophthalmic drugs (n=3 tacrolimus skin ointment for the external eyelids and n=3 non-steroidal and steroidal anti-inflammatory eye drops or eye ointment). New-onset DAOSD was seen in 25% (n=5/20) of the DAOSD patients at week 28.

Among the 85.5% (n=59/69) of the patients without DAOSD or with controlled DAOSD at week 28, 55.9% (n=33/59) of the patients were using any ophthalmic medication, of whom 40.7% (n=24/59) were treated with anti-inflammatory ophthalmic drugs (n=15/69 with tacrolimus skin ointment for the external eyelids and n=9/69 with non-steroidal and steroidal anti-inflammatory eye drops or eye ointment).

Altogether, these results show that only 10/69 (14.5%) of the patients had persisting or new-onset DAOSD after 28 weeks of treatment with dupilumab, suggesting that early treatment, especially anti-inflammatory ophthalmic medication, reduces the DAOSD severity.

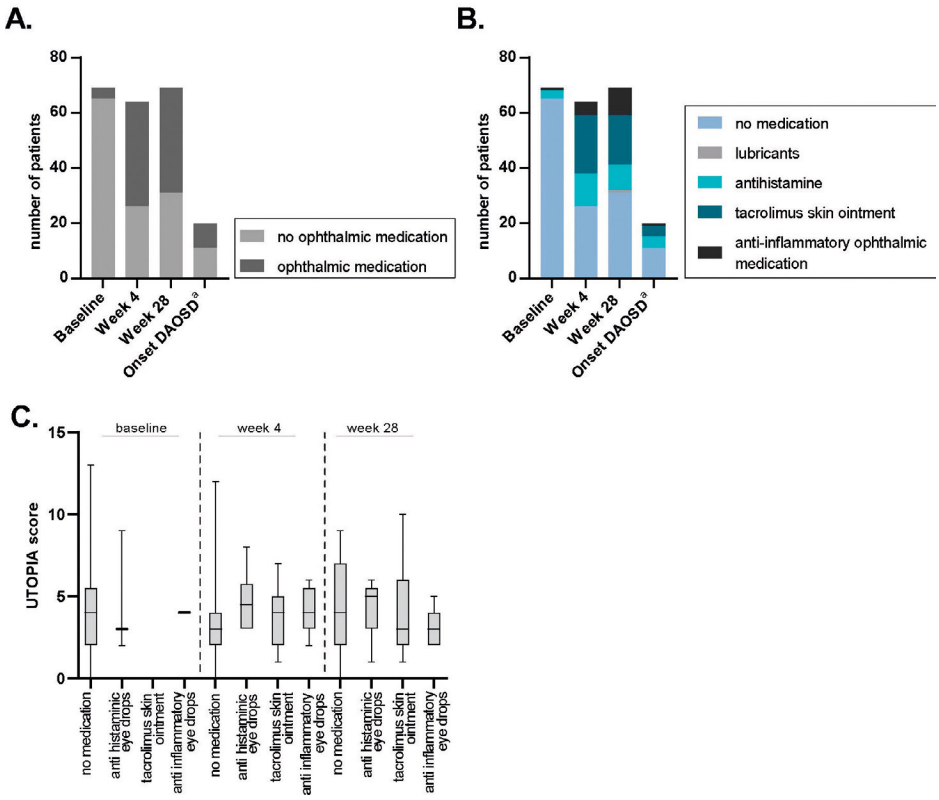

FIGURE 4. Use of ophthalmic medication during dupilumab treatment (with a minimum drug use of once every day).

The most potent medication per patient is shown. **A.** Number of patients treated with ophthalmic medication at baseline (n=4/69, 5.8%), at week 4 (n=38/64, 59.4%), at week 28 (n=38/69, 55.1%) during dupilumab treatment, and at the onset of dupilumab-associated ocular surface disease (DAOSD) (n=9/20, 45.0%). **B.** Number of patients treated with ophthalmic medication per medication group, at week 4, 28, and at the onset of DAOSD during dupilumab treatment. **C**. The severity of the ocular inflammation assessed by the Utrecht Ophthalmic and Inflammatory Allergic disease (UTOPIA) score at baseline, week 4 and 28 per ophthalmic medication group.

Abbreviations: DAOSD = dupilumab-associated ocular surface disease; OSD = ocular surface disease; UTOPIA = Utrecht Ophthalmic and Inflammatory Allergic disease score.

[a] Increase in UTOPIA score of ≥3 points compared to baseline.

DISCUSSION

This prospective study shows that OSD is very common in moderate-to-severe AD patients before the start of dupilumab treatment. DAOSD (UTOPIA ≥3 points increase from baseline) was observed in 28.9% (n=20/69) of the dupilumab-treated AD patients, of whom 5/20 (25%) patients also had limbal inflammation. A stable low number of GCs with a decrease in the percentage of CK19-CD45- MUC5AC+ cells were observed in DAOSD patients. After 28 weeks of treatment with dupilumab, only 50% (n=10/20)

of the patients with DAOSD had persisting or new-onset DAOSD, suggesting that early treatment reduces the severity of DAOSD.

Clinical trials and daily practice studies of dupilumab-treated AD patients reported DAOSD incidences of up to 34%, which is more or less comparable to our incidence.[2-4] The most frequently observed ophthalmological characteristics were conjunctivitis (both bulbar and tarsal), blepharitis, and Meibomian gland dysfunction, both before and during dupilumab treatment, which is partly in line with previous literature.[16-18] However, the majority of studies regarding DAOSD are not based on ophthalmological data, but on patient-reported diagnosis. Bortoluzzi et al.[19] found no correlation between an OSD questionnaire focusing on OSD symptoms and the ophthalmological examination, indicating that patient-reported diagnosis is less reliable. This is consistent with our results, as about half of the patients with OSD did not report any symptoms, both before and during dupilumab treatment. Nevertheless, most patients did report OSD symptoms in case of severe OSD and at the onset of DAOSD. In addition, at the onset of DAOSD, both blepharitis and tarsal inflammation were the most frequently observed ophthalmic characteristics, and limbitis was seen in 25% (n=5/20) of the patients. Severe chronic OSD with involvement of the limbus may lead to limbal stem cell deficiency with possible irreversible long-term effects and follow-up.[6] In order to distinguish between DAOSD and AD-associated OSD during treatment with dupilumab, knowledge of OSD severity before the start of dupilumab is very important.

Additionally, patients with more severe OSD before the start of dupilumab may require more potent ophthalmic medication, and ophthalmic examination is recommended to evaluate effectiveness. Our results show that patients who received anti-inflammatory ophthalmic treatment (e.g. tacrolimus skin ointment for the external eyelids or anti-inflammatory eye drops or eye ointment) had less severe OSD during dupilumab treatment, which is in line with previous studies.[7,17] Furthermore, our results show that after 28 weeks of treatment with dupilumab, only 14.5% (n=10/69) of the patients had a ≥3 points UTOPIA increase compared to baseline, suggesting that frequent ophthalmologic examinations and early treatment of (DA)OSD are effective in reducing the severity.

Currently, the exact patho-mechanism of DAOSD is still unclear. Previous case studies investigated conjunctival biopsies of AD patients with DAOSD, and found GC scarcity in patients with DAOSD (n=6), and improvement of GC density after discontinuation of dupilumab (n=1).[10,20] However, low GC numbers were already observed in moderate-to-severe AD patients before the start of dupilumab. During dupilumab treatment,

our findings show low but stable GC numbers in dupilumab-treated AD patients, which seems to be related to the severity of OSD (both before and during dupilumab treatment). In addition, a significant decrease in percentage of CK19-CD45-MUC5AC+ cells was observed during dupilumab treatment, suggesting that the function of GCs may be affected by dupilumab, but that the number of GCs is not reduced by dupilumab. GCs are specialized cells that secrete and produce mucins for the tear film, have protective and lubricant functions, and play an important role in the mucosal immune system by producing immune regulatory factors.[21-23] The decrease that we observed in the percentage of CK19-CD45-MUC5AC+ cells accompanied with stable GC numbers during dupilumab treatment compared to baseline, indicates that the OSD observed during treatment with dupilumab differs from the (episodic) allergic conjunctivitis, in which GC hyperplasia and mucin hypersecretion are found.[22] In other ocular diseases, such as keratoconjunctivitis sicca, the number of GCs is negatively correlated with the extent of inflammation.[22] We also found less GCs in patients with more severe OSD, suggesting that this negative correlation may also be present in our patients. This negative correlation was observed before the start of dupilumab, during dupilumab treatment, and at the onset of DAOSD, suggesting that the lower GC numbers in patients with more severe OSD may be severity related.

Recently, it was shown that IL4Rα, the target for dupilumab, is present on conjunctival GCs.[24] Additionally, Barnett et al. found a relative deficiency of MUC5AC in tear fluid of AD patients with DAOSD, which is in line with our results.[13] In our study, the number of GCs slightly increased over time, while the percentage of CK19-CD45-MUC5AC+ cells decreased significantly, suggesting functional changes of GCs during treatment with dupilumab, possibly leading to DAOSD. In addition, the decrease in percentage of CK19-CD45-MUC5AC+ cells might also lead to the dry eye sensation, which has been reported as sign of DAOSD.[25] The tear film, which protects and lubricate the ocular surface, consists of three layers: a lipid layer produced by the Meibomian glands, an aqueous layer produced by the lacrimal gland, and a mucus layer produced by the GCs.[26] Since the Schirmer's test remained stable, or even increased during dupilumab treatment, the dry eye sensation cannot be the consequence of insufficient tear production, but seems to be attributed to less mucus production by GCs and/or dysfunction of the Meibomian glands. Due to the ocular inflammation, artificial tears may not be sufficient in treating DAOSD, and anti-inflammatory treatment is needed to control the (DA)OSD.

Since early (anti-inflammatory) treatment was prescribed in patients with moderate-to-severe OSD and/or OSD symptoms, it remains unknown what the effect of dupilumab on GCs would have been if these patients would not have received ophthalmic

treatment. The possible effect of anti-inflammatory ophthalmic treatment on GCs makes it more difficult to examine the individual effect of dupilumab on GC numbers and on the percentage of CK19-CD45-MUC5AC+ cells. For example, cyclosporine A eye drops, which is an anti-inflammatory treatment, have been shown to increase GC density in dry eye disease patients.[27] Interestingly, oral cyclosporine A is one of the systemic treatment options for moderate-to-severe AD patients, which could explain the lower relative risk of developing or worsening of OSD in AD patients treated with conventional immunosuppressive systemic therapies (including cyclosporine A) compared to AD patients treated with dupilumab.[28] Additionally, improvement of DAOSD was seen in an AD patient after switching from dupilumab treatment to upadacitinib, which is a Janus Kinase inhibitor with a broader mode of action.[29-31]

This study has some limitations. First, flow cytometry analysis of the CK19-CD45-MUC5AC+ cells were conducted in a small cohort, and only few samples were available from patients with DAOSD. However, despite this, we did find significant differences in the total group, providing new insights in the patho-mechanism of DAOSD. Larger studies are needed to verify our results. Second, a substantial amount of patients were treated with ophthalmological treatment during dupilumab treatment, potentially leading to less severe DAOSD. As some patients started ophthalmic treatment at baseline, this may have influenced the natural course of the development of DAOSD during dupilumab treatment, resulting in an underestimation of the incidence of DAOSD in our study. However, DAOSD was assessed at the first visit that patients had an increase in their UTOPIA of ≥3 points from baseline, and only one of the patients with DAOSD used anti-inflammatory ophthalmic medication at onset of DAOSD. For that reason, the ophthalmological treatment did probably less influence our results of the DAOSD sub analyses.

Currently, we are not able to identify patients that are at risk of developing DAOSD. Our data suggest that patient-reported diagnosis is not always reliable. Ophthalmic examination before the start of dupilumab is recommended, since many moderate-to-severe AD patients have OSD before the start of dupilumab, and early treatment and recognition of (DA)OSD reduces the severity of DAOSD. If not feasible, we recommend to start tacrolimus skin ointment (1 mg/g) for the external eyelids once daily in patients that report symptoms of OSD during dupilumab treatment, since patients who received this treatment showed a decrease in their ocular inflammation in this study. In addition, tacrolimus skin ointment can be safely used for prolonged time, whereas corticosteroid eye drops or eye ointment may affect intraocular pressure leading to a higher risk of glaucoma and cataract.[17,32] If this treatment is not sufficient in treating DAOSD, low-threshold referral to an ophthalmologist is highly recommended.

In conclusion, OSD is common in moderate-to-severe AD patients before the start of dupilumab. The severity of DAOSD reduces with intensive ophthalmological follow-up and early treatment. Lower numbers of GCs are seen in patients with more severe OSD before and during treatment with dupilumab. In addition, the percentage of CK19-CD45-MUC5AC+ cells decreased during dupilumab treatment, suggesting a reduced function of the conjunctival GCs as a result of dupilumab treatment.

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SUPPLEMENTARY

TABLE S1. Ophthalmological characteristics during dupilumab treatment

	Baseline (n=69)	Week 4† (n=64)	Week 28 (n=69)
UTOPIA score, median (IQR)	4.0 (2.0-5.0)	3.0 (3.0-5.0)	3.0 (2.0-5.0)
No OSD, n (%)	6 (8.7)	3 (4.7)	5 (7.2)
Mild OSD, n (%)	37 (53.6)	41 (64.1)	41 (59.4)
Moderate OSD, n (%)	20 (29.0)	18 (28.1)	19 (27.5)
Severe OSD, n (%)	6 (8.7)	2 (3.1)	4 (5.8)
DAOSD‡, n (%)	n/a	7 (10.9)	10 (14.5)
OSD symptoms, n (%)	37 (53.6)	33 (51.6)	38 (55.1)
Blepharitis, n (%)	48 (70.6)	46 (71.9)	43 (62.3)
Meibomian gland dysfunction, n (%)	44 (63.8)	37 (57.8)	41 (59.4)
Tarsal inflammation, n (%)	58 (84.1)	58 (90.6)	62 (89.9)
Bulbar inflammation, n (%)	33 (47.8)	31 (48.4)	32 (46.4)
Limbitis, n (%)	3 (4.3)	5 (7.8)	5 (7.2)
Limbal vascularization, n (%)	40 (58.0)	36 (56.3)	34 (49.3)
Corneal punctate, n (%)	21 (30.4)	12 (18.8)	22 (31.9)
Missing, n (%)	0 (0)	0 (0)	1 (1.5)
Hurricane flu pattern, n (%)	0 (0)	0 (0)	0 (0)
Missing, n (%)	0 (0)	0 (0)	1 (1.5)
TBUT OD, seconds, median (IQR)	8 (5-10)	7 (5-10)	7 (4-10)
TBUT OS, seconds, median (IQR)	10 (5-10)	8 (5-10)	7 (5-10)
Missing, n (%)	1 (1.5)	1 (1.5)	1 (1.5)
Schirmer OD, millimetre, median (IQR)	15 (12-21)	16 (8-24)	18 (11-27)
Schirmer OS, millimetre, median (IQR)	14 (10-21)	15 (9-23)	16 (11-27)
Missing, n (%)	0 (0)	0 (0)	1 (1.5)
Goblet cells per mm^2, median (IQR)	346 (182-628)	264 (107-590)	429 (178-871)
Missing, n (%)	2 (2.9)	0 (0.0)	0 (0.0)
% CK19- CD45- MUC5AC+ cells, median (IQR)	26.0 (15.4-36.2)	10.9 (7.6-29.5)	12.7 (8.1-22.6)
Missing, n (%)	21 (30.4)	34 (53.1)	22 (31.9)

† 5 cases missing due to COVID19.

‡ Increase in UTOPIA score of ≥3 points at this visit compared to baseline.

Abbreviations: CK19 = cytokeratin 19; DAOSD = dupilumab-associated ocular surface disease; MUC5AC = Mucin 5 AC; IQR = interquartile range; OSD = Ocular Surface Disease; TBUT = tear break-up time; UTOPIA = Utrecht Ophthalmic Inflammatory and Allergic disease.

TABLE S2. Ophthalmological characteristics at DAOSD

	Baseline (n=20)	Onset DAOSD† (n=20)	P-value
UTOPIA score, median (IQR)	2.0 (0.5-3.0)	6 (5.0-9.0)	<0.001
No OSD, n (%)	5 (25.0)	0 (0.0)	
Mild OSD, n (%)	11 (55.0)	4 (20.0)	
Moderate OSD, n (%)	4 (20.0)	10 (50.0)	
Severe OSD, n (%)	0 (0.0)	6 (30.0)	
OSD symptoms, n (%)	9 (45.0)	18 (90.0)	0.004
Blepharitis, n (%)	10 (50.0)	19 (95.0)	0.004
Meibomian gland dysfunction, n (%)	9 (45.0)	17 (85.0)	0.008
Tarsal inflammation, n (%)	14 (70.0)	19 (95.0)	0.063
Bulbar inflammation, n (%)	5 (25.0)	17 (85.0)	<0.001
Limbitis, n (%)	0 (0.0)	5 (25.0)	0.063
Limbal vascularization, n (%)	7 (35.0)	14 (70.0)	0.016
Corneal punctate, n (%)	2 (10.0)	9 (40.0)	0.070
Hurricane flu pattern, n (%)	0 (0.0)	0 (0.0)	N/A
Missing, n (%)	0 (0.0)	0 (0.0)	
TBUT OD, seconds, median (IQR)	9.0 (5.0-10.0)	7 (4.5-10.0)	0.293
TBUT OS, seconds, median (IQR)	10.0 (5.0-10.0)	10 (4.5-10.0)	0.952
Missing TBUT, n (%)	1 (5.0)	0 (0)	
Schirmer OD, millimetre, median (IQR)	14 (11-25)	19 (11-29)	0.093
Schirmer OS, millimetre, median (IQR)	14 (9-24)	19 (11-32)	0.023
Missing Schirmer, n (%)	0 (0)	1 (5.0)	
Goblet cells per mm^2, median (IQR)	325 (110-613)	310 (162-594)	0.794
Missing, n (%)	0 (0)	0 (0)	
% CK19- CD45- MUC5AC+ cells, median (IQR)	29.6 (19.4-39.4)	20.8 (11.9-27.1)	0.091
Missing, n (%)	8 (40.0)	13 (65.0)	

† Increase in UTOPIA score of ≥3 points compared to baseline.

Abbreviations: CK19 = cytokeratin 19; DAOSD = dupilumab-associated ocular surface disease; MUC5AC = Mucin 5 AC; IQR = interquartile range; OSD = Ocular Surface Disease; TBUT = tear break-up time; UTOPIA = Utrecht Ophthalmic Inflammatory and Allergic disease.

TABLE S3. Number of patients treated with ophthalmic medication per medication group, at week 4, 28, and at the onset of DAOSD during dupilumab treatment.

	Baseline (n=69)	Week 4 (n=64†)	Week 28 (n=69)	Onset DAOSD‡ (n=20)
Medication group, n (%)				
No medication	65 (94.2)	26 (40.6)	31 (44.9)	11 (55.0)
Lubricants	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)
Antihistamine eye drops	3 (4.3)	12 (18.9)	9 (13.0)	4 (20.0)
Tacrolimus skin ointment for the external eyelids	0 (0.0)	21 (32.8)	18 (26.1)	4 (20.0)
Non-steroidal and steroidal anti-inflammatory eye drops or eye ointment	1 (1.4)	5 (7.8)	10 (14.5)	1 (5.0)

The most potent medication per patient is shown in this table. Abbreviations: DAOSD = dupilumab-associated ocular surface disease.

† Due to COVID19 there were 5 missing cases at week 4 of dupilumab treatment.

‡ Increase in UTOPIA score of ≥3 points compared to baseline.

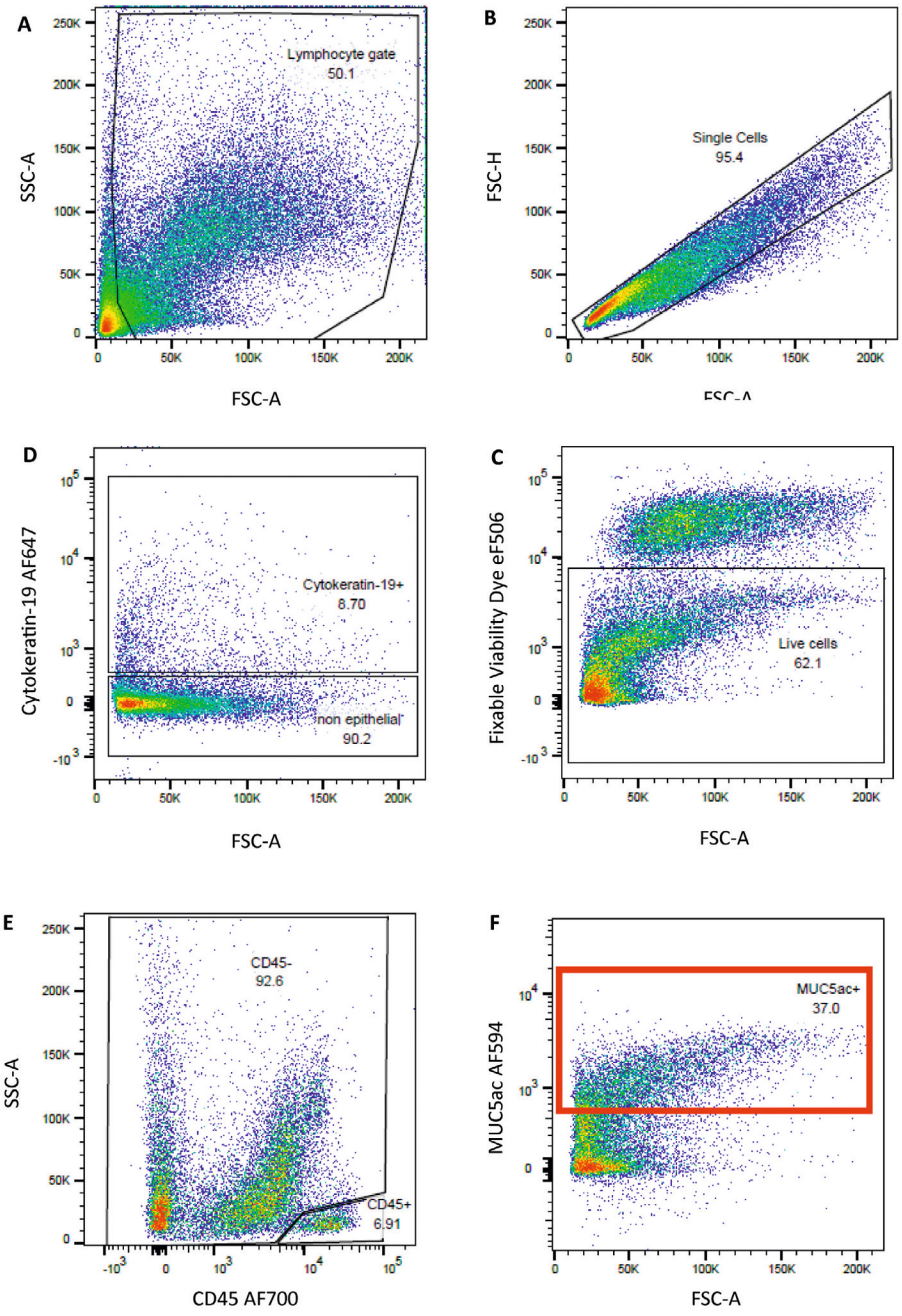

FIGURE S1. The different steps to gate Mucin 5AC (MUC5AC)+ cells are depicted.
Debris was excluded (**A**), single cells were gated (**B**), auto fluorescence and dead cells were excluded (**C**), exclusion of epithelial cells (cytokeratin-19+) (**D**) and CD45+ cells (**E**), and finally MUC5AC+ cells were gated (**F**).

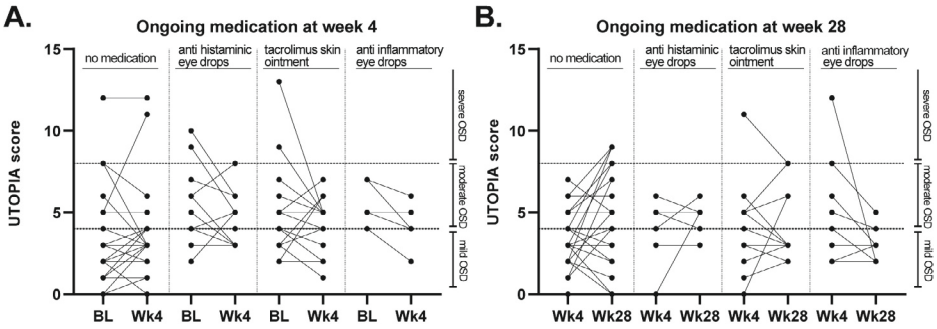

FIGURE S2. Effect of ophthalmic treatment on ocular inflammation during dupilumab treatment.

A. Ocular surface disease (OSD) severity assessed by the Utrecht Ophthalmic and Inflammatory Allergic disease (UTOPIA) score per ophthalmic treatment ongoing at week 4. **B.** OSD severity assessed by the UTOPIA score per ophthalmic treatment ongoing at week 28. Abbreviations: baseline = BL; OSD = ocular surface disease; Utrecht Ophthalmic Inflammatory and Allergic disease = UTOPIA; wk = week.

CHAPTER 5

Long-term follow-up and treatment outcomes of conjunctivitis during dupilumab treatment in patients with moderate-to-severe atopic dermatitis

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CLINICAL IMPLICATIONS

Ophthalmologist-confirmed conjunctivitis during dupilumab treatment was observed in 33 of 167 (19.8%) of patients with atopic dermatitis. Most patients still suffered from mild-to-moderate conjunctivitis during long-term follow-up despite treatment. Dose adjustment or discontinuation of dupilumab was needed in 10 of 33 (30%) and 3 of 33 (9%) patients, respectively.

Dupilumab is the first biologic treatment for atopic dermatitis (AD) and its effectiveness and safety are proven.[1] Although conjunctivitis is the most frequently reported side effect during dupilumab treatment in both clinical trials and daily practice, data on the clinical course of conjunctivitis during long-term use of dupilumab are lacking.[1-3] This prospective daily practice study evaluates ophthalmological characteristics and long-term treatment outcomes of ophthalmologist-confirmed conjunctivitis during dupilumab treatment in moderate-to-severe AD patients. During a 12-month evaluation period, 167 moderate-to-severe AD patients were treated with dupilumab 300 mg every 2 weeks at the University Medical Center Utrecht, the Netherlands. Patients reporting ophthalmological symptoms who could not be controlled with lubricant drops and/or tacrolimus skin ointment (1mg/g) for the external eyelids were referred to an ophthalmologist. Further (anti-inflammatory) ophthalmological treatment was prescribed by the ophthalmologist, and individually chosen per patient.

Conjunctivitis was reported in 66/167 (39.5%) patients, of whom 33 were referred to an ophthalmologist. Ophthalmologist-confirmed conjunctivitis was reported in 33/167 (19.8%) patients (17 female; mean age 45.7 years, standard deviation (SD) 14.3; mean Eczema Area Severity Index (EASI) at baseline 21.7 (SD 9.5), Table S1 in the Supplementary). History of (allergic) conjunctivitis was present in 24/33 (72.7%) patients. None of the 33 patients reported conjunctivitis symptoms at start of dupilumab. In the 33 referred patients, patient-reported eye symptoms, such as redness, tearing and itching, developed within a median of 33 days (interquartile range (IQR) 28.0-61.0) after starting dupilumab. Ophthalmological characteristics were examined and graded in terms of severity by an experienced ophthalmologist following the UTrecht OPhthalmic Inflammatory and Allergic disease (UTOPIA) ocular surface score (Table 1). Overall conjunctivitis severity was based on grading of different ophthalmological characteristics (Figure 1A-B).

During the first ophthalmological consultation, mild, moderate, and severe conjunctivitis were diagnosed in 22 (66.7%), 7 (21.2%), and 4 (12.1%) of the 33 referred patients, respectively (Figure 1B). Most frequently reported ophthalmological characteristics were tarsal and bulbar conjunctivitis, and blepharitis (in 28 (84.8%), 25 (75.8%), and 22 (66.7%) patients, respectively). Six (18.2%) patients presented with limbitis (Figure 1A).

TABLE 1. UTrecht OPhthalmic Inflammatory and Allergic disease (UTOPIA) ocular surface score

Ophthalmological characteristics	Severity*			
	None	**Mild**	**Moderate**	**Severe**
Blepharitis	No blepharitis	Bubbles, mild hyperemia of the eyelid	Hyperemia and mild swelling of the eyelid	Severe hyperemia, thickening, keratinization, scarring of the eyelid
Meibomian gland dysfunction	No Meibomian gland dysfunction	Bubbles, after pressing an oily substance is formed	Plugs or bubbles, after pressing an thicker substance is formed	Plugs or scarring, after pressing no substance is formed
Tarsal conjunctivitis	No tarsal conjunctivitis	Mild swelling and hyperemia, mild papillae	Larger papillae, moderate swelling and hyperemia	Moderate characteristics and/or keratinization, ulceration, sclerosis
Bulbar conjunctivitis	No bulbar conjunctivitis	Mild swelling and hyperemia	Moderate swelling and hyperemia in all quadrants	Severe swelling and hyperemia, mucus and excessive tearing, photophobia.
Limbitis	No limbitis	Mild swelling and hyperemia	Evident swelling/hyperemia over >3 clock hours	Severe swelling/hyperemia, conjunctival vascularization extending the normal limbus edge
Limbal vascularization	No abnormal limbal vascularization	Fine vascularization along the limbus	Moderate vascularization to the limbus >3 clock hours, or fine vascularization extending the normal limbus barrier	Strong vascularization extending the normal limbus barrier in >3 clock hours
Corneal punctate	No corneal punctate	Some punctate limited to the interpalpebral region	Fiddled punctate, extending the interpalpebral region or strongly present in the interpalpebral region	Significantly diffuse punctate and/or confluent
Hurricane pattern	No hurricane pattern	Elongated small and narrow punctate along the limbus to <0.25 radius and <1 clock hour	Long and thin punctate in hurricane pattern, up to <0.5 radius and <3 clock hours	Evident hurricane pattern >3 clock hours and/or 0.5 radius (cross pupil)
Overall severity of the conjunctivitis	None / mild / moderate / severe conjunctivitis**			

* None = 0 points; mild = 1 point; moderate = 2 points; severe = 3 points

** 0 = no conjunctivitis; 1-4 = mild conjunctivitis (unless the score consists of only Meibomian gland dysfunction and punctate, then the total score is 0); 5-8 = moderate conjunctivitis; ≥9 = severe conjunctivitis

The most frequently prescribed ophthalmological treatments during follow up included corticosteroid eye drops, tacrolimus skin ointment for the external eyelids, and lubricant drops (in 24 (72.7%), 25 (75.8%), and 26 (78.8%) patients, respectively, Table S2 in the Supplementary). During follow-up (mean 17.5 (SD +/- 3.4) months, dosing interval of dupilumab was prolonged to 300 mg every three to five weeks in 10/33 (30%) patients because of conjunctivitis, resulting in improvement of eye symptoms in six patients and remission in one patient. Discontinuation of dupilumab due to ocular pathology was necessary in 3/33 (9.1%) patients, showing improvement or remission in all cases (Figure 1C). Ineffectiveness of dupilumab led to discontinuation in 2/33 (6.1%) patients.

After follow-up, 24/28 (86%) patients who continued dupilumab treatment were still suffering from conjunctivitis (Figure 1B). New-onset limbitis during follow-up was seen in eight more patients (8/27, 29.6%) patients; in six cases despite ophthalmic anti-inflammatory treatment.

The conjunctivitis outcome during a follow-up of 17.6 months (SD +/-3.5), was evaluated for 28/33 (84.8%) patients who continued dupilumab, by comparing the first conjunctivitis severity category with the latest follow-up category (Figure 1D). Outcomes were categorized into worsened (worsening with ≥1 category), stable (unchanged category), improved (improvement with ≥1 category) or complete remission (no conjunctivitis). Complete remission was seen in 4/28 (14%) patients; of these, two were still using anti-inflammatory eye drops or tacrolimus ointment for the external eyelids. Improvement of conjunctivitis occurred in 7/28 (25%) patients, of which six were still using anti-inflammatory eye drops. Uncontrolled conjunctivitis, meaning stable or worsened conjunctivitis, was seen in 17/28 (61%) patients. Ophthalmic anti-inflammatory therapy was prescribed for all of these 17 patients; however, 2/17 patients reported being non-compliant.

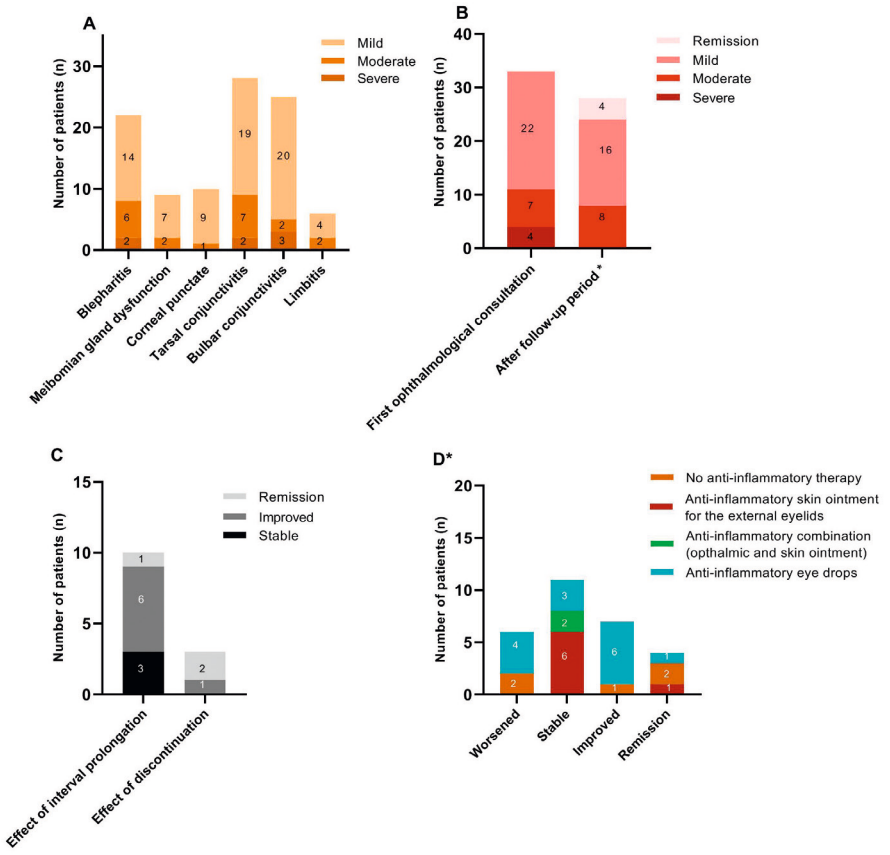

FIGURE 1. Results of 33 atopic dermatitis patients diagnosed with conjunctivitis during dupilumab treatment.

A. Ophthalmic characteristics at the first ophthalmological consultation (n=33). **B.** Severity of conjunctivitis at the first consultation (n=33) and after follow-up (n=28). **C.** Effect of dose adjustment of dupilumab due to ocular pathology. **D.** Outcome and treatment of conjunctivitis after follow-up (n= 28)*. *Discontinued patients (n=5) were excluded.

Literature regarding conjunctivitis during dupilumab is limited by small sample sizes, short follow-up duration, and lack of thorough and standardized ophthalmological investigation. In contrast, all 33 patients of our study underwent standardized examination by an ophthalmologist followed by long-term follow-up.

Several pathomechanisms have been suggested to be responsible for the development or worsening of conjunctivitis during dupilumab treatment in AD patients, such as rosacea-like conjunctivitis, focal scarcity of intra-epithelial goblet cells, and relative

ocular undertreatment due to lower tissue distribution of dupilumab in the eyes.[2,4,5] The last hypothesis seems in contradiction with our finding that interval prolongation or discontinuation of dupilumab resulted in improvement of the conjunctivitis.

The management of conjunctivitis during dupilumab treatment is challenging. Previous case series and case reports have described several therapeutic options, including tacrolimus eye ointment, fluorometholone eye drops, cyclosporine eye drops, and lifitegrast eye drops, leading to improvement in most cases.[6-8] The majority of our patients received combination therapy and most patients remained dependent on ophthalmic medication. Anti-inflammatory eye drops and/or tacrolimus ointment for the external eyelids were prescribed most often.

In contrast to clinical trial data, reporting that most conjunctivitis cases recovered or resolved while continuing dupilumab treatment, our results show more persistent ophthalmological signs and symptoms despite adequate ophthalmic treatment. Remarkably, 8/33 (24.2%) patients developed limbitis during follow-up; in six cases despite adequate ophthalmic anti-inflammatory treatment. Limbal stem cells are vital for corneal healing and the barrier function of the limbus. Chronic limbitis may lead to irreversible limbal stem cell deficiency, which could lead to irreversible long-term visual loss, making adequate monitoring of conjunctivitis necessary.[9]

This study has some limitations. Firstly, since all patients were seen in an AD expertise center, the population consisted of more severe AD patients. As severity of AD may be related with the development of conjunctivitis during dupilumab treatment, this may have affected the results.[2] Secondly, not all patients may have been compliant with ophthalmic treatment, which might have resulted in undertreatment of the conjunctivitis. Lastly, ophthalmological examination by an ophthalmologist was not performed before starting dupilumab; therefore, pre-existing ophthalmological pathology cannot be excluded.

In conclusion, this study shows ophthalmologist-confirmed conjunctivitis in 33/167 (19.8%) AD patients treated with dupilumab in a one-year period. During long-term ophthalmological follow-up, the majority of these patients still suffered from mild-to-moderate conjunctivitis despite treatment. Dose adjustment or discontinuation of dupilumab due to ocular pathology was needed in 10/33 and 3/33 of the patients, respectively.

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SUPPLEMENTARY

TABLE S1. Baseline table

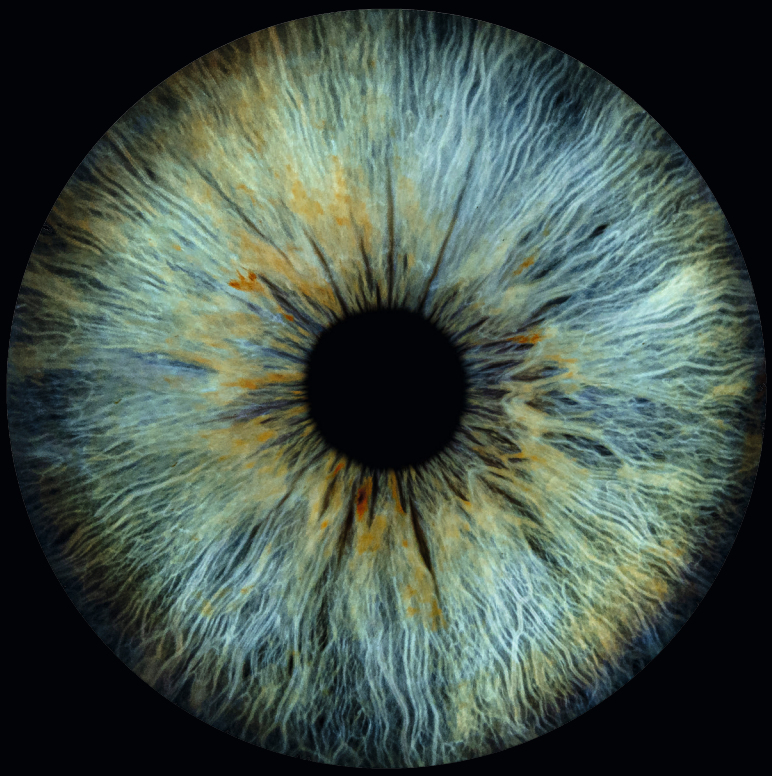
	Total group (n=33)
Sex, female, n (%)	17 (51.5)
Age (years) at start of dupilumab, mean (SD)	45.7 (14.3)
Age of primary onset AD	
Childhood, n (%)	28 (84.8)
Adolescence, n (%)	4 (12.1)
Adult, n (%)	1 (3.0)
Number of prior immunosuppressive systemic treatments for AD (used for at least 3 months), median (IQR)	2.0 (1.0 – 4.0)
Hospitalized for AD ever, n (%)	27 (81.8)
Atopic comorbidities	29 (87.9)
Allergic asthma, n (%)	23 (69.7)
Allergic rhinitis, n (%)	23 (69.7)
Allergic conjunctivitis, n (%)	24 (72.7)
Food allergy, n (%)	21 (63.6)
AD related parameters at start dupilumab	
EASI score baseline, mean (SD)	21.7 (9.5)
TARC (pg/ml), median, (IQR)	2856 (1271 – 8000)
Eosinophils (x10^9/L), median (IQR)	0.38 (0.26 – 0.72)
AD related parameters at referral to the ophthalmologist	
EASI score, mean (SD)	8.0 (5.8)
TARC (pg/ml), median (IQR)	625 (413 – 938)
Eosinophils (x10^9/L), median (IQR)	0.62 (0.30 – 1.30)
Number of days between start dupilumab and development of eye symptoms, median (IQR)	33.0 (28.0 – 61.0)
Number of days between start dupilumab and referral to the ophthalmologist, median (IQR)	94.0 (54.5 – 147.5)
Number of ophthalmological consultations, median, (IQR)	4.0 (2.5 – 8.0)
Total follow-up period (both dermatological and ophthalmological) (months), median (IQR)	22.0 (18.0 – 24.0)
Follow-up period since ophthalmological baseline(months), mean, (SD)	17.5 (3.4)
History of ocular disease (excluding allergic conjunctivitis)	11 (33.3)
History of atopic keratoconjunctivitis, n (%)	5 (45.5)
Active conjunctivitis at start dupilumab, n (%)	0 (0.0)
Rosacea	
History of rosacea, n (%)	4 (12.1)
Rosacea flare during follow-up, n (%)	6 (18.2)
Development of head-neck dermatitis during follow-up, n (%)	2 (6.1)

Data are n (%) unless otherwise indicated. Childhood is <12 years, adolescence is 12-17 years old and adult is >18 years old. AD = atopic dermatitis; SD = standard deviation; IQR = interquartile range; EASI = Eczema Area Severity Index; TARC = Thymus- and Activation-Regulated Chemokine

TABLE S2. Treatment for conjunctivitis, number of total prescribed treatments during follow-up

Prescribed therapies as treatment for conjunctivitis during follow-up	n=33
Lubricant drops	26 (78.8)
Anti-inflammatory therapy for the external eyelids	25 (75.8)
Antihistamine eye drops	14 (42.4)
Corticosteroid eye drops	24 (72.7)
Other anti-inflammatory therapy (eye drops/eye ointment)	12 (36.4)
Combined anti-inflammatory and antimicrobial therapy (eye drops/ eye ointment)	10 (30.3)
Other therapy	3 (9.1)

Data are n (%) unless otherwise indicated. Multiple therapies per patient.
Anti-inflammatory treatment for the external eyelids included tacrolimus skin ointment; corticosteroid eye drops included fluormetholone, dexamethasone, hydrocortisone, softacor, prednisolone; antihistamine eye drops included ketotifen; other anti-inflammatory therapy (eye drops/ eye ointment) included tacrolimus eye ointment, cyclosporine A eye drops; combined anti-inflammatory and antimicrobial treatment (eye drops/ eye ointment) are terracortril, tobradex; other therapies are cross-linking, bandage lens with chloramphenicol.



CHAPTER 6

Switching from dupilumab to tralokinumab in atopic dermatitis patients with ocular surface disease: preliminary case series

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KEY MESSAGES

- This prospective case series describes dermatological and ophthalmological characteristics of four atopic dermatitis patients.
- Patients had dupilumab-associated ocular surface disease (DAOSD) and were therefore switched to tralokinumab.
- In conclusion, some patients with DAOSD may benefit from switching to tralokinumab treatment.

To the editor,

Since 2018, new biological therapies that target pathways involved in the pathogenesis of atopic dermatitis (AD) are available to treat patients with moderate-to-severe AD.[1,2] Dupilumab inhibits interleukin (IL)-4 and IL-13 signalling by targeting the IL-4 receptor alpha and has proven its efficacy and safety.[2] However, high rates of dupilumab-associated ocular surface disease (DAOSD) have been reported during treatment.[2] In phase-3 trials with tralokinumab, a biological therapy that specifically targets IL-13, less ocular surface disease (OSD) has been reported compared to phase-3 trials with dupilumab (7.5% vs. 8.6-22.1%, respectively).[1,2] Nevertheless, the first phase-3 dupilumab trials also showed low rates of DAOSD and in addition, phase-3 dupilumab trial data cannot be compared one-to-one with the phase-3 tralokinumab trial data , since no head-to-head study is yet performed. Due to the more specific working mechanism of tralokinumab compared to dupilumab, it can be hypothesized that patients with DAOSD might benefit from switching to tralokinumab. Therefore, we compared ocular symptoms, ocular inflammation, and ophthalmic medication use in AD patients with DAOSD who were subsequently treated with tralokinumab.

This prospective, monocentric, observational case series included four patients with moderate-to-severe AD (median age 40.0 years (interquartile range (IQR) 27.3-66.3), 50% male (n=2/4)) treated at the University Medical Center Utrecht between March 2020 and December 2022. Patients discontinued dupilumab treatment due to development of DAOSD and were switched to tralokinumab. Informed consent was provided, and this study was considered as non-interventional by the Medical Research Ethics Committee. Patients were examined by both a dermatologist and ophthalmologist prior to the start, after 4, and after 28 weeks of treatment with dupilumab and tralokinumab following a standardized protocol, in which presence of OSD symptoms (red eyes, watery eyes, pruritus, pain, photophobia, and burning sensation) was evaluated. If needed – i.e. in case of new-onset OSD or worsening of pre-existent OSD, and after starting ophthalmic treatment for OSD –, additional ophthalmologic visits were performed. The Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score, focusing on the eyelids, conjunctiva (both bulbar and tarsal), and the limbus, was assessed at all ophthalmological visits by the same ophthalmologist.[3] Higher UTOPIA scores reflect more severe OSD, as described previously.[3] The UTOPIA score of the most affected eye was used for analyses. Additionally, past or current use of ophthalmic medication use was reported at each visit. The patients characteristics are available via https://zenodo.org/record/7509282.

Median Eczema Area and Severity Index (EASI) before initiation of dupilumab treatment were higher compared to EASI before starting tralokinumab treatment (21.6 (IQR 5.7-26.7) vs. 4.6 (IQR 1.2-7.3, respectively)), which can be explained by the fact that all patients started treatment with tralokinumab in the wash-out period of dupilumab. Although EASI was slightly higher at week 28 when treated with tralokinumab compared to dupilumab (Table 1), patients were satisfied with the effect of treatment on the AD. Additionally, at week 28 of tralokinumab treatment, less OSD symptoms were reported compared with dupilumab treatment in patients 2 and 4 (Table 1). During the course of tralokinumab treatment, UTOPIA scores decreased in patients 1, 2 and 3, indicating that ocular inflammation improved after switching from dupilumab to tralokinumab (Figure 1). However, patient 1 developed DAOSD after a longer period of treatment with dupilumab, suggesting that tralokinumab-associated OSD (TAOSD) may occur later. Nevertheless, patient 1 was able to taper down the frequency of hydrocortisone eye drops to once daily leading to controlled ocular inflammation. Patient 2 showed clear improvement in ocular inflammation during treatment with tralokinumab, which was achieved despite discontinuation of ophthalmic medication. Patient 3 showed an improvement in his UTOPIA score during dupilumab treatment by using dexamethasone eye drops twice daily. However, this patient switched to tralokinumab treatment because of fear of long-term side effects of high dose dexamethasone. Patient 3 still required dexamethasone eye drops during treatment with tralokinumab, although less frequently than during treatment with dupilumab. In patient 4, the UTOPIA score increased during the first weeks of treatment with tralokinumab, after which an almost equal UTOPIA score was achieved with the same ocular therapy as during treatment with dupilumab (Figure 1, Table 1).

TABLE 1. Patient characteristics during dupilumab and tralokinumab treatment at baseline and week 28

	Dupilumab treatment		Tralokinumab treatment	
	Baseline	**Week 28**	**Baseline**	**Week 28**
Patient 1, ♀, age 27 years				
UTOPIA score	0	1‡	5	3
EASI score	16.7	2.4	3.2	3.8
Number of symptoms	0/6	1/6	2/6	4/6
Ophthalmic medication use	None	-Tacrolimus skin ointment for external eyelids as needed	-Hydrocortisone eye drops 1/day ODS -Lubricant eye drops as needed	-Hydrocortisone eye drops 1/day ODS -Lubricant eye drops as needed
Patient 2, ♂, age 52 years				
UTOPIA score	5	8	9	3
EASI score	26.7	4.2	6.0	7.1
Number of symptoms	3/6	4/6	5/6	2/6
Ophthalmic medication use	None	-Standard treatment† -Hydrocortisone eye drops 2/day ODS	-Dexamethasone eye drops 3/day ODS -Ketotifen eye drops 2/day ODS	-Lubricant eye ointment as needed
Patient 3, ♂, age 71 years				
UTOPIA score	12	2	7	3
EASI score	26.5	0.9	0.5	1.2
Number of symptoms	5/6	1/6	0/6	1/6
Ophthalmic medication use	-Hydrocortisone, oxytetracycline and polymyxine B eye ointment 1/day ODS	-Dexamethasone eye drops 2/day ODS	-Dexamethasone eye drops 2/day ODS	-Dexamethasone eye drops 1/day ODS
Patient 4, ♀, age 28 years				
UTOPIA score	5	8	6	7
EASI score	2.1§	6.4	7.7	8.1
Number of symptoms	4/6	5/6	4/6	3/6
Ophthalmic medication use	-Lubricant eye ointment ante noctem	-Standard treatment†	-Standard treatment† -Hydrocortisone, oxytetracycline and polymyxine B eye ointment 2/day ODS	-Standard treatment†

UTOPIA score of the most severely affected eye is reported. † Standard treatment includes tacrolimus skin ointment for the external eyelids 1/day, ketotifen eye drops 2/day, and lubricant eye drops as necessary. ‡ Patient 1 developed DAOSD after 1 year and 6 months of dupilumab treatment (UTOPIA score 8). For that reason, dupilumab treatment was switched to tralokinumab therapy.§ Patient 4 started with dupilumab treatment despite having an EASI of 2.1 since the use of intensive topical corticosteroid therapy could not be reduced and ciclosporin A was contra-indicated due to severe side effects during previous use.
Abbreviations: EASI = Eczema and Area Severity Index; IGA = Investigator Global Assessment; ODS = Oculus Dexter et Sinister/Right and Left eye; OS = Oculus Sinister/Left eye.

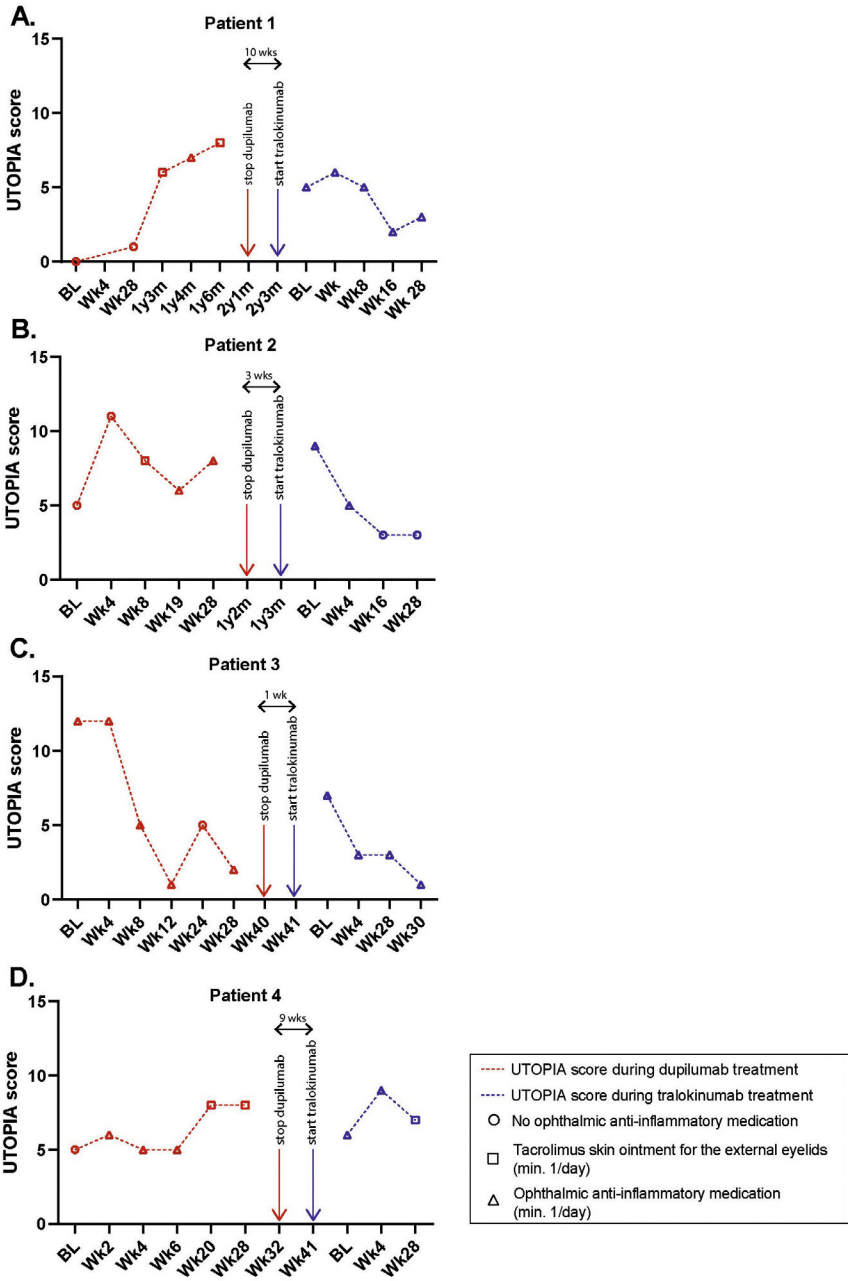

FIGURE 1. Ocular inflammation based on the Utrecht Ophthalmic Inflammatory and Allergic Disease (UTOPIA) score during dupilumab and tralokinumab treatment.
An open dot or triangle indicates the use of anti-inflammatory ophthalmic treatment (tacrolimus skin ointment for the external eyelids, steroid eye drops, or steroid eye ointment) at the marked time-point. UTOPIA score of the most severely affected eye is reported. Abbreviations: UTOPIA = Utrecht Ophthalmic Inflammatory and Allergic disease score; wk = week; y = year.

Our results suggest that some patients with DAOSD may benefit from switching to tralokinumab as fewer symptoms were reported and less ocular inflammation (i.e. lower UTOPIA scores) was observed in 3 out of 4 cases. Also, less ophthalmic steroids were needed during tralokinumab treatment in all four patients, leading to a lower risk of corticosteroid-induced glaucoma and cataract.[4]

As it is known that severe chronic DAOSD with involvement of the limbus may lead to irreversible limbal stem cell deficiency requiring long-term follow-up, this could also apply to TAOSD.[5] Therefore, physicians should not be reluctant to prescribe anti-inflammatory ophthalmic medication. As we recently described that 90% of the moderate-to-severe AD patients already have OSD before starting treatment, it is important to monitor patients ophthalmologically from the start of treatment with dupilumab or tralokinumab in order to distinguish between DAOSD/TAOSD and AD-associated OSD.[6]

It has been hypothesized that scarcity of goblet cells (GC) may play a role in the development of DAOSD.[7] As results of a recent in-vitro study suggested that the proliferation of GCs is influenced by both IL-4 and IL-13, less TAOSD may develop due to the more specific working mechanism of tralokinumab by affecting IL-13 signalling only.[8] In addition, the dual blockade of IL-4 and IL-13 signalling by dupilumab could lead to Th1/Th17 skewing, which is thought to contribute to the development of DAOSD as well.[1]

This study has some limitations. First, all patients were in their dupilumab wash-out period when starting tralokinumab, making it more difficult to distinguish between DAOSD and TAOSD at the early time points. However, the effect of tralokinumab monotherapy on ocular inflammation could still be evaluated due to the follow-up visits. As this study only examined four patients, larger comparable studies are needed to verify our results.

In conclusion, this prospective case series showed that some patients with DAOSD may benefit from switching from dupilumab to tralokinumab treatment.

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SUPPLEMENTARY

TABLE S1. Patient characteristics

	Patient 1	Patient 2	Patient 3	Patient 4
Gender	Female	Male	Male	Female
Age at start of treatment with dupilumab (years)	27	52	71	28
Onset of AD	Childhood	Childhood	Childhood	Childhood
Other atopic diseases	AA+; AR+; AC+; FA+	AA+; AR+; AC+; FA+	AA+; AR+; AC+; FA-	AA+; AR+; AC+; FA+
Previous history of eye diseases	-	Tear duct surgery ODS	Ectropion OS Cataract extraction with lens implantation ODS	Corneal erosion OS
EASI at start of dupilumab	16.7	26.7	26.5	2.1[§]
EASI at start of tralokinumab	3.2	6.0	0.5	7.7

[§] Patient 4 started with dupilumab treatment despite having an EASI of 2.1 since the use of intensive topical corticosteroid therapy could not be reduced and ciclosporin A was contra-indicated due to severe side effects during previous use.

Abbreviations: AA = Allergic Asthma; AR = Allergic Rhinitis; AC = Allergic Conjunctivitis ; EASI = Eczema Area Severity Index score; FA = Food Allergy; + = present, - = not present; OS = Oculus Sinister/Left eye; ODS = Oculus Dexter et Sinister/Right and Left eye

CHAPTER 7

Non-infectious uveitis secondary to dupilumab treatment in atopic dermatitis patients shows a pro-inflammatory molecular profile

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ABSTRACT

Severe uveitis is a rare complication of interleukin-4 receptor alpha blocking by dupilumab in atopic dermatitis (AD) patients. The aim of this study was to describe a case series of five moderate-to-severe AD patients who developed uveitis during dupilumab treatment, and to compare the proteomic profile of aqueous humor (AqH) of dupilumab-associated uveitis (n=3/5 available samples) with non-infectious uveitis (n=27) and cataract controls (n=11). Included patients were treated at the University Medical Center Utrecht (the Netherlands). Active dupilumab-associated uveitis complicated by serous detachment, cystoid macular edema, or secondary glaucoma developed within a median of 6.0 months (interquartile range 2.3-16.5 months) after starting dupilumab. Uveitis resolved after discontinuation of dupilumab and/or treatment with local or systemic corticosteroids. Proteomic profiling of AqH revealed that the molecular profile of dupilumab-associated uveitis resembled that of non-infectious uveitis. In conclusion, dupilumab-associated uveitis is a severe adverse event of dupilumab therapy, requiring urgent referral to an ophthalmologist.

INTRODUCTION

Dupilumab, a monoclonal antibody inhibiting interleukin (IL)-4 and IL-13 signaling by targeting the alpha subunit of the IL-4 receptor (IL-4Rα), is a biological used for the treatment of patients with moderate-to-severe atopic dermatitis (AD). Clinical trials and daily practice studies have proven dupilumab to be a highly effective and safe intervention for AD.[1,2] Despite its unprecedented success in controlling skin inflammation, in rare cases, dupilumab treatment can cause severe vision-threating intraocular inflammation, which is clinically highly reminiscent of non-infectious uveitis.[3-5] However, the underlying disease mechanisms of uveitis secondary to dupilumab therapy remain uncharacterized. Here, we described the characteristics of AD patients who developed uveitis during dupilumab treatment and compared the molecular profile of aqueous humor (AqH) by targeted proteomics of AD patients who developed uveitis during dupilumab treatment with a cohort of non-infectious uveitis patients and non-inflammatory cataract controls.

MATERIALS AND METHODS

This retrospective study included dupilumab-treated moderate-to-severe AD patients that visited the departments of Ophthalmology and Dermatology at the University Medical Center Utrecht (UMCU) (the Netherlands) between November 2016 and July 2021. Each patient started with 600mg dupilumab subcutaneously, followed by biweekly injections of 300mg. Clinical data were obtained from the electronic medical records. Human leucocyte antigen B27 (HLA-B27) typing was conducted in patients as part of the routine diagnostic work-up for uveitis. All patients provided informed consent.

To investigate the underlying immune pathways in dupilumab-associated uveitis, we used the available remainders of AqH from AD patients that were obtained by diagnostic anterior chamber paracentesis to exclude infectious causes of uveitis by PCR. Remainders of AqH were available from only three of the included patients. We compared these to AqH samples that were also remainders of diagnostic procedures from 27 treatment-free non-infectious uveitis patients (diagnosis by SUN criteria[6]) and 11 cataract controls (without history of inflammatory eye disease).

For targeted proteomics of the ocular microenvironment, AqH was subjected to proximity extension assay (PEA) technology.[7,8] Ninety-two proteins of the Immuno-oncology panel (Olink Proteomics) were measured according to the manufactures instruction. Proteins with a limit of detection warning were excluded. In short, PEA technology makes use of antibody sets that are linked with matching DNA-oligonucleotides per protein of interest. These oligonucleotides hybridize when brought into proximity after binding the protein and are extended by DNA

polymerase, thereby forming PCR targets. The PCR targets are quantified by real-time PCR. Obtained protein concentrations are expressed in normalized protein expression (NPX) values, which are in a log2 scale.

Bonferroni adjusted *P* values (Padj) from Likelihood ratio tests were used to test differences within the three groups. Principal component analysis was conducted in R (version 4.0.3) using the *factoextra* package.[9] Pathway enrichment analysis were conducted by *clusterProfiler R* package[10] using Reactome as a reference database.[11]

RESULTS

Between November 2016 and July 2021, a total of 522 moderate-to-severe AD patients were treated with dupilumab at the UMCU (the Netherlands). Five adult AD patients (n=5/522, 1%) developed uveitis during dupilumab treatment and were included in this study (Table 1). All cases had AD onset at (early) childhood and were treated with dupilumab because of uncontrolled and persistent moderate-to-severe AD. None of the patients had a history of inflammatory eye disease. All cases were HLA-B27-negative, except for patient 1 who also used adalimumab for treatment of ankylosing spondylitis at the time of sampling. Active uveitis (according to the SUN criteria[6]) developed within 2 weeks to 24 months (average 8.5 months) after starting dupilumab and was complicated by serous detachment reminiscent of *Vogt-Koyanagi-Harada* (VKH) syndrome (Figure 1, Patient 3), cystoid macular edema (Patient 5), or secondary glaucoma (Patient 4 and 5). Dupilumab treatment was discontinued because of uveitis severity in four cases. Uveitis resolved in all cases after successful treatment with corticosteroid eye drops and/or peri-ocular steroids injection and/or systemic corticosteroids (e.g. oral corticosteroids, intravenous corticosteroids).

Proteomic profiling of AqH available from patient 1, 3 and 4 was conducted and compared to AqH samples from 27 treatment-free patients with active non-infectious uveitis (HLA-B27 positive in n= 9/27, 33%), and 11 cataract controls (without history of inflammatory eye disease, Table S1). We detected 77 (out of 92 profiled, 15 were excluded because they were below the limit of detection) immune mediators in AqH (Table S2). Note that IL-4 and IL-13 concentrations were too low to be measured (i.e. below the quantification threshold) in all patient groups. Principal component analysis of the 77 proteins revealed that the dupilumab-associated uveitis patients clustered away from cataract controls, but together with the non-infectious uveitis patients, indicating an inflammatory profile more comparable to non-infectious uveitis (Figure 2A). Pathway enrichment analysis using *Reactome* of the 35 different significantly expressed immune mediators between dupilumab-associated uveitis and the other groups (*Padj* <0.05) showed an enrichment for the *"Interleukin-4 and Interleukin-13*

signaling" pathway (*Padj*= 3.7 x 10^{-11}) (Figure 2B).[11,12] Many pro-inflammatory proteins, such as IP10/CXCL10, TNFRSF21, CCL17, TNFRSF9/CD137, IL-6, and IL-8 were increased in both non-infectious uveitis and dupilumab-associated uveitis compared to cataract controls (Table S2 and Figure 2C).

TABLE 1. Patient characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	Male	Female	Male	Male	Female
Age, years	46	32	42	56	51
EASI at start of dupilumab	9.3	12	52.8	13.8	21.6
Onset of AD	Baby age	Childhood	Childhood	Baby age	Baby age
Other atopic diseases	AA+; AR+; AC+; FA-	AA+; AR+; AC+; FA-	AA-; AR-; AC+; FA-	AA+; AR+; AC+; FA-	AA+; AR+; AC+; FA+
Previous history of eye diseases	None	None	Blind left eye (unknown cause)	Multiple corneal scars due to AD	Cataract surgery ODS, chronic allergic conjunctivitis ODS
Previous relevant history	2012 Ankylosing spondylitis	Epilepsy Hypertension	None	During dupilumab treatment glaucoma OD	None
Relevant medication at start dupilumab	Adalimumab 40mg/ 2 weeks	Lacosamide 50mg Amlodipine 5mg	None	None	Ketotifen ODS 2x/day, oculotect unidose ODS
Duration of dupilumab treatment before the occurrence of the adverse event	0.5 months	9 months	6 months	24 months	CME after 4 months
Adverse event	Acute anterior uveitis OD	Anterior uveitis OS	Pan uveitis ODS (like in VKH syndrome)	Anterior uveitis OD	Anterior uveitis with CME and severe secondary glaucoma
Laboratory tests	HLA-B27: positive ANA: negative	HLA-B27: negative ANA: negative ACE: normal	HLA-B27 negative ANA: bordeline	HLA-B27 negative ANA: negative	HLA-B27 negative ANA: negative

TABLE 1. (Continued)

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Corticosteroid treatment of the adverse event	Topical and systemic (oral)	Topical	Systemic (intravenous) and oral mycophenolate mofil	Systemic (oral)	No steroids
Dupilumab treatment	Discontinued	Dosage interval prolongation to 300mg every 3 weeks	Discontinued	Discontinued	Discontinued
Outcome of the adverse event	Resolved	Resolved	Resolved	Resolving	Stable

Abbreviations: AA = Allergic Asthma; AR = Allergic Rhinitis; AC = Allergic Conjunctivitis ; ANA = Antinuclear Antibody; CME = Cystoid Macular Edema; CMV = Cytomegalovirus; EASI = Eczema Area Severity Index score; FA = Food Allergy; HLA-B27 = Human Leukocyte Antigen – B27; VKH = Vogt-Koyanagi-Harada syndrome

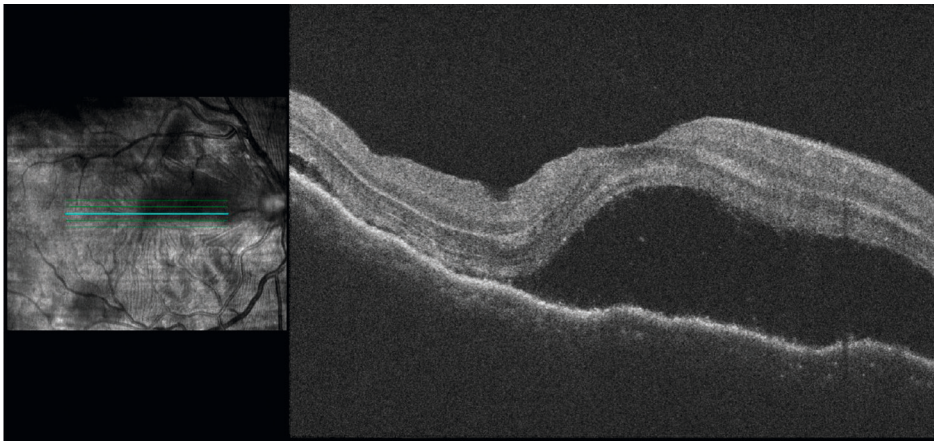

FIGURE 1. Optical coherence tomography image of patient 3.
A serous detachment is visible in the macular area.

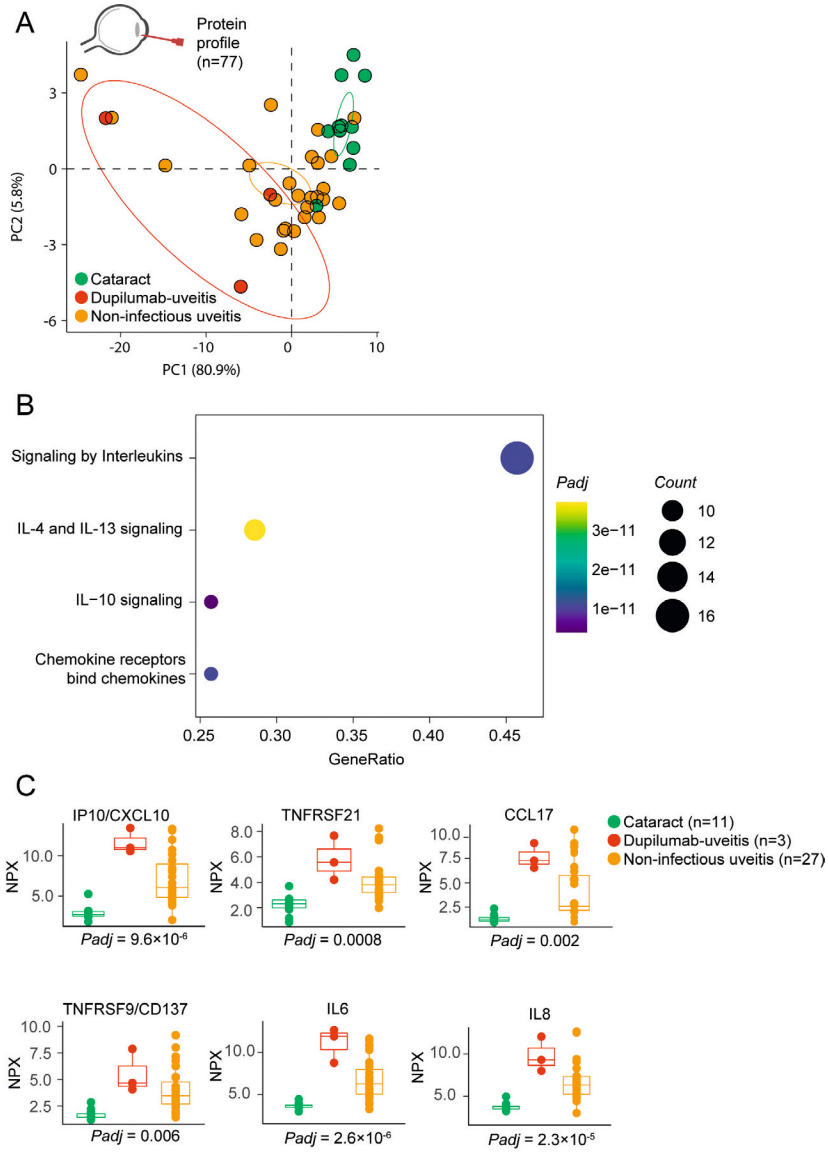

FIGURE 2. Molecular profile of aqueous humor of dupilumab-associated uveitis.
A. Principal component analysis of 77 detectable immune mediators in aqueous humor (AqH) from dupilumab-associated uveitis patients, patients with non-infectious uveitis, and controls with cataract (without history of inflammatory eye disease). The ellipses indicate the 95% confidence interval **B.** Pathway enrichment analysis of the 35 different significantly expressed immune mediators between dupilumab-associated uveitis and the other groups (*Padj* <0.05). **C.** Scatterplots for T-cell related immune mediators IP10/CXCL10, TNFRSF21, CCL17, TNFRSF9/CD137, IL-6, and IL-8. The NPX indicates the normalized protein expression data from *Olink*. The Bonferroni-adjusted P values from a likelihood ratio test are shown (see also Table S2). Abbreviations: CCL17 = C-C Motif Chemokine Ligand 17, IL = interleukin, IP10/CXCL10 = C-X-C motif chemokine ligand 10 (CXCL10) also known as Interferon gamma-induced protein 10 (IP-10), TNFRSF9/CD137 = tumor necrosis factor receptor superfamily member 9 or CD137, TNFRSF21 = Death receptor 6, also known as tumor necrosis factor receptor superfamily member 21.

DISCUSSION

This retrospective study shows that uveitis can occur as an adverse event of dupilumab treatment in AD patients and can be complicated by serous detachment reminiscent of VKH syndrome, cystoid macular edema, or secondary glaucoma. To the best of our knowledge, this is the first study that investigated the proteomic profiling of AqH of dupilumab-associated uveitis and compared these data with non-infectious uveitis and cataract patients. Proteomic profiling of AqH supported a severe intraocular inflammatory profile in the included AD patients remarkably similar to non-infectious uveitis.

AD does not predispose to non-infectious uveitis, but is associated with conjunctivitis and eyelid dermatitis.[13] Safety trials of dupilumab did not report on the development of uveitis as adverse event.[1] Although there is limited information available to determine the incidence of dupilumab-associated uveitis, we found that only 1% (n=5/522) of the dupilumab-treated AD patients in the UMCU developed uveitis, suggesting it is most likely a rare complication. Two patients had possible predisposing factors for the development of uveitis, which were HLA-B27-positive ankylosing spondylitis and VKH syndrome. To date, reports on dupilumab-associated uveitis have been limited to few case reports and small case series. In these cases, uveitis typically resolved after discontinuation of dupilumab, in line with our observations.[3-5]

While the exact disease mechanism of dupilumab-associated uveitis remains to be elucidated, previous work proposed that upregulation of interferon-gamma (IFN-γ) and the Th1 response may be associated with its development.[3] The immunologic profile of AD is mediated by IL-4 and IL-13 signaling, which is supported by the high success of dupilumab in treating AD.[1,2] In contrast, IL-4 and IL-13 alleviate eye inflammation in experimental animal models of uveitis, suggesting that IL-4/IL-13 signaling antagonizes uveitogenic pathways.[14,15] This is supported by the fact that cytokines such as IL-4 and IL-13 antagonize the expression of IFN-γ and TNF-alpha, which are cytokines associated with non-infectious uveitis.[16,17] Therefore, blocking IL-4 and IL-13 signaling by dupilumab may promote non-infectious uveitis in genetically predisposed individuals. In agreement with this, in our study, the levels of IFN-γ and TNF-alpha were highest in dupilumab-associated uveitis, but this difference did not reach statistical significance. As a result of dupilumab-associated uveitis, other cytokines classically associated with Th1 responses were significantly elevated, including interferon gamma-induced protein 10 (CXCL10) and the Th1-skewing cytokine IL-12, suggesting Th1-favorable conditions (Table S2). However, many other cytokines also showed significant elevations, suggesting that complex immune responses may result from signaling between networks of cytokines, involving multiple types of immune cells, as recently demonstrated by single-cell transcriptomics (scRNAseq) of non-infectious

uveitis.[18] For further research on this enigmatic eye disease, scRNAseq analyses of eye fluid biopsies of patients with dupilumab-associated uveitis will provide the required insight into the composition of the immune cells involved.

Based on this study, we conclude that uveitis can occur as a rare adverse event of dupilumab treatment in AD patients. Clinicians treating dupilumab-treated AD patients should be aware of uveitis secondary to dupilumab as a rare but severe adverse event. Urgent referral to an ophthalmologist is needed if patients develop symptoms of uveitis during dupilumab therapy.

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SUPPLEMENTARY

TABLE S1. Demographics of non-infectious uveitis controls and cataract controls

	Non-infectious Uveitis (n=27)	Cataract Controls (n=11)
Age, years, median (IQR)	42.5 (31.0-54.0)	73.0 (64.0-79.0)
Men, n (%)	11 (40.7)	4 (36.4)

TABLE S2. Median and interquartile ranges (IQR) of all proteins measured in eye fluid (aqueous humor) for each disease group. Obtained results are expressed in normalized protein expression (output Olink proteomic platform).

	Cataract controls (n=11)	Non-infectious uveitis (n=27)	Dupilumab uveitis (n=3)	Bonferroni adjusted *P* value from Likelihood ratio test
IL8	2.48 (2.07-2.63)	5.68 (4.22-6.97)	9.3 (7.71-12.67)	2.24×10^{-05}
TNFRSF9	1.46 (1.44-1.95)	3.46 (2.67-5.17)	4.66 (4.07-7.87)	0.006484
TIE2	1.99 (1.5-2.25)	2.53 (1.77-3.03)	4.22 (2.54-6.46)	0.822172
MCP-3	1.76 (1.4-2.11)	2.18 (1.78-4.9)	5.2 (2.65-10.86)	0.821555
CD40-L	2.06 (1.71-2.48)	2.29 (2.1-2.47)	2.26 (2.05-2.63)	1
CD244	2.09 (1.73-2.42)	2.47 (2.16-3.12)	3.55 (2.32-5.18)	1
EGF	0.76 (0.42-0.9)	0.93 (0.6-1.08)	1.1 (0.83-1.85)	1
ANGPT1	0.46 (0.04-0.59)	0.89 (0.51-1.58)	1.44 (0.94-4.29)	0.219268
IL7	3.36 (2.91-3.82)	3.95 (3.19-5.02)	5.06 (3.01-6.44)	1
PGF	3.08 (2.84-3.53)	5.96 (4.62-7.51)	8.81 (5.7-11.67)	4.65×10^{-05}
IL6	2.66 (2.43-2.78)	5.73 (4.14-8.16)	12.31 (8.62-13.19)	2.55×10^{-06}
MCP-1	10.55 (8.85-10.9)	11.72 (11.09-12.54)	13.92 (12.42-14.18)	1.53×10^{-06}
CRTAM	1.97 (1.89-2.1)	2.22 (1.88-2.6)	2.36 (2.12-5.28)	1
CXCL11	1.23 (1.09-1.34)	1.71 (1.28-3.12)	3.44 (2.82-10.22)	1
MCP-4	1.72 (1.5-1.82)	2.66 (1.9-6.31)	4.88 (3.57-11.43)	0.434039
TRAIL	1.73 (1.5-1.99)	2.48 (2.13-2.98)	4.31 (2.67-7.43)	0.320447
CXCL9	2.66 (2.38-3.18)	4.45 (3.53-6.81)	7.49 (5.9-12.3)	0.020634
CD8A	1.82 (1.61-1.89)	2.19 (1.84-3.49)	4.14 (2.64-9.4)	1
CAIX	1.49 (1.26-1.87)	2.64 (1.91-3.35)	4.7 (3.93-8.42)	0.015895
MUC-16	1.24 (1.03-1.37)	1.37 (1.13-1.57)	1.86 (1.63-3.62)	1
ADA	1.61 (1.54-1.8)	2.34 (2.08-3.53)	4.43 (3.39-6.61)	0.035239
CD4	1.83 (1.52-2.26)	2.26 (1.86-2.73)	2.75 (2.5-4.77)	1
Gal-9	3.77 (3.47-3.88)	5.43 (5.03-6.39)	7.4 (6.57-9.49)	4.52×10^{-06}
VEGFR-2	3.07 (2.76-3.21)	4.49 (3.98-5.02)	6.14 (4.86-6.76)	4.2×10^{-05}
CD40	4.24 (3.88-4.62)	5.91 (5.51-7.01)	7.75 (7.04-11.19)	0.000729
IL18	1.87 (1.71-2.22)	3.69 (2.77-4.72)	6.11 (4.28-8.86)	0.005646
GZMH	1.68 (1.43-1.76)	1.88 (1.62-2.57)	2.37 (1.42-6.62)	1

TABLE S2. (Continued)

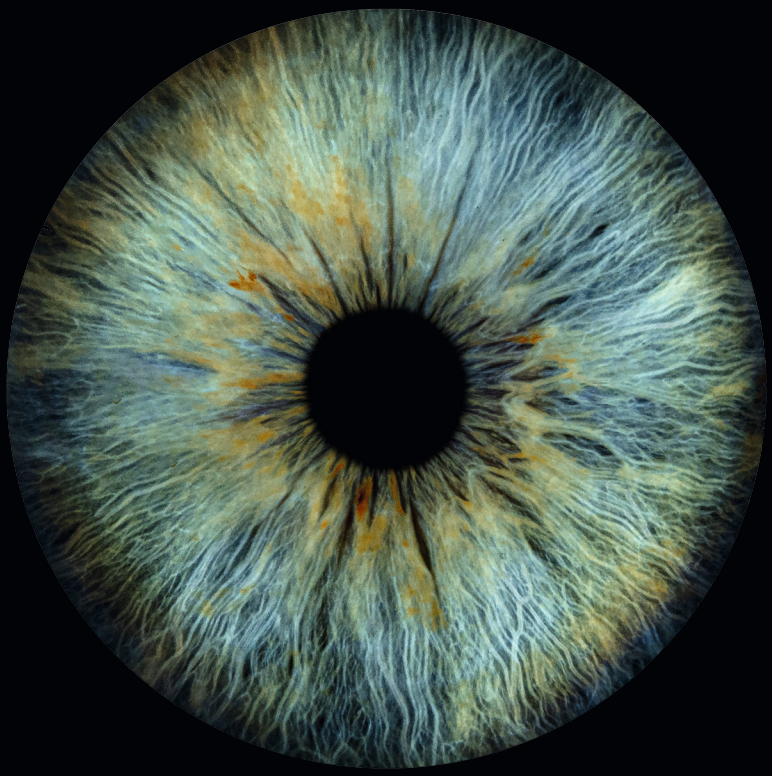
	Cataract controls (n=11)	Non-infectious uveitis (n=27)	Dupilumab uveitis (n=3)	Bonferroni adjusted *P* value from Likelihood ratio test
LAP TGF-beta-1	2.35 (1.88-2.61)	3.59 (3.04-4.47)	6.12 (4.22-8.62)	0.010663
CXCL1	2.09 (1.92-2.59)	5.03 (3.59-5.94)	7.78 (7.48-13.44)	0.000588
TNFSF14	1.99 (1.65-2.5)	2.56 (2.21-2.95)	3.37 (2.68-6.17)	1
TWEAK	7.73 (7.12-7.94)	8.52 (8.11-8.85)	8.71 (8.63-9.02)	4.74×10^{-06}
PDGF subunit B	0.07 (0.01-0.21)	0.59 (0.28-1.08)	1.1 (0.94-2.4)	0.194346
PDCD1	1.67 (1.52-1.93)	2.3 (1.95-2.97)	3.36 (2.34-5.57)	0.238754
FASLG	1.46 (1.29-1.54)	3.63 (2.5-5.09)	4.36 (2.8-8.05)	0.002224
CCL19	2.47 (2.21-2.83)	3.8 (3.08-6.61)	8.37 (5.71-11.81)	0.008045
MCP-2	1.91 (1.67-2.16)	3.64 (2.67-5.41)	6.5 (5.2-11.53)	0.071093
CCL4	2.24 (1.97-2.48)	3.53 (2.72-4.35)	6.2 (4.54-7.55)	0.119391
IL15	3.07 (2.66-3.48)	4.65 (4.04-5.57)	6.47 (5.54-8.01)	0.000291
Gal-1	2.46 (2.13-2.59)	3.1 (2.38-4.68)	5.94 (3.46-7.52)	0.096302
PD-L1	2.63 (2.22-2.77)	3.61 (3.38-4.8)	5.06 (5.05-9.74)	0.024566
CD27	1.93 (1.74-2.36)	5.18 (3.85-7.22)	4.86 (4.73-8.03)	8.42×10^{-06}
CXCL5	2.21 (1.59-2.44)	2.68 (2.04-4.26)	6.32 (3.33-9.83)	0.831444
HGF	6.18 (5.12-7.06)	8.75 (7.95-9.99)	11.29 (9.96-11.84)	1.62×10^{-07}
GZMA	2.17 (1.94-2.35)	4.67 (2.72-6.66)	4.61 (2.31-10.05)	0.050393
HO-1	1.92 (1.6-2.27)	4.05 (2.68-5.23)	8.82 (5.23-11.91)	0.004553
CX3CL1	0.68 (0.52-0.88)	0.96 (0.6-1.22)	1.4 (0.94-5.61)	1
CXCL10	2.56 (2.31-3.02)	5.99 (4.65-9.38)	10.98 (10.57-13.48)	9.61×10^{-06}
CD70	1.18 (1.01-1.24)	1.1 (1.02-1.5)	1.36 (1.28-4.37)	1
IL10	2.01 (1.94-2.18)	2.3 (2.03-2.55)	3.03 (2.83-6.3)	1
TNFRSF12A	1.27 (1.01-1.53)	2.38 (1.79-3.59)	5.08 (4.1-6.69)	0.000208
CCL23	1.36 (1.08-2.01)	3.62 (2.51-4.53)	6.34 (3.86-9.94)	0.048647
CD5	1.23 (1.05-1.39)	2.13 (1.49-3.97)	3.01 (2.96-7.09)	0.77478
CCL3	2.25 (1.92-2.76)	3.88 (2.94-5.06)	6.58 (5.19-8.44)	0.027155
MMP7	4.81 (3.28-6.17)	7.8 (6.46-8.68)	10.24 (6.95-10.5)	0.001273
NCR1	1.51 (1.07-1.65)	1.54 (1.37-1.76)	1.58 (1.38-4.35)	1
DCN	3.38 (1.96-4.51)	5 (4.54-5.3)	5.32 (4.97-5.42)	1.31×10^{-05}
TNFRSF21	2.32 (1.88-2.64)	3.82 (3.06-4.5)	5.57 (4.2-7.66)	0.000835
TNFRSF4	2.92 (2.42-3.13)	3.39 (3.04-3.88)	4.42 (3.77-6.68)	0.793328
MIC-A/B	2.2 (1.96-2.42)	2.84 (2.3-3.91)	1.63 (1.55-5.6)	1
CCL17	1.26 (1.07-1.46)	2.58 (2.17-6.17)	7.3 (6.56-9.1)	0.002128
ANGPT2	1.55 (1.41-1.68)	2.01 (1.66-2.7)	4.81 (2.8-7.87)	0.293033
IFN-gamma	2.03 (1.74-2.29)	6.05 (2.18-8.9)	7.22 (5.39-16.99)	0.134632
LAMP3	1.96 (1.55-2.18)	2.01 (1.78-2.3)	2.48 (2.22-4.25)	1

TABLE S2. (Continued)

	Cataract controls (n=11)	Non-infectious uveitis (n=27)	Dupilumab uveitis (n=3)	Bonferroni adjusted *P* value from Likelihood ratio test
CASP-8	1.78 (1.63-2.03)	2.23 (1.91-3.24)	6.64 (3.51-9.28)	0.096735
ICOSLG	1.64 (1.47-1.81)	2.78 (2.09-3.58)	4 (3.22-6.17)	0.006889
MMP12	2.07 (1.55-2.57)	3.55 (2.6-5)	8.56 (2.88-8.85)	0.032112
CXCL13	1.92 (1.84-2.19)	3.42 (2.41-5.54)	7.87 (7.33-10.39)	0.001049
PD-L2	1.61 (1.53-1.81)	1.75 (1.54-1.95)	2.07 (1.73-4.3)	1
VEGFA	7 (6.52-7.51)	8.92 (7.91-9.86)	10.56 (8.66-12.91)	0.006396
LAG3	1.09 (0.96-1.41)	1.86 (1.28-2.53)	2.54 (1.99-6.61)	1
CCL20	2.28 (2.07-2.59)	2.9 (2.33-3.54)	7.84 (4.6-13.54)	0.159833
TNF	1.89 (1.73-2.07)	2.25 (1.93-2.92)	3.11 (2.46-11.86)	0.924308
KLRD1	1.24 (1.09-1.45)	1.68 (1.39-2.11)	2.43 (1.79-7.58)	0.717819
GZMB	1.21 (0.84-1.37)	1.51 (1.26-2.6)	1.92 (1.87-8.3)	1
CD83	1.41 (1.39-1.62)	1.83 (1.58-2.1)	2.54 (2.4-3.72)	0.165186
IL12	2.81 (2.45-3.17)	4.24 (3.62-5.35)	5.1 (4.99-8.34)	0.001145
CSF-1	6.2 (5.62-6.47)	6.9 (6.36-7.51)	8.47 (7.26-10.67)	0.056849

PART IV

The pathomechanism of dupilumab-associated ocular surface disease in moderate-to-severe atopic dermatitis patients



CHAPTER 8

High dupilumab levels in tear fluid of atopic dermatitis patients with moderate-to-severe ocular surface disease

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ABSTRACT

Background

The patho-mechanism of ocular surface disease (OSD) in dupilumab-treated atopic dermatitis (AD) patients remains unclear. The aim of this study is to measure dupilumab levels in tear fluid and serum, and relate these findings to the severity of OSD during dupilumab treatment in AD patients.

Methods

This prospective study included dupilumab-treated moderate-to-severe AD patients who were seen by a dermatologist and an ophthalmologist before the start of dupilumab (baseline), and after 4 and 28 weeks of dupilumab treatment. Dupilumab levels in tear fluid and serum were measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Additionally, a pilot study was conducted to measure dupilumab on conjunctival epithelial cells using flow cytometry and LC-MS/MS.

Results

At baseline, 89.6% (n=43/48) of the patients had OSD, with 50.0% having moderate-to-severe OSD. After 28 weeks of dupilumab treatment, the median dupilumab tear fluid levels were 0.55 mg/L (IQR 0.35-1.31) and 0.29 mg/L (IQR 0.16-0.60) in patients with moderate-to-severe OSD and patients with no or mild OSD, respectively (p=0.02). Dupilumab levels could be detected on conjunctival epithelial cells of 5 AD patients treated with dupilumab for 4 weeks.

Conclusion

Patients with moderate-to-severe OSD had higher dupilumab tear fluid levels compared to patients with no or mild OSD, indicating that dupilumab reaches the ocular surface. Dupilumab was also detected in conjunctival cell suspensions and was found to directly bind CD45- conjunctival epithelial cells. This suggests that AD-induced changes of the conjunctival epithelium may play a role in the development of OSD as well as increased local drug availability.

INTRODUCTION

Atopic dermatitis (AD) is a chronic, inflammatory, itchy skin disease with a prevalence up to 10% in adults.[1,2] The first biologic therapy that has been introduced to treat moderate-to-severe AD is dupilumab. Dupilumab is a fully human monoclonal IgG4 antibody that is directed against the interleukin (IL)-4 receptor-alpha (IL-4Rα) subunit, inhibiting the binding of IL-4 and IL-13.[3] It has proven its effectiveness in both clinical trials and daily practice studies.[3-5] The most frequently reported adverse event during dupilumab treatment in AD patients is dupilumab-associated ocular surface disease (DAOSD), which has been reported in up to 34% of the patients.[4-6] Remarkably, in dupilumab trials in other type-2 inflammatory diseases, such as asthma and chronic rhinosinusitis with nasal polyposis, no increased rates of DAOSD were reported.[6] Recently we found that 90% of the moderate-to-severe AD patients had clinical characteristics of ocular surface disease (OSD) before the start of dupilumab, suggesting that AD patients may have a predisposition to develop DAOSD.[7] It might be possible that this pre-existent OSD is aggravated during dupilumab treatment and is then diagnosed as DAOSD.

Several hypothesis are suggested to be responsible for the development of DAOSD, such as focal scarcity of conjunctival goblet cells (GCs). This might be a result of the IL-13 blocking effect, leading to reduced GC hyperplasia.[6,8] It is also hypothesised that DAOSD incidence may decrease in patients with higher serum dupilumab concentrations, and that local under-treatment of dupilumab in the eyes might play a role in the development of DAOSD.[6,9] At this moment, the exact patho-mechanism of DAOSD remains unclear.

To the best of our knowledge, no data are available on dupilumab levels in tear fluid of dupilumab-treated AD patients. The aim of this study was to measure dupilumab levels in tear fluid and serum, and relate this to the severity of OSD during dupilumab treatment in AD patients.

MATERIALS AND METHODS

Study design and patients

This prospective monocenter observational cohort study included adult patients with moderate-to-severe AD between February 2020 and September 2021 from the University Medical Center Utrecht (UMCU), the Netherlands. Included patients were not using systemic immunosuppressive therapies for at least two weeks prior to initiation of dupilumab treatment.

All patients were examined by a dermatologist and an ophthalmologist before the start of dupilumab (baseline), and after 4 and 28 weeks of dupilumab treatment. At baseline, a full ophthalmological examination was performed after which patients received a 600mg loading dose of dupilumab, followed by 300mg injections every other week. If patients developed symptoms of OSD and/or worsening of pre-existing OSD during dupilumab treatment, an extra ophthalmological examination was performed. In some patients, the week 4 or week 28 visit corresponded with the occurrence of OSD symptoms reported by patients and/or worsening of pre-existing OSD during dupilumab treatment. The ophthalmologist started OSD treatment if patients had signs and symptoms of OSD, which might have influenced the OSD severity during dupilumab treatment. Treatment options included tacrolimus skin ointment for the eyelids, eye drops (including lubricants, antihistaminic, and corticosteroids), or eye ointment (including lubricants and corticosteroids).The selected therapy depended on the severity of OSD. All patients provided written informed consent for this study that was approved by the Institutional Review Board of the UMCU.

Data collection

Dermatological and ophthalmological examination (baseline and during dupilumab treatment)

Clinical and dermatological data included patient characteristics, severity of AD based on the Eczema Area and Severity Index (EASI) and the Investigator's Global Assessment (IGA), and the presence or absence of other atopic comorbidities. In addition, ophthalmologic examination was performed according to the standardized Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score, focussing on the severity of the inflammation of the conjunctiva, both tarsal and bulbar, the eyelids, and the limbus.[10] An overall severity classification of no (UTOPIA score 0), mild (UTOPIA score 1-4), moderate (UTOPIA score 5-8), or severe OSD (UTOPIA score ≥9) was reported per patient per visit. Severity of OSD was based on the eye with the highest severity within a patient. During ophthalmological examination, topical anaesthesia eye drops (0.4% oxybuprocaine hydrochloride) were dripped in both eyes. Tear production was measured by a Schirmer's test.[11] Subsequently, Schirmer's strips were air-dried for at least 1 hour and stored at -80 °C until further analyses.

Processing of the Schirmer's strips

Schirmer's strips were eluted to obtain tear fluid for further processing. The elution buffer included PBS, Tween20 0.50%, BSA 1%, and 1 EDTA-free Protease Inhibitor Cocktail tablet (Sigma). Every Schirmer's strip was cut into small pieces and placed in one well of a Falcon plate. Next, 100 µl elution buffer was added per well, the plate was sealed and incubated over night at 4°C on a shaker (230 rpm). The next day, a quick spin down was done for 1 minute (1500 rpm). A MaxiSorp plate was taped under the

MultiScreen filter plate, and the supernatant was collected. A spin down at 2100g was conducted during 5 minutes for two times. The eluted tear fluid from both eyes were combined into a new Falcon plate used and the eluted tear fluid was stored at -80 °C until further analyses.

Measurement of dupilumab levels in tear fluid and serum
Dupilumab concentrations in both tear fluid and serum samples were measured with liquid chromatography tandem mass spectrometry (LC-MS/MS) according to our in-house developed method[12], with the following minor modifications for tear fluid measurements. In short, 10µL sample was pipetted in 1mL 96-well plate and 10µL SIL IFX was added followed by 10µL bovine serum and 70µL TRIS (50mM, pH 8, 0.5% OG). Then, 100µL saturated ammonium sulfate was added to each sample followed by 1 minute mixing at 1350 RPM. The 96 well plate was centrifuged at 4000 G for 5 minutes. After the supernatant was decanted the pellet was reduced, alkylated, digested and measured following our in-house developed method.[12]

Measurement of dupilumab on conjunctival epithelial cells
A pilot study was conducted to investigate the effect of dupilumab tear fluid levels on conjunctival cells. Conjunctival impression cytology (CIC) was collected from 3 control AD patients (not treated with dupilumab) and from 5 AD patients treated with dupilumab for 4 weeks. CIC was obtained as described previously, and stored in 100µL PBS (Sigma) at -80°C until further analysis.[7] LC-MS/MS was used to measure dupilumab in CIC suspensions.

Additionally, in 4 different AD patients that were being treated with dupilumab for 4 weeks, flow cytometry analysis of CIC (collected in PBS (Sigma) containing 0,05% (w/v) paraformaldehyde (Alfa Aesar)) was conducted. Within 3 weeks of sample collection, cells were extracted by gentle manual agitation using a 0.70µM easystrainer (Greiner Bio-One) for 2 minutes. Surface staining of CD45 AF700 and anti-IgG4-biotin, as a marker for dupilumab binding, was performed for 25 minutes at 4°C, followed by 25 minutes incubation of the second antibody streptavidin-APC at 4°C. Surface staining of IL-4Rα (CD124) PE was performed for 25 minutes at 37°C, using an optimization protocol after testing different temperatures. Data acquisition was performed on a FACS Fortessa flow cytometer (BD Biosciences) and data was analysed using FlowJo Software (Tree Star Inc.)

Statistical analysis

Patient characteristics were described using absolute numbers (N) and percentages for categorical variables and median with interquartile ranges (IQR) for non-normally distributed continuous variables. Differences in dupilumab levels between no or mild

OSD and moderate-to-severe OSD were calculated using the Mann-Whitney U test. Correlations between dupilumab levels in tear fluid and serum, and UTOPIA scores were described using Spearman's test. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were conducted with SPSS Statistics version 26.0.0.1 (IBM Corp. IBM SPSS Statistics for Windows). Figures were created by Prism (version 9.3.0 GraphPad Software).

RESULTS

Baseline results

Patient characteristics

A total of 48 moderate-to-severe dupilumab-treated AD patients were included (Table 1). Median baseline EASI score was 16.4 (IQR 10.9-21.8). Before start of dupilumab, mild, moderate, and severe OSD were reported in 19/48 (39.6%), 17/48 (35.4%), and 7/48 (14.6%) patients, respectively. Only 5/48 (10.4%) patients had no OSD at baseline.

Dupilumab levels in tear fluid and serum at week 4 and week 28

To investigate whether dupilumab is able to reach the ocular surface, we have measured dupilumab levels in tear fluid. At week 4 of dupilumab treatment, mild, moderate, and severe OSD were reported in 25/48 (52.1%), 12/48 (25.0%), and 4/48 (8.3%) patients, respectively. Data of 4 patients were missing due to COVID-19. After 4 weeks of dupilumab treatment, patients with moderate-to-severe OSD at week 4 had comparable dupilumab tear fluid levels to patients with no or mild OSD at week 4 (0.25 mg/L (IQR 0.19-0.61) vs. 0.22 mg/L (IQR 0.13-0.55), p=0.35) (Figure 1A, Table S1). No correlation was found between dupilumab tear fluid levels at week 4 and UTOPIA score at week 4 of dupilumab treatment (Spearman's correlation 0.238, p=0.124).

At week 28 of dupilumab treatment, mild, moderate, and severe OSD were reported in 25/48 (52.1%), 16/48 (33.3%), and 5/48 (10.4%) patients, respectively. After 28 weeks of dupilumab treatment, significant higher dupilumab tear fluid levels were found in patients with moderate-to-severe OSD at week 28 compared to patients with no or mild OSD at week 28 (0.55 mg/L (IQR 0.35-1.31) vs. 0.29 mg/L (IQR 0.16-0.60), respectively, p=0.02) (Figure 1B, Table S1). Additionally, a significant correlation between dupilumab tear fluid levels at week 28 and UTOPIA score at week 28 was found (Spearman's correlation 0.505, p<0.001).

TABLE 1. Patient characteristics at baseline

	Total cohort (n=48)
Age (years), median (IQR)	38 (27-48)
Men, n (%)	24 (50.0)
Age of onset of AD, n (%)	
Childhood	44 (91.7)
Adolescence	2 (4.2)
Adult	2 (4.2)
History of self-reported episodic acute allergic conjunctivitis, n (%)	39 (81.3)
Allergic asthma, n (%)	23 (47.9)
Allergic rhinitis, n (%)	36 (75.0)
Food allergy, n (%)	27 (56.3)
History of rosacea, n (%)	2 (4.2)
EASI score, median (IQR)	16.4 (10.9-21.8)
IGA score, median (IQR)	3 (3-4)
AD eyelid involvement in the past year, n (%)	32 (66.7)
AD facial involvement in the past year, n (%)	45 (93.8)
TARC (pg./ml), median (IQR)	1553 (802-2402)
Peripheral blood eosinophils (×109/L), median (IQR)	0.25 (0.15-0.44)
Eosinophilia (≥0.45×109/L), n (%)	11 (22.9)
Severity of OSD before the start of dupilumab†, n (%)	
No OSD	5 (10.4)
Mild OSD	19 (39.6)
Moderate OSD	17 (35.4)
Severe OSD	7 (14.6)

† Severity of OSD is based on eye with the highest severity within a patient.
Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IGA scale, Investigator's Global Assessment Scale; IQR, interquartile range; OSD, Ocular Surface Disease; TARC, thymus and activation-regulated chemokine

In contrast to dupilumab tear fluid levels, dupilumab serum levels after 4 weeks of dupilumab treatment were significantly lower in patients with moderate-to-severe OSD at week 4 compared to patients with no or mild OSD at week 4 (62.1 mg/L (IQR 52.0-77.0) vs. 79.4 mg/L (IQR 63.2-110.5), p=0.043) (Figure 1C, Table S1). After 28 weeks of treatment with dupilumab, no significant differences were found in dupilumab serum levels in patients with moderate-to-severe OSD at week 28 compared to patients with no or mild OSD at week 28 (Figure 1D, Table S1). Both at week 4 and week 28 of dupilumab treatment, no correlations were found between serum dupilumab levels

and UTOPIA scores (Spearman's correlation -0.286, p=0.066 at week 4 and Spearman's correlation 0.019, p=0.907 at week 28).

Dupilumab tear fluid levels in patients with new onset OSD or worsening of pre-existing OSD

Dupilumab levels in tear fluid at the onset of OSD (n=3, 1 missing sample) or in case of worsening of pre-existing OSD (n=14, 5 missing samples) were measured in 17 patients, samples of 6 patients were missing. Patients were divided into having mild OSD (n=3, 2 missing samples) or moderate-to-severe OSD (n=14, 4 missing samples). Significantly higher dupilumab levels were found in patients with moderate-to-severe OSD compared to patients with mild OSD (1.07 mg/L (IQR 0.28-2.69) vs. 0.16 mg/L (IQR 0.09-0.55), p=0.047) (Figure 1E, Table S1).

Taken together this demonstrates that although dupilumab serum levels are lower (at week 4) or similar (at week 28) in patients with moderate-to-severe OSD compared to patients with no or mild OSD, local dupilumab levels in tear fluid do increase with increasing OSD severity.

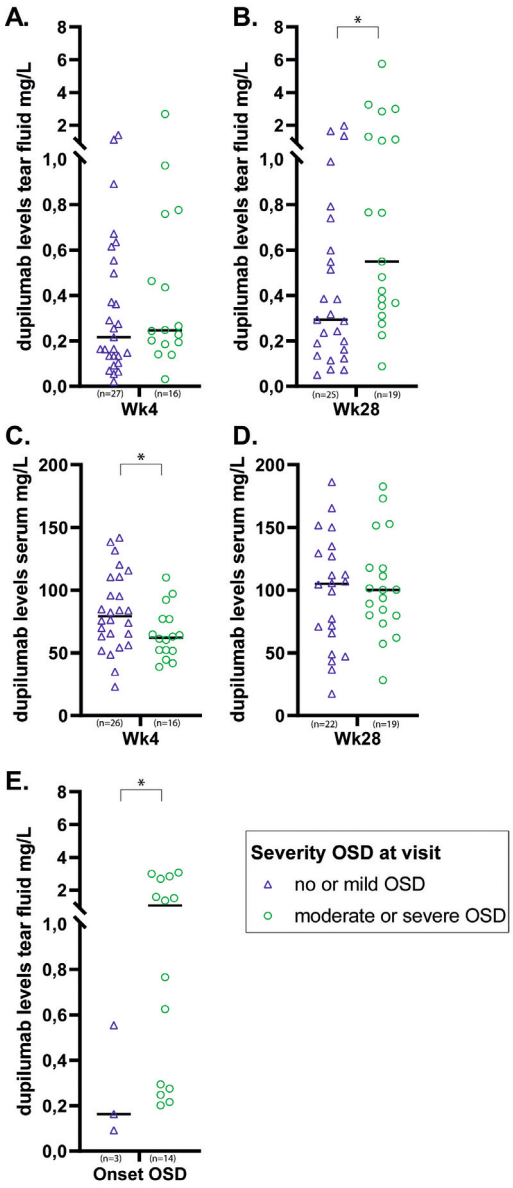

FIGURE 1. Dupilumab levels in tear fluid and serum.

Severity of ocular surface disease (OSD) is based on the moment of tear fluid collection, and on the eye with the highest severity within a patient. Bold lines display the median. Differences were calculated with Mann-Whitney U tests. **A**. Tear fluid dupilumab at week 4 of dupilumab (n=43), **B.** Tear fluid dupilumab at week 28 of dupilumab (n=44), **C**. Serum dupilumab at week 4 of dupilumab (n=42), **D**. Serum dupilumab at week 28 of dupilumab (n=41). **E.** Tear fluid dupilumab at the additional ophthalmological examination which was scheduled due to development of OSD symptoms and/or worsening of ocular inflammation (onset OSD or worsening of pre-existent OSD) (n=17, 6 samples are missing). * Indicates statistical significance.

Dupilumab on conjunctival cells

The presence of dupilumab in tear fluid does not prove its binding and potential direct biological effect on conjunctival cells. Therefore we investigated dupilumab binding on conjunctival cells in a pilot study by analysing CIC suspensions with LC-MS/MS and flow cytometry (Gating strategy in Figure S1, patient characteristics are shown in Table S2). Dupilumab levels could indeed be detected in the conjunctival cell suspensions of 5 AD patients treated with dupilumab for 4 weeks compared to 3 AD controls (Figure 2A).

Flow cytometry analysis confirmed IgG4 (dupilumab) binding on CD45- epithelial cells in 4 AD patients treated with dupilumab for 4 weeks (Figure 2B). Furthermore, Median Fluorescence Intensity (MFI) of IL-4Rα on CD45- epithelial cells decreased after 4 weeks of dupilumab treatment compared to MFI of IL-4Rα on CD45- epithelial cells at baseline (Figure 2C). These data indicate that dupilumab present at the ocular surface may have a direct effect on the conjunctival epithelium by blocking IL-4Rα.

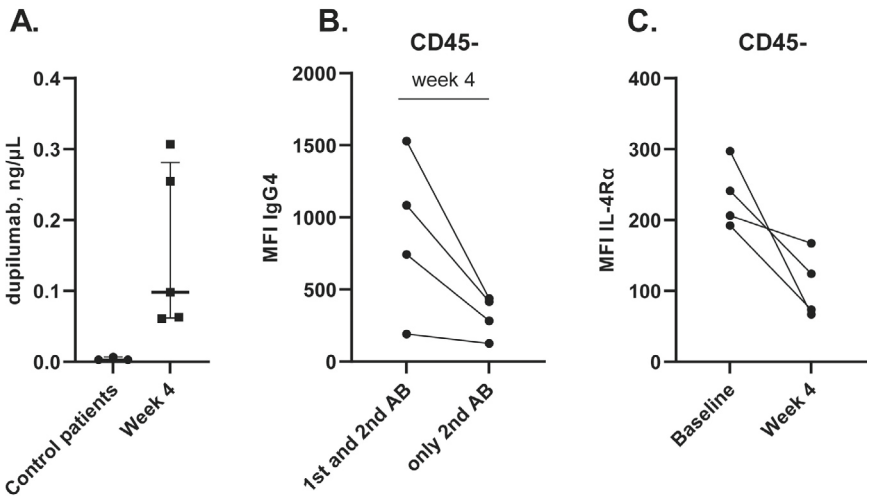

FIGURE 2. Dupilumab measured in conjunctival cells obtained by conjunctival impression cytology.

A. Dupilumab measured in conjunctival cell suspensions from 5 dupilumab-treated AD patients (at week 4) and 3 AD control patients (not treated with dupilumab) by using LC-MS/MS. **B**. Median Fluorescence Intensity (MFI) of IgG4 (= anti-dupilumab) in CD45- epithelial cells from 4 dupilumab-treated AD patients (at week 4) compared to the control staining including only the secondary antibody (AB) streptavidine-APC. **C**. MFI of IL-4Rα on CD45- cells from 4 AD patients before dupilumab treatment (baseline) and after 4 weeks of treatment.

DISCUSSION

This prospective study shows that OSD is very frequent in moderate-to-severe AD, and that dupilumab-treated AD patients with moderate-to-severe OSD had higher dupilumab tear fluid levels compared to patients with no or mild OSD during dupilumab treatment. Additionally, dupilumab was detected in conjunctival cell suspensions of five AD patients after 4 weeks of treatment with dupilumab.

At week 4 of dupilumab treatment, lower serum dupilumab levels were found in patients with moderate-to-severe OSD. However, the steady-state is achieved after 16 weeks of dupilumab treatment, which may explain the variation in the week 4 levels.[13] After 28 weeks of dupilumab treatment, dupilumab serum levels of patients with no or mild OSD at week 28 were similar to dupilumab serum levels of patients with moderate-to-severe OSD at week 28, and no correlation was found between serum dupilumab levels and UTOPIA scores at week 28. However, tear fluid dupilumab levels were higher in patients with moderate-to-severe OSD at week 28 of dupilumab treatment. These findings are in contradiction with the hypothesis that development of DAOSD is related to local under-treatment by dupilumab in the eyes, based on an inverse relationship between serum dupilumab levels and conjunctivitis.[9] Furthermore, the hypothesis of Akinlade et al.[6], who suggested that conjunctivitis incidence might decrease with higher dupilumab concentrations is also not in line with our findings. Based on our results, higher serum levels of dupilumab do not prevent development of OSD. This is also supported by our previous findings that prolongation of the dosing interval of dupilumab or discontinuation of dupilumab resulted in improvement of DAOSD.[10] In addition, we recently investigated dupilumab serum levels in moderate-to-severe AD patients after 16 weeks of treatment, and did not find an association between development of DAOSD and serum dupilumab levels.[14] However, our current study indicates that higher levels of dupilumab in tear fluid might be related to the severity of OSD during dupilumab treatment, and UTOPIA scores at week 28 significantly correlated with dupilumab tear fluid levels at week 28. It is well possible that pre-existent OSD increases local barrier permeability and dupilumab availability. This is supported by observations by Sebbag et al.[15] who investigated the impact of mild or severe conjunctivitis on lacrimal drug levels of oral prednisolone in six dogs. They reported a larger amount of prednisolone in eyes with conjunctivitis compared to control eyes, and found significantly higher levels of prednisolone in eyes of dogs with severe conjunctivitis compared to mild conjunctivitis. Sebagg et al.[15] suggested that increased permeability of conjunctival vessels due to conjunctivitis might lead to leakage of plasma constituents into the tear component, which is called the blood-tear barrier. Yokoi et al.[16] investigated the barrier function of the ocular surface epithelium in AD patients, and demonstrated impaired epithelium barrier function in the eyes of

patients with AD and blepharoconjunctivitis. These findings suggest that AD patients with moderate-to-severe OSD might have a disrupted blood-tear barrier, leading to significantly higher dupilumab tear fluid levels during treatment compared to patients with no or mild OSD. This leads to the question whether the increased dupilumab tear fluid levels affect local epithelial cell homeostasis and/or development.

We could detect dupilumab in conjunctival cell suspensions of dupilumab-treated AD patients after 4 weeks of treatment with dupilumab. Additional analysis showed IgG4 binding, indicative of dupilumab binding since dupilumab was also measured in the conjunctival cell suspensions, on CD45- epithelial cells after 4 weeks of treatment. The decrease in IL-4Rα staining, which is the receptor targeted by dupilumab, on CD45- cells after 4 weeks of dupilumab treatment indicates direct binding of dupilumab on the IL-4Rα of conjunctival cells. The question is whether this has any biological and/or clinical consequences. A previous study by Ueta et al. showed that functional IL-4Rα is expressed on human conjunctival epithelium.[17] Recently, Hansen et al.[18] demonstrated that human GCs also express IL-4Rα and that both IL-4 and IL-13 are important for their homeostasis. Previously, we demonstrated scarcity of conjunctival GCs in patients who developed DAOSD.[8] Based on our current data we hypothesize that dupilumab-induced changes of conjunctival epithelium development may play a role in the development of OSD during dupilumab treatment, and that local dupilumab levels could have a worsening effect on DAOSD by interfering with GC development. Further research is needed to study the implications of the binding of dupilumab on the conjunctival epithelial cells.

A limitation of our study is that some patients received ophthalmological treatment for their OSD which may have led to less severe OSD. However, patients were divided into having no or mild OSD or moderate-to-severe OSD based on the clinical examination which was performed at every visit. Severity was based on the clinical UTOPIA score, and was independent of the use of ophthalmological treatment. For that reason, the ophthalmological treatment did probably not influence our results.

In conclusion, dupilumab-treated AD patients with moderate-to-severe OSD had higher dupilumab tear fluid levels compared to patients with no or mild OSD. This might be explained by a disrupted blood-tear barrier. Finally, dupilumab binding on conjunctival epithelial cells was found, indicating that dupilumab reaches the ocular surface. Further research is needed to investigate the implications of these increased levels of dupilumab.

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SUPPLEMENTARY

TABLE S1. Dupilumab levels in tear fluid and serum during dupilumab treatment categorized by OSD severity at the collection moment

	No or mild OSD at tear fluid collection	Moderate-to-severe OSD at tear fluid collection	P-value
4 weeks after start dupilumab (n=44[†])			
Tear fluid			
Number of patients	27	16	
Missing	1	0	
Dupilumab level in tear fluid (mg/L), median (IQR)	0.22 (0.13-0.55)	0.25 (0.19-0.61)	0.353
Serum			
Number of patients	26	16	
Missing	2	0	
Dupilumab level in serum (mg/L), median (IQR)	79.4 (63.2-110.5)	62.1 (52.0-77.0)	0.043
28 weeks after start dupilumab (n=48)			
Tear fluid			
Number of patients	25	19	
Missing	2	2	
Dupilumab level in tear fluid (mg/L), median (IQR)	0.29 (0.16-0.60)	0.55 (0.35-1.31)	0.021
Serum			
Number of patients	22	19	
Missing	5	2	
Dupilumab level in serum (mg/L), median (IQR)	105.1 (65.7-129.6)	100.3 (79.8-117.9)	0.814
Onset or worsening DAOSD (n=23)			
Tear fluid			
Number of patients	3	14	
Missing	2	4	
Dupilumab level (mg/L), median (IQR)	0.16 (0.09-0.55)	1.07 (0.28-2.69)	0.047

Severity of OSD is based on eye with the highest severity within a patient. P-values were calculated with Mann-Whitney U tests. Abbreviations: DAOSD, dupilumab-associated ocular surface disease; IQR, interquartile range; OSD, Ocular surface disease.

[†] 4 patients did not complete their week 4 visit due to the COVID19 pandemic.

TABLE S2. Patient characteristics of patients in which dupilumab binding on conjunctival cells obtained by conjunctival impression cytology was measured

	Dupilumab measured in CIC suspensions (n=5)	Dupilumab measured in CIC suspensions of AD controls (n=3)	IgG4 and IL-4Rα measured in CIC suspensions (n=4)
Age (years), median (IQR)	28.0 (22.5 – 48.0)	39.0 (24.0 – N/A)	50.5 (27.3 – 64.0)
Men, n (%)	2 (40.0)	2 (66.7)	3 (75.0)
EASI score baseline, median (IQR)	10.5 (1.8 – 19.7)	14.5 (13.1 – N/A)	14.8 (7.9 – 21.6)

Abbreviations: AD, atopic dermatitis; CIC, conjunctival impression cytology; EASI, Eczema

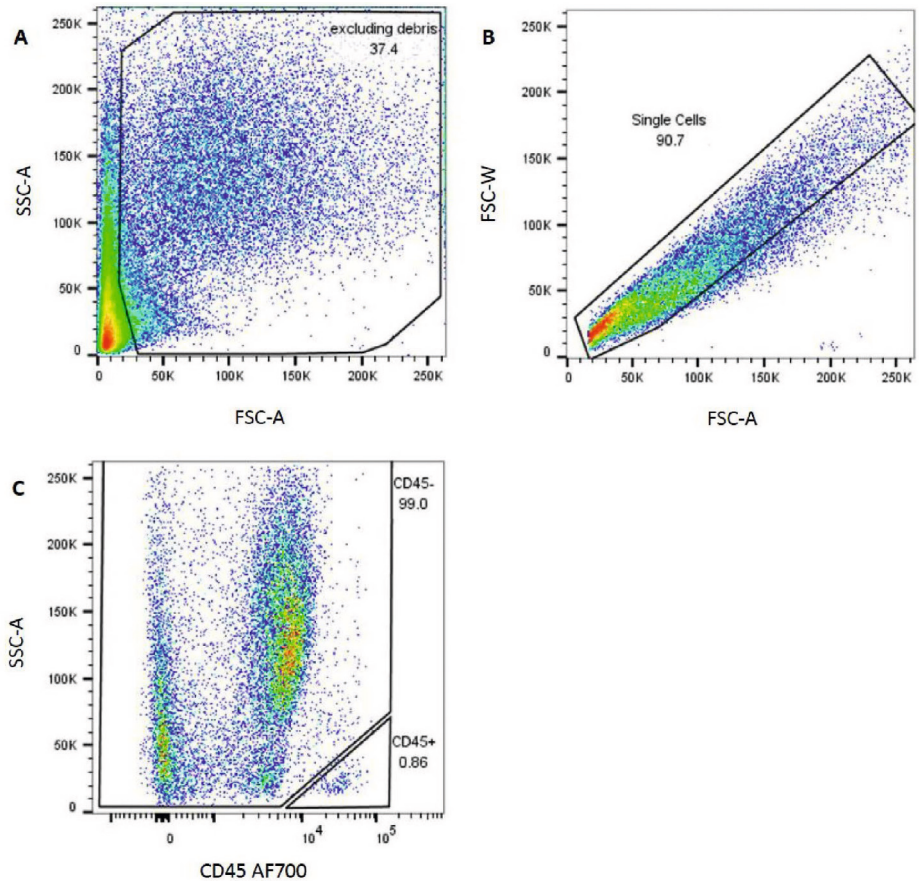

FIGURE S1. The different steps to gate CD45- cells are depicted.
Debris was excluded (**A**), single cells were gated (**B**) and finally CD45- cells were gated (**C**). Median Fluorescence Intensity (MFI) of IL-4Rα PE and IgG4 biotin – streptavidin APC was determined within CD45- population.

CHAPTER 9

Biomarkers in tear fluid of dupilumab-treated moderate-to-severe atopic dermatitis patients

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KEY MESSAGES

- This study, funded by dupilumab's manufacturer, measured tear fluid biomarker levels from dupilumab-treated atopic dermatitis (AD) patients.
- Patients with moderate-to-severe ocular surface disease before the start of dupilumab treatment had increased AD-related severity tear fluid biomarkers.
- No differences in Th1 or Th17-associated tear fluid biomarker levels were observed during dupilumab treatment.

To the Editor,

Atopic dermatitis (AD) patients treated with dupilumab, a biologic therapy targeting the shared receptor component for interleukin (IL)-4 and IL-13, frequently reported dupilumab-associated ocular surface disease (DAOSD) as a side effect.[1] Additionally, the majority of AD patients have ocular surface disease (OSD) before starting dupilumab, suggesting that AD patients may be predisposed to develop DAOSD.[2] The exact pathomechanism of DAOSD remains unclear, and little information is available regarding conjunctival inflammation. Therefore, our aim was to characterize conjunctival inflammation by measuring tear fluid biomarker levels and relate this to OSD severity before and during dupilumab treatment in AD patients.

This prospective study included moderate-to-severe AD adult patients between February 2020 and March 2021 from the University Medical Center Utrecht, the Netherlands. Dupilumab was dosed according to the label (300mg every two weeks). Examination was performed by a dermatologist and an ophthalmologist at the start of dupilumab (baseline), and after 4 and 28 weeks of dupilumab treatment. An additional ophthalmological examination was performed in patients who developed DAOSD. During dupilumab treatment, the ophthalmologist could start OSD treatment, and selected therapy depended on OSD severity. Written informed consent was provided and the study was approved by the Institutional Review Board.

Data regarding AD severity and atopic comorbidities were collected. Additionally, the standardized Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score was performed, providing an overall severity classification of no, mild, moderate, or severe OSD, based on the eye with the highest severity within a patient.[3] Tear fluid was collected by using Schirmer's strips, that were air-dried for ≥1 hour and stored at -80°C. Schirmer's strips were eluted to obtain tear fluid for biomarker measurements, and tear fluid from both eyes was combined. Biomarker levels (IL-22, pulmonary and activation-regulated chemokine (PARC), periostin, thymus and activation-regulated chemokine (TARC), Granzyme B, IL-6, Interferon gamma (IFN-γ), CXCL10, IL-12, tumor necrosis factor alpha (TNF-α), IL-17, IL-23, IL-4, IL-5, IL-13) in tear fluid were measured by multiplex technology (xMAP; Luminex)[4] at baseline, and after 4 and 28 weeks of dupilumab treatment. Eight healthy controls (non-atopic, no use of ophthalmic medication) were included. Samples above or below the assay limits of detection were given values equivalent to the lower limit of quantification divided by 2, or the upper limit of quantification multiplied by 2.[4] No samples were above the assay limits of detection in our study. Statistical analyses were conducted with SPSS Statistics version 26.0.0.1 (IBM Corp. IBM SPSS Statistics for Windows). Correlations were analysed using Spearman's test. A heat map and correlation matrix were created using R version

4.0.3. Figures were created by Prism (version 9.3.0 GraphPad Software). Supplemental materials including Spearman's correlations, tear fluid biomarkers per severity, and a heat map including baseline tear fluid biomarkers are available at: https://doi.org/10.5281/zenodo.7445605.

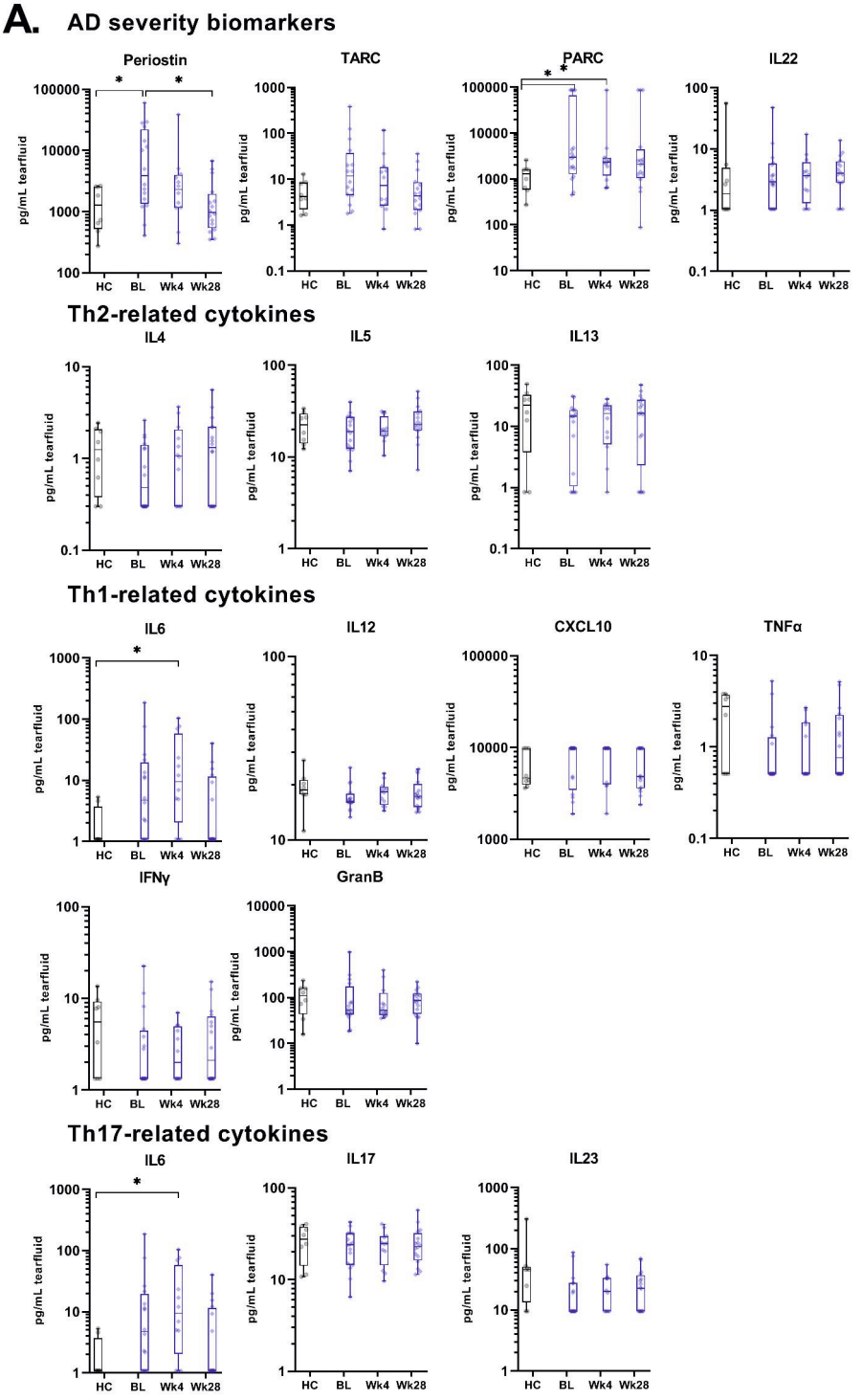
Sixteen patients (median baseline Eczema Area and Severity Index (EASI) score 16.5 (IQR 13.9-24.5)) were included (Table 1). At baseline, no, mild, moderate, and severe OSD were reported in 3/16 (18.8%), 7/16 (43.8%), 3/16 (18.8%), and 3/16 (18.8%) patients, respectively. Significantly higher PARC and periostin tear fluid levels were found in moderate-to-severe AD patients (n=16) compared to healthy controls (n=8) at baseline (Figure 1A). Additionally, significant higher baseline IL-22, TARC, and periostin tear fluid levels were measured in patients with moderate-to-severe OSD compared to patients with no or mild OSD (Figure 1B). At baseline, both TARC and IL-22 tear fluid levels were significantly correlated with EASI scores (both total EASI and head neck EASI), and head neck EASI scores significantly correlated with UTOPIA scores. In conclusion, AD-related severity tear fluid baseline biomarkers were significantly higher in patients with moderate-to-severe OSD compared to patients with no or mild OSD.

All patients responded to dupilumab treatment (median EASI 6.8 (IQR 3.1-9.8) and 2.3 (IQR 1.5-4.4) at week 4 and 28, respectively). During dupilumab treatment, median UTOPIA scores slightly increased (non-significantly) from 2.5 (IQR 1.0-6.8) at baseline, to 3.0 (IQR 2.0-5.8) and 4.0 (IQR 2.0-7.8) at week 4 and 28, respectively. After 4 weeks (n=12, 4 missing due to COVID-19 pandemic) and 28 weeks (n=16) of dupilumab treatment, tear fluid levels of TARC and periostin decreased compared to baseline (Figure 1A). Although not all statistically significant, IL-22, TARC, and periostin tear fluid levels remained higher in patients with moderate-to severe OSD compared to patients with no or mild OSD during dupilumab treatment (Figure 1B). Both TARC and IL-22 tear fluid levels significantly correlated with EASI scores at week 4, but not at week 28 of dupilumab treatment. Head neck EASI scores correlated significantly with IL-22 tear fluid levels at week 4, but not correlated with UTOPIA scores at week 4 and 28. Levels of Th1- and Th17-related tear fluid cytokines (IL-6, IFN-γ, CXCL10, IL-12, TNF-α, IL-17, and IL-23) and Th2-related tear fluid cytokines (IL-4, IL-5, IL-13) remained stable during dupilumab treatment and no differences were found in these cytokines between patients with no or mild OSD and patients with moderate-to-severe OSD.

TABLE 1. Patient characteristics at baseline

	Total cohort (n=16)
Age (years), median (IQR)	36 (26-46)
Men, n (%)	8 (50.0)
Age of onset of AD, n (%)	
Childhood	15 (93.8)
Adolescence	0 (0)
Adult	1 (6.3)
History of self-reported episodic acute allergic conjunctivitis, n (%)	14 (87.5)
Allergic asthma, n (%)	8 (50.0)
Allergic rhinitis, n (%)	13 (81.3)
Food allergy, n (%)	5 (31.3)
History of rosacea, n (%)	0 (0)
EASI score, median (IQR)	16.5 (13.9-24.5)
IGA score, median (IQR)	3 (3-4)
AD eyelid involvement in the past year, n (%)	8 (50.0)
AD facial involvement in the past year, n (%)	14 (87.5)
TARC (pg./ml), median (IQR)	1954 (1111-3607)
Peripheral blood eosinophils ($\times 10^9$/L), median (IQR)	0.37 (0.16-0.52)
Eosinophilia ($\geq 0.45 \times 10^9$/L), n (%)	6 (37.5)
Severity of OSD before the start of dupilumab†, n (%)	
No OSD	3 (18.8)
Mild OSD	7 (43.8)
Moderate OSD	3 (18.8)
Severe OSD	3 (18.8)

† Severity of OSD is based on eye with the highest severity within a patient. Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IGA scale, Investigator's Global Assessment Scale; IQR, interquartile range; OSD, Ocular Surface Disease; TARC, thymus and activation-regulated chemokine.


A.
AD severity biomarkers
Periostin
TARC
PARC
IL22
Th2-related cytokines
IL4
IL5
IL13
Th1-related cytokines
IL6
IL12
CXCL10
TNFα
IFNγ
GranB
Th17-related cytokines
IL6
IL17
IL23
pg/mL tearfluid
HC
BL
Wk4
Wk28

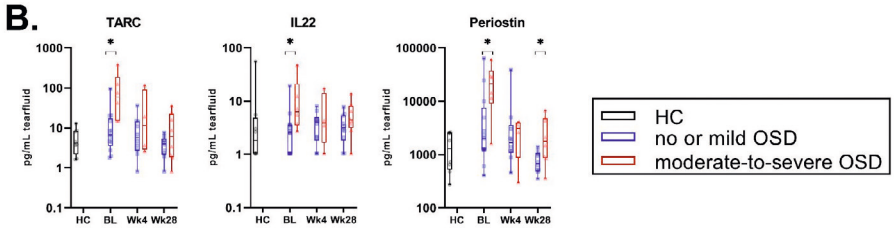


FIGURE 1. Biomarkers measured in tear fluid of moderate-to-severe AD patients before and during dupilumab treatment.

Boxplots with median and interquartile ranges (healthy controls (n=8) in grey). Whisker plots display the minimum and maximum. Differences were calculated with Mann-Whitney U tests. **A.** Biomarkers measured in the total cohort before the start of dupilumab (n=16) and after 4 weeks (n=12) and 28 weeks (n=16) of dupilumab treatment. **B.** TARC, IL-22, and Periostin measured in tear fluid of moderate-to-severe AD patients before the start of dupilumab (n=16) and after 4 weeks (n=12) and 28 weeks (n=16) of dupilumab treatment per OSD severity. Severity of OSD is based on eye with the highest severity within a patient at the selected time point.

Abbreviations: GranB, granzyme B; HC, healthy control; IL, interleukin; IFNγ, interferon gamma; TNFα, tumor necrosis factor alpha; PARC, pulmonary and activation-regulated chemokine; TARC, thymus and activation-regulated chemokine.

* Indicates statistical significance.

However, 7/16 (44%) patients showed ≥3 points increase in UTOPIA score during dupilumab treatment (i.e. DAOSD onset) compared to baseline. At DAOSD onset, the median UTOPIA score was significantly higher compared to baseline (6.0 (IQR 3.0-8.0) vs. 2.0 (IQR 0.0-2.0), p=0.017, respectively (n=7)). Significantly higher granzyme B and lower periostin tear fluid levels were found at DAOSD onset compared to baseline (127.3pg/mL (IQR 97.1-195.1) vs. 48.8pg/mL (IQR 43.2-77.2), p=0.028, and (1038pg/mL (781-3967) vs. 2344pg/mL (IQR 1265-10071), p=0.028, respectively). This suggest a role for T cell cytotoxicity in OSD worsening during dupilumab treatment. At week 28 of dupilumab treatment, only 4/16 (25%) patients had ≥3 points increase in UTOPIA score compared to baseline, suggesting that early OSD treatment may be effective in reducing DAOSD severity.

Before starting dupilumab, 81.2% of the patients had OSD, and patients with moderate-to-severe OSD had increased tear fluid levels of IL-22, TARC, and periostin compared to patients with no or mild OSD. The increased periostin might contribute to the pathogenesis of ocular allergic diseases.[5] Furthermore, baseline TARC and IL-22 tear fluid levels were significantly correlated with baseline EASI scores. Interestingly, TARC and IL-22 are known biomarkers for clinical severity of AD, and more severe AD is found in patients with more severe OSD.[2,6] This suggests that high tear fluid baseline levels of TARC and IL-22 in patients with moderate-to-severe OSD are related to AD severity.

Due to the impaired epithelial barrier function in eyes of AD patients, as suggested by Yokoi et al.[7], the AD-related severity tear fluid biomarkers might be increased in AD patients with more severe OSD and play a role in the local barrier disruption. Furthermore, the head neck EASI score significantly correlated with the UTOPIA score at baseline, suggesting more severe OSD in patients with more severe head neck AD at baseline. Dogru et al.[8] previously reported significantly more severe metaplasia of the ocular surface in patients with facial atopy. For that reason, the local barrier disruption of the eyes might not be the only explanation for the higher biomarker levels since these cytokines may also be locally produced by activated conjunctival epithelial cells. Taken together, moderate-to-severe AD patients may be predisposed having AD-related OSD prior to dupilumab treatment.

During dupilumab treatment, no specific differences in Th1 or Th17-associated biomarker tear fluid levels were found, comparable with previous literature.[9] However, granzyme B tear fluid levels were significantly higher at DAOSD onset compared to baseline (n=7 of which 6 available samples). Increased granzyme B, indicating cytotoxic T cell activity, is previous observed in conjunctival biopsies of DAOSD patients, and comparable with our results.[1]

AD-related severity tear fluid biomarkers decreased after 4 and 28 weeks of dupilumab treatment, while the UTOPIA score slightly increased. However, moderate-to-severe OSD patients remained to have slightly higher tear fluid levels of TARC, IL-22, and periostin compared to patients with no or mild OSD. No correlation was found between these markers and EASI scores at week 28, and between EASI scores (both total EASI score and head neck EASI score) and UTOPIA scores, indicating that OSD severity is not related to AD severity during dupilumab treatment. These data suggest that dupilumab may play a dual generally protective and locally aggravating role in OSD. The question remains what the pathomechanism is of DAOSD in AD patients. Scarcity of conjunctival goblet cells due to dupilumab treatment has been hypothesized to induce local inflammation and DAOSD.[1,3] Prospective studies are needed to learn more about the pathomechanism of DAOSD.

A limitation of our study is that only tear fluid biomarkers were measured, and therefore cannot be compared with serum levels. Second, some patients received treatment for their OSD, potentially leading to less severe OSD. However, OSD severity was based on the ophthalmological examination which was conducted every visit, and was independent of ophthalmological treatment.

In conclusion, OSD is associated with increased tear fluid levels of AD-related severity biomarkers in patients with moderate-to-severe OSD before starting dupilumab. These biomarkers decreased during dupilumab treatment, but remained slightly higher in patients with moderate-to-severe OSD. No specific differences in Th1 or Th17-associated biomarkers were found in tear fluid during dupilumab treatment, despite the slightly (not significantly) increasing UTOPIA score. Further research in larger cohorts is needed to verify our results.

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SUPPLEMENTARY

TABLE S1. Spearman's correlations

Spearman's correlations biomarkers in tear fluid	N	Spearman's rho	P-value
IL-22 in tear fluid and EASI score			
At baseline	16	0.552	0.027
At week 4	12	0.709	0.010
At week 28	16	0.139	0.608
TARC in tear fluid and EASI score			
At baseline	16	0.576	0.019
At week 4	12	0.830	0.001
At week 28	16	0.090	0.741

Abbreviations: EASI, Eczema Area and Severity Index; IL, Interleukin; TARC, thymus and activation-regulated chemokine

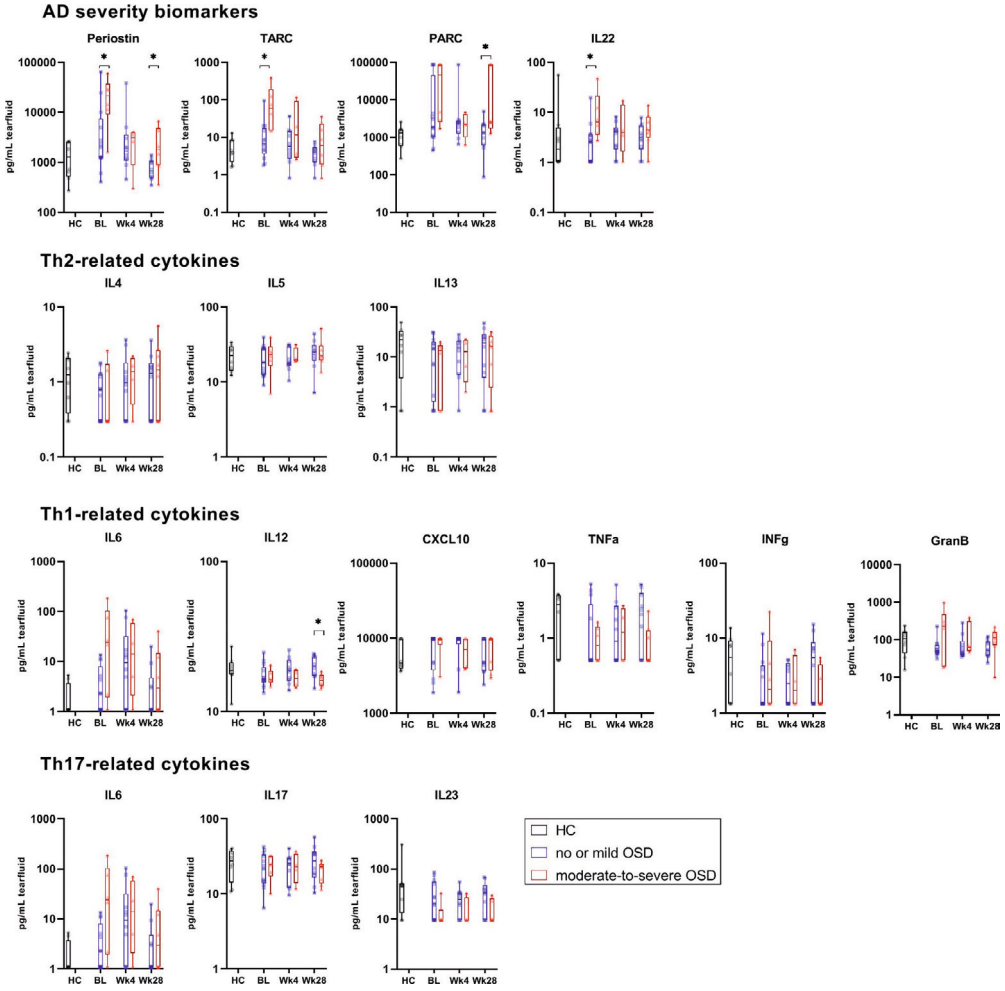


FIGURE S1. All other biomarkers measured in tear fluid of 16 moderate-to-severe AD patients before the start of dupilumab and after 4 and 28 weeks of dupilumab treatment per OSD severity.

Severity of OSD is based on eye with the highest severity within a patient. Boxplots with median and interquartile ranges (patients in blue, healthy controls (n=8) in grey). Whisker plots display the minimum and maximum. Differences were calculated with Mann-Whitney U tests. Abbreviations: GranB, granzyme B; HC, healthy control; IL, interleukin; IFNg; interferon gamma; TNFa, tumor necrosis factor alpha; PARC, pulmonary and activation-regulated chemokine. * Indicates statistical significance.

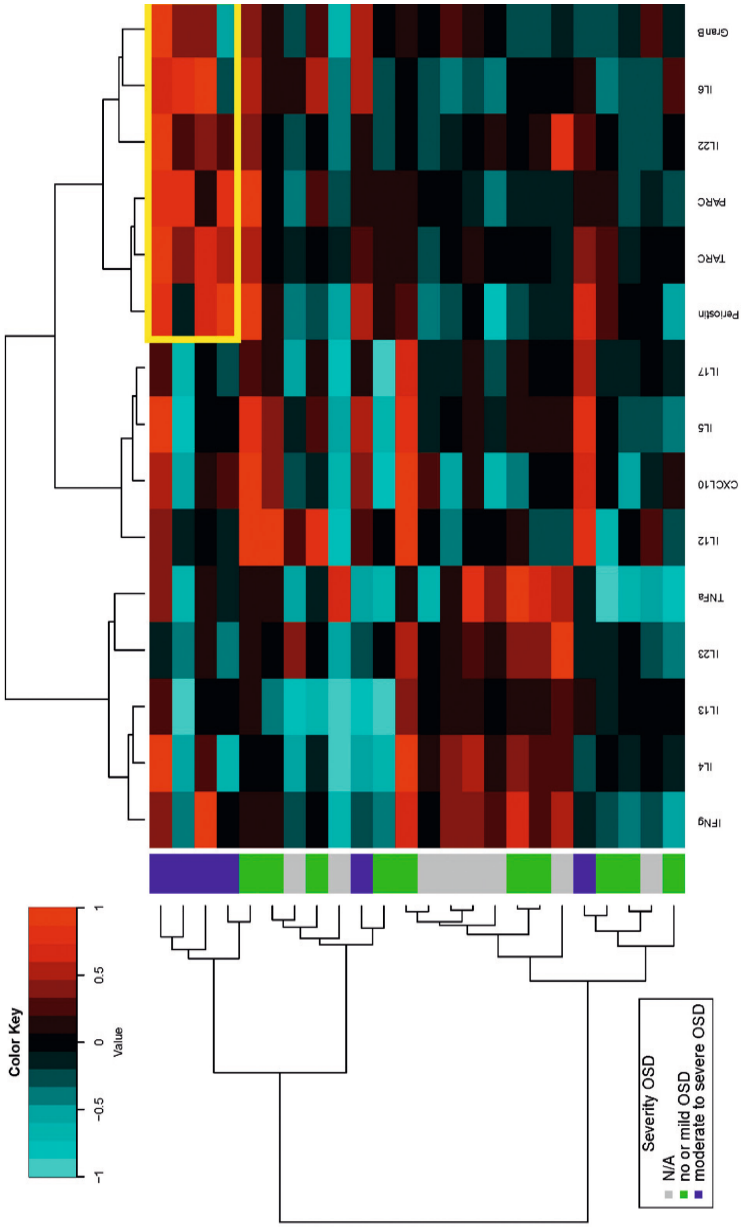
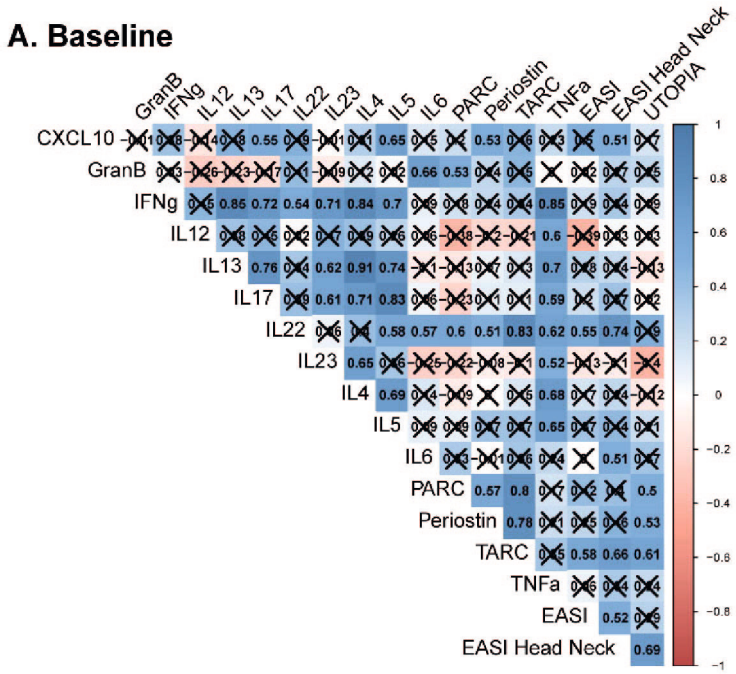
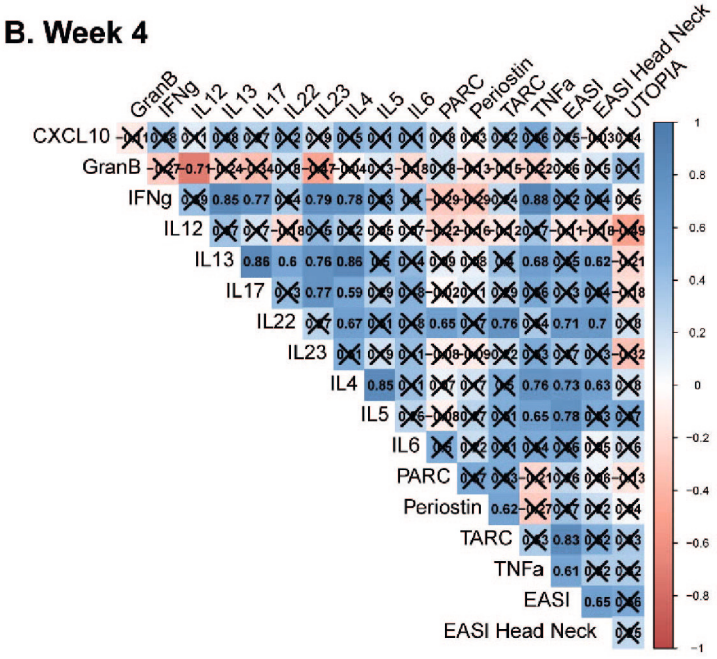


FIGURE S2. Heat map of cytokines in tear fluid at baseline.

The yellow square highlights the higher cytokines in patients with moderate-to-severe OSD. Grey indicates the healthy controls, green indicates the patients with no or mild OSD, and blue indicates patients with moderate-to-severe OSD. Abbreviations: GranB, granzyme B; IL, interleukin; IFNg; interferon gamma; TNFa, tumor necrosis factor alpha; OSD, ocular surface disease; PARC, pulmonary and activation-regulated chemokine.


A. Baseline
GranB
IFNg
IL12
IL13
IL17
IL22
IL23
IL4
IL5
IL6
PARC
Periostin
TARC
TNFa
EASI
EASI Head Neck
UTOPIA
CXCL10
GranB
IFNg
IL12
IL13
IL17
IL22
IL23
IL4
IL5
IL6
PARC
Periostin
TARC
TNFa
EASI
EASI Head Neck
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1


B. Week 4
GranB
IFNg
IL12
IL13
IL17
IL22
IL23
IL4
IL5
IL6
PARC
Periostin
TARC
TNFa
EASI
EASI Head Neck
UTOPIA
CXCL10
GranB
IFNg
IL12
IL13
IL17
IL22
IL23
IL4
IL5
IL6
PARC
Periostin
TARC
TNFa
EASI
EASI Head Neck
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

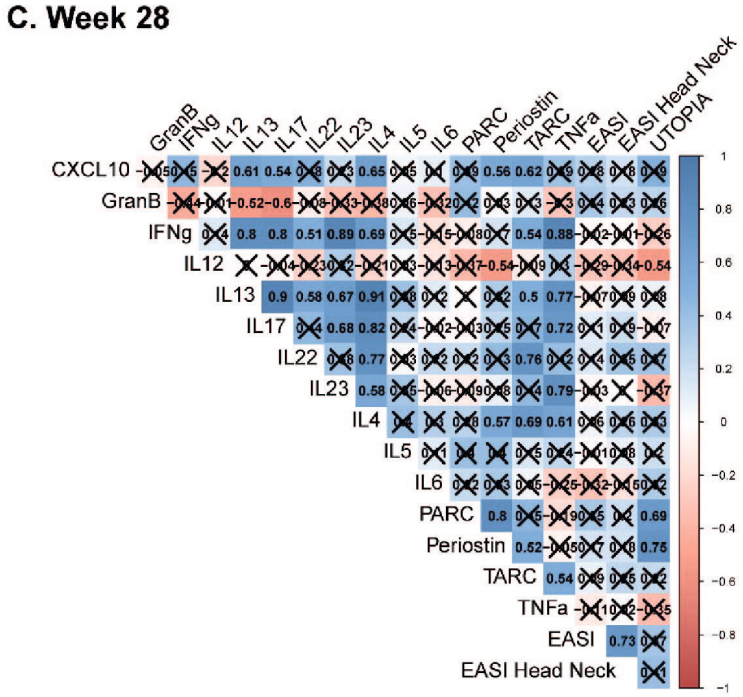
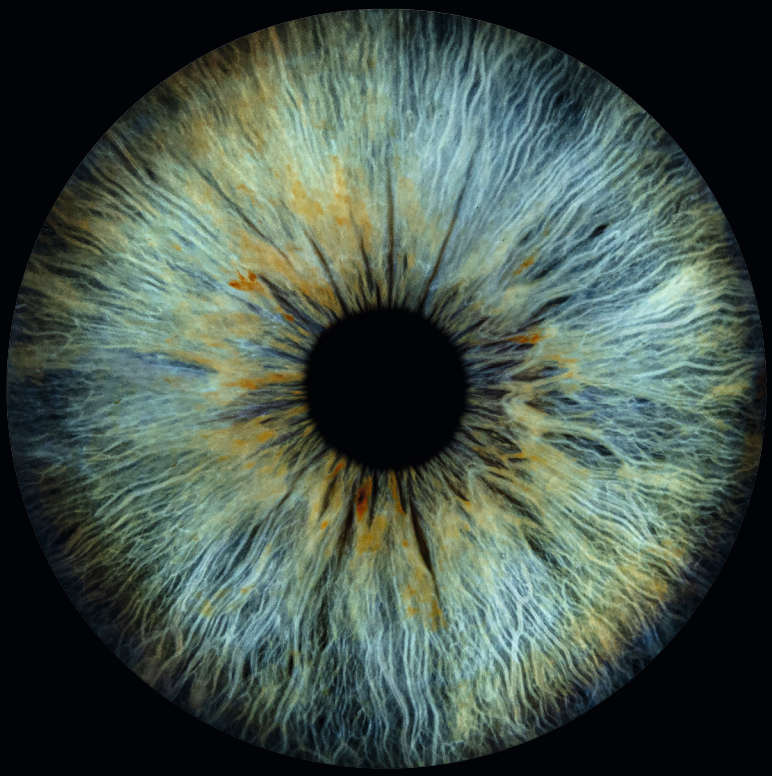


FIGURE S3. Spearman's correlations.

X indicates that there is no statistical significant correlation (p>0.05). **A.** Before the start of dupilumab. **B.** After 4 weeks of treatment with dupilumab. **C.** After 28 weeks of treatment with dupilumab.

Abbreviations: EASI, Eczema Area and Severity Index; GranB, granzyme B; HC, healthy control; IL, interleukin; IFNg; interferon gamma; PARC, pulmonary and activation-regulated chemokine; TNFa, tumor necrosis factor alpha; UTOPIA, Utrecht Ophthalmic Inflammatory and Allergic disease score.



CHAPTER 10

Unravelling the immunological characteristics of conjunctival inflammation in atopic dermatitis patients treated with dupilumab

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INTRODUCTION

Dupilumab-associated ocular surface disease (DAOSD) is the most commonly reported side effect in dupilumab-treated moderate-to-severe atopic dermatitis (AD) patients.[1] Dupilumab targets the interleukin (IL)-4 receptor alpha, thereby affecting the IL-4 and IL-13 signalling. It has been hypothesized that scarcity of conjunctival goblet cells (GC) may be responsible for the development of DAOSD.[2] In addition, the immune response in the conjunctival epithelium appears to play a central role in the pathogenesis of DAOSD, as the study by Bakker et al. reported a panel of infiltrating immune cells in conjunctival biopsies from 6 patients with DAOSD, including an increased CD4+/CD8+ ratio and a local Th1 inflammatory response.[3] Normally, the intraepithelial lymphocytes in the conjunctival epithelium are predominantly CD8+ T-cells.[4,5] Interestingly, ocular surface disease (OSD) is common in moderate-to-severe AD patients before the start of dupilumab treatment, and is accompanied by low conjunctival GC numbers.[6] However, there are no detailed studies on the immune cell composition of the conjunctival epithelium in AD patients with OSD before and during dupilumab treatment.

Therefore, the aim of this study is to investigate the immunological changes of the immune cells populating the superficial conjunctival epithelium to better understand the pathomechanism of (DA)OSD in AD patients.

METHODS

This prospective cohort study included dupilumab-treated moderate-to-severe AD patients from the University Medical Center Utrecht in the Netherlands between September 2020 and October 2021. All patients were seen by both an ophthalmologist and a dermatologist before and during dupilumab treatment (baseline, week 4, week 28). An additional ophthalmological visit was scheduled if patients developed signs and symptoms of DAOSD. The ophthalmic examination was based on the standardized Utrecht Ophthalmic Inflammatory and Allergic Disease (UTOPIA) score, including assessment of the severity of inflammation of the conjunctiva (both bulbar and tarsal), eyelids, and limbus.[7] The severity of OSD per eye was classified as no (UTOPIA score 0), mild (UTOPIA score 1-4), moderate (UTOPIA score 5-8), or severe OSD (UTOPIA score ≥9), following the severity ranges of the UTOPIA score. DAOSD was defined as the first visit during dupilumab treatment at which an increase in UTOPIA score of ≥3 points from baseline was observed.

Conjunctival impression cytology (CIC) was performed to collect cellular material from the first one to three superficial cell layers of the conjunctival tissue. The CIC from the right eye was analysed by flow cytometry, as described previously.[6] From a selected group of patients (Table S1, S2) flow cytometry data were available. The

percentages of Cytokeratin 19- (CK19) CD45+CD3+ cells, CD3+CD4+ cells, CD3+CD8+ cells, CD3+Ki67+ cells, CD4+Ki67+ cells, CD8+Ki67+ cells, CD4+ Granzyme B (GzmB)+ cells, and CD8+ GzmB+ cells were analysed. Twelve controls (non-atopic, no use of ophthalmic medications) were included as healthy controls (HCs).

Differences over time were analysed by using the Wilcoxon test. The Mann Whitney-U test was conducted to analyse differences within the severity groups. The gating strategy is shown in Figure S1.

RESULTS

A total of 49 dupilumab-treated moderate-to-severe AD patients (median age 38 years (interquartile range (IQR) 27-52), 46.9% (n=23/49) men) were included. At baseline, the median Eczema Area and Severity Index (EASI) was 14.0 (IQR 10.8-17.0). Of all patients, 95.9% (n=47/49) already had characteristics of OSD before starting dupilumab treatment (Table 1).

Comparable percentages of CD45+CD3+ cells were found between AD patients and HCs at baseline (Figure 1A). However, a higher CD4+/CD8+ ratio was observed in AD patients at baseline than in HCs, which remained stable during dupilumab treatment (Figure 1B). The percentage of CD3+Ki67+ cells was slightly higher in AD patients at baseline than in HCs, and increased non-significantly during dupilumab treatment (Figure 1C). In some patients, the CD8+ T-cells proliferation increased >20% during dupilumab compared to baseline (Figure 1D). At baseline, an increased percentage of CD4+Ki67+ cells was observed, which may explain the higher CD4/CD8+ ratio in AD patients at baseline (Figure 1B, S2). Examination of the cytotoxic activity of CD8+ T-cells showed a significantly lower percentage of CD8+GzmB+ cells in AD patients compared with HCs at baseline, which remained stable during dupilumab treatment (Figure 1E). GzmB+ cells were predominantly expressed within the CD8+ subsets, both before and during dupilumab treatment (Figure S2).

TABLE 1. Patient characteristics at baseline

	Total cohort (n=49)
Age (years), median (IQR)	38 (27-52)
Men, n (%)	23 (46.9)
Age of onset of AD, n (%)	
Childhood	45 (91.8)
Adolescence	4 (8.2)
Adult	0 (0.0)
History of self-reported episodic acute allergic conjunctivitis, n (%)	33 (67.3)
Allergic asthma, n (%)	29 (59.2)
Allergic rhinitis, n (%)	35 (71.4)
Food allergy, n (%)	29 (59.2)
History of rosacea, n (%)	2 (4.1)
EASI score at baseline, median (IQR)	14.0 (10.8-17.0)
IGA score at baseline, median (IQR)	3 (2-3)
AD eyelid involvement in the past year, n (%)	38 (77.6)
AD facial involvement in the past year, n (%)	47 (95.9)
TARC (pg./ml), median (IQR)	1332 (787-1905)
Severity of OSD before the start of dupilumab, n (%)	
No OSD	2 (4.1)
Mild OSD	28 (57.1)
Moderate OSD	16 (32.7)
Severe OSD	3 (6.1)

Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IGA scale, Investigator's Global Assessment Scale; IQR, interquartile range; OSD, Ocular Surface Disease; TARC, thymus and activation-regulated chemokine.

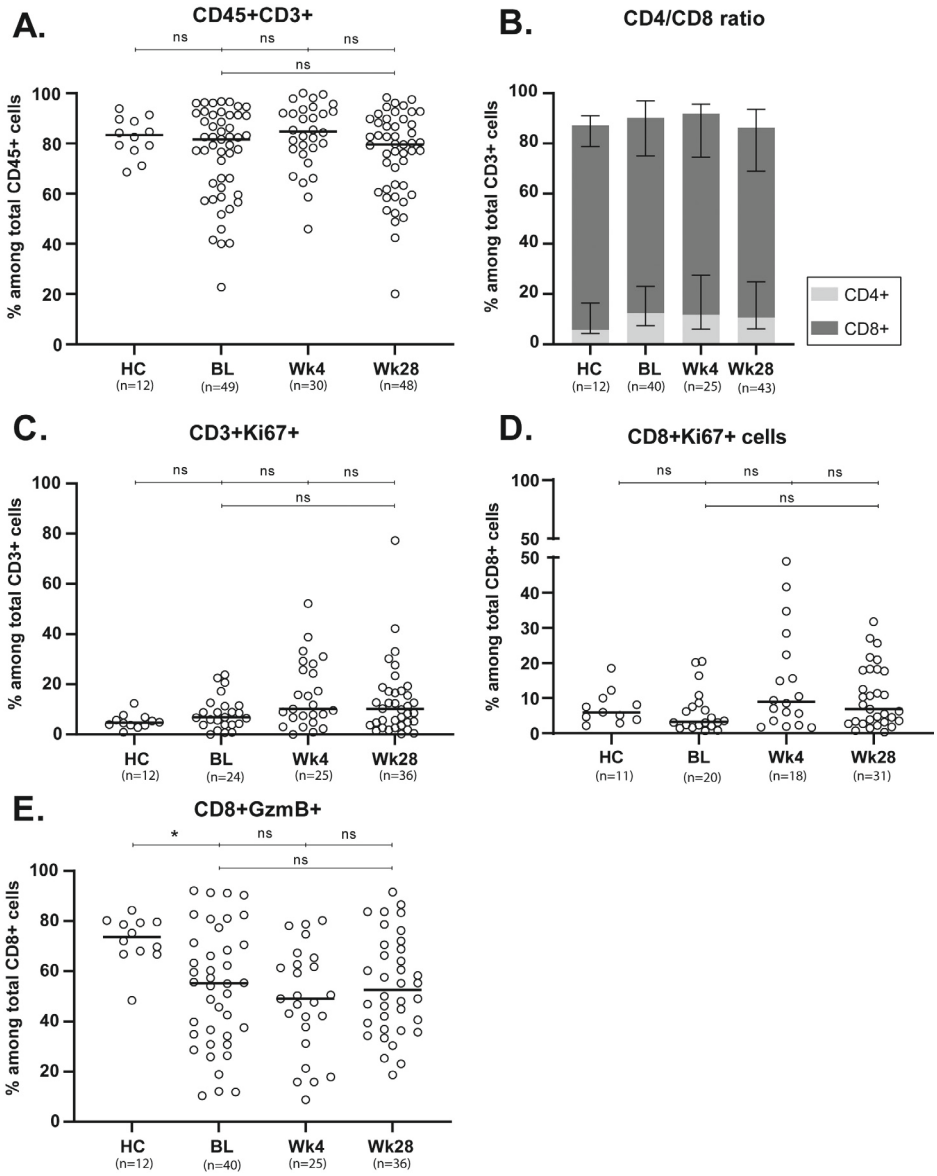

FIGURE 1. Flow cytometry results of the total cohort per time point.
The percentage (%) of CD45+CD3+ cells (**A**), CD4+CD8+ ratio (**B**), CD3+Ki67+ cells (**C**), CD8+Ki67+ cells (**D**) CD8+GzmB+ cells (**E**) in healthy controls, at baseline, week 4, and week 28 of dupilumab treatment.
* Indicates statistical significance.

Data were also analysed by severity of OSD, and patients were classified as having no or mild OSD or moderate-to-severe OSD. At baseline, the percentage of CD45+CD3+ T-cells was significantly lower in patients with moderate-to-severe OSD compared

to patients with no or mild OSD (Figure 2A). No significant differences in CD3+ T-cell distribution were seen during dupilumab treatment (Figure 2A). However, a slightly higher CD4+/CD8+ ratio was seen in patients with moderate-to-severe OSD compared to patients with no or mild OSD during dupilumab treatment (Figure 2B). In addition, higher percentages of CD3+Ki67+ cells and CD8+GzmB+ cells were seen in patients with moderate-to-severe OSD compared to patients with no or mild OSD during dupilumab treatment (Figure 2C, D).

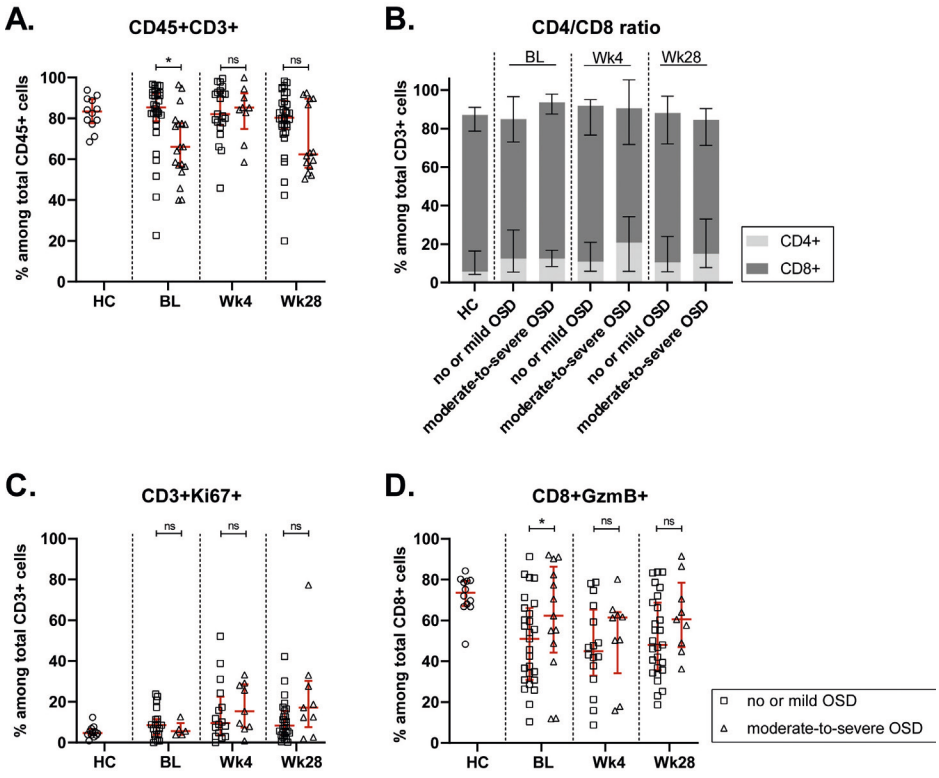

FIGURE 2. Flow cytometry results of the total cohort per time point per severity of the ocular surface disease.

The percentage (%) of CD45+CD3+ cells (**A**), CD4+CD8+ ratio (**B**), CD3+Ki67+ cells (**C**), CD8+GzmB+ cells (**D**) in healthy controls, at baseline, week 4, and week 28 of dupilumab treatment.

* Indicates statistical significance.

A total of 24.5% (n=12/49) patients showed an increase in UTOPIA score of ≥3 points from baseline, which was defined as DAOSD. Flow cytometry data at the onset of DAOSD were available from 83.3% (n=10/12) of these patients (Table S1). The median time between the start of dupilumab treatment and the onset of DAOSD was 9.5 weeks (IQR 4.3-30.0). No significant changes in total CD3+ T-cells, CD8+GzmB+ cells, and in CD3+Ki67+ cells were observed at the onset of DAOSD compared to baseline (Figure

3A, C, D). Patients who developed DAOSD had a higher CD4+/CD8+ ratio at baseline compared to HCs (Figure 3B). At the onset of DAOSD, this ratio was slightly increased compared to baseline (Figure 3B).

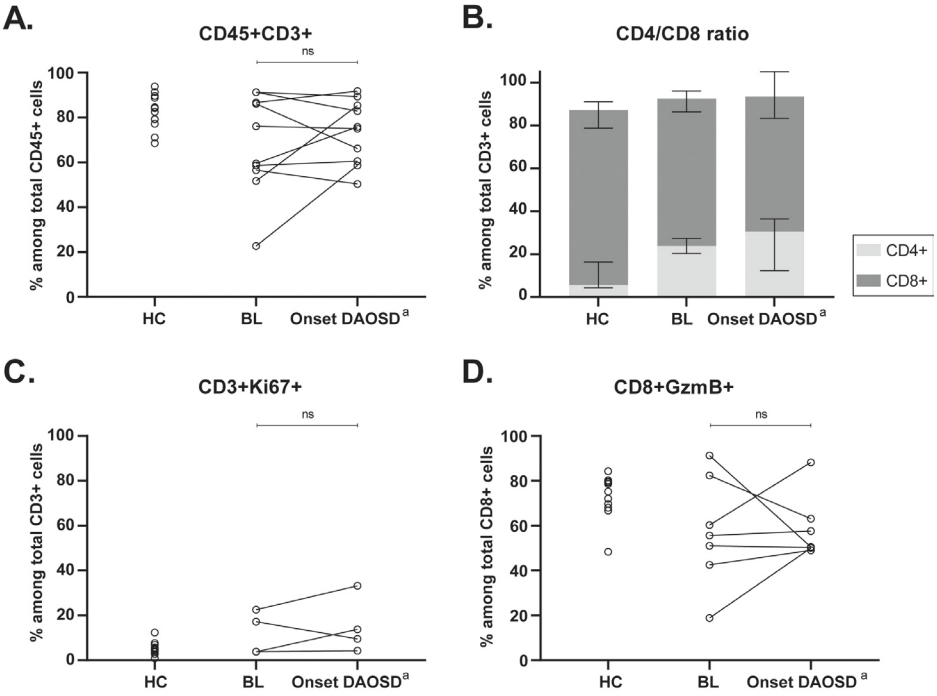

FIGURE 3. Flow cytometry results of the patients that developed dupilumab-associated ocular surface disease.

A. The % of CD45+CD3+ cells in healthy controls (HC, n=12), at baseline (BL), and at the onset of DAOSD (n=10). **B.** The CD4+CD8+ ratio in HCs (n=12), at BL, and at the onset of DAOSD (n=7). **C.** The % of CD3+Ki67+ cells in HCs (n=12), at BL, and at the onset of DAOSD (n=4). **D.** The % of CD8+GzmB+ cells in HCs (n=12), at BL, and at the onset of DAOSD (n=7).

DISCUSSION

This prospective study investigated the immunological changes of superficial conjunctival cells to better understand the pathomechanism of (DA)OSD. Compared to HCs and AD patients at baseline, patients with moderate-to-severe OSD had a higher CD4+/CD8+ ratio during dupilumab treatment. Patients with moderate-to-severe OSD during dupilumab treatment also had higher percentages of CD3+Ki67+ cells and CD8+GzmB+ cells than patients with no or mild OSD. In addition, a slightly higher CD4+/CD8+ ratio was observed at the onset of DAOSD compared to baseline.

The slightly increased percentage of CD3+Ki67+ cells in AD patients during dupilumab treatment suggests that there may be an activation state with local proliferation of T-cells. However, CD4+Ki67+ expression is slightly lower during dupilumab treatment compared to baseline, suggesting that the increase in CD4+ cells during dupilumab treatment is probably due to infiltration rather than expansion (Figure S2). On the other hand, CD8+Ki67+ increased slightly during dupilumab treatment, suggesting both expansion and infiltration of these cells (Figure S2). Previously, Yokoi et al. suggested that moderate-to-severe AD patients with blepharoconjunctivitis could have an impaired conjunctival barrier function.[8] This may explain why CD3+CD4+ cells were able to reach the conjunctival epithelia, leading to the higher CD4+/CD8+ ratio in AD patients compared to HCs. Patients with more severe OSD during dupilumab treatment could have a more impaired conjunctival barrier function, which may explain the higher CD4+/CD8+ ratio found in patients with moderate-to-severe OSD compared to patients with no or mild OSD.

Barabino et al. analysed CIC samples from patients with dry eye disease (n=15) and HCs (n=15) by flow cytometry, and reported higher CD4+ T-cells in the conjunctival cells of patients with dry eye disease than in HCs.[9] They suggested that CD4+ cells infiltrating the conjunctival epithelium may include Th1 cells, and that activation of CD4+ cells may induce secretion of interferon gamma (IFN-γ).[9,10] Furthermore, it may be possible that the CD8+ T-cells in the conjunctiva of (dupilumab-treated) AD patients also express IFN-γ, as Hijnen et al. previously showed that CD8+ T-cells in AD lesions produce IFN-γ.[11] However, CD8+ cells in the conjunctiva were also present in HCs, which were probably inactive and therefore did not produce IFN-γ. In addition, Bakker et al. found significantly higher IFN-γ in the infiltrates of 6 conjunctival biopsies from patients with DAOSD.[3] Taken together, we hypothesize that the increased CD4+ T-cells and possibly also the CD8+ T-cells, both of which were found in the superficial conjunctival layers of AD patients, in dupilumab-treated AD patients, and in patients with more severe OSD, might lead to higher expression of IFN-γ.

In addition, previous studies reported an inverse correlation between IFN-γ expression and the GC density in the bulbar conjunctiva.[12,13] It is hypothesized that GC scarcity plays a role in the development of DAOSD, and in addition, lower GC numbers were previously found in moderate-to-severe AD patients.[6] The higher percentage of CD4+ T-cells in AD patients before and during dupilumab treatment compared to HCs may indicate higher IFN-γ expression, which could contribute to a decrease in conjunctival GC density.[2,6] Conjunctival GCs normally play an important immunomodulatory role by suppressing the maturation and production of several cytokines, including IFN-γ.

The reduced number of GCs that are found in moderate-to-severe AD patients before the start of dupilumab, may result in less suppression of IFN-γ. It is hypothesized that dupilumab reduces the function of GCs by inhibiting IL-13, which may enhance the reduced suppression of IFN-γ and subsequently lead to conjunctival inflammation during dupilumab treatment. Further studies on IFN-γ expression of conjunctival T-cells in (dupilumab-treated) AD patients are needed to verify this hypothesis.

In our study, the percentage of CD8+GzmB+ cells was lower in AD patients than in HCs. However, tear fluid biomarker analysis showed no significant difference in GzmB levels between AD patients and HCs.[14] It is possible that GzmB degranulates in patients with AD and OSD, resulting in significantly lower percentages of CD8+GzmB+ cells compared to HCs, but comparable levels in tear fluid. In addition, a higher percentage of CD8+GzmB+ cells (although not significant at all-time points) was seen in patients with moderate-to-severe OSD compared to patients with no or mild OSD, both before and during dupilumab treatment. This might indicate increased cytotoxic activity in patients with more severe OSD, as also described by Bakker et al., who detected GzmB in conjunctival biopsies from 6 patients with DAOSD.[3] Furthermore, at the onset of DAOSD, only 2/7 patients showed a decrease in their percentage of CD8+GzmB+ cells compared to baseline, whereas the other 5/7 patients showed similar or increased percentages. In addition, we recently found that patients with DAOSD had significantly higher GzmB tear fluid levels at the onset of DAOSD compared to baseline.[14] However, both cohorts were small, so more data are needed to learn more about the potential role of GzmB in the pathomechanism of DAOSD.

This study has some limitations. First, only the superficial conjunctival layers were examined in this study, and some samples consisted of very few conjunctival cells. This raises the question of whether immunological changes or infiltration can be fully studied in the first superficial conjunctival layers. However, we have shown that it is possible to detect infiltrating cells in the superficial layers of the conjunctiva. The study by Bakker et al. examined conjunctival biopsies and found that the infiltrates were usually not located in the superficial conjunctival layers.[3] In addition, they found no increased inflammatory cells in control areas of the biopsies compared to areas with the infiltrate.[3] However, as biopsies are an invasive technique, this can only be studied in small cohorts. Since CIC sampling is a non-invasive technique, it can provide information from a larger cohort of patients. Future research will include conjunctival organoids, which will provide more information about pathogenic mechanisms in the deeper conjunctival cell layers. Another limitation is that only a small cohort of DAOSD patients was analysed and data were missing at some time points. Inclusion of more

patients is needed to learn more about the immunological cells of the superficial conjunctival layers of AD patients with DAOSD.

In conclusion, we hypothesize that the immune response in the conjunctival epithelium plays a role in the pathogenesis of (DA)OSD, as the number of CD4+ T-cells was higher in AD patients before and during dupilumab treatment compared to HCs. Based on findings from previous studies, we hypothesize that this may lead to increased IFN-γ expression, which subsequently reduces the number of conjunctival GCs.

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SUPPLEMENTARY

TABLE S1. Number of patient samples analysed per visit.

	Baseline (n=49)	Week 4 (n=49)	Week 28 (n=49)	Onset DAOSD (n=12)
CD45+CD3+ data, n (%)	49 (100.0)	30 (61.2)	48 (98.0)	10 (83.3)
CD3+CD4+ data, n (%)	40 (81.6)	25 (51.0)	43 (87.8)	10 (83.3)
CD3+CD8+ data, n (%)	40 (81.6)	25 (51.0)	43 (87.8)	10 (83.3)
CD8+GzmB+ data, n (%)	40 (81.6)	25 (51.0)	36 (73.5)	10 (83.3)
CD3+Ki67+ data, n (%)	24 (49.0)	25 (51.0)	36 (73.5)	10 (83.3)

Abbreviations: DAOSD, dupilumab-associated ocular surface disease; GzmB, granzyme B.

TABLE S2. Number of patient samples selected on available CD4+ data.

	Baseline (n=11)	During dupilumab treatment (n=27)
CD4+Ki67+ data, n (%)	8 (72.2)	27 (100.0)
CD8+Ki67+ data, n (%)	8 (72.2)	26 (93.3)
CD4+GzmB+ data, n (%)	11 (100.0)	27 (100.0)
CD8+GzmB+ data, n (%)	11 (100.0)	27 (100.0)

Abbreviations: DAOSD, dupilumab-associated ocular surface disease; GzmB, granzyme B.

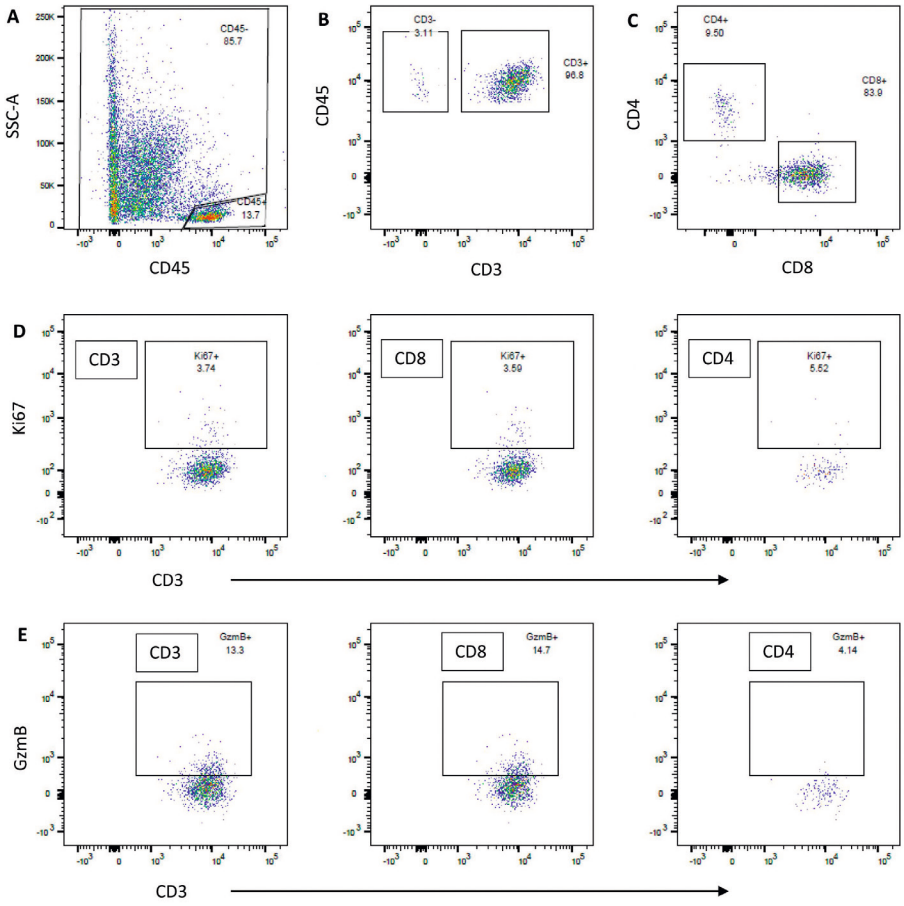

FIGURE S1. Flow cytometry gating strategy for T-cells.
Representative gating strategy for CD45+ cells (**A**), CD3+ T-cells (**B**) and CD4+ and CD8+ T-cells (**C**). Granzyme B (GzmB) (**D**) and Ki67+ (**E**) expression is depicted within total CD3+, CD8+ and CD4+ T-cells.

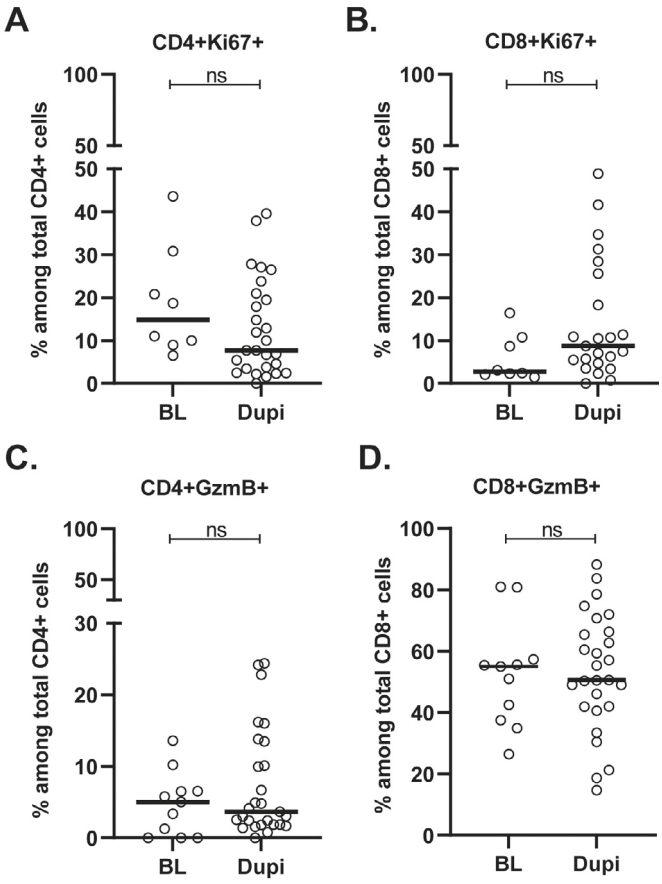

FIGURE S2. Flow cytometry results from patients whose CD4+ data were available.
The percentage (%) of CD4+Ki67+ cells **(A)**, CD8+Ki67+ cells **(B)**, CD4+ GzmB+ cells **(C)**, and CD8+ GzmB + cells **(D)** at baseline (BL, n=11) and during dupilumab treatment (dupi, n=27). As CD4+ data were not available of all patients at all timepoints, data during dupilumab treatment were combined. Ns= non-significant.

10

PART V

Discussion and appendices

CHAPTER 11

General discussion

Ocular surface disease (OSD), which can be used as an umbrella term for various ocular diseases such as conjunctivitis, blepharitis, and dry eyes, is a common finding in patients with moderate-to-severe atopic dermatitis (AD) before and during dupilumab treatment (Figure 1). The aims of the research presented in this thesis are to: 1) further identify the clinical and ophthalmological characteristics of OSD and its pathomechanism in patients with moderate-to-severe AD; 2) clarify the clinical and ophthalmological characteristics of dupilumab-associated ocular surface disease (DAOSD), investigate risk factors for its development, and learn more about the management of DAOSD and its long-term follow-up; 3) describe the pathomechanism of OSD during dupilumab treatment in patients with moderate-to-severe AD by analysing conjunctival impression cytology samples and tear fluid.

The implications, clinical recommendations, and suggestions for future research regarding the main findings of this thesis are discussed in this chapter.

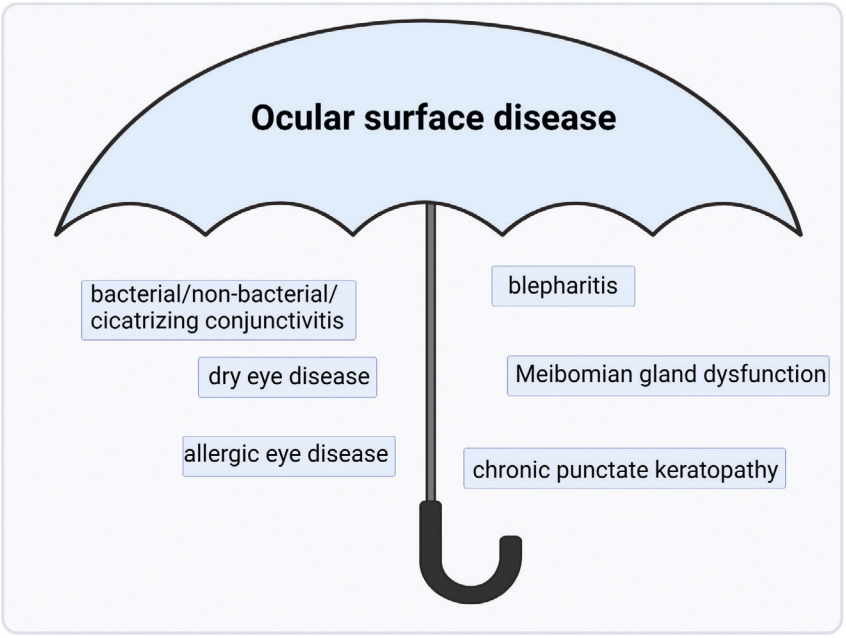

FIGURE 1. Eye conditions that fall under the term ocular surface disease.[1]
Figure created in Biorender.

MAIN FINDINGS OF THIS THESIS

Ocular surface disease in moderate-to-severe atopic dermatitis patients and its pathomechanism

- Before starting dupilumab treatment, 60/70 (90.0%) patients already had characteristics of OSD, and OSD severity was related to AD severity. – **Chapter 2**
- AD patients with OSD had low conjunctival goblet cell (GC) numbers – **Chapter 2**
- Higher levels of AD-related severity tear fluid biomarkers were found in patients with moderate-to-severe OSD compared to patients with no or mild OSD. – **Chapter 9**

Ocular surface disease as adverse event in moderate-to-severe atopic dermatitis patients treated with dupilumab

- Pre-existing ocular pathology (e.g. in combination with ophthalmic medication or eyelid eczema) could be associated with the development of DAOSD. – **Chapter 3**
- In a prospective study, 28.9% (n=20/69) of the patients were found to have DAOSD based on ophthalmic examination. Only 10/20 patients had persistent or new-onset DAOSD at week 28 of dupilumab treatment, suggesting that the severity of DAOSD improves with intensive ophthalmological follow-up and early treatment. – **Chapter 4**
- However, most patients still suffered from mild-to-moderate (DA)OSD during long-term follow-up (mean 17.5 months) despite ophthalmic treatment, as reported in a prospective case series (n=33). Dose adjustment or discontinuation of dupilumab due to ocular pathology was needed in 10/33 (30.0%) and 3/33 (9.0%) patients, respectively. – **Chapter 5**
- Some AD patients with DAOSD may benefit from switching to tralokinumab treatment, according to a prospective case series (n=4). – **Chapter 6**
- Dupilumab-associated uveitis is a serious and rare adverse event of dupilumab treatment with a molecular profile similar to that of non-infectious uveitis. – **Chapter 7**

The pathomechanism of dupilumab-associated ocular surface disease in moderate-to-severe atopic dermatitis patients

- The number of conjunctival GCs remained low or slightly increased during dupilumab treatment, while the expression of Mucin 5AC (MUC5AC) decreased, suggesting an impaired function of the conjunctival GCs as a result of dupilumab treatment. – **Chapter 4**

- Patients with moderate-to-severe OSD had higher dupilumab tear fluid levels compared to patients with no or mild OSD, demonstrating that dupilumab reaches the ocular surface. – **Chapter 8**
- Dupilumab was detected in conjunctival cell suspensions and was found to bind directly to CD45- conjunctival epithelial cells. – **Chapter 8**
- No differences in T helper (Th) 1- or Th17-associated tear fluid biomarker levels were observed during dupilumab treatment. – **Chapter 9**
- Patients with DAOSD had significantly higher granzyme B tear fluid levels compared to baseline, suggesting cytotoxic activity. – **Chapter 9**
- The immune response in the conjunctival epithelium may play a role in the pathogenesis of (DA)OSD, as the percentage of CD4+ T-cells was higher in AD patients before and during dupilumab treatment compared to healthy controls. – **Chapter 10**

The study design of chapters 2, 4, 6, 8, 9, and 10 is shown in Figure 2.

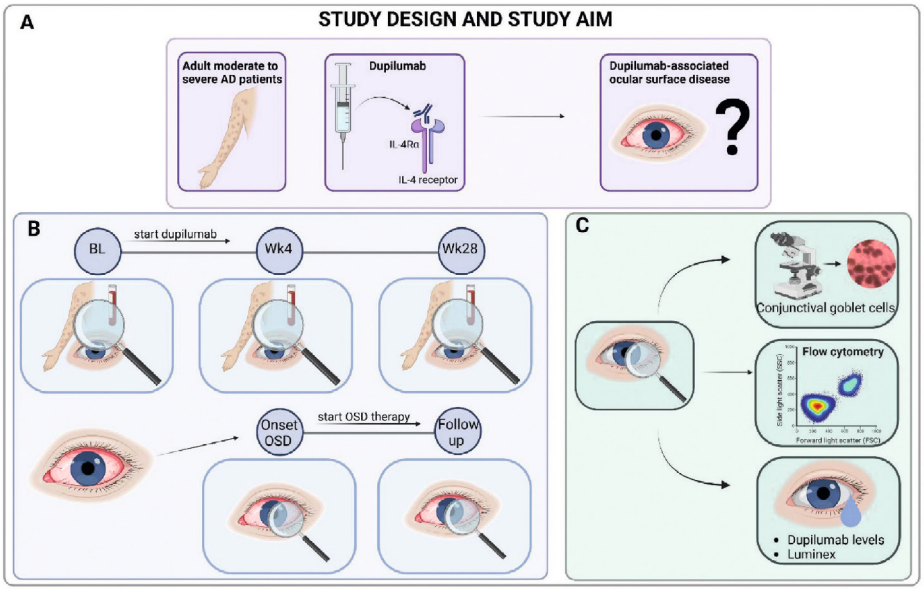

FIGURE 2. Study design of the prospective study.
A. Aim of the prospective study. **B.** Time points of the different visits and the conducted investigations. **C.** Additional analysis to the clinical ophthalmological examination. Abbreviations: atopic dermatitis, AD; BL, baseline; interleukin, IL; ocular surface disease, OSD; week, wk. The figure is partly taken from Figure 1 in Chapter 4 and is created in Biorender.

OCULAR SURFACE DISEASE IN MODERATE-TO-SEVERE ATOPIC DERMATITIS PATIENTS AND ITS PATHOMECHANISM

AD is known to be associated with ocular comorbidities.[2] Uncontrolled AD around the eyes may lead to chronic scratching and rubbing, which contributes to the risk of developing ocular symptoms.[2] For example, blepharoconjunctivitis can develop in patients with AD when S. aureus bacteria are spread from the skin to the eyes through frequent rubbing.[2] Ravn et al. found an overall prevalence of conjunctivitis in 31.7% of the AD patients, compared to 13.3% among controls.[3] In moderate-to-severe AD patients, the reported prevalence of conjunctivitis was even higher, up to 39.6%.[3] Other commonly reported ocular diseases in patients with moderate-to-severe AD include eyelid eczema, superficial punctate keratopathy, allergic conjunctivitis, atopic keratoconjunctivitis, cataract, increased risk of retinal detachment, keratoconus, and viral ocular infections.[2,4]

The development of ocular side effects during dupilumab treatment, the first biologic treatment for moderate-to-severe AD, highlights the importance of better understanding OSD in AD, and has renewed the interest in ocular comorbidity in patients with AD.[5]

Ocular surface disease in moderate-to-severe AD patients: diagnosis and severity measurement

In **chapter 2**, we investigated OSD in patients with moderate-to-severe AD before the start of dupilumab treatment. Patients included in this prospective study were seen by both a dermatologist and an ophthalmologist. To objectively assess the severity of OSD, we have introduced the Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score. The UTOPIA score is a standardized ophthalmic examination that includes the severity of inflammation of the eyelids (both blepharitis and Meibomian gland dysfunction), the conjunctiva (both tarsal and bulbar), and the limbus (Figure 3). For each eye, an overall severity category of no (UTOPIA 0), mild (UTOPIA 1-4), moderate (UTOPIA 5-8), or severe (UTOPIA ≥9) OSD is given based on the grading of the individual ophthalmic characteristics (Figure 3). The higher the UTOPIA score, the more severe the patient's OSD.

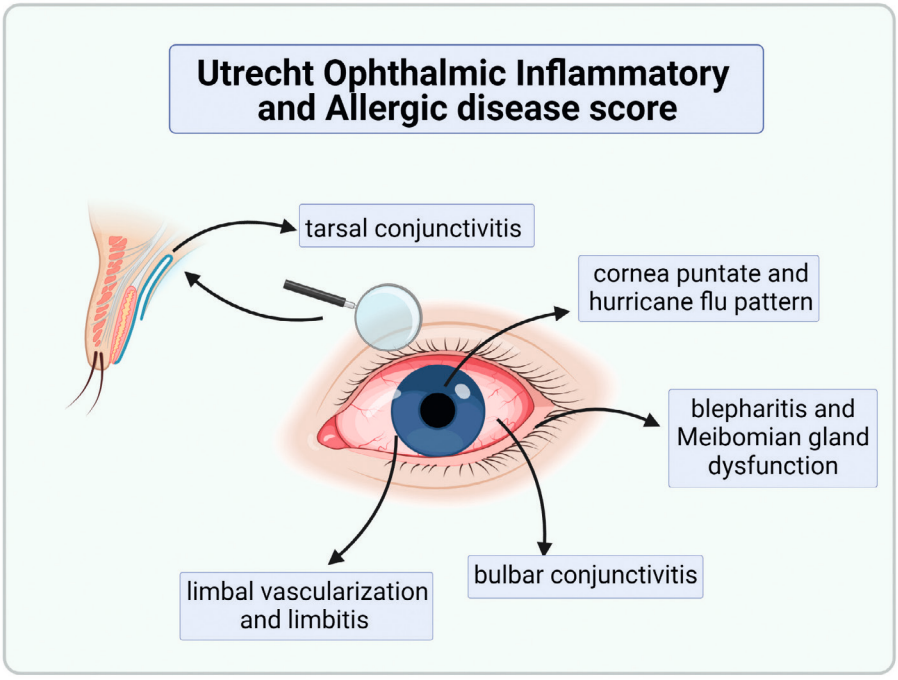

FIGURE 3. The individual characteristics of the Utrecht Ophthalmic Inflammatory and Allergic disease score (UTOPIA-score).
The severity ranges are shown in chapter 5. Figure created in Biorender.

Current literature uses different terminology to describe diseases of the ocular surface (e.g. conjunctivitis and blepharoconjunctivitis, both of which are part of OSD), making it difficult to compare studies. In addition, the characteristics of allergic conjunctivitis are different from those of AD-related OSD. Therefore, different terms are used when referring to specific studies in this chapter. Furthermore, several methods are used in the current literature to diagnose OSD, including patient-reported diagnosis, physician-confirmed diagnosis (without examination by an ophthalmologist), and ophthalmologist-confirmed diagnosis (with or without the use of an objective scoring system). Furthermore, many studies do not describe how OSD is diagnosed, or how to differentiate between different degrees of severity of ocular inflammation. This highlights the importance of using a standardized ophthalmic examination. By using a standardized score, such as the UTOPIA score, the severity of OSD can be measured over time, the severity of ocular side effects can be assessed, and the effect of ophthalmic therapies can be evaluated in more detail.

Chapter 2 shows that the majority of patients with moderate-to-severe AD already have OSD before starting dupilumab treatment (90.0%, n=60/70). Of all patients, 32/70 (45.7%) had mild OSD, 24/70 (34.3%) had moderate OSD, and 7/70 (10.0%) had severe OSD. Only 7/70 (10.0%) patients had no signs of OSD. In line with our results, Dogru et al. described papillary hypertrophy of the tarsal conjunctiva and blepharitis in most of the patients with moderate-to-severe AD (76.2% and 71.4%, respectively).[6] A study by Touhouche et al. also performed an ophthalmic examination in moderate-to-severe AD patients before starting dupilumab.[7] Interestingly, they observed papillae (a sign of tarsal conjunctivitis) in 17/46 (37.0%) patients, which is less than our finding in **chapter 2** of tarsal conjunctivitis in 57/70 (81.4%) patients.[7] However, Touhouche et al. did not describe clear definitions of the ophthalmic examination and the grading of the papillary severity, making comparison with the findings in **chapter 2** difficult.[7]

The importance of performing a standardized ophthalmological examination when investigating OSD in AD patients is emphasized by the fact that half of the patients with signs of OSD in **chapter 2** did not report any ocular symptoms (e.g. redness, itching, excessive tear fluid, burning sensation, pain, and photophobia). This shows that patient-reported OSD is less reliable, leading to possible underdiagnoses of OSD. Bortoluzzi et al. also found this discrepancy, as they reported lack of correlation between the Ocular Surface Disease Index (OSDI), which focuses in part on symptoms of OSD, and the clinical evaluation of the ocular surface.[8] Low OSDI scores were observed in patients with severe ocular surface involvement.[8]

Associations between OSD and AD

Despite the knowledge that OSD is common in moderate-to-severe AD patients, ophthalmic examination in these patients is seldomly performed in daily practice before starting dupilumab, which is essential to determine the difference between AD-related OSD and DAOSD.[9] However, as Foley et al. pointed out, the UTOPIA score may not be a suitable tool for the dermatologist, as this examination is performed with a slit lamp and requires ophthalmic knowledge and experience.[10] This emphasizes the need to learn more about the associations between OSD and dermatological, ophthalmological, and clinical factors, in order to identify which patients need an ophthalmic examination and ophthalmic treatment.

Nationwide Danish registries examined hospital diagnosis codes to learn more about the prevalence and risk of conjunctivitis (which was not differentiated into conjunctivitis subtypes) in mild and severe AD.[11,12] An increased lifetime incidence of conjunctivitis was observed in patients with active AD compared to patients with inactive AD (but with a previous diagnosis of AD).[11] In addition, patients with severe

AD had a higher risk of developing conjunctivitis than patients with mild AD.[11,12] **Chapter 2** describes that patients with moderate-to-severe OSD have more severe AD, based on higher Eczema Area and Severity Index (EASI), higher Investigator Global Assessment (IGA), and higher serum Thymus and Activation-Regulated Chemokine (TARC) levels than patients with no or mild OSD. Additionally, in **chapter 2**, significantly more patients with moderate-to-severe OSD reported the presence of eyelid and facial eczema in the past year compared to patients with no or mild OSD. Dogru et al. also found an association between OSD and eyelid or facial eczema in AD patients.[6] In addition, this study observed higher grades of conjunctival squamous metaplasia, which may occur as a result of chronic ocular inflammation, in patients with facial atopy and suggested that this may be the basis for the clinical morbidity of corneal complications in AD patients.[6,13] Altogether, the presence of eyelid or facial eczema and more severe AD may indicate an increased risk of (undiagnosed) OSD, from which dermatologists should be aware so patients can be referred to an ophthalmologist.

The association between the presence of eyelid and facial eczema (based on the head neck EASI) and OSD severity (measured by the UTOPIA score) is evaluated in **chapter 9**. The head neck EASI correlated with the UTOPIA score, indicating that patients with eyelid or facial eczema have significantly more severe OSD.

Conjunctival inflammation characteristics of OSD in AD patients

In **chapter 9**, we investigated tear fluid biomarkers in 16 moderate-to-severe AD patients before starting dupilumab treatment. Significantly higher IL-22 and TARC tear fluid levels were measured in patients with moderate-to-severe OSD compared to patients with no or mild OSD, as reported in **chapter 9**. Interestingly, TARC and IL-22 are known biomarkers for clinical severity of AD.[14] Since we have shown in **chapter 2** that more severe AD was related to more severe OSD, and **chapter 9** found a significant correlation between both total EASI and head neck EASI (including eyelid eczema) and tear fluid levels of TARC and IL-22, the high levels of TARC and IL-22 in the tear fluid of patients with moderate-to-severe OSD may be related to the severity of AD.[14] The relation between these biomarkers in tear fluid and the severity of AD might be explained by an impaired barrier function of the ocular surface epithelium. Yokoi et al. showed that the ocular surface barrier is impaired in AD patients with blepharoconjunctivitis, which is characterized by inflammation of the eyelid margin and the conjunctiva (e.g. blepharitis and conjunctivitis, respectively).[15,16] As reported in **chapter 2**, all patients with moderate-to-severe OSD had blepharitis and tarsal conjunctivitis, suggesting that these patients had a similar barrier dysfunction as described by Yokoi et al.[15]

Taken together, these results show that AD patients with moderate-to-severe OSD have high levels of AD-related tear fluid biomarkers, possibly due to the AD-related impaired conjunctival barrier function and/or local production by activated conjunctival epithelial cells, which are related to the severity of AD. This indicates that these patients may be predisposed to AD-related OSD, which is different from allergic conjunctivitis.

Conjunctival goblet cells and MUC5AC in moderate-to-severe AD patients

The assumption that patients with moderate-to-severe AD are predisposed to OSD, is also supported by the lower number of conjunctival GCs in these patients compared to healthy controls (**chapters 2 and 4**). Conjunctival impression cytology (CIC), a non-invasive technique to collect the first few cell layers of the conjunctival epithelium, was used to investigate the number of GCs and their function. Conjunctival GCs are normally found in the conjunctival tissue and have multiple functions. One of the main mucins produced by these GCs is MUC5AC, which has a protective function for the ocular surface.[17] In addition, conjunctival GCs improve the stability of the tear film and maintain the integrity of the mucosal barrier. GCs also have an important function in the mucosal immune system through the production of immunoregulatory factors, for example by suppressing the maturation and production of several cytokines, including interferon-gamma (IFN-γ).[18-20] In line with our findings, Dogru et al. previously reported significantly lower conjunctival GC densities in AD patients compared to controls.[6] In addition, **chapters 2 and 4** show that conjunctival GC counts were lower in AD patients with more severe OSD, suggesting that the number of GCs in AD patients is negatively correlated with the severity of the inflammation.

To investigate the function of the conjunctival GCs in AD-related OSD, MUC5AC secretion was examined in **chapters 2 and 4**. CIC samples from moderate-to-severe AD patients were analysed by using flow cytometry. Higher MUC5AC expression was found in patients with more severe OSD, while their GC numbers were lower compared to patients with no or mild OSD. Furthermore, **chapter 4** shows a non-significant but higher expression of MUC5AC in AD patients compared to healthy controls. Previous literature also reports that increased expression of MUC5AC, despite reduced conjunctival GC numbers, could be a defence response to compensate for the impaired ocular surface.[21]

In addition, we have found a higher CD4/CD8+ ratio in conjunctival epithelial cells in moderate-to-severe AD patients compared to healthy controls (**chapter 10**), possibly due to the reduced conjunctival barrier function in AD, allowing CD4+ cells to reach

the conjunctival epithelia.[15] It has been suggested that CD4+ cells that infiltrate the conjunctival epithelium may include Th1 cells, which could induce IFN-γ secretion.[18,22] In addition, CD8+ T-cells in the conjunctiva of AD patients may also express IFN-γ, as Hijnen et al. previously demonstrated that CD8+ T-cells in AD lesions produce IFN-γ.[23] Expression of IFN-γ is inversely correlated with GC density of the bulbar conjunctiva, suggesting that increased IFN-γ expression may lead to a lower number of GCs in moderate-to-severe AD patients.[18] A lower number of GCs could reduce the ocular surface protection due to decreased MUC5AC secretion, which may result in an impaired conjunctival barrier function leading to ocular inflammation. In addition, higher percentages of granzyme B, which may be cytotoxic for GCs, were found in patients with moderate-to-severe OSD compared to patients with no or mild OSD (**chapter 10**). Taken together, we hypothesize that this results in a self-reinforcing chain of events that influence each other, as shown in Figure 4.

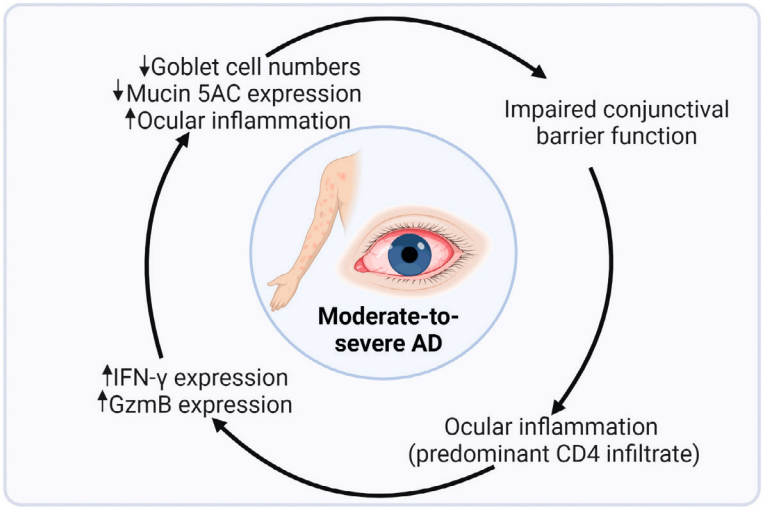

FIGURE 4. Hypothesis regarding the decreased goblet cell numbers and the ocular inflammation found in moderate-to-severe atopic dermatitis patients.
Abbreviations: AD, atopic dermatitis; GzmB, granzyme B; IFN-γ, interferon-gamma. Figure created in Biorender.

In other ocular diseases, such as graft-versus-host disease, the number of GCs is also negatively correlated with the severity of inflammation.[19] In contrast, GC hyperplasia and mucin hypersecretion have been reported in conjunctival papilloma, chronic injuries, atopic keratoconjunctivitis, and allergic conjunctivitis.[19,24] The Th2 cytokine IL-13 normally increases conjunctival GC proliferation and reduces GC apoptosis.[17] It has been suggested that exposure to allergens stimulates GC proliferation and mucin

secretion.[19] The low conjunctival GC densities that are observed in the moderate-to-severe AD patients with OSD indicate that AD-related OSD has a different pathogenesis than allergic conjunctivitis (Figure 5).

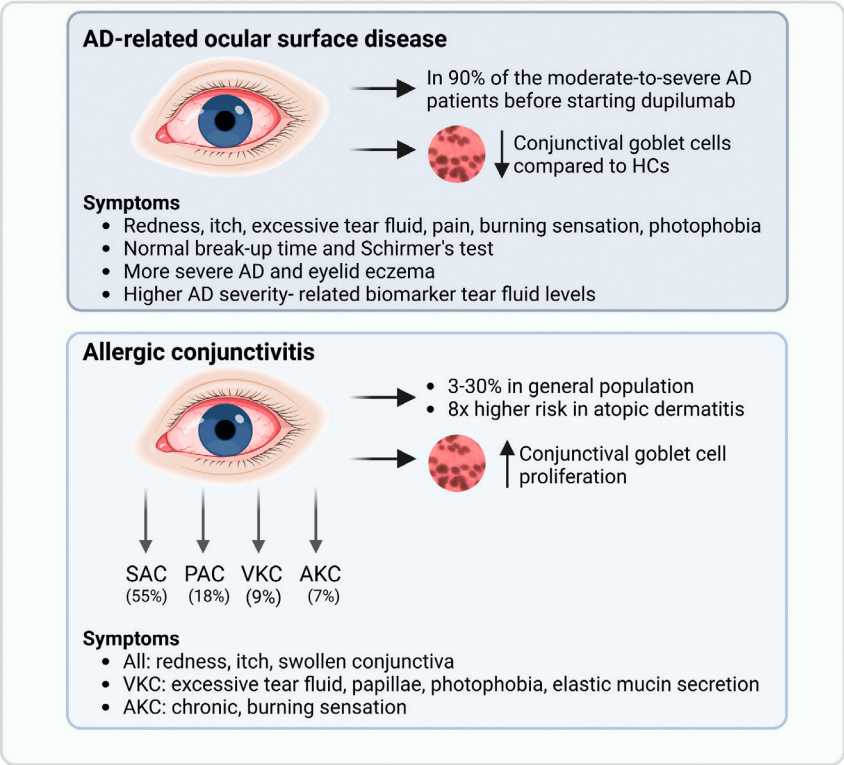

FIGURE 5. AD related ocular surface disease and allergic conjunctivitis: two different entities.

Characteristics of AD-related ocular surface disease compared to allergic conjunctivitis.[19,24-30] Abbreviations: AD, atopic dermatitis; AKC, atopic keratoconjunctivitis; HCs, healthy controls; PAC, perennial allergic conjunctivitis; SAC, seasonal allergic conjunctivitis; VKC, vernal keratoconjunctivitis. Figure created in Biorender.

To conclude, OSD is very frequent in patients with moderate-to-severe AD and is associated with low conjunctival GC numbers, possibly due to their (AD-related) conjunctival inflammation that may be further aggravated by the AD-related impaired conjunctival barrier function. In addition, AD patients with moderate-to-severe OSD more often have severe AD, eyelid eczema in the past year, and higher levels of AD-severity related tear fluid biomarkers compared to patients with no or mild OSD (Figure 5). The question arises as to what effect dupilumab will have on this pre-existing OSD, which will be discussed in the other parts of this general discussion.

OCULAR SURFACE DISEASE AS ADVERSE EVENT IN MODERATE-TO-SEVERE ATOPIC DERMATITIS PATIENTS TREATED WITH DUPILUMAB

Risk factors for the development of DAOSD

As DAOSD is the most commonly reported side effect in dupilumab-treated AD patients, it is desirable to identify which patients are at risk of developing DAOSD. **Chapter 3** identifies risk factors for the development of DAOSD in patients with moderate-to-severe AD by performing univariate and multivariate logistic regression analyses. This large prospective daily practice study included a total of 469 patients, in whom DAOSD was reported in 152/469 (32.4%) patients. Univariate analyses showed an association between AD eyelid involvement in the past year and the development of DAOSD, which was also found by Touhouche et al.[7] In **chapters 2 and 9,** both describing a prospective study of 70 and 16 AD patients respectively**,** we report that eyelid eczema was more common in patients with moderate-to-severe OSD compared to patients with no or mild OSD before starting dupilumab, and that eyelid eczema was associated with higher AD-related severity tear fluid biomarkers.

This raises the question whether patients with pre-existing OSD are predisposed to developing DAOSD. **Chapter 3** reports that having a history of any eye disease (excluding self-reported allergic conjunctivitis) combined with the use of ophthalmic medication before starting dupilumab treatment is a risk factor for the development of DAOSD. Katsuta et al. also reported that AD patients with DAOSD were more likely to have a history of ocular complications, suggesting that patients who develop DAOSD may already have an (undiagnosed) ocular disease that possibly worsens during treatment with dupilumab, which was also mentioned by Popiela et al.[31,32]. Taken together, this highlights the need to perform an ophthalmic examination before starting dupilumab treatment to identify and, if necessary, treat pre-existing ocular pathology.

Unlike some other studies that have examined risk factors for DAOSD, we found no association between AD severity at baseline and the development of DAOSD (**chapter 3**).[5,33] However, dupilumab-treated AD patients with a larger decrease in EASI within the first 16 weeks of treatment were more likely to develop DAOSD. This could indicate that a better clinical efficacy of dupilumab in AD may be associated with a higher risk of developing DAOSD.

Taken together, the risk factor analyses in **chapter 3** show that pre-existing ocular pathology (e.g. in combination with ophthalmic medication or eyelid eczema) may be associated with the development of DAOSD.

Signs and symptoms of DAOSD

To learn more about potential risk factors, more knowledge is needed regarding ophthalmic characteristics of DAOSD. The study presented in **chapter 4** examines the frequency and severity of DAOSD in a prospective study (n=69) to further investigate the ophthalmic characteristics of DAOSD. OSD was present in 91.3% (n=63/69) of the patients before starting dupilumab treatment. Both before and during dupilumab treatment, tarsal inflammation of the conjunctiva was the most frequently observed characteristic of OSD, which is in line with previous literature.[6,34,35] DAOSD (i.e. ≥3 points UTOPIA increase from baseline) was observed in 20/69 (28.9%) patients and developed after a median of 12.5 weeks (IQR 4.3-30.8 weeks). This is relatively later than other studies, which reported the onset of DAOSD after 2 to 8 weeks of dupilumab treatment.[5,31] A possible explanation for this difference is that most of the patients in **chapter 4** had pre-existing OSD before starting dupilumab treatment, and some of them started ophthalmic treatment. In addition, DAOSD was based on ophthalmic examination and not on patient-reported diagnosis, which may also explain the difference.

Most of the patients in **chapter 4** who developed DAOSD had no or mild OSD before starting dupilumab (n=5/20 (25.0%) and n=11/20 (55.0%), respectively). As a result, only few of these patients received ophthalmic drugs at the onset of DAOSD (e.g., only 1 patient received anti-inflammatory eye drops). While none of the 20 patients who developed DAOSD had limbitis at baseline, 5/20 (25.0%) patients showed limbitis at the onset of DAOSD. Severe chronic (DA)OSD with limbal involvement, defined as limbitis, may be the result of chronic ocular inflammation. Limbitis could lead to limbal stem cell deficiency with possible irreversible long-term effects, such as conjunctivalization (i.e. conjunctival tissue covering the cornea), and requires long-term follow-up.[36,37]

Remarkably, not all patients with (DA)OSD report symptoms before and during dupilumab treatment (**chapters 2 and 4**), suggesting that the patient-reported diagnosis is less reliable. Only 37/69 (53.6%), 33/64 (51.6%), and 38/69 (55.1%) patients reported symptoms of OSD at baseline, and after 4 weeks and 28 weeks of treatment with dupilumab, respectively. It might be possible that low corneal sensitivity may play a role in the low symptomatic ocular surface condition in AD patients with OSD, as reduced corneal sensitivity scores have been observed in patients with atopic keratoconjunctivitis, dry eye disease, and Sjögren's syndrome.[8,38-40] Adatia et al. stated that reduced corneal sensation was correlated with the severity and chronicity of dry eye disease in patients with Sjögren's syndrome, suggesting that subjective symptoms may decrease over time due to its chronicity.[40] Interestingly, **chapter 4** shows that patient-reported diagnosis seemed to be more reliable in the case of

DAOSD, as 18/20 (90.0%) patients with DAOSD reported ocular symptoms at the onset of DAOSD. Based on these findings, we hypothesize that AD patients with AD-related OSD may report fewer symptoms due to its chronicity. In addition, we hypothesize that AD patients with DAOSD, including new-onset DAOSD or worsening of pre-existing OSD during dupilumab treatment, may report symptoms more adequately as it has a more acute onset. However, it is also possible that AD patients with DAOSD were more focused on reporting ocular symptoms, since they were examined ophthalmologically both at baseline and during dupilumab treatment.

Treatment of DAOSD

Chapter 4 describes the effect of different ophthalmic treatments on (DA)OSD and their prescription frequency. Patients were examined ophthalmologically (both before and during dupilumab treatment), and ophthalmic treatment was started if needed (e.g., in case of pre-existing OSD or worsening of (DA)OSD). Only 5.8% (n=4/69) of the patients were already using ophthalmic medications before starting dupilumab treatment, showing that OSD in AD is often undetected leading to undertreatment. As dermatologists do not usually ask their AD patients about eye complaints, and AD patients often do not report OSD symptoms, there is a higher risk of long-term consequences of (undiagnosed) OSD in AD patients. Since many patients (n=63/69, 91.3%) appeared to have OSD at the first ophthalmological examination in **chapter 4**, 59.4% (n=38/64) of the patients started ophthalmic treatment at baseline and continued to use this after 4 weeks of dupilumab treatment. At week 28 of dupilumab treatment, 55.1% (n=38/69) of the patients were using ophthalmic therapy. Therefore, it may be possible that the patients studied in **chapter 4** have less severe OSD due to this early ophthalmic treatment.

A total of 20 patients developed DAOSD in **chapter 4**, which was controlled in 50.0% (n=10/20) of the patients at week 28 (i.e. patients with a previous increase in UTOPIA of ≥3 points from baseline, but no longer at week 28). Six of these 10 patients were receiving anti-inflammatory ophthalmic treatment (e.g. tacrolimus skin ointment or corticosteroid eye drops), suggesting that early treatment improves the severity of DAOSD. Of the 85.5% (n=59/69) patients with no or controlled DAOSD at week 28, 40.7% (n=24/59) patients received anti-inflammatory ophthalmic treatment. These patients had lower UTOPIA scores compared to patients treated with antihistamine eye drops or without treatment, indicating that anti-inflammatory therapies are effective in the treatment of ocular surface inflammation.

Popiela et al. retrospectively investigated the presentation, management, and long-term consequences of DAOSD in 28 dupilumab-treated AD patients.[31] This

study reported DAOSD in 32.1% (n=9/28) of the patients, and a good response to corticosteroid eye drops was observed in 7 of these patients.[31] Nahun et al. investigated a retrospective cohort of 37 dupilumab-treated AD patients, and found rapid resolution of signs and symptoms in 4 patients with DAOSD (specifically with blepharoconjunctivitis) after treatment with tacrolimus skin ointment (twice daily for 3 weeks).[41] Tacrolimus skin ointment has also shown efficacy in the treatment of OSD in other previous studies.[42,43] In addition, we have shown improvement in ocular inflammation in dupilumab-treated AD patients who were treated with tacrolimus skin ointment on the external eyelids (**chapter 4**). Combined with the finding that the presence of eyelid eczema in the past year is associated with the development of DAOSD (**chapter 3**), this suggests that early treatment of eyelid eczema could potentially lead to a reduced risk of developing DAOSD. However, as both dermatologists and patients are reluctant to use topical corticosteroids on the eyelids, eyelid eczema is often undertreated.[44] Nevertheless, it is important to treat eyelid eczema, especially as it appears to be associated with AD-related OSD (Figure 5). If long-term treatment is required, tacrolimus skin ointment can be safely used on the eyelids for prolonged periods and is effective in treating eyelid eczema.[42,44] We therefore recommend tacrolimus skin ointment for the treatment of eyelid eczema.

Long-term follow up of DAOSD

Overall, the long-term management of DAOSD can be challenging. Ophthalmic corticosteroids are effective in the treatment of DAOSD, but as these medications can cause long-term side effects (e.g. cataract and glaucoma), data on the long-term follow-up of patients with DAOSD are needed.[44] The study presented in **chapter 5** investigates patients with an ophthalmologist-confirmed DAOSD and their long-term follow-up (with a mean follow-up duration of 17.5 months). Self-reported DAOSD was found in 66/167 (39.5%) dupilumab-treated AD patients, of which 33/66 (50.0%) patients were referred to an ophthalmologist because their patient-reported symptoms could not be controlled with artificial tears and/or tacrolimus skin ointment (0.1%) for the external eyelids. Most of these 33 patients still had mild-to-moderate DAOSD after long-term follow-up, despite anti-inflammatory ophthalmic treatment. This is comparable to the findings of Popiela et al., who described that 67.0% (n=6/9) of the dupilumab-treated AD patients remained on ophthalmic corticosteroids after a mean follow-up of 16 months.[31]

Long-term data of dupilumab trials (phase 1 to 3) in AD patients showed that after 3 years of treatment with dupilumab, 20.7% (45/217 events) of the DAOSD events still persisted, and that the majority of DAOSD cases resolved with ophthalmic treatment (e.g. ophthalmic corticosteroids).[45,46] In addition, 87.3% (775/888 cases) of the DAOSD

cases were resolved after 4 years of treatment.[47] These results contradict our findings, as we found that most patients still suffered from mild-to-moderate DAOSD during long-term follow-up despite anti-inflammatory ophthalmic treatment, of which long-term use (in the case of ophthalmic corticosteroids) is not recommended (**chapter 5**). In addition, we even found worsening of DAOSD during follow-up (median follow-up time 17.6 months), as new-onset limbitis was observed in 8 patients (n=8/27, 29.6%). The differences between our results and the data from the phase 3 trials may be explained by the fact that the phase 3 trials investigated patient-reported diagnosis, which appears to be less reliable. Moreover, the studies that investigated long-term data from the dupilumab trials excluded patients who discontinued dupilumab treatment due to side effects, like DAOSD.[45-47] However, the follow-up in the dupilumab trials was longer than the follow-up in **chapter 5**, which may also explain the differences and suggest that DAOSD may improve after a longer period of treatment. Long-term real-world data on DAOSD are needed to compare with data from the dupilumab trials, which we will be looking at in the future.

Effect of dose adjustment of dupilumab on DAOSD

Chapter 5 examines the effect of dose adjustment of dupilumab on DAOSD. Prolongation of the dosing interval to 300 mg dupilumab every 3 to 5 weeks due to ocular pathology was needed in 10/33 (30.0%) patients, resulting in improvement of ocular inflammation in 6 patients and remission in 1 patient. Dupilumab was discontinued due to ocular pathology in 3/33 (9.1%) patients, leading to improvement or remission in all cases. Patruno et al. found improvement in ocular symptoms in 46.6% (n=7/15) of the patients with DAOSD after prolongation of the dosing interval to 300 mg every 3 to 4 weeks, without worsening of AD.[48] In addition, Spekhorst et al. reported persistent AD control in patients who tapered the dosage of dupilumab due to controlled AD.[49] The beneficial effect of dose reduction on DAOSD seems to be in contrast with the study by Akinlade et al., who pooled data on dupilumab concentrations of phase 3 dupilumab trials from baseline to week 16 and suggested that the incidence of DAOSD may decrease with higher serum concentrations of dupilumab.[5] However, these studies reported patient-reported DAOSD. In addition, the first phase 3 dupilumab trials (SOLO 1 and 2) reported lower rates of DAOSD than other phase 3 trials and real-world data, possibly because less was known about DAOSD at that time.[5,33] Since prolongation of the dosing interval may reduce the severity of DAOSD, and does not lead to exacerbation in AD patients with controlled disease, this could be an effective strategy to reduce the severity of DAOSD.[49]

Switching to a different treatment for AD due to DAOSD

If DAOSD cannot be controlled with anti-inflammatory treatment or dose reduction of dupilumab treatment, switching to a different targeted therapy to treat AD may be a solution. Phase 3 trials of tralokinumab, a biologic therapy that specifically targets IL-13, reported fewer OSD as adverse event compared to phase 3 trials of dupilumab (7.5% vs. 8.6-22.1%, respectively).[5,50] However, low percentages of DAOSD were also observed in the early dupilumab trials. Additionally, a head-to-head trial, which would be needed to compare OSD rates between these biologic therapies, has not yet been conducted. Consistent with dupilumab phase 3 trials in other type 2 diseases, no increased rates of OSD were reported in asthma patients treated with tralokinumab, suggesting that AD patients may be predisposed to develop both DAOSD and tralokinumab-associated OSD (TAOSD).[51] As tralokinumab specifically targets IL-13, it is hypothesized that this may lead to less OSD compared to dupilumab, which affects both IL-4 and IL-13 signalling.[50] However, the pathomechanism leading to TAOSD and DAOSD remains unclear.[50]

The prospective case series in **chapter 6** describes the effect of switching from dupilumab to tralokinumab treatment in 4 AD patients with DAOSD. Ocular inflammation (assessed by the UTOPIA score), OSD symptoms, and ophthalmic medication use were evaluated. Improvement of inflammation was seen in 3/4 patients and all patients reduced their use of ophthalmic medication during tralokinumab treatment, suggesting that some patients with DAOSD may benefit from switching to tralokinumab. Pezollo et al. investigated the effect of tralokinumab in 12 patients who did not respond to dupilumab treatment.[52] They reported no serious adverse events and observed that the previous DAOSD did not relapse during tralokinumab treatment (n=4/12).[52] However, these patients were not examined ophthalmologically, making it difficult to compare these results with our findings. Further studies are needed to learn more about the effect of switching to tralokinumab treatment in patients with DAOSD.

Other ophthalmic side effects during dupilumab treatment

In addition to DAOSD, a rare but more serious ophthalmological side effect of dupilumab in AD patients is dupilumab-associated uveitis. Studies on dupilumab-associated uveitis are limited to a few case reports and small case series. In these patients, dupilumab-associated uveitis was clinically similar to non-infectious uveitis and resolved after discontinuation of dupilumab treatment.[53-55] Interestingly, AD does not predispose to non-infectious uveitis, suggesting that the development of uveitis during dupilumab treatment may be dupilumab-related.[2] **Chapter 7** describes the clinical characteristics of dupilumab-associated uveitis in 5 AD patients. The uveitis resolved after discontinuation of dupilumab (n=4) and/or treatment with local or

systemic corticosteroids (n=1), in line with previous studies.[53-55] In addition, the proteomic profile of aqueous humor (AqH) of dupilumab-associated uveitis (n=3/5 available samples) was compared with non-infectious uveitis (n=27) and cataract controls (n=11). Principal component analysis of the profile of 77 proteins detected in AqH showed that dupilumab-associated uveitis patients had a pro-inflammatory profile comparable to non-infectious uveitis.

The pathomechanism of AD is characterized by a Th2 cell immune response, while autoimmune uveitis, which is a group of diseases without a known infectious trigger (e.g. non-infectious uveitis), is mainly characterized by a Th1 or Th17 cell response.[33,56-58] Interestingly, other Th1/Th17 related adverse events are observed in AD patients during dupilumab treatment, such as psoriasis, enthesitis, and rosacea.[57,59] Bridgewood et al. stated that IL-4 and IL-13 normally inhibit the production of IL-23 and IL-17, leading to downregulation of the Th1/Th17 pathway activation.[57] They hypothesized that due to dupilumab the IL-23 and IL-17 production is upregulated, leading to upregulation of the Th1/Th17 pathway activation which can result in the Th1/Th17 related adverse events, such as dupilumab-associated uveitis.[57] **Chapter 7** reports a pro-inflammatory profile comparable to non-infectious uveitis, but no specific Th1/Th17 profile was observed. However, the number of analysed patients was low (n=3), so larger studies are needed to learn more about dupilumab-associated uveitis. However, as dupilumab-associated uveitis can cause severe vision-threatening intraocular inflammation, we recommend urgent referral to an ophthalmologist in the event of vision loss combined with pain.

Summary and clinical implications

In summary, DAOSD occurs in approximately one-third of the dupilumab-treated AD patients. Ocular symptoms are most commonly reported by patients with severe OSD and at the onset of DAOSD. However, many moderate-to-severe AD patients with OSD often report no ocular symptoms, both before and during dupilumab treatment. In addition, pre-existing ocular pathology (e.g. in combination with ophthalmic medication or eyelid eczema) is associated with the development of (patient-reported) DAOSD. These points highlight the need for a standardized ophthalmic examination, such as the UTOPIA score. Future research could focus on the risk of developing ophthalmic-confirmed DAOSD to better identify patients at risk of developing DAOSD by investigating the individual baseline characteristics of OSD (e.g. blepharitis and tarsal conjunctivitis) and its association to developing DAOSD.

Early ophthalmic treatment reduces the severity of DAOSD, and anti-inflammatory ophthalmic drugs are most effective. However, long-term use of ophthalmic

corticosteroids should be avoided because of side effects such as glaucoma and cataracts.[44] Contrary to corticosteroid ophthalmic therapies, tacrolimus skin ointment does not elevate the intraocular pressure.[42,44] Therefore, we recommend prescribing tacrolimus skin ointment (0.1%) for the external eyelids as initial treatment for DAOSD. If this is not sufficient, referral to an ophthalmologist for examination and treatment with anti-inflammatory ophthalmic drugs is recommended. In addition, as extending the dosing interval may reduce DAOSD severity, and does not lead to exacerbation in AD patients with controlled disease, this could be an effective strategy to reduce the severity of DAOSD.[49] Lastly, switching to another biologic or small molecule might be effective for some patients with DAOSD.

In addition to DAOSD, more serious ocular side events may occur in dupilumab-treated AD patients, such as dupilumab-associated uveitis. As this can cause severe vision-threating intraocular inflammation, we recommend urgent referral to an ophthalmologist in case of (acute) pain and loss of vision.

Figure 6 summarises the recommendations for referral to an ophthalmologist in case of ophthalmic symptoms during dupilumab treatment.

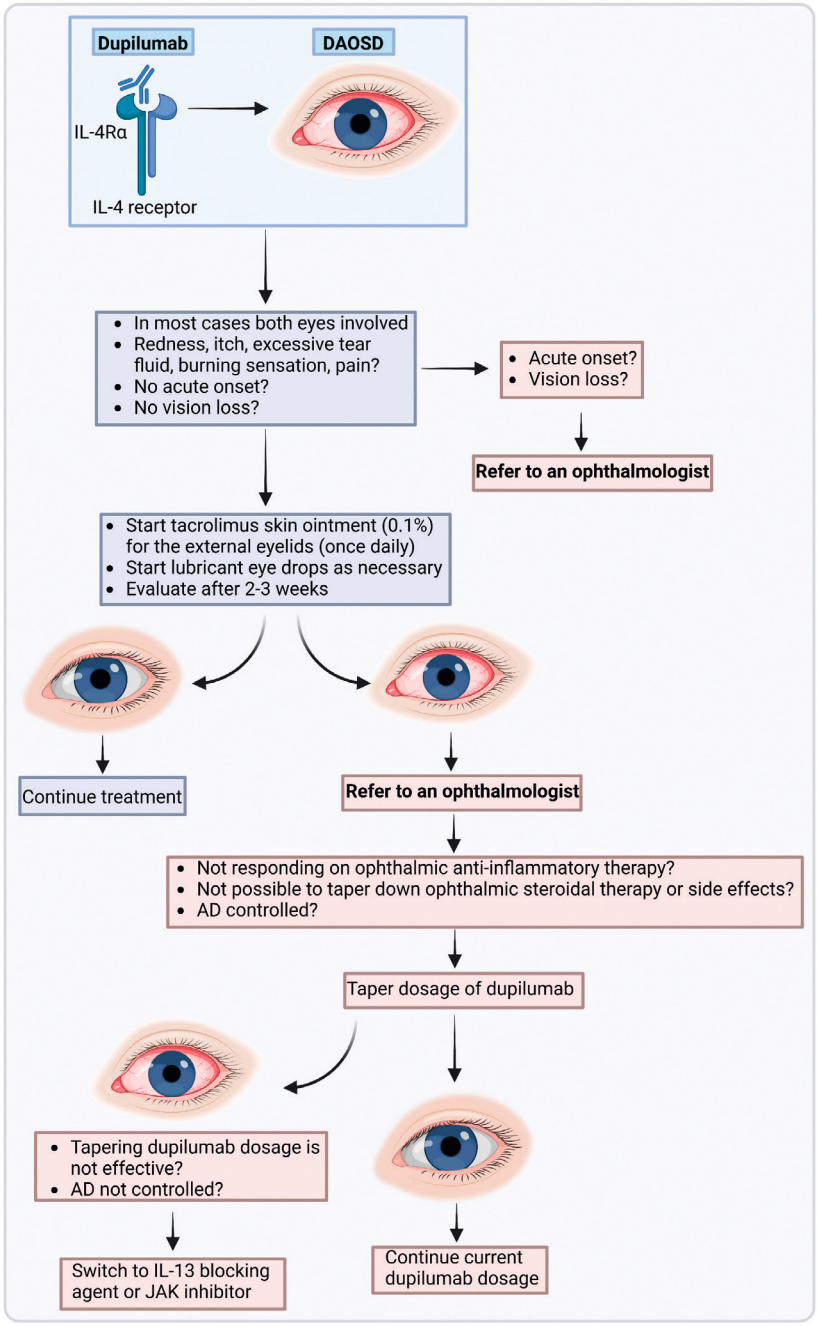

FIGURE 6. Recommendations regarding referral to an ophthalmologist in case of dupilumab-associated ocular pathology.

Abbreviations: AD, atopic dermatitis; DAOSD, dupilumab-associated ocular surface disease; IL, interleukin; JAK, janus kinase inhibitor. Figure created in Biorender.

THE PATHOMECHANISM OF DUPILUMAB-ASSOCIATED OCULAR SURFACE DISEASE IN MODERATE-TO-SEVERE ATOPIC DERMATITIS PATIENTS

Dupilumab tear fluid levels and OSD severity in AD patients

The exact pathomechanism of DAOSD in AD patients remains unclear. Several hypotheses have been suggested, such as undertreatment of the eyes by dupilumab.[60] This hypothesis was based on an inverse relationship between serum dupilumab levels at week 16 and the development of DAOSD. The study presented in **chapter 8** measures dupilumab levels in tear fluid and serum by using liquid chromatography with tandem mass spectrometry (LC-MS/MS), and relates these findings to the severity of OSD during dupilumab treatment in AD patients (n=48). At baseline, 89.6% (n=43/48) of the patients had OSD, with 50.0% having moderate-to-severe OSD. At week 28 of dupilumab treatment, median dupilumab tear fluid levels were significantly higher in patients with moderate-to-severe OSD compared to patients with no or mild OSD, while serum levels were not related to OSD severity. These results disprove the hypothesis that under-treatment of the eyes by dupilumab leads to the development of DAOSD.

Dupilumab is a monoclonal IgG4 antibody that inhibits the IL-4 and IL-13 signalling. Elevated levels of IgG in tear fluid have been found in several ocular diseases.[61-67] For example, increased levels of total IgG in tear fluid were observed in patients with vernal conjunctivitis and in patients with acute adenoviral conjunctivitis.[62,65] While IgG plays an important role in the immune response to microbial organisms and toxins in serum, the exact function of IgG in tears remains unknown.[68] However, it is known that IgG crosses the blood-tear barrier as a result of ocular inflammation and increased vascular permeability.[69] It has also been suggested that measuring IgG levels in tear fluid may be a useful marker of the extent of inflammation and vascular permeability.[69] A study that examined tears from 20 chickens showed that IgG is indeed transferred from serum to tear fluid.[70] In addition, Yokoi et al. reported an impaired conjunctival barrier in AD patients with blepharoconjunctivitis.[15] Taken together, the altered vascular permeability and reduced AD-related conjunctival barrier function in AD patients with OSD may lead to leakage of IgG (including the IgG4 antibody dupilumab) from the vessels into the tear fluid, resulting in the detection of dupilumab in the tear fluid. As tear fluid IgG levels may be a marker for the extent of inflammation, this may also explain why higher levels of dupilumab are found in the tear fluid of patients with more severe OSD.

Relation between serum dupilumab levels and the development of DAOSD

It is hypothesized that the incidence of DAOSD may decrease with higher dupilumab serum concentrations.[5] This is in contrast to the results presented in **chapter 8**, which showed no relationship between serum dupilumab levels and OSD severity after 28 weeks of treatment with dupilumab. The difference may be explained by the fact that we assessed the severity of (DA)OSD by an ophthalmic examination, whereas Akinlade et al. based DAOSD on patient-reported symptoms.[5]

In line with the results presented in **chapter 8**, Spekhorst et al. showed no relationship between serum dupilumab levels and the development of DAOSD in 295 dupilumab-treated AD patients.[71] A broad range of serum dupilumab levels at week 16 was found, suggesting that the treatment response to dupilumab may depend on the target availability of IL-4 receptor alpha (IL-4Rα).[71] High dupilumab levels could lead to complete saturation of IL-4Rα, whose expression varies between patients, suggesting that the required serum level of dupilumab to affect IL-4Rα differs among patients.[71] Interestingly, in-vitro studies have shown that IL-4Rα is also present on conjunctival epithelial cells and on conjunctival GCs.[72,73] To investigate this further, we conducted a pilot study measuring dupilumab on conjunctival epithelial cells using flow cytometry and LC-MS/MS (**chapter 8**). Dupilumab levels were detected on conjunctival epithelial cell suspensions (n=5) and reduced conjunctival IL-4Rα expression was observed after 4 weeks of treatment with dupilumab (n=4). Since dupilumab-treated AD patients with more severe OSD had high levels of dupilumab in their tear fluid (**chapter 8**), it is possible that this leads to complete saturation of IL-4Rα on conjunctival cells, and may result in a relative individual overdose of dupilumab in the eyes. This is supported by the findings presented in **chapter 5**, describing a prospective case series of 33 dupilumab-treated AD patients with ophthalmic confirmed DAOSD. Prolonging the dupilumab dosing interval resulted in less signs and symptoms (as assessed by the UTOPIA score), possibly as a result of reduced dupilumab levels in the eyes. Taken together, these results indicate that dupilumab reaches the ocular surface, and as dose reduction of dupilumab may lead to lower dupilumab tear fluid levels, the increased local drug availability in the eyes may play a role in the development of DAOSD. Furthermore, as Hansen et al. reported that IL-4Rα is present on conjunctival GCs, we hypothesize that local dupilumab levels in the eyes could worsen (DA)OSD by interfering with the conjunctival GC development.[73]

Effect of dupilumab on conjunctival goblet cells and Mucin 5AC

GC scarcity may play a role in the development of DAOSD. Bakker et al. found low conjunctival GC numbers in conjunctival biopsies from 6 AD patients with DAOSD.[74] In addition, Voorberg et al. reported recurrence of GCs after discontinuation of dupilumab (n=1).[75] However, **chapter 2** describes that moderate-to-severe AD patients already have lower conjunctival GCs compared to healthy controls before the start of dupilumab treatment. The study presented in **chapter 4** investigates the effect of dupilumab on GC numbers and its function in a prospective study (n=69). The number of conjunctival GCs increased slightly during dupilumab treatment while the percentage of Cytokeratin 19 (CK19)-CD45-MUC5AC+ cells decreased significantly. In addition, DAOSD was observed in 28.9% (n=20/69) of the patients, in whom the number of GCs remained stable and the percentage of CK19-CD45-MUC5AC+ cells decreased at the onset of DAOSD compared to baseline. A relative deficiency of MUC5AC in the tear fluid of dupilumab-treated patients was also reported by Barnett et al.[35] Taken together, these findings suggest that the function of the conjunctival GCs may be impaired as a result of dupilumab treatment, which is reflected by decreased MUC5AC expression.

Remarkably, patients treated with dupilumab for other type 2 diseases (e.g. asthma and chronic rhinosinusitis with nasal polyps) do not show increased rates of DAOSD.[5] Interestingly, both asthma patients and patients with chronic rhinosinusitis with nasal polyps have GC hyperplasia in their airway epithelia and nasal epithelia, respectively.[76-78] Therefore, it might be possible that the conjunctival GC numbers are also relatively high in these patients, thereby, the impaired GC function induced by dupilumab will not lead to DAOSD in patients with asthma or with chronic rhinosinusitis with nasal polyps. However, it is currently unknown whether patients with asthma or with chronic rhinosinusitis with nasal polyps (both without having AD) have pre-existing OSD and/or have a normal conjunctival GC density, which may be an interesting topic for future research.

Eosinophils and their potential role in the development of DAOSD

One of the characteristics of type 2 inflammation in AD and asthma is an increased number of eosinophils, defined as eosinophilia. Dupilumab blocks IL-4/IL-13-induced eosinophil migration into tissues, resulting in transient eosinophilia in both dupilumab-treated AD and dupilumab-treated asthma patients.[79,80] Several studies have reported an association between the development of DAOSD and the transient eosinophilia in dupilumab-treated AD patients.[32,81,82] In addition, **chapter 3** shows a significantly stronger increase in peripheral blood eosinophils at week 16 compared to baseline in AD patients who developed DAOSD compared to those who did not. An increase in

eosinophils during dupilumab treatment is associated with eosinophilia at baseline.[83] In addition, eosinophilia is associated with more severe AD.[84] As AD-related OSD and eosinophilia are more common in more severe AD, the eosinophilia found in DAOSD patients during dupilumab treatment may be related to the AD-severity and not to DAOSD. Interestingly, Bakker et al. found eosinophils in the stromal infiltrate in conjunctival biopsies from AD patients with DAOSD, but these eosinophils were not degranulated or activated.[74] In contrast, activated eosinophils are present in conjunctival tissue of patients with allergic conjunctivitis and are absent in epithelium of non-atopic controls.[85-87] Furthermore, dupilumab-treated asthma patients did not develop DAOSD, while transient eosinophilia also occurred during dupilumab treatment.[5,80] Taken together, it seems unlikely that transient peripheral blood eosinophilia due to dupilumab treatment is directly involved in the pathogenesis of DAOSD in AD patients.

Conjunctival inflammation during dupilumab treatment

To characterize the conjunctival inflammation during dupilumab treatment, we investigated tear fluid biomarker levels in dupilumab-treated AD patients (n=16) before and during dupilumab treatment (**chapter 9**). Bakker et al. found increased granzyme B in conjunctival biopsies of 6 patients with DAOSD, and suggested that this could have a cytotoxic effect on conjunctival GCs.[88] Granzyme B is released by cytotoxic T lymphocytes and natural killer cells, and destroys tumour cells or virus cells by activating of apoptosis.[89] Consistent with the findings of Bakker et al., **chapter 9** shows significantly higher granzyme B fluid levels at the onset of DAOSD (i.e. ≥3 points UTOPIA increase from baseline) compared to baseline. Additionally, **chapter 10** presents a study that measures CIC samples by flow cytometry (n=49) and shows a higher percentage of CD8+ granzyme B+ cells (although not significant at all visits) in patients with moderate-to-severe OSD compared to patients with no or mild OSD. However, small cohorts were studied and more data are needed to learn more about the potential role of granzyme B in the development of DAOSD.

Interestingly, **chapter 9** also demonstrates significantly higher tear fluid levels of IL-22, TARC, and periostin, known as AD-related severity biomarkers, in patients with moderate-to-severe OSD compared to patients with no or mild OSD before starting dupilumab.[14] This suggests that patients with moderate-to-severe AD could have AD-related OSD, which differs from allergic conjunctivitis. Tear fluid levels of TARC and periostin decreased during dupilumab treatment but remained higher in patients with moderate-to severe OSD compared to patients with no or mild OSD during dupilumab treatment, suggesting that these biomarkers are related to OSD severity. A prospective study of Ariëns et al. investigated serum biomarkers of 36 dupilumab-treated patients

after 16 weeks of treatment with dupilumab. They found a significant decrease in serum levels of IL-22, TARC, and periostin after 16 weeks of dupilumab treatment compared to baseline.[33] This suggests that dupilumab treatment leads to a decrease in the AD-related severity biomarkers in both tear fluid and serum. This may indicate a beneficial effect of dupilumab on AD-related OSD. On the other hand, **chapter 4** showed an impairment of GC function by dupilumab, suggesting that dupilumab could have both a positive and negative effect on OSD in AD patients. We hypothesize that this imbalance may shift in favour of the beneficial effect after a longer treatment period with dupilumab, as long-term data from the dupilumab trials showed that most cases of DAOSD resolved over time (Figure 7).[45-47]

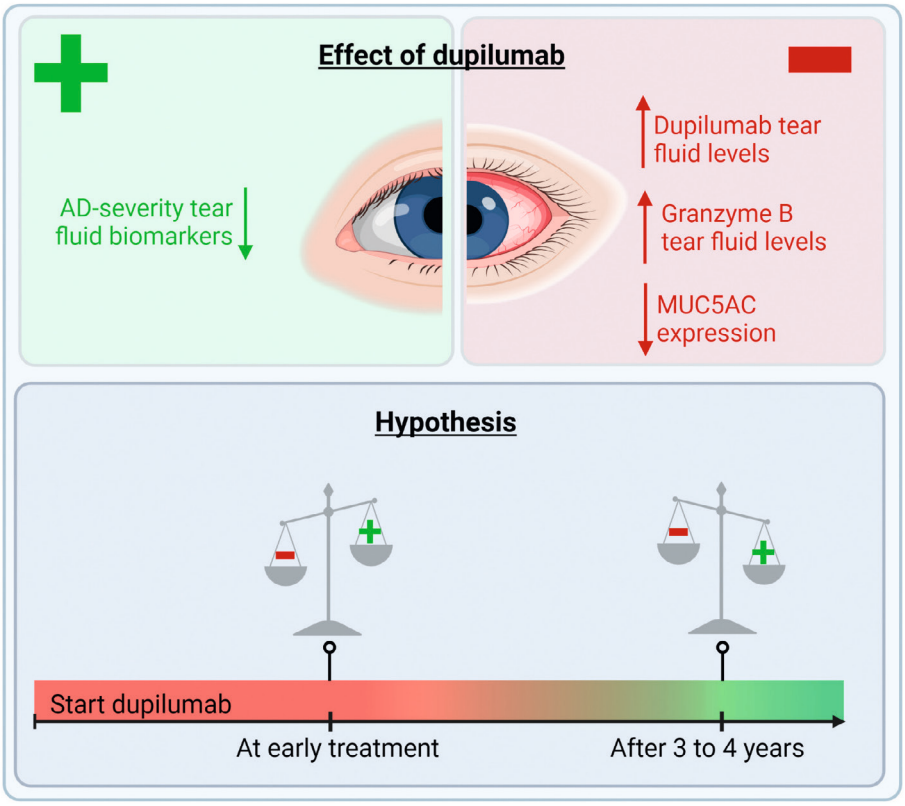

FIGURE 7. Hypothesised positive and negative ocular effects associated with dupilumab. Figure created in Biorender. Abbreviations: AD, atopic dermatitis; MUC5AC, mucin 5AC.

Consistent with previous literature, the levels of Th1-, Th2-, and Th17-related tear fluid cytokines remained stable during dupilumab treatment and no differences were found in these cytokines between patients with no or mild OSD and patients with moderate-

to-severe OSD (**chapter 9**).[90] The findings in **chapter 9** differ from the increased local Th1-related cytokine production found in conjunctival biopsies of 6 patients with DAOSD.[88] A possible explanation for this difference might be that the cytokines found in the conjunctival biopsies do not reach the tear fluid.[88] In addition, the conjunctival biopsies in the study by Bakker et al. were collected before the start of ophthalmic anti-inflammatory treatment, whereas the patients in **chapter 9** could have been treated with anti-inflammatory ophthalmic drugs at the time of tear fluid collection, which may also explain the different findings.[88] Furthermore, an increased IFN-γ expression in conjunctival biopsies of these 6 patients with DAOSD was found.[88] Previous literature has shown that IFN-γ negatively affects conjunctival GC proliferation.[73,88] In addition, **chapter 10** demonstrates a slightly higher CD4+/CD8+ ratio in dupilumab-treated AD patients with moderate-to-severe OSD compared to patients with no or mild OSD. As hypothesized in the first part of this chapter, an increased CD4+/CD8+ ratio may lead to higher IFN-γ expression, which may partly explain the low GC counts found in patients with more severe OSD.

Our original hypothesis for the development of DAOSD stated that blocking of IL-4 might lead to reduced proliferation of Th2 cells and to reduced inhibition of the proliferation of Th1 and Th17 cells (Figure 8A). However, tear fluid analyses did not show any differences in Th1- and Th17- related biomarkers during dupilumab treatment, which is not in accordance with our original hypothesis. Due to the small cohort that was studied and the fact that patients could be treated with ophthalmic anti-inflammatory drugs, it cannot be fully ruled out that the shift towards Th1/Th17 cells is associated with the development of DAOSD (Figure 8B), and further research is needed. In addition, we originally hypothesized that the blocking effect of dupilumab on IL-13 might lead to reduced GC hyperplasia, leading to less GCs and less mucus production (Figure 8A). The studies presented in this thesis show that the function, and not the number of conjunctival GCs is indeed affected by dupilumab, as MUC5AC expression is reduced. As a recent in-vitro study by Hansen et al. showed that human conjunctival GCs express receptors for IL-4 and IL-13 signalling and that GC proliferation is promoted by both IL-4 and IL-13, the impaired GC function may be the result of dupilumab blocking both IL-4 and IL-13 signalling.[73] However, the role of IL-4 in the proliferation of conjunctival GCs is less clear than that of IL-13, and its contribution to the development of DAOSD requires further investigation (Figure 8B). Lastly, we found higher tear fluid levels of granzyme B at the onset of DAOSD compared to baseline in patients with DAOSD, suggesting a cytotoxic effect on conjunctival GCs.

In summary, we hypothesize that the blockade of IL-4 and IL-13 signalling by dupilumab leads to impairment of GC function, contributing to the development of DAOSD

and enhancing the cascade of events shown in Figure 4. However, as the role of IL-4 in the development of DAOSD and in the conjunctival GC proliferation is not fully understood, further studies are needed.

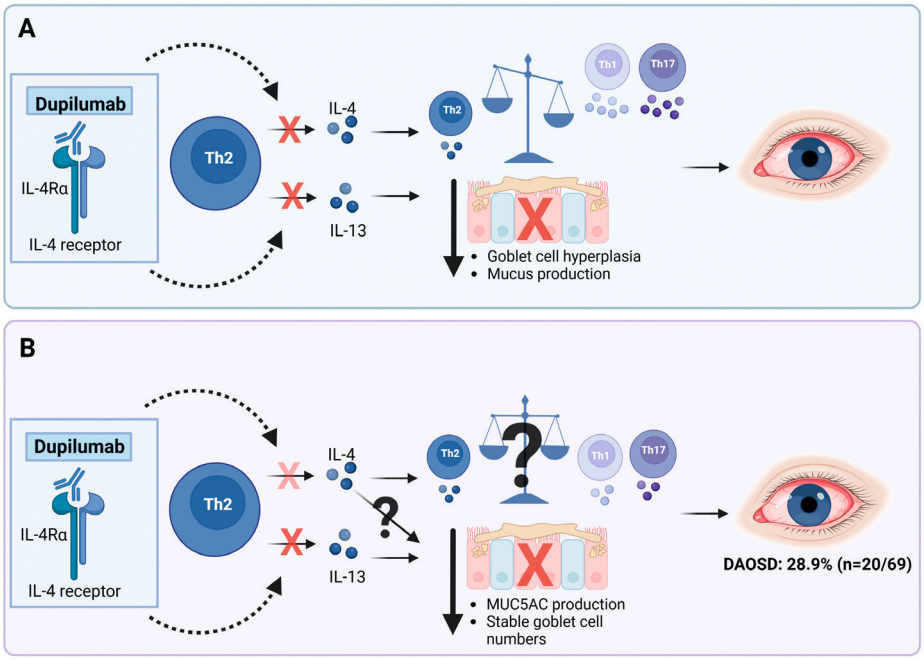

FIGURE 8. Hypothesis on the pathomechanism of dupilumab-associated ocular surface disease (DAOSD).

A. Original hypothesis on DAOSD development that was studied in the prospective studies described in this thesis. Dupilumab blocks IL-4 and IL-13 signalling. Blocking of IL-4 leads to less proliferation of Th2 cells, and to less inhibition of the proliferation of Th1 and Th17 cells. This might lead to a disbalance between these cells, leading to a Th-1 inflammatory response. The blocking effect of dupilumab on IL-13 might lead to less GC hyperplasia, leading to less GCs and less mucus production. **B**. Revised hypothesis on DAOSD development including the results of the prospective studies described in this thesis. The results of this thesis show that Mucin 5AC production by conjunctival goblet cells is affected by dupilumab (by blocking the signalling of IL-13 and possibly also of IL-4). The hypothesis is that blocking of IL-4 may lead to less proliferation of Th2 cells, and to less inhibition of the proliferation of Th1 and Th17 cells. Tear fluid biomarkers showed no differences in Th1- and Th17- related biomarkers during dupilumab treatment, but higher granzyme B was found in tear fluid of patients with DAOSD. However, due to the small cohort that was investigated, this hypothesis cannot be fully confirmed.

Abbreviations: AD, atopic dermatitis; DAOSD, dupilumab-associated ocular surface disease; IL, interleukin; MUC5AC, Mucin 5AC; Th, T helper. Figure created in Biorender.

The effect of ophthalmic treatment on conjunctival cells

The question arises what effect ophthalmic treatment has on the conjunctival inflammatory cells and on the conjunctival GCs. Virtanen et al. investigated the effect of tacrolimus skin ointment (0.03%) on CIC samples from 10 patients with atopic blepharoconjunctivitis and reported a significant decrease in eosinophils, neutrophils, lymphocytes, and squamous cell metaplasia after 6 weeks of treatment.[43] No differences in the GC density were found after treatment with tacrolimus skin ointment.[43] In 18 patients with mild-to-moderate contact lens-induced papillary conjunctivitis, an increase in conjunctival GCs was found after 6 weeks of tacrolimus treatment (0.05%).[91] It may be possible that the dosage of 0.03% tacrolimus is too low, and at least 0.05% or 0.1% tacrolimus is needed to increase GC numbers. We hypothesize that as GC numbers increase a result of tacrolimus treatment, the MUC5AC expression may increase as well, leading to improved ocular surface protection and subsequently less ocular inflammation.

In addition, cyclosporin A and ophthalmic corticosteroids are also effective in increasing GC numbers in several ophthalmic diseases, such as dry eye disease and contact lens-induced papillary conjunctivitis.[91,92] Cyclosporin A eye drops showed reduced conjunctival epithelial cell apoptosis and decreased IFN-γ expression in a mouse model of dry eye disease, suggesting that this may improve both ocular inflammation and conjunctival GC proliferation.[93] Additionally, a different mouse model showed that cyclosporin A eye drops led to upregulation of MUC5AC, suggesting an improvement in GC function.[94]

On the other hand, the use of artificial tears (4 times daily for 4 weeks) did not increase GC density, as reported in a study of 6 dry eye patients.[95] Due to the ocular inflammation in DAOSD, artificial tears only may not be sufficient in treating DAOSD, which was also suggested by Tauqeer et al.[96] Nevertheless, artificial tears may be beneficial in some patients by improving symptoms (even for a short term) and tear film stability, especially in patients with dry eyes.[97]

Taken together, anti-inflammatory ophthalmic treatments appear to increase GC numbers (and possibly also improve the GC function) and reduce conjunctival inflammation, which may break down the vicious cycle shown in Figure 4.

Some of the patients who were included in our studies were treated with anti-inflammatory ophthalmic medication during dupilumab treatment. This may have influenced our results, making it more difficult to investigate the pathomechanism of DAOSD. As our studies were based on daily practice data (Figure 2), we believed it

would be unethical not to start ophthalmic treatment when needed (e.g. in case of ocular symptoms or worsening of OSD). In addition, most of the patients with OSD had mild OSD and did not report any ocular symptoms, suggesting that this would not have been diagnosed without an ophthalmological examination. In addition, we have defined DAOSD as an increase in UTOPIA score of ≥3 points from baseline to clarify the definition. Despite the early initiation of ophthalmic treatment, 20/69 patients developed DAOSD (**chapter 4**). This number could be higher if patients were not started on ophthalmic treatment at baseline, which could have led to an underestimation of DAOSD in the study presented in **chapter 4**. The only possibility to study the effect of dupilumab on the eyes without the influence of ophthalmic treatment, is to include more patients without OSD at baseline, which seems to be an unrepresentative population as 90% of the moderate-to-severe AD patients have OSD before starting dupilumab treatment (**chapter 2**). In addition, it may be interesting to include children with moderate-to-severe AD who start with dupilumab treatment, as they will have a shorter duration of atopy and may also have fewer AD-related OSD at baseline. However, collecting CIC samples from children may be challenging. Together this summarises the difficulties of conducting research into the pathomechanism of DAOSD in dupilumab-treated AD patients. Therefore, research using conjunctival organoids is being started and may provide more insight into the pathomechanism of DAOSD.

PERSPECTIVES FOR OCULAR SURFACE DISEASE IN ATOPIC DERMATITIS

This thesis shows that OSD is very common in moderate-to-severe AD patients before starting dupilumab treatment. Relative high rates of worsening of OSD in AD patients are mostly reported during dupilumab treatment, whereas the conventional systemic therapies and other new targeted therapies (e.g. IL-13 blocking therapies only or Janus kinase inhibitors (JAKi)) show less or no worsening of OSD.[50,98-100] Switching from dupilumab to JAKi could improve the severity of DAOSD.[99] It may be possible that conventional systemic therapies and JAKi also suppress the AD-related ocular surface inflammation, leading to lower rates of OSD. The relatively low incidence of OSD reported in the tralokinumab and lebrikizumab AD trials may be explained by the fact that they only block IL-13, possibly resulting in less impairment of the conjunctival GC function as both IL-4 and IL-13 may play a role in the conjunctival GC proliferation (Figure 8B). It may be possible that IL-4 plays an additional role in decreasing the MUC5AC expression and therefore the development of DAOSD. The findings in **chapter 6** suggest that some AD patients with DAOSD may benefit from switching to tralokinumab. but larger comparable studies between dupilumab and the IL-13 blocking biologics need to be conducted to investigate this hypothesis.

Currently, we are not able to fully identify patients that are at risk of developing DAOSD. Ophthalmic examination before starting dupilumab is recommended as many moderate-to-severe AD patients have OSD before starting dupilumab, and early treatment and diagnosis of (DA)OSD reduces the severity of DAOSD. As this may not be feasible due to the increasing number of AD patients indicated for new AD treatments and the limited capacity in the department of ophthalmology, we recommend starting tacrolimus skin ointment (0.1%) once daily for the external eyelids and artificial tears in patients who report symptoms of OSD before and during dupilumab treatment, as patients with more severe OSD and new-onset DAOSD report their symptoms in general accurately. If tacrolimus skin ointment and artificial tears are not sufficient in the management of DAOSD, low-threshold referral to an ophthalmologist is strongly recommended. Urgent referral to an ophthalmologist is required for (acute) pain and loss of vision, which may indicate dupilumab-associated uveitis (Figure 6).

Overall, it may be advisable for all patients with moderate-to-severe AD who are starting dupilumab treatment to use tacrolimus skin ointment (0.1%) once daily for the external eyelids in combination with artificial tears as a preventive strategy. However, more research is needed to investigate its potential beneficial effects.

Suggestions for future research

The prospective study of OSD in dupilumab-treated AD patients, which started in February 2019, is ongoing and is still including patients. Future research could use the (ophthalmological) data from this prospective study to further investigate risk factors for the development of DAOSD by investigating the individual characteristics of OSD (e.g. blepharitis and tarsal conjunctivitis) and its association to developing DAOSD. This may also provide more information which targeted AD-treatment is recommended for AD patients with pre-existing moderate-to-severe OSD. In addition, the effect of 1 or 2 years of treatment with dupilumab on the conjunctival GCs could be investigated for the long term prognosis. Further studies could examine the pathomechanism of DAOSD in more detail by studying the effect of dupilumab on conjunctival organoids. As new treatments are available for moderate-to-severe AD, it is interesting to study the effect of JAKi and IL-13 blocking treatments on GCs and on pre-existing OSD in AD patients. Moreover, real-world data on the incidence of OSD as an adverse event of these new therapies would be valuable. In addition, it could be useful to investigate the effect of switching from dupilumab to JAKi or an IL-13 blocking agent on DAOSD in a larger cohort.

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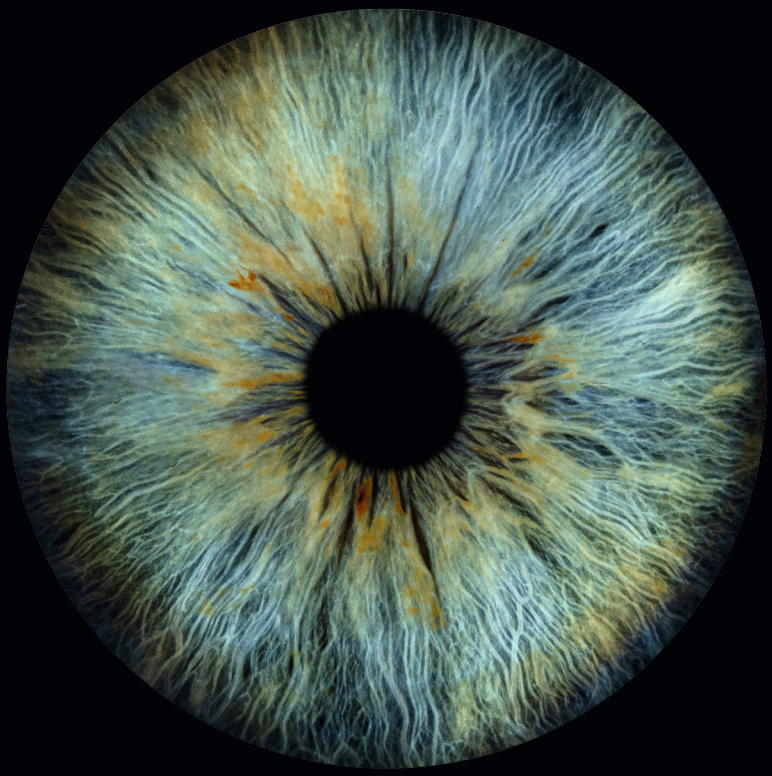

CHAPTER 12

English summary
Nederlandse samenvatting

ENGLISH SUMMARY

Atopic dermatitis (AD) is a chronic skin disease that affects up to 10% of the adults. AD patients often have other atopic comorbidities, such as asthma, allergic rhinitis, food allergy, and allergic conjunctivitis. In addition, ocular surface disease (OSD), which can be used as an umbrella term for various eye diseases such as conjunctivitis and blepharitis, is very common in moderate-to-severe AD patients. Dupilumab is the first biologic treatment for moderate-to-severe AD and inhibits the interleukin (IL)-4 and IL-13 signalling. The most commonly reported side effect during dupilumab treatment in AD patients is dupilumab-associated ocular surface disease (DAOSD). This highlights the importance of better understanding OSD in AD patients, and has renewed interest in it.

The research described in this thesis aimed to: 1) further identify the clinical and ophthalmological characteristics of OSD and its pathomechanism in moderate-to-severe AD patients; 2) clarify the clinical and ophthalmic characteristics of DAOSD, investigate risk factors for its development, and learn more about the management of DAOSD and its long-term follow-up; 3) describe the pathomechanism of OSD during treatment with dupilumab in moderate-to-severe AD patients.
In the final chapter the main findings are re-evaluated for implications, clinical recommendations, and suggestions for future research.

OSD in moderate-to-severe AD patients and its pathomechanism

To better understand the characteristics of OSD and its pathomechanism in moderate-to-severe AD patients, a prospective study including 70 moderate-to-severe AD patients was conducted (**chapter 2**). Patients were categorized by the severity of OSD based on the Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score, which focusses on the inflammation of the eyelids, conjunctiva, and the limbus. In addition, conjunctival impression cytology (CIC) samples were collected to investigate the first few conjunctival cell layers to learn more about the conjunctival goblet cells (GCs) and its main secretory mucin, Mucin 5AC (MUC5AC). OSD was observed in 63/70 (90.0%) patients, of which 32/70 (45.7%) had mild OSD, 24/70 (34.3%) had moderate OSD, and 7/70 (10.0%) had severe OSD. Patients with moderate-to-severe OSD had more severe AD and reported more eyelid or facial eczema in the past year compared to patients with no or mild OSD. Only half of the patients with OSD reported ocular symptoms, indicating that patient-reported diagnosis is less reliable. AD patients with OSD had lower conjunctival GC numbers compared to healthy controls. Furthermore, as the severity of OSD increased, lower GC numbers were observed. However, higher MUC5AC expression was found in patients with more severe OSD, possibly as a defence response. To conclude, 90% of the moderate-to-severe AD patients had OSD with low

conjunctival GC numbers before starting dupilumab, leading to the question of what effect dupilumab will have on pre-existing OSD.

OSD as adverse event in moderate-to-severe AD patients treated with dupilumab

Since DAOSD is the most commonly reported side effect in dupilumab-treated AD patients, it is desirable to identify which patients are at risk of developing DAOSD. **Chapter 3** identified risk factors for the development of self-reported DAOSD in a prospective study including 469 moderate-to-severe AD patients treated with dupilumab. Self-reported DAOSD was found in 152/469 (32.4%) patients. A significant association between eyelid eczema in the past year and the development of DOASD was found. In addition, a significant association between having a history of any eye disease (excluding self-reported allergic conjunctivitis) combined with the use of ophthalmic medication before the start of dupilumab treatment and the development of DAOSD was observed. In conclusion, this study showed that pre-existent ocular pathology (e.g. in combination with ophthalmic medication or eyelid eczema) is associated with developing (self-reported) DAOSD.

To further examine the ophthalmic characteristics of (DA)OSD, a prospective study presented in **chapter 4** investigated the frequency and severity of (DA)OSD (by means of the UTOPIA score), and the effect of ophthalmic treatment in 69 moderate-to-severe AD patients before and during dupilumab treatment. In addition, the effect of dupilumab on the conjunctival GCs was studied, since it is hypothesised that scarcity of conjunctival GCs may play a role in developing DAOSD. OSD was present in 91.3% (n=63/69) of the patients before the start of dupilumab. The median number of conjunctival GCs slightly increased during dupilumab treatment in the included patients, while the percentage of Cytokeratin 19 (CK19)- CD45- MUC5AC+ cells decreased significantly, suggesting an impairment of the GC function due to dupilumab treatment. DAOSD (i.e. ≥3 points UTOPIA increase from baseline) was observed in 28.9% (n=20/69) of the patients, in whom GC numbers remained stable and the percentage of CK19-CD45-MUC5AC+ cells decreased at onset of DAOSD compared with baseline. After 28 weeks of dupilumab treatment, new-onset or ongoing DAOSD was only seen in 14.5% (n=10/69) of the patients. Of the 85.5% (n=59/69) patients with no or controlled DAOSD at week 28, 40.7% (n=24/59) patients received anti-inflammatory ophthalmic drugs (e.g. tacrolimus skin ointment, corticosteroid eye drops). Taken together, these results show that the severity of DAOSD reduces with early ophthalmic treatment. The conjunctival GC function may be affected by dupilumab treatment, as reflected by the decrease in %CK19-CD45-MUC5AC+ cells, while the absolute number of GCs remains stable.

The long-term ophthalmological follow up based on the UTOPIA score and ophthalmic treatments for DAOSD were investigated in a prospective case series (**chapter 5**). Self-reported DAOSD was observed in 66/167 (39.5%) dupilumab-treated AD patients, of whom 33 were referred to an ophthalmologist. No ophthalmic examination was available before starting dupilumab (baseline), but none of the 33 patients reported ocular symptoms at the start of dupilumab treatment. Most patients (n=24/28, 86%) still suffered from mild-to-moderate ophthalmic-confirmed DAOSD during long-term follow up (mean follow up duration of 17.5 months) despite anti-inflammatory ophthalmic treatment. In 10/33 (30%) patients, dose adjustment of dupilumab was necessary due to DAOSD, resulting in remission or improvement of DAOSD in the majority of cases (n=1/10 and n=6/10, respectively). Dupilumab was discontinued because of ocular pathology in 3/33 (9%) patients, resulting in remission or improvement in all cases. Together, these findings show that treatment of DAOSD can be challenging and that DAOSD may persist over a long period of time. Dose adjustment of dupilumab might lead to improvement of DAOSD.

If DAOSD cannot be controlled with anti-inflammatory ophthalmic medication or dose adjustment of dupilumab treatment, switching to a different treatment for AD, such as another biological treatment or a Janus Kinase inhibitor (JAKi), might be a solution. In patients who prefer treatment with a biological agent or when the use of JAKi is contraindicated, tralokinumab could be a possible alternative, which specifically targets IL-13. It is hypothesized that this may lead to less OSD compared to dupilumab. A prospective case series examined the effect of switching from dupilumab to tralokinumab treatment in AD patients with DAOSD (n=4, **chapter 6**). This study evaluated ocular inflammation, symptoms, and ophthalmic treatment. Ocular inflammation improved in 3/4 patients, and all patients needed less ophthalmic medication during tralokinumab treatment. These results suggest that some patients with DAOSD may benefit from switching to tralokinumab treatment.

The pathomechanism of DAOSD in moderate-to-severe AD patients

The exact pathomechanism of DAOSD in dupilumab-treated AD patients remains unclear. One of the hypotheses is that there is an inverse relationship between dupilumab serum levels and the development of DAOSD. In **chapter 8** we prospectively measured dupilumab levels in tear fluid and serum of 48 moderate-to-severe AD patients, and related these findings to the severity of OSD during dupilumab treatment. Dupilumab levels in tear fluid and serum were measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). At baseline, 89.6% (n=43/48) of the patients had OSD, of which 50.0% had moderate-to-severe OSD. At week 28 of dupilumab treatment, the median dupilumab tear fluid levels were significantly higher

in patients with moderate-to-severe OSD compared to patients with no or mild OSD, while serum dupilumab levels were similar. Additionally, a pilot study was conducted to measure dupilumab on conjunctival epithelial cells using flow cytometry (n=4) and LC-MS/MS (n=5). Dupilumab was detected in the conjunctival cell suspensions (n=5/5) and was found to directly bind CD45- conjunctival epithelial cells (n=4/4). Taken together, these findings show that development of DAOSD is not caused by lack of dupilumab reaching the eyes, but that increased local drug availability may play a role in the development of DAOSD.

To learn more about the conjunctival inflammation of (DA)OSD, tear fluid biomarkers were measured by multiplex technology (xMAP; Luminex) in 16 dupilumab-treated moderate-to-severe AD patients at baseline, week 4, and week 28 (**chapter 9**). Significantly higher IL-22, thymus and activation-regulated chemokine (TARC), and periostin levels, also known as AD-related severity biomarkers, were found in tear fluid of patients with moderate-to-severe OSD compared to patients with no or mild OSD at baseline. TARC and periostin tear fluid levels decreased during dupilumab. Levels of Th1- and Th17-related tear fluid cytokines (IL-6, INF-γ, CXCL10, IL-12, TNF-α, IL-17, and IL-23) and Th2-related tear fluid cytokines (IL-4, IL-5, IL-13) remained stable during dupilumab treatment, and no differences were found between patients with no or mild OSD and patients with moderate-to-severe OSD. However, significantly higher granzyme B (GzmB) and lower periostin tear fluid levels were found at DAOSD onset compared to baseline. In conclusion, dupilumab AD-severity tear fluid markers decreased during treatment and high GzmB tear fluid levels were found at the onset of DAOSD. Further research in larger cohorts is needed to verify these results.

To better understand the pathomechanism of (DA)OSD, a study on the immunological changes of immune cells populating the superficial conjunctival epithelium is presented in **chapter 10**. Flow cytometry data of conjunctival cells obtained by CIC were collected before and during dupilumab treatment (n=49 AD patients and n=12 healthy controls). Severity of (DA)OSD was assessed by the UTOPIA score. The percentages of CK19-CD45+CD3+ cells, CD3+CD4+ cells, CD3+CD8+ cells, CD3+Ki67+ cells, CD4+Ki67+ cells, CD8+Ki67+ cells, CD4+GzmB+ cells, and CD8+GzmB+ cells were analysed. A higher CD4+/CD8+ ratio and a lower percentage of CD8+GzmB+ cells were seen in AD patients at baseline compared to healthy controls, which both remained stable during dupilumab treatment. Additionally, a slightly higher CD4+/CD8+ ratio, and higher percentages of CD3+Ki67+ cells and CD8+GzmB+ cells were seen in patients with moderate-to-severe OSD compared with no or mild OSD during dupilumab treatment. We hypothesize that the immune response in the conjunctival epithelium plays a role in the pathogenesis of (DA)OSD, as the number of CD4+ T-cells

was higher in AD patients before and during dupilumab treatment compared with healthy controls. Previous studies suggested that CD4+ cells (and possibly also CD8+ cells) produce interferon-gamma (IFN-γ), which negatively affect GC proliferation. Therefore, we hypothesize that increased CD4+ cells may lead to increased IFN-γ expression, which could reduce the number of GCs. Further studies are needed to verify this.

Dupilumab-associated uveitis as adverse event in atopic dermatitis patients

In addition to DAOSD, dupilumab-treated AD patients may develop dupilumab-associated uveitis, which is a more severe and rare ocular adverse event. **Chapter 7** retrospectively investigated this in 5 dupilumab-treated AD patients. Clinical characteristics were described and the proteomic profile of aqueous humor (AqH) of dupilumab-associated uveitis (n=3/5 available samples) was compared with non-infectious uveitis (n=27) and cataract controls (n=11). Active dupilumab-associated uveitis complicated by serous detachment, cystoid macular edema, or secondary glaucoma developed within a median of 6.0 months (interquartile range 2.3-16.5 months) after starting dupilumab treatment. Uveitis resolved after discontinuation of dupilumab (n=4/5) and/or treatment with local or systemic corticosteroids (n=1/5). Principal component analysis of the profile of 77 detected proteins in AqH revealed that the dupilumab-associated uveitis patients were distinct from cataract controls, but showed a pro-inflammatory profile comparable to non-infectious uveitis. As dupilumab-associated uveitis is a severe adverse event of dupilumab treatment, urgent referral to an ophthalmologist is recommended.

Future perspectives for ocular surface disease in atopic dermatitis

Chapter 11 discussed the most important results of this thesis in the context of other currently available literature on DAOSD, leading to recommendations for patient care and suggestions for future research. Future research could investigate the individual characteristics of OSD (e.g. blepharitis and tarsal conjunctivitis) as risk factors for the development of DAOSD, which may also provide more information on which targeted AD-treatment is recommended for AD patients with pre-existing OSD. As the pathomechanism of DAOSD in AD patients is still not fully understood, further studies could examine this in more detail by studying the effect of dupilumab on conjunctival organoids. Furthermore, the long-term effect of dupilumab treatment on

the conjunctival GCs could be investigated. In addition, it could be interesting to study the effect of the new AD treatments (other biological treatments or JAKi) on (DA)OSD.

As many patients with moderate-to-severe AD have pre-existing OSD, tacrolimus skin ointment for the external eyelids and artificial tears can be prescribed. If this is not sufficient, low-threshold referral to an ophthalmologist is recommended, as early treatment and diagnosis of (DA)OSD reduces its severity. In case of (acute) pain or loss of vision, urgent referral to an ophthalmologist is required.

NEDERLANDSE SAMENVATTING

Constitutioneel eczeem (CE) is een chronische, inflammatoire huidziekte die voorkomt bij 10% van de volwassenen. Naast CE hebben deze patiënten vaak ook andere atopische aandoeningen, zoals astma, allergische rhinitis, voedselallergie en allergische conjunctivitis. Bovendien komt *ocular surface disease* (OSD), een verzamelnaam voor verschillende ontstekingen van het oogoppervlakte zoals conjunctivitis en blefaritis, vaak voor bij patiënten met matig tot ernstig CE. Dupilumab is het eerste biological voor de behandeling van matig tot ernstig CE, en remt de signaaltransductie van interleukine (IL)-4 en IL-13. De meest voorkomende bijwerking van dupilumab is *dupilumab-associated ocular surface disease* (DAOSD). Door deze bijwerking van dupilumab is er vernieuwde interesse ontstaan in oogklachten bij CE patiënten, zowel voorafgaand aan de behandeling met dupilumab (OSD) als tijdens deze behandeling (DAOSD). En uit deze interesse is dit proefschrift tot stand gekomen.

De onderzoeken in dit proefschrift hadden verschillende onderzoeksdoelen. Allereerst wilden we meer te weten komen over kenmerken van OSD en het onderliggende mechanisme bij patiënten met matig tot ernstig CE. Daarnaast wilden we de klinische en oogheelkundige kenmerken van DAOSD beschrijven, risicofactoren voor het ontwikkelen van DAOSD onderzoeken, het effect van oogheelkundige behandeling evalueren en de langdurige follow-up observeren. Bovendien onderzochten we het onderliggende mechanisme van DAOSD in patiënten met matig tot ernstig CE. In het laatste hoofdstuk werden de belangrijkste bevindingen opnieuw besproken, waarbij klinische aanbevelingen en suggesties voor toekomstig onderzoek werden geformuleerd.

De klinische kenmerken en het onderliggende mechanisme van OSD bij patiënten met matig tot ernstig CE

Om de klinische kenmerken en het onderliggende mechanisme van OSD bij matig tot ernstige CE-patiënten beter in kaart te brengen, hebben we een prospectief onderzoek uitgevoerd (n=70) (**hoofdstuk 2**). Patiënten werden oogheelkundig onderzocht en de OSD werd gescoord middels de *Utrecht Ophthalmic Inflammatory and Allergic disease* (UTOPIA) score, die zich richt op inflammatie van de oogleden, conjunctiva en de limbus. Aan de hand van conjunctivale impressie cytologie (CIC) werden de eerste cellagen van het conjunctiva epitheel verzameld, om vervolgens de hoeveelheid conjunctivale slijmbekercellen en de Mucine 5AC (MUC5AC) productie te onderzoeken. De meeste CE patiënten (n=63/70 (90,0%)) hadden OSD, waarvan 32/70 (45,7%) milde OSD, 24/70 (34,3%) matige OSD en 7/70 (10,0%) ernstige OSD. Patiënten met matig tot ernstige OSD hadden ernstiger CE en rapporteerden vaker eczeem in het gelaat of op de oogleden in het afgelopen jaar in vergelijking met

patiënten zonder of met milde OSD. Slechts de helft van de patiënten met OSD rapporteerde klachten, wat erop wijst dat de patiënt gerapporteerde diagnose minder betrouwbaar is. Onderzoek van de CIC-monsters liet zien dat CE-patiënten met OSD minder conjunctivale slijmbekercellen hebben dan gezonde controles. Bovendien objectiveerden we minder slijmbekercellen naarmate de ernst van de OSD toenam. Samenvattend heeft 90% van de patiënten met matig tot ernstig CE al OSD met weinig conjunctivale slijmbekercellen voor de start van behandeling met dupilumab. Dit leidt tot de vraag wat voor effect dupilumab heeft op deze pre-existente OSD.

OSD als bijwerking bij patiënten met matig tot ernstig CE die worden behandeld met dupilumab (DAOSD)

In hoofdstuk 3 t/m 6 hebben we de risicofactoren voor het ontwikkelen van DAOSD, de kenmerken van DAOSD, de follow-up op lange termijn en de (oogheelkundige) behandeling van DAOSD onderzocht.

Hoofdstuk 3 beschrijft een prospectief onderzoek naar risicofactoren voor de ontwikkeling van DAOSD in 469 CE-patiënten. Van deze patiënten ontwikkelden 152/469 (32.4%) anamnestisch DAOSD. Dit onderzoek liet onder andere een verhoogd risico zien op de ontwikkeling van DAOSD bij patiënten die in het afgelopen jaar ooglideczeem hadden. Daarnaast vonden we een associatie tussen de ontwikkeling van de DAOSD en het hebben van een oogziekte in de medische voorgeschiedenis (waarbij een voorgeschiedenis van allergische conjunctivitis niet werd meegenomen) gecombineerd met het gebruik van oogheelkundige medicatie voor de start van behandeling (baseline). Concluderend liet dit onderzoek zien dat pre-existente oculaire pathologie (bv. in combinatie met oogheelkundige medicatie of ooglideczeem) geassocieerd is met de ontwikkeling van (anamnestische) DAOSD.

Om meer te weten te komen over de oogheelkundige kenmerken van DAOSD werd een prospectief onderzoek gedaan naar de frequentie en de ernst van DAOSD, en het effect van oogheelkundige behandeling in 69 patiënten met matig tot ernstig CE (**hoofdstuk 4**). Aangezien een van de hypotheses is dat schaarste van conjunctivale slijmbekercellen een rol speelt bij het ontstaan van DAOSD, onderzochten we ook het effect van dupilumab op de slijmbekercellen. OSD was reeds aanwezig in 91,3% (n=63/69) van de patiënten vóór de start van dupilumab. Het aantal conjunctivale slijmbekercellen nam licht toe tijdens behandeling met dupilumab, terwijl het percentage cytokeratine 19 (CK19)- CD45- MUC5AC+ cellen significant daalde. Dit laat zien dat de functie van de slijmbekercellen afneemt tijdens de behandeling met dupilumab. DAOSD (≥3 punten toename van de UTOPIA-score t.o.v. baseline) werd vastgesteld bij 28,9% (n=20/69) van de patiënten, van wie het aantal slijmbekercellen

stabiel bleef en het percentage CK19-CD45-MUC5AC+-cellen afnam op het moment van het ontstaan van DAOSD in vergelijking met baseline. Na 28 weken behandeling met dupilumab werd bij slechts 14,5% (n=10/69) van de patiënten nieuwe of persisterende DAOSD gezien. Van de 85,5% (n=59/69) patiënten zonder DAOSD of met gecontroleerde DAOSD op week 28, kreeg 40,7% (n=24/59) ontstekingsremmende oogheelkundige medicatie. Samenvattend laten deze resultaten zien dat de ernst van (DA)OSD afneemt als er al vroeg wordt gestart met oogheelkundige behandeling. De daling van het percentage CK19-CD45-MUC5AC+ cellen laat zien dat de functie van de slijmbekercellen van de conjunctiva verslechtert tijdens dupilumab behandeling.

In een prospectief onderzoek onderzochten we de oogheelkundige kenmerken aan de hand van de UTOPIA score en de lange termijn behandeluitkomsten van oogheelkundig bevestigde DAOSD bij patiënten met matig tot ernstig CE (**hoofdstuk 5**). DAOSD werd anamnestisch gerapporteerd door 66/167 (39,5%) met dupilumab behandelde CE-patiënten, waarvan er 33 werden doorverwezen naar een oogarts. Er was geen oogheelkundig onderzoek gedaan vóór de start van dupilumab, maar alle 33 patiënten rapporteerden geen oogklachten bij de start van dupilumab behandeling (baseline). De meeste patiënten (n=24/28, 86%) hadden na de langdurige follow-up van gemiddeld 17,5 maanden persisterende milde tot matige DAOSD ondanks het gebruik van ontstekingsremmende oogheelkundige medicatie. Vanwege DAOSD werd het doseringsinterval van dupilumab verlengd bij 10/33 (30%) patiënten, wat bij de meeste patiënten resulteerde in remissie dan wel verbetering van DAOSD (n=1/10 en n=7/10, respectievelijk). Dupilumab werd gestaakt vanwege oculaire pathologie bij 3/33 (9%) patiënten, wat in alle gevallen leidde tot verbetering of remissie van de DAOSD. Concluderend tonen deze resultaten aan dat DAOSD soms moeilijk behandelbaar is en dat het langdurig kan persisteren. Dosisreductie van dupilumab kan leiden tot verbetering van DAOSD.

Indien DAOSD onvoldoende verbeterd met ontstekingsremmende oogheelkundige medicatie of intervalverlenging van de dupilumab, kan worden overgestapt op een andere behandeling voor CE, zoals een ander biological of een Janus Kinase-remmer (JAKi). Tralokinumab is een alternatief voor patiënten die de voorkeur geven aan een biological of wanneer een JAKi is gecontra-indiceerd. Tralokinumab richt zich specifiek op IL-13, en de hypothese is dat dit daardoor leidt tot minder OSD. De prospectieve case-serie weergeven in **hoofdstuk 6** beschrijft het effect van de overstap op tralokinumab behandeling op DAOSD bij 4 CE-patiënten. In dit onderzoek werden de oculaire inflammatie, de anamnestische oogklachten en de oogheelkundige behandeling gerapporteerd. Er werd een verbetering van de oculaire inflammatie gezien bij 3/4 van de patiënten, en alle patiënten hadden minder oogheelkundige

medicatie nodig tijdens de behandeling met tralokinumab. Deze resultaten suggereren dat sommige patiënten met DAOSD baat kunnen hebben bij de overstap van dupilumab naar tralokinumab behandeling.

Het onderliggende mechanisme van DAOSD bij patiënten met matig tot ernstig CE

Het onderliggende mechanisme van DAOSD bij CE-patiënten die worden behandeld met dupilumab is nog niet volledig opgehelderd. Eén hypothese is dat er een omgekeerde relatie is tussen de serumspiegels van dupilumab en de ontwikkeling van DAOSD. In **hoofdstuk 8** onderzochten we prospectief de dupilumab spiegels in traanvocht en serum gerelateerd aan de ernst van de OSD tijdens dupilumab behandeling bij 48 patiënten met matig tot ernstig CE. Dupilumab spiegels in traanvocht en serum werden gemeten met *liquid chromatography coupled with tandem mass spectrometry* (LC-MS/MS). Op baseline had 89,6% (n=43/48) van de patiënten OSD, waarbij 50,0% matige tot ernstige OSD had. Na 28 weken behandeling met dupilumab waren de mediane dupilumab traanvochtspiegels significant hoger bij patiënten met matig tot ernstige OSD in vergelijking met patiënten zonder of met milde OSD, terwijl de dupilumab serumspiegels vergelijkbaar waren. Daarnaast onderzochten we in een pilotstudie de binding van dupilumab op epitheelcellen van de conjunctiva met behulp van flow cytometrie (n=4) en LC-MS/MS (n=5). Dupilumab werd gedetecteerd in conjunctiva cel suspensies (n=5/5) en bond aan CD45- epitheelcellen van de conjunctiva (n=4/4). Samenvattend laten deze resultaten zien dat DAOSD niet wordt veroorzaakt door te weinig dupilumab in het oog, maar dat een verhoogde lokale beschikbaarheid van dupilumab een mogelijke rol speelt in het ontstaan van DAOSD.

Om meer te weten te komen over DA(OSD) werden biomarkers gemeten middels multiplex technologie (*xMAP; Luminex*) in het traanvocht van 16 dupilumab-behandelde matig tot ernstige CE-patiënten (**hoofdstuk 9**). Op baseline werden significant hogere IL-22, *thymus and activation-regulated chemokine* (TARC) en periostin waardes, ook bekend als CE-gerelateerde ernst biomarkers, gevonden in het traanvocht van patiënten met matig tot ernstige OSD in vergelijking met patiënten zonder OSD of met milde OSD. De traanvocht levels van TARC en periostin daalden tijdens dupilumab behandeling. Th1 en Th17-gerelateerde traanvochtcytokines (IL-6, INF-γ, CXCL10, IL-12, TNF-α, IL-17, en IL-23) en Th2-gerelateerde traanvochtcytokines (IL-4, IL-5, IL-13) bleven stabiel tijdens dupilumab behandeling, en er werden geen verschillen gevonden in deze cytokines tussen patiënten zonder OSD of met milde OSD en patiënten met matig tot ernstige OSD. Wel werden er significant hogere granzyme B (GzmB) en lagere periostin traanvocht levels gevonden op het moment van het ontstaan van DAOSD vergeleken met de baseline waardes. Samenvattend

laten deze resultaten zien dat de CE-gerelateerde ernst traanvocht biomarkers daalden tijdens dupilumab behandeling en dat er hogere GzmB en periostin traanvocht levels waren op het moment van het ontstaan van DAOSD. Onderzoek in grotere cohorten is nodig om deze resultaten te verifiëren.

Om het onderliggende mechanisme van (DA)OSD beter te begrijpen, werd in **hoofdstuk 10** gekeken naar de immunologische veranderingen van het conjunctiva epitheel. CIC-samples werden geanalyseerd door flow cytometrie op baseline, week 4 en week 28. Er werden 49 CE-patiënten en 12 gezonde controles geïncludeerd, en de ernst van (DA)OSD werd beoordeeld middels de UTOPIA-score. De percentages van de cellen CK19-CD45+CD3+, CD3+CD4+, CD3+CD8+, CD3+Ki67+, CD4+Ki67+, CD8+Ki67+, CD4+GzmB+, en CD8+GzmB+ werden geanalyseerd. Op baseline werd er wel een hogere CD4+/CD8+ ratio en een lager percentage CD8+GzmB+ cellen gezien bij CE-patiënten vergeleken met gezonde controles, die stabiel bleef tijdens dupilumab behandeling. Bovendien werd er tijdens dupilumab behandeling een iets hogere CD4+/CD8+ ratio, een hoger percentage CD3+Ki67+ cellen en een hoger percentage CD8+GzmB+ cellen gezien bij patiënten met matige tot ernstige OSD in vergelijking met patiënten zonder OSD of met milde OSD. Op basis van deze resultaten is onze hypothese dat de immuunrespons in het epitheel van de conjunctiva een mogelijke rol speelt in het onderliggende mechanisme van (DA)OSD, aangezien het aantal CD4+ T-cellen voor en tijdens de dupilumab behandeling hoger was bij CE-patiënten in vergelijking met gezonde controles. In eerdere onderzoeken werd gesuggereerd dat CD4+ cellen mogelijk interferon gamma (IFN-γ) produceren, wat een negatief effect heeft op de proliferatie van de slijmbekercellen. Wij denken daarom dat het verhoogde percentage van de CD4+ cellen kan leiden tot een verhoogde IFN-γ productie, die vervolgens leidt tot vermindering van het aantal conjunctivale slijmbekercellen.

Dupilumab-geassocieerde uveïtis als bijwerking van dupilumab

Naast DAOSD is dupilumab-geassocieerde uveïtis een ernstige en zeldzame oculaire bijwerking van dupilumab die kan ontstaan bij CE-patiënten. **Hoofdstuk 7** onderzocht retrospectief de klinische kenmerken (n=5) en het proteomische profiel (n=3/5 beschikbare monsters) van 5 CE-patiënten met dupilumab-geassocieerde uveitis. Het voorste oogkamervocht (AqH) van dupilumab-geassocieerde uveïtis (n=3) werd vergeleken met niet-infectieuze uveïtis (n=27) en controle patiënten met cataract (n=11). Actieve dupilumab-geassocieerde uveïtis werd gecompliceerd door sereuze loslating, cystoïde maculair oedeem of secundair glaucoom en ontwikkelde zich binnen een mediaan van 6,0 maanden (interkwartielbereik 2,3-16,5 maanden) na het starten van de dupilumab behandeling. De dupilumab-geassocieerde uveïtis verbeterde na het staken van dupilumab (n=4/5) en/of behandeling met lokale

of systemische corticosteroïden (n=1/5). Principale componenten analyse van 77 eiwitten in AqH toonde aan dat de dupilumab-geassocieerde uveïtis patiënten een vergelijkbaar pro-inflammatoir profiel vertoonden met niet-infectieuze uveïtis. Aangezien dupilumab-geassocieerde uveïtis een ernstige bijwerking van dupilumab is, is een dringende verwijzing naar een oogarts noodzakelijk.

Toekomst perspectieven voor OSD bij patiënten met constitutioneel eczeem

In **hoofdstuk 11** worden de belangrijkste resultaten van dit proefschrift besproken in de context van andere literatuur over DAOSD, wat leidt tot aanbevelingen voor de dagelijkse praktijk en suggesties voor toekomstig onderzoek. In de toekomst zouden individuele kenmerken van (DA)OSD (bijvoorbeeld blefaritis en tarsale conjunctivitis) als risicofactoren voor het ontwikkelen van DAOSD kunnen worden onderzocht, wat ook meer richting zou kunnen geven aan de keuze voor de juiste CE-behandeling voor CE-patiënten met pre-existente OSD. Aangezien het onderliggende mechanisme van DAOSD bij CE-patiënten nog steeds niet geheel duidelijk is, kan dit verder worden onderzocht door het effect van dupilumab op conjunctivale organoïden te bestuderen. Tevens zou het langetermijneffect van de behandeling met dupilumab op de conjunctivale slijmbekercellen kunnen worden onderzocht. Daarnaast zou het ook interessant zijn om het effect van andere nieuwe CE-medicijnen (andere biologicals of JAKi) op slijmbekercellen, pre-existente OSD bij CE-patiënten en op DAOSD na de overstap op één van de nieuwe behandelingen te onderzoeken.

Aangezien veel patiënten met matige tot ernstig CE pre-existente OSD hebben, kan tacrolimus huidzalf voor de oogleden in combinatie met kunsttranen worden voorgeschreven. Als dit onvoldoende werkt wordt laagdrempelige verwijzing naar een oogarts aanbevolen, omdat vroegtijdige behandeling en diagnose van (DA)OSD de ernst hiervan kan verminderen. In geval van (acute) pijn of visusdaling is een dringende doorverwijzing naar een oogarts nodig.

CHAPTER 13

List of abbreviations

Contributing authors

Acknowledgements

List of publications

Curriculum vitae

LIST OF ABBREVIATIONS

95% CI	95% confidence intervals
AD	Atopic dermatitis
ANA	Antinuclear antibody
AqH	Aqueous humor
BUT	Break-up time
CIC	Conjunctival impression cytology
CK19	Cytokeratin 19
CME	Cystoid macular edema
CMV	Cytomegalovirus
DAOSD	Dupilumab-associated ocular surface disease
EASI	Eczema Area and Severity Index
FDR	False Discovery Rate
GC	Goblet cell
HLA-B27	Human Leukocyte Antigen – B27
HOME	Harmonising Outcome Measures for Eczema
IGA	Investigator's Global Assessment
IL	Interleukin
IL-4Rα	Interleukin-4 receptor alpha
IFN-g	Interferon gamma
IQR	Interquartile ranges
LC-MS/MS	Liquid chromatography tandem mass spectrometry
METC	Medical Research Ethics Committee
MFI	Median Fluorescence Intensity
Mm	Millimetres
mm2	Millimetre squared
MUC5AC	Mucin 5AC
NPX	Normalized protein expression
OD	Oculus dexter (right eye)
OR	Odds ratio
OS	Oculus sinister (left eye)
OSD	Ocular surface disease
OSDI	Ocular Surface Disease Index
PARC	Pulmonary and activation-regulated chemokine
PAS	Periodic Acid-Schiff
PEA	Proximity extension assay
SD	Standard deviation
TAOSD	Tralokinumab-associated ocular surface disease

TARC	Thymus and activation-regulated chemokine
Th	T helper
TNF-a	Tumor necrosis factor alpha
UMCU	University Medical Center Utrecht
UTOPIA	Utrecht Ophthalmic Inflammatory and Allergic disease
VKH	Vogt-Koyanagi-Harada syndrome

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LIST OF PUBLICATIONS

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CURRICULUM VITAE

Roselie Achten werd geboren op 12 mei 1994 te Nijmegen. Na het behalen van haar atheneum diploma aan het Canisius College in Nijmegen in 2012, begon zij in hetzelfde jaar aan de studie geneeskunde aan de Universiteit Utrecht. In het laatste jaar van de master geneeskunde heeft zij onder begeleiding van dr. Marlies de Graaf onderzoek gedaan naar de impact van constitutioneel eczeem op kinderen en ouders op de afdeling Dermatologie in het Universitair Medisch Centrum Utrecht (UMCU). Na het behalen van haar artsenexamen in 2019, is zij aansluitend aangenomen als arts-onderzoeker op deze afdeling. Ze mocht het onderzoek naar oogklachten bij patiënten met constitutioneel eczeem uitvoeren onder begeleiding van promotoren prof. dr. Marjolein de Bruin-Weller en prof. dr. Joke de Boer, en copromotoren dr. Marlies de Graaf en dr. Judith Thijs. De bevindingen van dit onderzoek hebben geleid tot dit proefschrift. Per 1 april 2023 is zij gestart met de opleiding tot dermatoloog in het UMCU. Roselie woont samen met Joost Albers in Utrecht.