Received: 10 January 2023 | Revised: 7 February 2023 | Accepted: 24 February 2023

DOI: 10.1111/all.15717

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

ORIGINAL ARTICLE

Allergen-Specific Immunotherapy and Biologics

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

Roselie Achten[1] | Judith Thijs[1] | Marlot van der Wal[2] | Chantal van Luijk[3] | Marlies de Graaf[1] | Daphne Bakker[1] | Joke de Boer[3] | Femke van Wijk[2] | Marjolein de Bruin-Weller[1]

[1]Department of Dermatology and Allergology, National Expertise Center for Atopic Dermatitis, University Medical Center Utrecht, Utrecht, the Netherlands

[2]Center for Translational Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

[3]Department of Ophthalmology, University Medical Center Utrecht, Utrecht, the Netherlands

Correspondence
Roselie Achten, Department of Dermatology and Allergology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands.
Email: r.e.achten@umcutrecht.nl

Funding information
Sanofi

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.

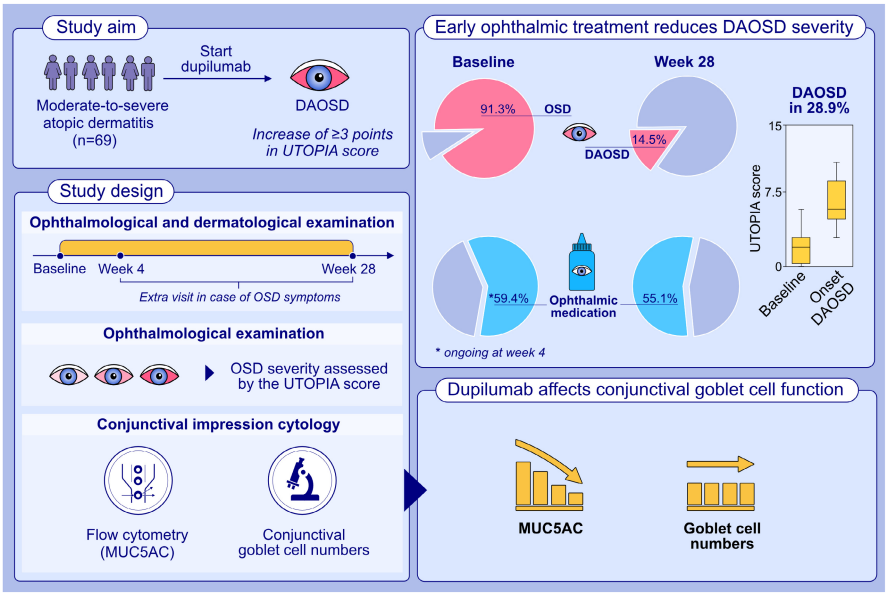
Results: Ocular surface disease (OSD) was present in 91.3% (n = 63/69) patients at baseline. DAOSD was observed in 28.9% (n = 20/69) patients, in whom GC numbers remained stable and the percentage of CK19-CD45-MUC5AC+ cells decreased at onset of DAOSD compared with baseline. After 28 weeks of dupilumab 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: Ocular surface disease is common in moderate-to-severe AD patients before starting dupilumab. During treatment with dupilumab DAOSD severity

Abbreviations: AD, atopic dermatitis; CIC, conjunctival impression cytology; CK-19, Cytokeratin 19; DAOSD, dupilumab-associated ocular surface disease; EASI, Eczema Area and Severity Index; GC, Goblet cell; IGA, Investigator's Global Assessment; IL, interleukin; IL-4Rα, IL-4 receptor α; IQR, interquartile ranges; mm^2, millimetre squared; MUC5AC, mucin 5AC; OSD, ocular surface disease; TARC, thymus and activation-regulated chemokine; TBUT, tear break-up time; UMCU, University Medical Centre Utrecht; UTOPIA, Utrecht Ophthalmic Inflammatory and Allergic disease.

improves with early ophthalmic treatment. The decrease in percentage of CK19-CD45-MUC5AC+ cells during dupilumab treatment suggests an impairment of the GC function due to dupilumab treatment.

KEYWORDS
atopic dermatitis, dupilumab, dupilumab-associated ocular surface disease, goblet cell



GRAPHICAL ABSTRACT

We investigated the frequency and severity of DAOSD, ophthalmic treatment response, and the effect of dupilumab on conjunctival GCs in 69 moderate-to-severe AD patients. Many AD patients had OSD before starting dupilumab, and 59.4% started ophthalmic treatment, resulting in less severe (DA)OSD. 28.9% (n = 20/69) patients developed DAOSD, with stable but low GC numbers and reduced GC function compared to baseline.

Abbreviations: AD, atopic dermatitis; DAOSD, dupilumab-associated ocular surface disease; GC, goblet cell; MUC5AC, mucin 5AC; OSD, ocular surface disease; UTOPIA, Utrecht Ophthalmic Inflammatory and Allergic disease

1 | 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.[13] reported a relative deficiency of Mucin 5 AC (MUC5AC), which is the main mucin produced by the conjunctival GCs, in tear fluid of dupilumab-treated AD patients with

DAOSD. 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.

2 | METHODS

2.1 | 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.

2.2 | 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.

Atopic dermatitis (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

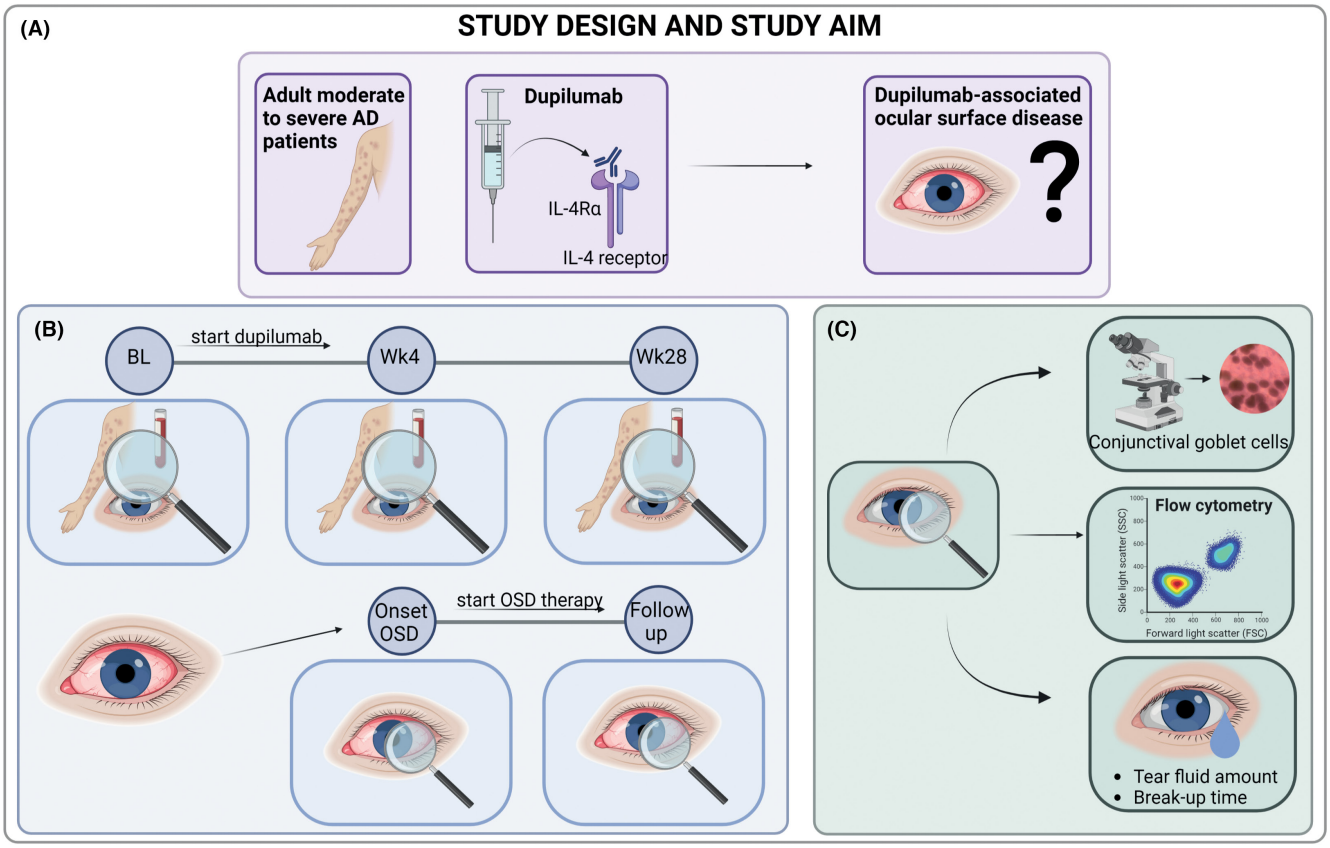


FIGURE 1 Study design of the prospective study. (A) Aim of the study. (B) Time points of the different visits and the conducted investigations. (C) Additional analysis to the clinical ophthalmological examination. AD, atopic dermatitis; BL, baseline; IL, interleukin; OSD, ocular surface disease; wk,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

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 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]

2.3 | 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.

2.4 | 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 .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.

3 | RESULTS

3.1 | Patient characteristics at baseline

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.

3.2 | 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 COVID-19 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), $p < .001$, 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 and 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).

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).

3.3 | Goblet cell numbers and mucin production

Conjunctival impression cytology 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

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)
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[a], *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)

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]Severity of OSD is based on eye with the highest severity within a patient.

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.

3.4 | 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).

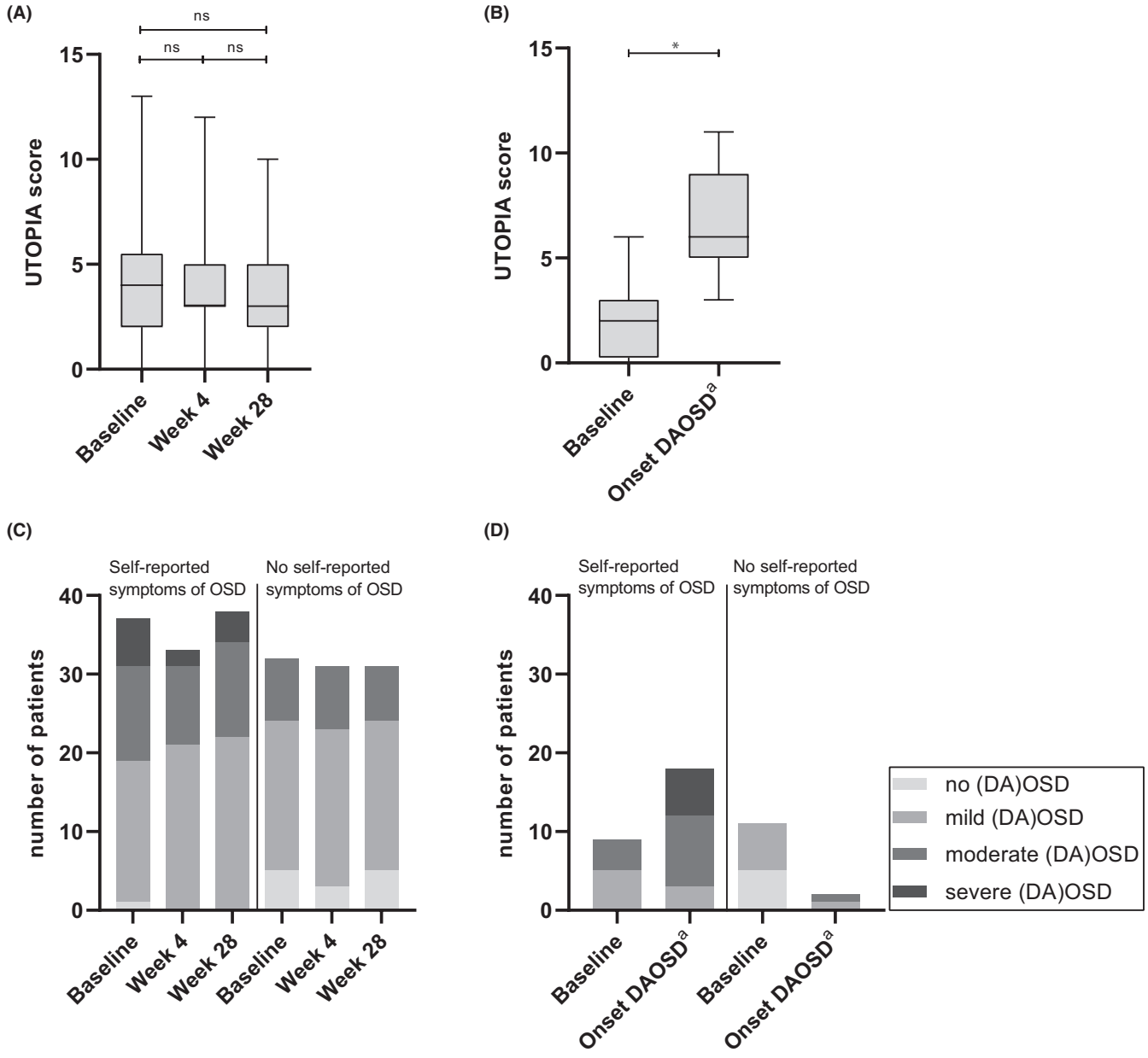


FIGURE 2 Clinical characteristics of ocular surface disease before and during dupilumab treatment. (A) Utrecht Ophthalmic Inflammatory and Allergic disease (UTOPIA) score of the total cohort before the start of dupilumab (baseline, *n* = 69), and after 4 (*n* = 64, 5 missing cases due to COVID19) and 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]. ns, non-significant; OSD, ocular surface disease; UTOPIA, Utrecht Ophthalmic Inflammatory and Allergic disease. *Indicates statistical significance. [a]Increase in UTOPIA score of ≥3 points compared to baseline.

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

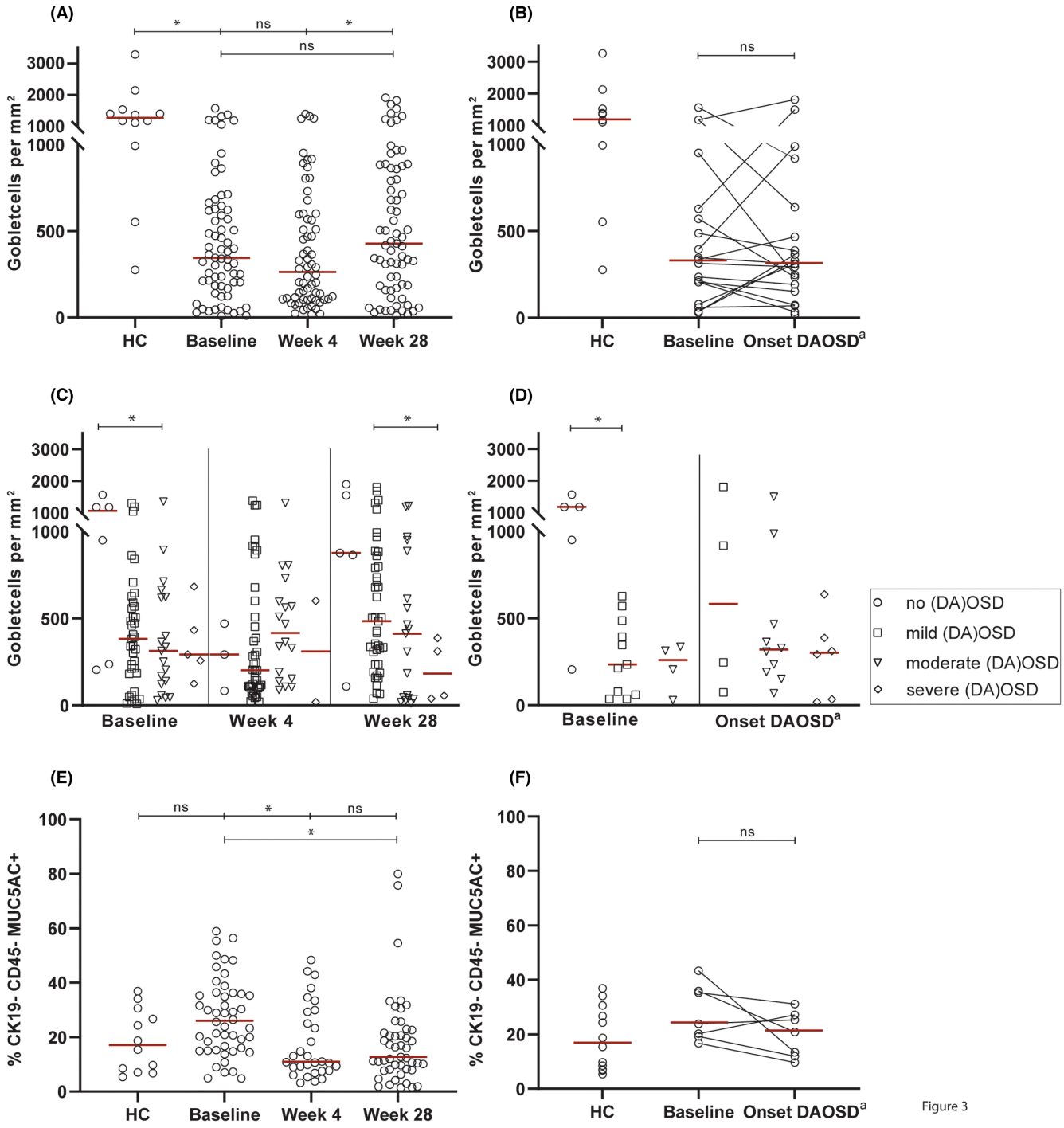


Figure 3

FIGURE 3 Results of conjunctival impression cytology from dupilumab-treated atopic dermatitis patients. The medians are displayed by the black and 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 ($p < .05$). (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 ($p < .05$). (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 ($n = 12$). CK19, cytokeratin19; DAOSD, dupilumab-associated ocular surface disease; MUC5AC, mucin 5AC; ns, non-significant; OSD, ocular surface disease; UTOPIA, Utrecht Ophthalmic Inflammatory and Allergic disease.*Indicates statistical significance. [a]Increase in UTOPIA score of ≥3 points compared to baseline.

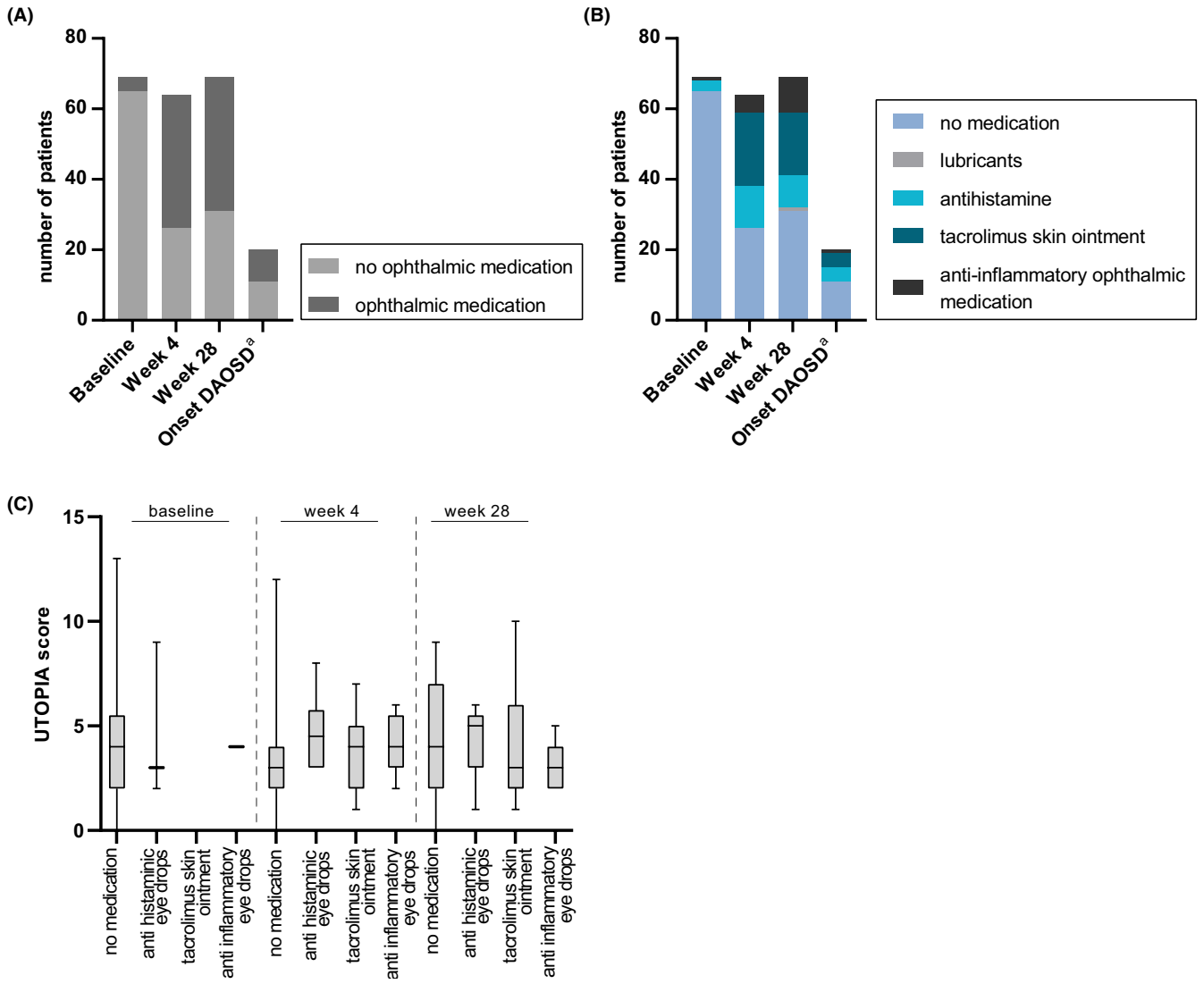


FIGURE 4 Use of ophthalmic medication during dupilumab treatment (with a minimum drug use of once every day). The most potent medication per patient is shown. (A) Number of patients treated with ophthalmic medication at baseline (n = 4/69, 5.8%), at Week 4 (n = 38/64, 59.4%), at Week 28 (n = 38/69, 55.1%) 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. 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.

(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.

4 | 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.[13] found a relative deficiency of MUC5AC in tear fluid of AD patients with DAOSD, which is in line with our results. 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.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data. All authors have been involved in drafting the manuscript or revising it critically and have given final approval of the version to be published.

ACKNOWLEDGEMENTS

We would like to thank Lieneke Ariëns and Lian van der Gang for critically reading the manuscript.

FUNDING INFORMATION

This investigator-initiated study was sponsored by Sanofi. The sponsor was not involved in the analyses, interpretation of the data and preparation of the manuscript.

CONFLICT OF INTEREST STATEMENT

Roselie Achten has nothing to disclose. Judith Thijs is a speaker for Sanofi, Janssen, Almirall and LEO Pharma. Marlot van der Wal has nothing to disclose. Chantal van Luijk is a speaker for Sanofi and Santen. Marlies de Graaf is a principal investigator, advisory board member and/or speaker for Sanofi, Regeneron Pharmaceuticals, LEO Pharma, AbbVie, Pfizer and Eli Lilly. Daphne Bakker is a speaker for Sanofi, Janssen and LEO Pharma. Joke de Boer has nothing to disclose. Femke van Wijk is a speaker and/or consultant for Janssen, Johnson & Johnson, and Takeda. Marjolein de Bruin-Weller is a consultant, advisory board member, and/or speaker for AbbVie, Almirall, Aslan, Arena, Eli Lilly, Galderma, Janssen, Leo Pharma, Pfizer, Regeneron Pharmaceuticals and Sanofi and obtained the study funding for this current study which is sponsored by Sanofi.

ORCID

Roselie Achten https://orcid.org/0000-0001-5617-8615
Judith Thijs https://orcid.org/0000-0003-2753-5235
Daphne Bakker https://orcid.org/0000-0002-0193-5794
Marjolein de Bruin-Weller https://orcid.org/0000-0002-1249-6993

REFERENCES

1. de Bruin-Weller M, Thaci D, Smith CH, et al. Dupilumab with concomitant topical corticosteroid treatment in adults with atopic dermatitis with an inadequate response or intolerance to ciclosporin a or when this treatment is medically inadvisable: a placebo-controlled, randomized phase III clinical trial (LIBERTY AD CAFE). *Br J Dermatol*. 2018;178(5):1083-1101. doi:10.1111/bjd.16156
2. Ariens LF, van der Schaft J, Spekhorst LS, et al. Dupilumab shows long-term effectiveness in a large cohort of treatment-refractory atopic dermatitis patients in daily practice: 52-weeks results from the Dutch BioDay registry. *J Am Acad Dermatol*. 2020;84:1000-1009. doi:10.1016/j.jaad.2020.08.127
3. Ariens LFM, van der Schaft J, Bakker DS, et al. Dupilumab is very effective in a large cohort of difficult-to-treat adult atopic dermatitis patients: first clinical and biomarker results from the BioDay registry. *Allergy*. 2020;75(1):116-126. doi:10.1111/all.14080
4. Akinlade B, Guttman-Yassky E, de Bruin-Weller M, et al. Conjunctivitis in dupilumab clinical trials. *Br J Dermatol*. 2019;181(3):459-473. doi:10.1111/bjd.17869
5. Achten R, Bakker D, Ariens L, et al. Long-term follow-up and treatment outcomes of conjunctivitis during dupilumab treatment in patients with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol Pract*. 2020;9:1389-1392.e2. doi:10.1016/j.jaip.2020.09.042
6. Mehta U, Farid M. Dupilumab induced limbal stem cell deficiency. *Int Med Case Rep J*. 2021;14:275-278. doi:10.2147/IMCRJ.S308583
7. Nahum Y, Mimouni M, Livny E, Bahar I, Hodak E, Leshem YA. Dupilumab-induced ocular surface disease (DIOSD) in patients with atopic dermatitis: clinical presentation, risk factors for development and outcomes of treatment with tacrolimus ointment. *Br J Ophthalmol*. 2020;104(6):776-779. doi:10.1136/bjophthalmol-2019-315010
8. Wu D, Daniel BS, Lai AJX, et al. Dupilumab-associated ocular manifestations: a review of clinical presentations and management. *Surv Ophthalmol*. 2022;67(5):1419-1442. doi:10.1016/j.survophthal.2022.02.002
9. Neagu N, Dianzani C, Avallone G, et al. Dupilumab ocular side effects in patients with atopic dermatitis: a systematic review. *J Eur Acad Dermatol*. 2022;36(6):820-835. doi:10.1111/jdv.17981
10. Bakker DS, Ariens LFM, van Luijk C, et al. Goblet cell scarcity and conjunctival inflammation during treatment with dupilumab in

patients with atopic dermatitis. *Br J Dermatol*. 2019;180(5):1248-1249. doi:10.1111/bjd.17538
11. Achten RE, Bakker DS, van Luijk CM, et al. Ocular surface disease is common in moderate-to-severe atopic dermatitis patients. *Clin Exp Allergy*. 2022;52(6):801-805. doi:10.1111/cea.14127
12. Achten R, Thijs J, van Luijk C, et al. Biomarkers in tear fluid of dupilumab-treated moderate-to-severe atopic dermatitis patients. *Clin Exp Allergy*. 2022;52:801-805. doi:10.1111/cea.14269
13. Barnett BP, Afshari NA. Dupilumab-associated mucin deficiency (DAMD). *Transl Vis Sci Technol*. 2020;9(3):29. doi:10.1167/tvst.9.3.29
14. Akpek EK, Amescua G, Farid M, et al. Dry eye syndrome preferred practice pattern®. *Ophthalmology*. 2019;126(1):P286-P334. doi:10.1016/j.ophtha.2018.10.023
15. Lee JH, Kee CW. The significance of tear film break-up time in the diagnosis of dry eye syndrome. *Korean J Ophthalmol*. 1988;2(2):69-71. doi:10.3341/kjo.1988.2.2.69
16. Felfeli T, Georgakopoulos JR, Jo CE, et al. Prevalence and characteristics of Dupilumab-induced ocular surface disease in adults with atopic dermatitis. *Cornea*. 2022;41(10):1242-1247. doi:10.1097/ICO.0000000000002866
17. Wollenberg A, Ariens L, Thurau S, van Luijk C, Seegraber M, de Bruin-Weller M. Conjunctivitis occurring in atopic dermatitis patients treated with dupilumab-clinical characteristics and treatment. *J Allergy Clin Immunol Pract*. 2018;6(5):1778-1780e1. doi:10.1016/j.jaip.2018.01.034
18. Tauqeer Z, Jinno SE, Chung CW, Massaro-Giordano M, Bunya VY. Clinical characteristics and treatment for Dupilumab-related ocular complications in atopic dermatitis patients. *Clin Ophthalmol*. 2022;16:947-958. doi:10.2147/OPTH.S336978
19. Bortoluzzi P, Ferrucci S, Galimberti D, et al. New insights on ocular surface disease in patients with atopic dermatitis treated with dupilumab. *Br J Dermatol*. 2021;186:186-187. doi:10.1111/bjd.20706
20. Voorberg AN, den Dunnen WFA, Wijdh RHJ, de Bruin-Weller MS, Schuttelaar MLA. Recurrence of conjunctival goblet cells after discontinuation of dupilumab in a patient with dupilumab-related conjunctivitis. *J Eur Acad Dermatol Venereol*. 2020;34(2):e64-e66. doi:10.1111/jdv.15914
21. Alam J, de Paiva CS, Pflugfelder SC. Immune – goblet cell interaction in the conjunctiva. *Ocul Surf*. 2020;18(2):326-334. doi:10.1016/j.jtos.2019.12.006
22. Swamynathan SK, Wells A. Conjunctival goblet cells: ocular surface functions, disorders that affect them, and the potential for their regeneration. *Ocul Surf*. 2020;18(1):19-26. doi:10.1016/j.jtos.2019.11.005
23. Gipson IK. Goblet cells of the conjunctiva: a review of recent findings. *Prog Retin Eye Res*. 2016;54:49-63. doi:10.1016/j.preteyeres.2016.04.005
24. Hansen PM, Tollenaere MAX, Hedengran A, et al. IL-4 and IL-13 both contribute to the homeostasis of human conjunctival goblet cells *in vitro*. *Allergy*. 2022;77(8):2555-2558. doi:10.1111/all.15326
25. Hébert M, Ruyu Qi S, You E, Mercier M, Laughrea P. Characterising the chronicity of dupilumab-associated ocular surface disease: an analysis of a retrospective case series. *BMJ Open Ophthalmol*. 2022;7:e000947. doi:10.1136/bmjophth-2021-000947
26. Pflugfelder SC, Stern ME. Biological functions of tear film. *Exp Eye Res*. 2020;197:108115. doi:10.1016/j.exer.2020.108115
27. Pflugfelder SC, De Paiva CS, Villarreal AL, Stern ME. Effects of sequential artificial tear and cyclosporine emulsion therapy on conjunctival goblet cell density and transforming growth factor-beta2 production. *Cornea*. 2008;27(1):64-69. doi:10.1097/ICO.0b013e318158f6dc
28. Schneeweiss MC, Kim SC, Wyss R, Schneeweiss S, Merola JF. Dupilumab and the risk of conjunctivitis and serious infection in patients with atopic dermatitis: a propensity score-matched cohort study. *J Am Acad Dermatol*. 2021;84(2):300-311. doi:10.1016/j.jaad.2020.09.084
29. Hayama K, Fujita H. Improvement of dupilumab-associated conjunctivitis after switching to upadacitinib in a patient with atopic dermatitis. *Dermatol Ther*. 2022;35(7):e15575. doi:10.1111/dth.15575
30. Wollenberg A, Beck LA, de Bruin WM, et al. Conjunctivitis in adult patients with moderate-to-severe atopic dermatitis: results from five tralokinumab clinical trials. *Br J Dermatol*. 2021;186:453-465. doi:10.1111/bjd.20810
31. Waldman RA, DeWane ME, Sloan SB. Does interleukin-4 inhibition play a role in dupilumab-associated conjunctivitis? *Br J Dermatol*. 2020;182(1):251. doi:10.1111/bjd.18450
32. Haeck IM, Rouwen TJ, Timmer-de Mik L, de Bruin-Weller MS, Bruijnzeel-Koomen CA. Topical corticosteroids in atopic dermatitis and the risk of glaucoma and cataracts. *J Am Acad Dermatol*. 2011;64(2):275-281. doi:10.1016/j.jaad.2010.01.035

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Achten R, Thijs J, van der Wal M, et al. Dupilumab-associated ocular surface disease in atopic dermatitis patients: Clinical characteristics, ophthalmic treatment response and conjunctival goblet cell analysis. *Allergy*. 2023;78:2266-2276. doi:10.1111/all.15717