Received: 17 October 2022 | Revised: 15 November 2022 | Accepted: 30 November 2022

DOI: 10.1111/cea.14269

RESEARCH LETTER

WILEY

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

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 (300 mg every 2 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, tumour necrosis factor alpha (TNF-α), IL-17, IL-23, IL-4, IL-5 and 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 the 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 (Appendix S1) 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 with healthy controls (n = 8) at baseline (Figure 1A). Additionally, significantly 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 weeks 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 weeks 4 and 28, respectively. After 4 weeks (n = 12, 4 missing due to COVID-19

Femke van Wijk and Marjolein de Bruin-Weller contributed equally to this study.

pandemic) and 28 weeks (n = 16) of dupilumab treatment, tear fluid levels of TARC and periostin decreased compared with 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 weeks 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

Key messages

- This study, funded by dupilumab's manufacturer, measured tear fluid biomarker levels from dupilumab-treated atopic dermatitis (AD) patients.
- Patients with more 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.

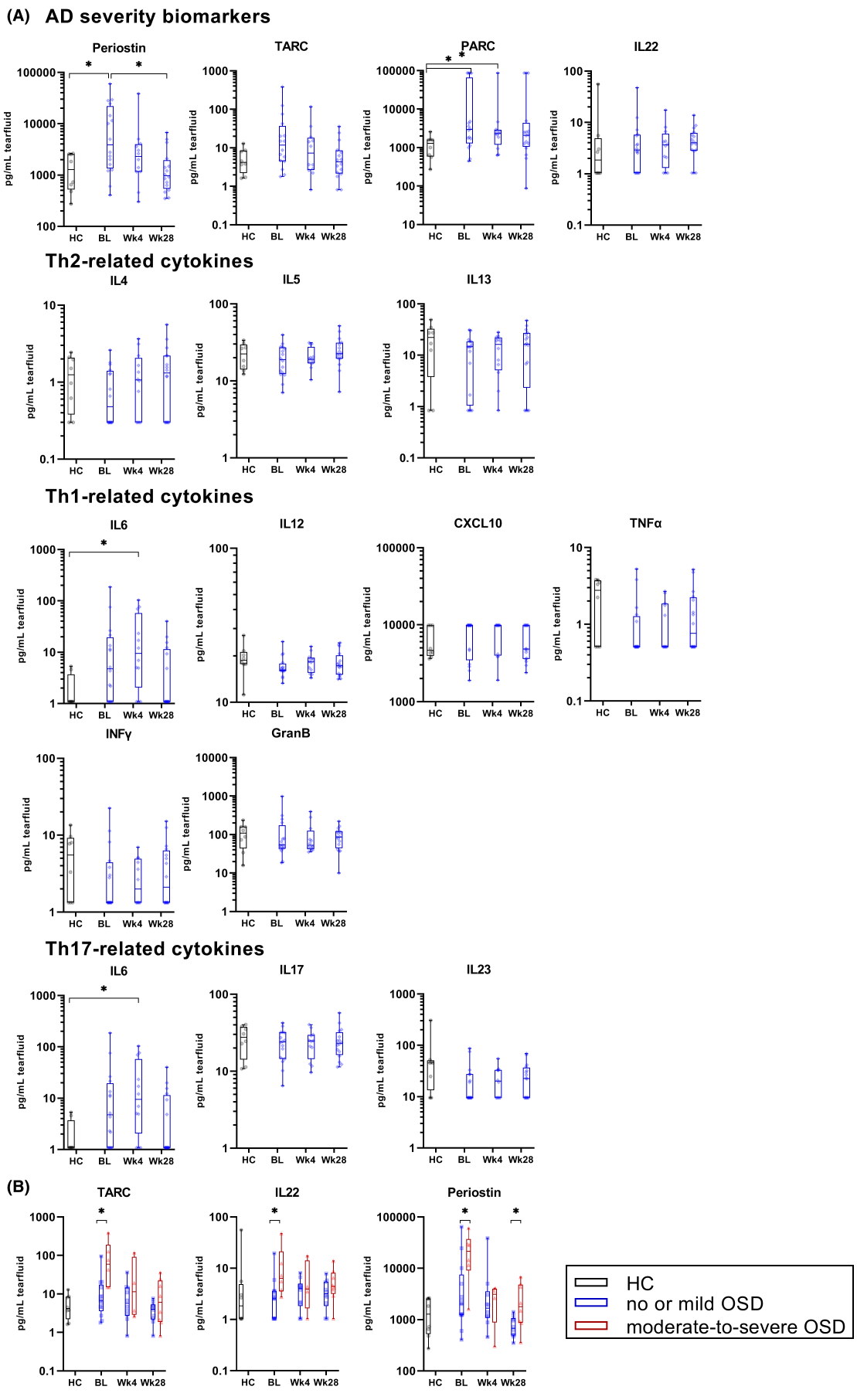
TABLE 1 Patient characteristics at baseline

	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[a], n (%)	
No OSD	3 (18.8)
Mild OSD	7 (43.8)
Moderate OSD	3 (18.8)
Severe OSD	3 (18.8)

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 the eye with the highest severity within a patient.

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 the eye with the highest severity within a patient at the selected time point. GranB, granzyme B; HC, healthy control; IL, interleukin; IFN-γ, interferon gamma; PARC, pulmonary and activation-regulated chemokine; TARC, thymus and activation-regulated chemokine; TNF-α, tumour necrosis factor alpha. *Statistical significance



treatment, and no differences were found in these cytokines between patients with no or mild OSD and patients with moderate-to-severe OSD.

However, 7/16 (44%) patients showed ≥3 points increase in UTOPIA score during dupilumab treatment (i.e.DAOSD onset) compared with baseline. At DAOSD onset, the median UTOPIA score was significantly higher compared with baseline (6.0 (IQR 3.0–8.0) vs. 2.0 (IQR 0.0–2.0), $p = .017$, respectively ($n = 7$)). Significantly higher granzyme B and lower periostin tear fluid levels were found at DAOSD onset compared with baseline (127.3 pg/ml (IQR 97.1–195.1) vs. 48.8 pg/ml (IQR 43.2–77.2), $p = .028$, and 1038 pg/ml (781–3967) vs. 2344 pg/ml (IQR 1265–10,071), $p = .028$, respectively). This suggests 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 with 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 the 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 with baseline ($n = 7$ of which 6 available samples). Increased granzyme B, indicating cytotoxic T cell activity, is previously 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.

AUTHOR CONTRIBUTIONS

RA, JT, EK, DB, FW and MB-W have made substantial contributions to the acquisition of data, or analysis and interpretation of data. All authors have made substantial contributions to the conception and design of this study and have been involved in drafting the manuscript or revising it critically. All authors have given final approval for the version to be published.

FUNDING INFORMATION

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

CONFLICT OF INTEREST

Roselie Achten has nothing to disclose. Judith Thijs is a speaker for Sanofi Genzyme and LEO Pharma. Chantal van Luijk is a speaker for Sanofi Genzyme and Santen. Edward Knol is a speaker and advisory board member for Sanofi Genzyme. Eveline Delemarre has nothing to disclose. Marlies de Graaf is a consultant, advisory board member and/or speaker for AbbVie, Eli Lilly, Leo Pharma, Novartis, Pfizer, Regeneron and Sanofi Genzyme. Daphne Bakker is a speaker for Sanofi Genzyme 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 and Sanofi Genzyme.

DATA AVAILABILITY STATEMENT

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

Roselie Achten[1]
Judith Thijs[1]
Chantal van Luijk[2]
Edward Knol[1,3]
Eveline Delemarre[3]
Marlies de Graaf[1]
Daphne Bakker[1]
Joke de Boer[2]
Femke van Wijk[3]
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]*Department of Ophthalmology, University Medical Center Utrecht, Utrecht, The Netherlands*
[3]*Center for Translational Immunology, University Medical Center Utrecht, Utrecht University, 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

ORCID

Roselie Achten https://orcid.org/0000-0001-5617-8615
Judith Thijs https://orcid.org/0000-0003-2753-5235
Chantal van Luijk https://orcid.org/0000-0001-7815-5449
Edward Knol https://orcid.org/0000-0001-7368-9820
Eveline Delemarre https://orcid.org/0000-0002-4310-0998
Marlies de Graaf https://orcid.org/0000-0001-9004-5111
Daphne Bakker https://orcid.org/0000-0002-0193-5794
Joke de Boer https://orcid.org/0000-0003-1712-3050
Femke van Wijk https://orcid.org/0000-0001-8343-1356
Marjolein de Bruin-Weller https://orcid.org/0000-0002-1249-6993

REFERENCES

1. Bakker DS, Ter Linde JJM, Amini MM, et al. Conjunctival inflammation in dupilumab-treated atopic dermatitis comprises a multicellular infiltrate with elevated T1/T17 cytokines: a case series study. *Allergy*. 2021;76:3814-3817.
2. 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;52:801-805.
3. 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*. 2021;9:1389-1392.e2.
4. Bakker DS, de Graaf M, Nierkens S, et al. Unraveling heterogeneity in pediatric atopic dermatitis: identification of serum biomarker based patient clusters. *J Allergy Clin Immunol*. 2022;149(1):125-134.
5. Fujishima H, Okada N, Matsumoto K, et al. The usefulness of measuring tear periostin for the diagnosis and management of ocular allergic diseases. *J Allergy Clin Immunol*. 2016;138(2):459-467.e2.
6. Thijs JL, Nierkens S, Herath A, et al. A panel of biomarkers for disease severity in atopic dermatitis. *Clin Exp Allergy*. 2015;45(3):698-701.
7. Yokoi K, Yokoi N, Kinoshita S. Impairment of ocular surface epithelium barrier function in patients with atopic dermatitis. *Br J Ophthalmol*. 1998;82(7):797-800.
8. Dogru M, Katakami C, Nakagawa N, Tetsumoto K, Yamamoto M. Impression cytology in atopic dermatitis. *Ophthalmology*. 1998;105(8):1478-1484.
9. Vuillemey L, Febvay C, Puzenat E, et al. Analysis of cytokines in tear fluid from atopic dermatitis patients with dupilumab-associated ocular adverse events. *J Eur Acad Dermatol Venereol*. 2022;36(3):e195-e197.

SUPPORTING INFORMATION

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