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The differential effects of dupilumab and Janus kinase 1-selective inhibitors on the skin microbiome in adult patients with moderate-to-severe atopic dermatitis using metagenomic sequencing: results from the BioDay registry

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Abstract

Background

The skin microbiome in atopic dermatitis (AD) is characterized by decreased bacterial diversity and increased *Staphylococcus aureus* colonization. Although dupilumab has been shown to improve the skin microbiome in adult AD patients, little is known about the effects of Janus kinase 1-selective inhibitors (JAK1-i). Therefore, the aim of this study was to evaluate the differential effects of dupilumab and JAK1-i on the skin microbiome of adult AD patients. Skin swabs were collected from dupilumab-treated patients (N = 10) lesional/facial at baseline (T0) and week 16 (T16), from JAK1-i-treated patients (N = 15) lesional/facial at T0, week 4 (T4) and week 28 (T28), and from healthy controls (HCs; N = 17) at a single time point. Shotgun metagenomic sequencing was used to assess microbial composition and diversity.

Results

During both treatments, the Eczema Area and Severity Index (EASI) scores significantly decreased over time (both $p < 0.01$), reflecting clinical response. In lesional/facial skin, *S. aureus* abundance significantly decreased after 16 weeks of dupilumab, whereas a nonsignificant decrease was observed after 28 weeks of JAK1-i. Microbial diversity increased in dupilumab-treated patients, approaching levels observed in HCs, while no such shift occurred during JAK1-i treatment. In both groups, commensal bacteria and fungi remained less abundant than in HCs.

Conclusions

Despite comparable clinical improvements, dupilumab induced a more pronounced shift in the skin microbiome towards healthy skin compared with JAK1-i.

BACKGROUND

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by a complex interplay between a predominantly T helper (Th)2-driven immune response, genetic predisposition, impaired skin barrier function, and an altered skin microbiome [1]. The inflammation in AD is driven by an increased expression of type 2 cytokines, which impair antimicrobial defense mechanisms by inhibiting antimicrobial peptide (AMP) synthesis [2]. Consequently, the skin microbiome of AD patients is characterized by decreased bacterial diversity and increased colonization with *Staphylococcus aureus*, which correlates with inflammation and disease severity [3].

Dupilumab is a fully human monoclonal antibody that targets the interleukin (IL)-4 receptor alpha (IL-4Rα) and thereby blocks both IL-4 and IL-13 signalling, two key drivers in the Th2-pathway. It is registered for treating patients with moderate-to-severe AD and it was previously shown that dupilumab leads to an

increased microbial diversity and a decreased *S. aureus* abundance in AD patients, correlating with clinical improvement [4–12]. However, these studies primarily focused on the bacteriome while limited data is available of dupilumab's effect on the fungal and yeast microbiome. These data would be of particular interest as facial side effects, such as head-neck dermatitis (HND) and red face, have been reported in patients treated with dupilumab [13, 14]. *Malassezia* species seem to be involved in these cases, as antifungal agents were effective as treatment [15]. However, the underlying mechanisms remain unclear.

More recently, Janus kinase 1-selective inhibitors (JAK1-i) were approved for treating patients with moderate-to-severe AD.[16–18] JAK1-i block the JAK-STAT pathway and thereby downregulate several cytokine signalling pathways leading to a decrease in AD severity and itch [19]. However, JAK1-i are associated with an increased risk of (skin) infections and acneiform eruptions in AD patients [20, 21]. To date, little is known about the effect of JAK1-i on the skin microbiome.

Therefore, the aim of our study was to evaluate the effects of dupilumab and JAK1-i on the skin microbiome of adult patients with moderate-to-severe AD. Secondly, we aimed to achieve a more comprehensive characterization of the skin microbiome by examining not only the bacteriome but also the abundance of fungi and yeast.

METHODS

Study design and population

This prospective, observational cohort study included adult patients (aged ≥ 18 years) with moderate-to-severe AD participating in the Dutch BioDay registry, treated at the University Medical Center Utrecht (UMCU) between May 2022 and September 2024.

In the dupilumab group, patients received a loading dose of 600mg subcutaneously, followed by 300mg every other week. Patients visited the outpatient clinic at baseline (T0) and after 16 weeks (T16). Patients treated with a JAK1-i received either upadacitinib (15/30 mg) or abrocitinib (100/200 mg), with the dosage based on disease severity and clinicians' evaluation. Outpatient clinic visits for the JAK1-i group took place at baseline (T0), and after 4 weeks (T4) and 28 weeks (T28) of treatment. These timepoints were chosen for the JAK1-i group to better characterize the dynamics of microbiome changes during treatment.

Systemic immunosuppressive and/or immunomodulating treatment was discontinued before dupilumab/JAK1-i initiation. No patients were in washout of any previous systemic medication. Patients with a clinically relevant skin infection (e.g. impetiginization) or topical and/or systemic antibiotic and/or antimycotic medication within 4 weeks prior to the start of dupilumab/JAK1-i treatment, were excluded. A matching number of nonatopic healthy subjects was included as a control group. The study was part of the BioDay registry, which was approved by the local Medical Research Ethics Committee (METC

Utrecht 18/239) of the UMCU as a noninterventional study and was performed according to the Declaration of Helsinki. All patients provided written informed consent.

Sample collection

Sterile flocked swabs were taken from both lesional and facial skin of each patient at T0 and T16 or T28. Facial skin was chosen as sampling site for nonlesional (NL) skin, as this also allowed potential HND and acneiform eruptions to be identified. For patients treated with JAK1-i, additional swabs were taken at T4. The swabs were wetted with a buffer (phosphate-buffered saline with 0,05% Tween®-20). The skin samples were collected by rubbing two separate swabs for 30 seconds on either the lesional or facial skin. The swabs were stored in sterile tubes and immediately snap frozen into liquid nitrogen and thereafter stored at -80°C until microbiome analysis. Additionally, swabs from the antecubital fossa and facial swabs were collected from healthy controls (HCs) at one single time point. AD severity was assessed by the Eczema Area and Severity Index (EASI) score, stratified by EASI≤7 versus EASI>7 (i.e. mild AD versus moderate-to-severe AD) [22].

DNA extraction and shotgun metagenomic sequencing

DNA extraction was performed using the Qiagen EZ2 PowerFecal Pro DNA/RNA Kit for automated DNA isolation on the Qiagen EZ2 platform according to manufacturer's instructions. In short, the two skin swabs per sample were thawed and resuspended in Power Bead tubes containing 700 ml CD1. After resuspension, 100 ml CD2 and 100 ml phenol-choloroform-isoamylalcohol were added. Mechanical lysis was performed using a Mini-Beadbeater-24 (Biospec, Rijswijk, the Netherlands). Samples underwent a bead-beating cycle at 3800 rpm for 4 min. Following bead-beating, samples were spin for 5 min at 18,000 g. Supernatant was transferred to the reagent cartridge according to the protocol and run on the EZ2 using the DNA protocol. Total DNA was quantified by Quant-iT™ PicoGreen dsDNA Assay kit (Invitrogen).

Shotgun metagenomic sequencing was performed by Macrogen Europe B.V. (Amsterdam, The Netherlands) using TruSeq Nano for library preparation on the NovaSeq X Plus platform (Illumina, San Diego, CA, USA) with paired-end 150 bp reads (PE150) with 10 Gb per sample.

Bioinformatic processing and data analysis

Raw reads were processed using the Humann 3.9 pipeline based on Beghini et al. (https://pmc.ncbi.nlm.nih.gov/articles/PMC8096432/), which includes the KneadData v.0.12.2 tool for quality control, adaptor trimming and removal of human genomic DNA using the Homo_sapiens_hg39_T2T_Bowtie2_v0.1 database (https://huttenhower.sph.harvard.edu/kneaddata), metaphlan 4.1 for taxonomic profiling using the metaphlan4_vJun23_202403 database.

All analyses were performed using R 4.4.1 (2024-06-14) in RStudio (version 2026.01.2) and the functions of the packages *miaViz* and *ggplot2* [23-26]. Alpha diversity was calculated using the *vegan* package. Beta diversity was visualized using Principal Coordinate Analysis (PCoA) on Bray-Curtis dissimilarities

using plot ordination functions from the *mia* package. Correlations were tested using PERMANOVA with the adonis function at 999 permutations of the *vegan* package. Differential abundance (DA) analysis was performed using three different statistical tools: analysis of Compositions of Microbiomes with Bias Correction 2 (ANCOM-BC2), Microbiome multivariable Associations with Linear models (MaAslin2), and Linear models for Differential Abundance analysis (LinDA) [27-29]. Since each tool may yield varying p-values, with some being more conservative in data handling than others, we opted to incorporate all three tools for these analyses. If two out of three tools showed significant Log Fold Changes (LFCs), the results were considered statistically relevant. Relative abundance plots for individual taxa were generated using GraphPad Prism (version 11.0.0). Statistical analysis was performed using the Kruskal-Wallis test.

Patient and treatment characteristics

Data on baseline characteristics regarding sex, age, age of AD onset, atopic comorbidities, and previous use of immunosuppressive/-modulating drugs were collected. Clinical effectiveness was assessed using the EASI score. Outcomes were described with mean, median, standard deviation (SD) or interquartile ranges (IQR). Statistical analyses were performed using IBM SPSS Statistics for Windows (version 31.0). A two-sided p-value < 0.05 was considered statistically significant.

RESULTS

Study cohort and treatment characteristics

In total, 25 AD patients were enrolled, including 10 patients treated with dupilumab with a median age of 36.5 years (IQR 20.0-52.3) and 15 patients treated with a JAK1-i (upadacitinib N=9 and abrocitinib N=6), with a median age of 30.0 years (IQR 23.0-38.0). The mean EASI significantly decreased in both groups, from 21.7 at T0 to 5.6 at T16 in the dupilumab group (p = 0.005) and from 21.4 (T0) to 6.3 at T4 and 6.5 at T28 (p = 0.001 and p = 0.003, respectively) in the JAK1-i group. All other patient and treatment characteristics are described in **Table 1**. Additionally, 17 HCs with a similar age and gender were enrolled.

Table 1. Baseline, patient and treatment characteristics

	Dupilumab cohort N=10	JAK1-i cohort[a] N=15	p-value	Control group N=17
Female, N (%)	5 (50.0)	5 (33.3)	0.44	8 (47.1)
Age (years), median (IQR)	36.5 (20.0 – 52.3)	30.0 (23.0 – 38.0)	0.74	28.0 (26.0 – 31.0)
Age of onset AD, N (%)			1.00	-
Childhood	9 (90.0)	13 (86.7)		
Adolescence	1 (10.0)	2 (13.3)		
Adulthood	0	0		
Atopic disease at baseline, N (%)				-
Allergic asthma	7 (70.0)	8 (53.3)	0.68	
Allergic rhinitis	9 (90.0)	12 (80.0)	0.63	
Allergic conjunctivitis	9 (90.0)	14 (93.3)	1.00	
Food allergy	5 (50.0)	8 (53.3)	1.00	
≥2 atopic comorbidities	9 (90.0)	12 (80.0)	0.63	
History of immunosuppressive/-modulating drugs				-
Cyclosporin A	8 (80.0)	14 (93.3)	0.54	
Methotrexate	4 (40.0)	6 (40.0)	1.00	
Biologics[b]	1 (10.0)	11 (73.3)	**0.01**	
JAK inhibitors[c]	1 (10.0)	6 (40.0)	0.18	
Other[d]	5 (50.0)	8 (53.3)	1.00	
EASI score, mean (SD)				-
Baseline	21.7 (14.8)	21.4 (13.7)	1.00	
Week 4	-	6.3 (5.7)	-	
Week 16	5.6 (4.8)	-	-	
Week 28	-	6.5 (6.5)	-	
Disease severity during treatment (EASI≤7), N (%)				-
Week 4	-	10 (66.7)	-	
	8 (80.0)	-	-	

Week 16	-	11 (73.3)	-
Week 28			

[a]JAK1-i: upadacitinib, N = 9 (15mg, N = 6 vs 30mg, N =3), abrocitinib N = 6 (200mg, N = 6). [b]Refers to dupilumab (N=10) and tralokinumab (N=2). [c]Refers to baricitinib (N=6) and/or upadacitinib (N=2). [d]Refers to prednisolone (N=15), alitretinoin (N=1), and/or mycophenolic acid (N=1). Washout criteria based on half-life times include <1 week for cyclosporine A, JAK inhibitors, and prednisolone; <4 weeks for long-acting immunosuppressive drugs (e.g. methotrexate), and <10 weeks for biologics.
Abbreviations: AD: atopic dermatitis, EASI: Eczema Area and Severity Index, IQR: interquartile range, JAK: Janus kinase, JAK1-i: Janus kinase selective-1 inhibitor, mg: milligrams; N: number; SD: standard deviation.

Effect of dupilumab versus JAK1-i treatment on lesional skin microbiome

The relative abundances of the top-20 most abundant species (including the 'Other' and 'Unclassified' groups) in lesional skin are depicted in **Figure 1a**. A clear shift in microbiome composition towards healthy skin was observed in both treatment groups. However, this shift was more pronounced in the dupilumab-treated group.

Comparison of the alpha and beta diversity between the two treatment groups confirmed the differences in microbial composition (**Fig. 1b-e**). For the dupilumab group, alpha diversity analysis revealed a nonsignificant increase between T0 and T16 based on Inverse Simpson (ISI) and Shannon index (SI), while a significant difference was observed for the SI between T0 and HCs ($p < 0.05$) (**Fig. 1b**). A similar, but significant increase in alpha diversity for both indexes was observed in the JAK1-i group after four weeks of treatment (ISI: $p < 0.05$; SI: $p < 0.01$). However, after 28 weeks, this effect diminished, with alpha diversity being significantly different (ISI: $p < 0.05$; SI: $p < 0.01$) compared to HCs (**Fig. 1c**). Beta diversity analysis revealed significant differences in the dupilumab group, which reflects the separate clustering of T0 samples from T16 and HC samples (**Fig. 1d**). A significant difference was also found for the JAK1-i group, but in this case, beside the T0 samples also the T4 and T28 samples grouped separately from HC samples (**Fig. 1e**), indicating no effect of JAK1-i on microbial composition.

Using HCs as a reference, all DA analysis tools identified that *S. aureus* was significantly more present in the dupilumab group at T0, with a LFC varying from 7.9 to 25, while it was not significantly different when comparing HCs to T16 (**Supplemental material; Fig. S1a**). In the JAK1-i treatment group, *S. aureus* was also significantly more present at T0, with a LFC ranging from 10 to 26 (**Supplemental material; Fig. S1b**). However, it remained significantly more present at T4 and T28, with LFCs varying from 7.2 to 21 and 7.4 to 20, respectively (**Supplemental material; Fig. S1b**).

These results were confirmed by a significant decrease in relative abundance for *S. aureus* from 0.39 at T0 to 0.002 at T16 ($p < 0.001$) in the dupilumab group compared to 0.0001 in HC, which was only significant for T0 ($p < 0.0001$) (**Fig. 2a**). At T0, the relative abundance of *S. aureus* was higher in the

JAK1-i group than in the dupilumab group. However, it also decreased from 0.56 at T0 to 0.19 at T4 and 0.26 at T28 in the JAK1-i group. Nevertheless, a significant difference was only observed when compared with HCs ($p < 0.0001$, $p < 0.05$, and $p < 0.01$, respectively) (**Fig. 2b**).

Furthermore, in both treatment groups, lower disease severity (i.e., EASI ≤ 7) was associated with lower mean relative abundance of *S. aureus* (**Supplemental material; Fig. S2a**). Regarding microbial diversity, no significant changes in alpha diversity were observed during dupilumab treatment. However, samples from patients with lower disease severity were more comparable to those from HCs (**Supplemental material; Fig. S2b**), consistent with the beta diversity analysis (**Supplemental material; Fig. S2d**). In contrast, during JAK1-i treatment, alpha diversity differed significantly according to disease severity, with samples from patients with EASI ≤ 7 resembling HCs and differing significantly from those with EASI > 7 (**Supplemental material; Fig. S2c**). Nevertheless, beta diversity remained separated into three distinct groups, suggesting little effect of JAK1-i on beta diversity (**Supplemental material; Fig. S2e**).

In contrast, in both treatment groups, skin commensal bacteria and fungi like *Malassezia globosa* and *Malassezia restricta* remained significantly lower compared to HCs (**Fig.** 2**c-h**). For example, relative abundance analysis of *Cutibacterium acnes* revealed significantly higher abundance in HCs compared to the dupilumab group (T0, $p < 0.0001$; T16, $p < 0.05$) and JAK1-i group (T0 and T28, $p < 0.01$) (**Fig. 2c-d**). Finally, the relative abundance of *M. restricta* and *M. globosa* remained significantly lower in both treatment groups compared to HCs ($p < 0.0001$ for both T0) (**Fig. 2e-h**). However, the overall abundance of both species is very low, so the clinical relevance remains unclear.

Altogether, these data show that 16 weeks of dupilumab treatment shifts the lesional skin microbiome towards a more healthy skin. JAK1-i treatment induced a less pronounced shift, mainly driven by changes in the relative abundance, as microbial diversity remained largely unchanged.

Effect of dupilumab versus JAK1-i treatment on facial skin microbiome

Facial skin was chosen as sampling site for NL skin, as this also allowed potential HND and acneiform eruptions to be identified. The numbers of available lesional and NL facial samples at each time point were as follows: T0: dupilumab, 5/10 lesional vs 5/10 NL; JAK1-i, 4/15 lesional vs 8/15 NL (not documented N = 3); T4: JAK1-i, 10/15 lesional vs 2/15 NL (not documented N = 3); T16: dupilumab, 6/10 lesional vs 4/10 NL; and T28: JAK1-i, 7/10 lesional vs 5/15 NL (not documented N = 3).

The relative abundances of the top-20 most abundant species (including the 'Other' and 'Unclassified' groups) in facial skin are depicted in **Figure 3a**. A small shift in microbiome composition towards healthy skin was observed in the dupilumab-treated group, however, this shift was less apparent compared to lesional skin. In contrast, no shift in microbiome composition was seen in the JAK1-i-treated group.

In contrast to lesional skin, comparison of the alpha and beta diversity between the two treatment groups showed no differences in microbial composition (**Fig. 3b-e**). For both treatment groups, the alpha diversity was similar at all timepoints to HCs (**Fig. 3b-c**). However, beta diversity analysis revealed

significant differences in the dupilumab group, with T0 samples grouping separately from T16 and HC samples (**Fig. 3d),** while in the JAK1-i group, HC samples grouped separately from samples taken at all timepoints (**Fig. 3e**), again, indicating no effect of JAK1-i on beta diversity.

Using HCs as a reference, all DA analysis tools identified that *S. aureus* was significantly more present in the dupilumab group at T0, with a LFC varying from 6.8 to 22, while it was not significantly different when comparing HCs to T16 (**Supplemental material; Fig. S3a**). In the JAK1-i treatment group, *S. aureus* was also significantly more present at T0, with a LFC ranging from 5.9 to 21 (**Supplemental material; Fig. S3b**). However, it remained significantly more present at T4 and T28, with LFCs varying from 5.2 to 18 and 5.6 to 18, respectively (**Supplemental material; Fig. S3b**).

These results were confirmed by a significant decrease in the relative abundance of *S. aureus* (median relative abundance T0 of 0.043 and T16 of 0.004) in the dupilumab-treated group; while remaining unchanged and significantly different compared to HCs in the JAK1-i group (median relative abundance of 0.064, 0.011 and 0.013 for T0, T4 and T28, respectively) (**Fig. 4a-b**).

In contrast, in both treatment groups, no significant changes were observed for either skin commensal bacteria, nor fungi like *M. globosa* and *M. restricta* (**Fig. 4c-h**). For example, relative abundance analysis of *C. acnes* was only significantly lower in the JAK1-i group at T0 compared to HCs ($p < 0.01$) but showed no significant changes at other time points or in the dupilumab treated group (**Fig. 4b-c**).

In summary, the facial skin microbiome also shifted towards a more healthy skin during dupilumab treatment, though these changes were less apparent than those in lesional skin. In contrast, there was no significant shift in relative abundance or microbial diversity in the facial skin during JAK1-i treatment.

DISCUSSION

This prospective observational study demonstrates the differential effects of dupilumab and JAK1-i on the skin microbiome of AD patients. While lesional skin microbiome shifted towards that of healthy skin during 16 weeks of dupilumab treatment, this shift was less pronounced during JAK1-i treatment. Moreover, no clear shift was observed in the facial skin microbiome of patients treated with JAK1-i. This study is the first to use metagenomic sequencing to characterize the skin microbiome, enabling a comprehensive assessment of both bacterial and fungal species.

The most prominent changes in the lesional skin microbiome were observed during dupilumab treatment, consistent with previous studies [5-8]. DA analysis in dupilumab-treated patients revealed a significant decrease in the mean relative abundance of *S. aureus*, which was nearly absent by week 16. Additionally, both alpha and beta diversity shifted towards HCs, indicating a healthier skin microbiome after 16 weeks of treatment. In contrast, lesional *S. aureus* abundance in JAK1-i-treated patients remained significantly different from HCs after 4 and 28 weeks of treatment. Although the JAK1-i group included significantly more biologic-experienced patients than the dupilumab group, suggesting a more difficult-to-treat group, baseline EASI scores did not differ significantly between both groups. Similar

findings were reported by Thomova et al., who investigated the effects of four different therapies (i.e. dupilumab, cyclosporine A, JAK-inhibitors, and topical corticosteroids) on clinical outcomes and the skin bacteriome in AD patients [11]. They found an almost complete depletion of *S. aureus* after three months of dupilumab treatment, whereas no changes were observed in patients treated with a JAK inhibitor. A possible explanation is that JAK inhibitors exert broader immunomodulatory effects compared to the more targeted mechanism of dupilumab, which may restore the skin microbiome more directly in AD patients. Supporting this hypothesis, tralokinumab, an IL-13 inhibitor, has also been shown to reduce *S. aureus* abundance in lesional skin, although this was assessed using qPCR rather than microbiome sequencing, whereas cyclosporine A, a broad immunosuppressive agent, did not result in similar changes [6, 30]. In contrast, recent studies have reported reductions in both *S. aureus* abundance and disease severity in patients treated with abrocitinib or upadacitinib, respectively [31, 32]. Furthermore, microbial diversity increased following abrocitinib treatment in both lesional and NL skin [32]. However, these findings were derived from a clinical trial, in which the study population may not always reflect real-world clinical practice. Moreover, the study compared microbial changes with a placebo-treated group and did not include HCs, limiting the interpretation of whether these changes represented restoration towards a healthy skin microbiome. Additionally, follow-up was limited to 12 weeks; whereas our results suggest that initial microbial restoration may occur, but that this effect diminishes during follow-up. Taken together, these findings suggest that the exact pathomechanisms by which dupilumab and JAK1-i influence the skin microbiome remain unclear and should be further investigated in future studies.

Similar effects were observed in the facial (preferably NL) skin microbiome of dupilumab-treated patients, with a significant decrease in *S. aureus* abundance and a shift in microbial diversity towards HCs after 16 weeks of treatment. Previous studies have reported similar reductions in *S. aureus* abundance in NL skin, although findings regarding changes in microbial diversity have been inconsistent, with some studies reporting no significant changes in alpha diversity and others showing increased diversity [5-9, 31, 33]. However, differences in sampling locations across studies make it difficult to compare results. Altogether, these findings suggest that facial/NL skin also shifts towards a more healthy skin during dupilumab treatment, although less pronounced than lesional skin. In contrast, JAK1-i treatment did not significantly alter microbial composition or diversity in facial skin, highlighting differences between these therapies despite comparable clinical effects. Consistent with findings in lesional skin, this suggests that JAK1-i treatment may improve clinical symptoms without fully restoring the skin microbiome. This indicates that underlying immune dysregulation may persist despite clinical improvement, as also suggested by previous studies showing persistent elevated disease-severity serum biomarker levels in patients treated with JAK1-i despite clinical response [34]. However, further research is needed to better understand these effects.

Facial side-effects, such as HND and acneiform eruptions, have been reported in AD patients treated with dupilumab and JAK1-i, respectively [13, 21]. Although the exact underlying pathomechanisms remain unclear, *Malassezia* species and *C. acnes* seem to be involved [35-37]. In the current study, *Malassezia* abundance increased during dupilumab treatment in both lesional and facial skin, but

remained significantly lower compared with HCs, with no patients developing clinical signs of HND. In contrast, Umemoto et al. reported a decrease in *Malassezia* colonization, specifically *M. restricta* and *M. globosa*, during 12 weeks of dupilumab treatment in facial lesional skin; they also did not observe HND in their study population [10]. HND has been more specifically associated with *M. furfur* rather than other *Malassezia* species [38]. Notably, *M. furfur* was not detected in our study population limiting the interpretation of our findings in relation to this side effect. During JAK1-i treatment, *Malassezia* abundance remained low and stable in lesional skin, while it increased in facial skin. Similarly, Li et al. reported increased *Malassezia* abundance in lesional skin, especially during JAK1-i treatment, and to a lesser extent during dupilumab treatment [39]. This suggests that the effects of systemic treatment on *Malassezia* are variable and may depend on the type of species involved. A potential explanation for HND may be that IL-4/IL-13 blockade induces a shift towards a Th17/Th22-mediated pathway, generating an immune response against *Malassezia* [40]. This may also explain why HND is less commonly reported during JAK1-i treatment, which exerts broader immunomodulatory effects. Acneiform eruptions are a commonly reported side effect of JAK1-i treatment in AD patients [41]. Although increased *C. acnes* has been suggested as a potential mechanism, *C. acnes* abundance remained low and stable during JAK1-i treatment in our study compared with HCs. This suggests that other mechanisms or microbial species may be involved.

Limitations of this study include the relatively small sample size of AD patients, which may have limited statistical power to detect differences between treatment groups. Nevertheless, a significant decrease in *S. aureus* abundance was observed despite the modest cohort size. In addition, treatments were assessed at different time points, limiting direct comparability between groups. However, both treatments were evaluated at time points at which clinical efficacy is typically expected to be established, as reflected by improvements in EASI scores. Finally, facial skin swabs were preferably collected from NL skin; however, this was not always feasible. Therefore, the findings regarding the facial skin microbiome cannot be fully generalized to the NL skin microbiome.

CONCLUSION

In conclusion, although both dupilumab and JAK1-i treatment resulted in a comparable clinical effect, reflected by improvement in EASI scores, both the lesional and facial skin microbiome of AD patients treated with dupilumab shifted towards that of healthy skin, while this effect was less pronounced during JAK1-i treatment. Moreover, no clear microbiome-based explanation for the susceptibility to side effects such as HND or acneiform eruptions was identified.

Abbreviations

AD – atopic dermatitis; AMP – antimicrobial peptide; DA – differential abundance; DUPI – dupilumab; EASI – Eczema Area and Severity Index; HCs – healthy controls; HND – head-neck dermatitis; IL – interleukin; IL-4Rα – interleukin-4 receptor alpha; IQR – interquartile range; JAK1-i – Janus kinase 1-selective inhibitor(s); LFCs – log fold changes; N – number; NL – nonlesional; PCoA – Principal

Coordinate Analysis; SD – standard deviation; Th – T helper; T0 – baseline; T4 – week 4; T16 – week 16; T28 – week 28

Declarations

Ethics approval and consent to participate

The study was part of the BioDay registry, which was approved by the Medical Research Ethics Committee Utrecht (METC Utrecht 18/239) of the University Medical Center Utrecht as a noninterventional study and was performed according to the Declaration of Helsinki. All patients provided written informed consent.

Consent for publication

Not applicable.

Clinical trial number

Not applicable.

Availability of data and material

The metagenomic sequencing data generated and/or analysed during the current study have been deposited in the European Nucleotide Archive repository under study accession PRJEB112498 (https://www.ebi.ac.uk/ena/browser/view/ PRJEB112498). However, the data are currently not publicly accessible due to a technical issue with the ENA system. We are in contact with ENA to resolve this issue and make the data publicly available as soon as possible.

Competing interests

FV has been a speaker for Sanofi. CB has been a speaker for AbbVie and Eli Lilly. MdG has been a consultant, advisory board member and/or speaker for AbbVie, ALK, Almirall, Eli Lilly, Galderma, Janssen, LEO Pharma, Novartis, Pfizer, Regeneron, and Sanofi. MdBW has been a consultant, advisory board member and/or speaker for AbbVie, Almirall, Aslan, Amgen, Eli Lilly, Galderma, Leo Pharma, Pfizer, Regeneron, Sanofi and Takeda. JT, DB, and AS report that there are no competing interests to declare.

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Authors' contributions

Conceptualization: FV, JT, CB, MdG, MdBW; Data curation: FV, JT; Formal analysis: FV, JT; Resources: DB, AS; Funding acquisition: MdG, MdBW; Investigation: FV, JT; Methodology: JT; Supervision: MdG, MdBW; Visualization: FV, JT; Writing – original draft: FV, JT; Writing – review & editing: CB, MdG, MdBW

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Figures

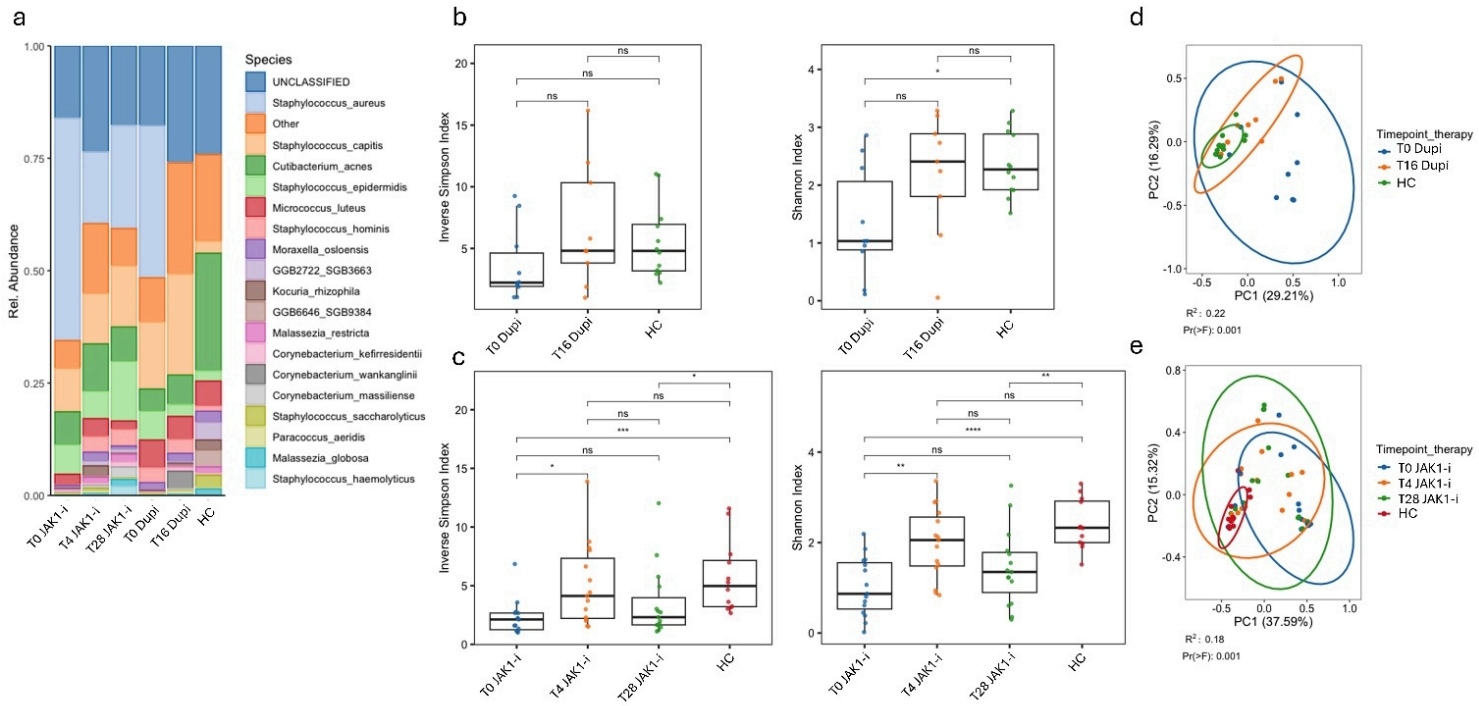


Figure 1

Microbiome changes in lesional skin from baseline to weeks 4, 16, and 28

a: mean relative abundance top-20 most abundant species (including the 'Other' and 'Unclassified' groups). b and c: alpha diversity expressed by Inverse Simpson and Shannon diversity index. d and e: beta diversity in Bray-Curtis dissimilarity PcoA plot.

Box and whisker plot represents the mean, minimum and maximum values. Significant p-values are indicated with * (p<0.05), ** (p<0.01), *** (p<0.001), **** (p<0.0001).

Abbreviations: Dupi: dupilumab, HC: healthy controls, JAK1-i: Janus kinase selective-1 inhibitor, ns: nonsignificant, Rel.: relative, T0: baseline, T3: 4 weeks of treatment, T16: 16 weeks of treatment, T28: 28 weeks of treatment.

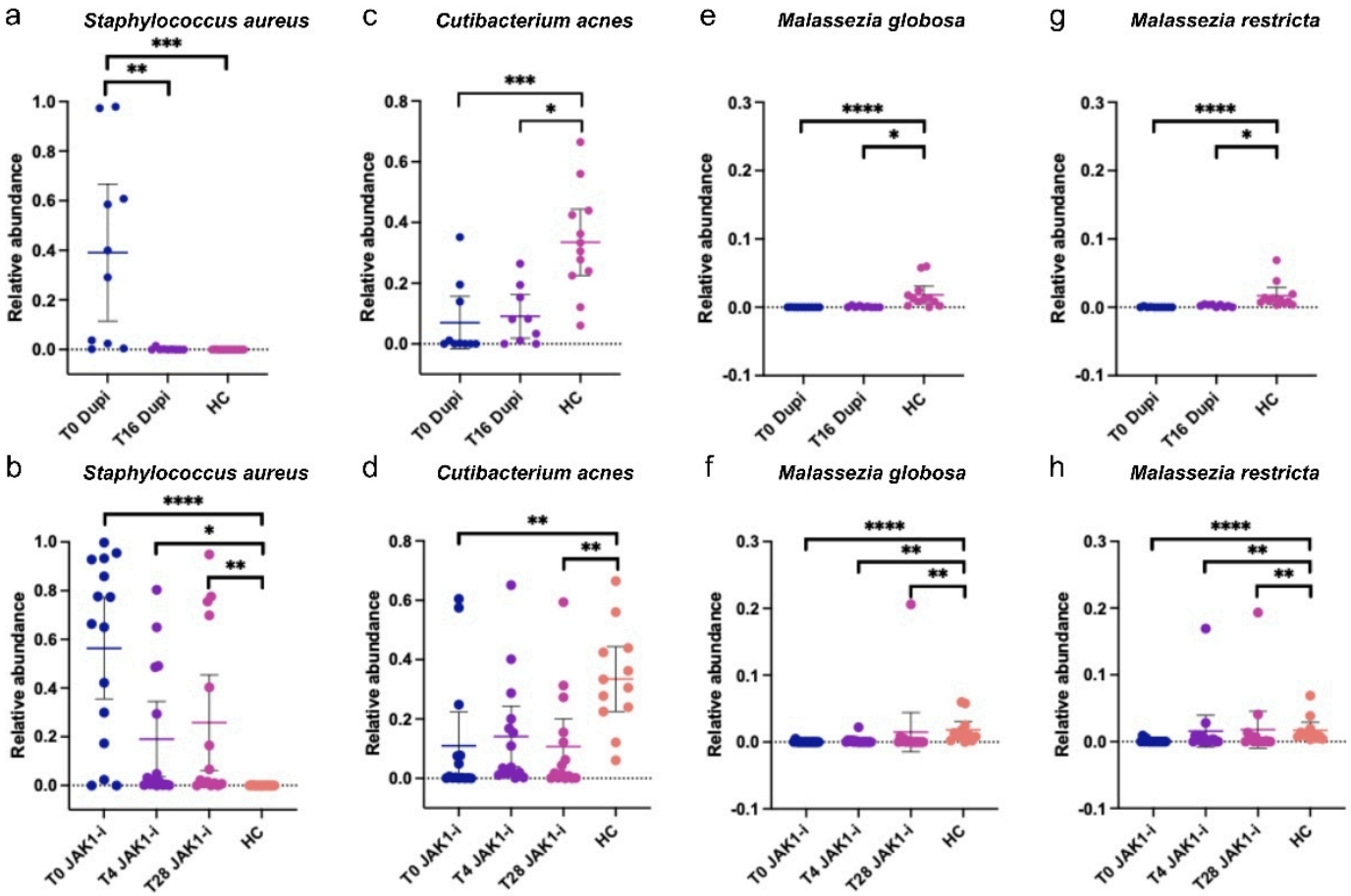


Figure 2

Differences in relative abundance among specific species in the lesional skin microbiome

Abbreviations: Dupi: dupilumab, HC: healthy controls, JAK1-i: Janus kinase selective-1 inhibitor, T0: baseline, T4: 4 weeks of treatment, T16: 16 weeks of treatment, T28: 28 weeks of treatment. Significant p-values are indicated with * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$).

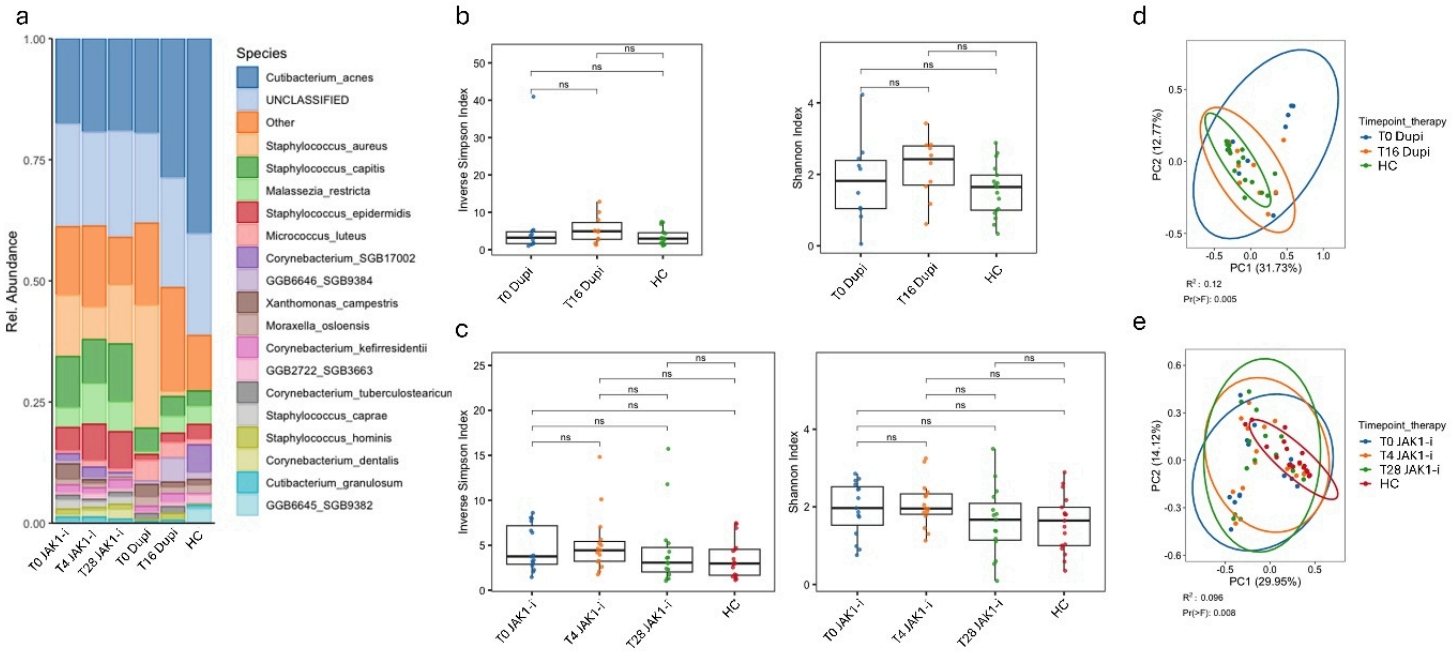


Figure 3

Microbiome changes in facial skin from baseline to weeks 4, 16, and 28

a: mean relative abundance top-20 most abundant species (including the ‘Other’ and ‘Unclassified’ groups). b and c: alpha diversity expressed by Inverse Simpson and Shannon diversity index. d and e: beta diversity in Bray-Curtis dissimilarity PcoA plot.

Box and whisker plot represents the mean, minimum and maximum values. Significant p-values are indicated with * (p<0.05), ** (p<0.01), *** (p<0.001), **** (p<0.0001).

Abbreviations: Dupi: dupilumab, HC: healthy controls, JAK1-i: Janus kinase selective-1 inhibitor, ns: nonsignificant, Rel.: relative, T0: baseline, T3: 4 weeks of treatment, T16: 16 weeks of treatment, T28: 28 weeks of treatment.

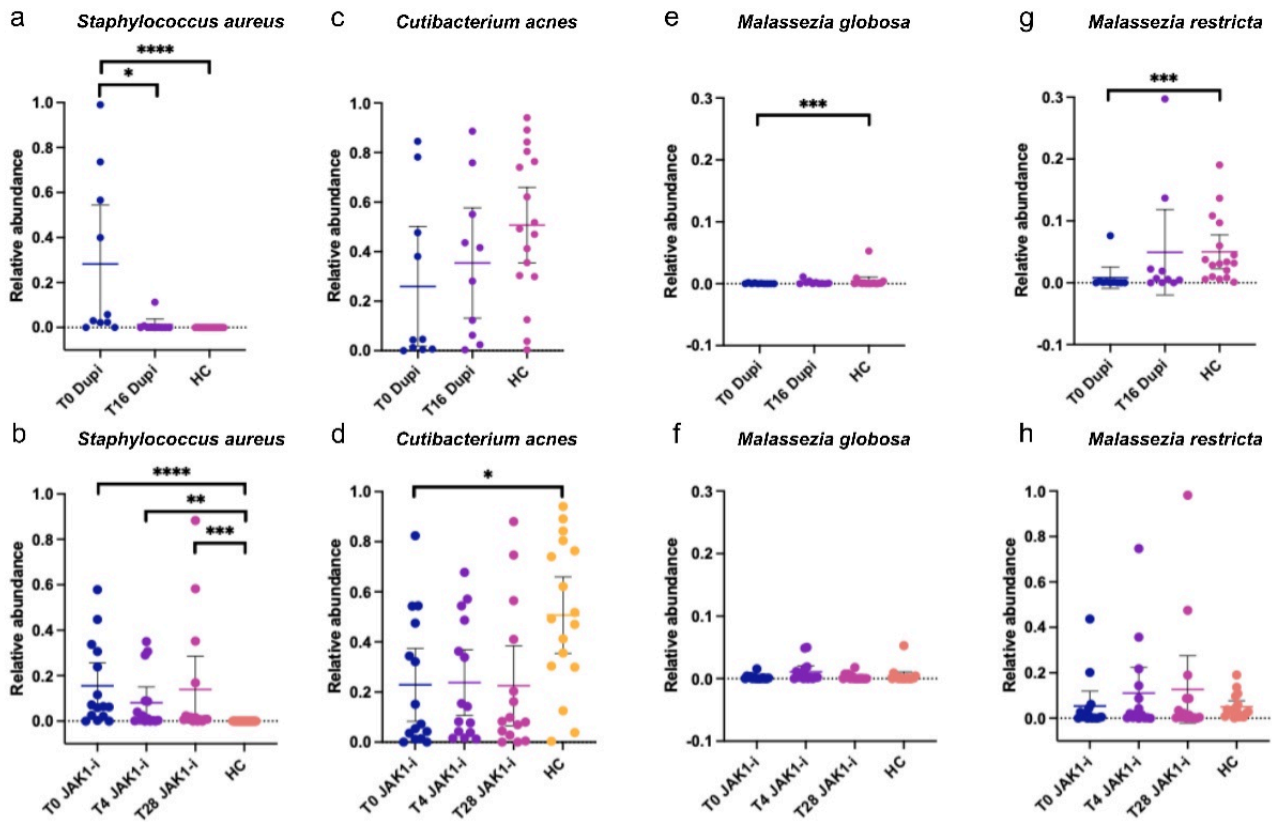


Figure 4

Differences in relative abundance among specific species in the facial skin microbiome

Abbreviations: Dupi: dupilumab, HC: healthy controls, JAK1-i: Janus kinase selective-1 inhibitor, T0: baseline, T4: 4 weeks of treatment, T16: 16 weeks of treatment, T28: 28 weeks of treatment. Significant p-values are indicated with * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$).

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