Skin Microbiome in Patients with Hand Eczema and Healthy Controls: A Three-week Prospective Study

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The pathogenesis of chronic hand eczema remains unclear. Insights into the skin microbiome in hand eczema and its potential relevance to disease severity may help to elucidate the underlying mechanisms of hand eczema. The aim of this study was to characterize the microbiome in patients with hand eczema and healthy controls. A 5-visit prospective study was conducted over a period of 3 weeks. At each visit, bacterial swabs were taken from the hands of patients with hand eczema and controls. The microbiome was examined using DNA extraction and 16S rRNA amplicon sequencing (V3–V4 regions). Fifty patients with hand eczema and 50 controls were included (follow-up rate=100%). The baseline bacterial α-diversity was reduced on the hands of patients with hand eczema compared with controls (effect size=−0.31; 95% confidence interval (95% CI) −0.50; −0.11; $p=0.003$). The dysbiosis on the patients' hands was stable over the study period, was associated with disease severity, and was characterized by reduced bacterial diversity and different bacterial community compositions.

Key words: hand eczema; hand dermatitis; microbiome; dysbiosis; *S. aureus*.

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SIGNIFICANCE

Chronic hand eczema is a frequently occurring disease, although the underlying mechanisms are unclear. The skin is densely colonized with different bacteria, collectively defined as the bacterial microbiome, which may influence skin health. This is one of the first studies to compare the composition of the bacterial microbiome on the hands of patients with hand eczema with those of healthy controls. Over a period of 3 weeks significant differences were found in the bacterial microbiome. The imbalance in the skin microbiome in chronic hand eczema requires further study, as it may be important for future treatment strategies.

Hand eczema (HE) is a common inflammatory skin disease, which has a 1-year prevalence of nearly 10% in the general population (1). HE often becomes chronic (2), and factors involved in the perpetuating course of HE need further exploration. *Staphylococcus aureus* is cultured from the hands of more than 50% of all patients with HE, and the colonization is strongly associated with disease severity (3–6). Furthermore, the anterior nares are a natural habitat for *S. aureus*, and nose-to-hand transmission may occur more frequently in patients with HE (6). However, whether skin colonization with *S. aureus* in HE is favoured by the impaired skin barrier (7), or whether part of the pathogenesis is based on expression of virulence factors, as seen in atopic dermatitis (AD) (8), remains unknown.

The human skin is colonized by a wide range of microorganisms. Several different definitions of the term "microbiome" exist (9); in this study it is defined as the microbial community assessed by 16S rRNA gene sequencing, which has frequently been used in previous skin microbiome research (10, 11). Despite general agreement on the importance of the skin microbiome in other inflammatory skin diseases (12, 13), it remains unexplored in HE. In AD, skin bacterial dysbiosis, i.e. a change in bacterial diversity compared with the healthy skin microbiome, is linked to disease severity (10, 14), the dominance of staphylococcal species (15) and, specifically, an overabundance of *S. aureus* at the expense of other staphylococci (12). Thus, the hypothesis in the current study was that the bacterial communities on the skin of patients with HE express similar characteristics, although less pronounced due to high extrinsic exposure of the hands. Insights into the skin microbiome of patients with HE could help elucidate the potential involvement of skin-colonizing bacteria in the pathogenesis and chronic course of the disease. Culture-based results regarding the 1-week prevalence of *S. aureus* and the temporal variation in *S. aureus* clonal complex types have been published previously (5, 6). The aims of the current study were to explore the skin and nasal microbiome of patients with HE compared with those of healthy controls, to determine the association with disease severity, and to investigate the temporal variations in a clinical real-life setting, using next-generation sequencing targeting 16S rRNA.

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MATERIALS AND METHODS

Population and study design

An exploratory prospective study was designed to investigate patients with chronic HE and healthy controls. Patients with chronic HE according to European Society of Contact Dermatitis guidelines (16) were recruited consecutively from a tertiary referral centre (Department of Dermatology, Bispebjerg Hospital, Denmark) from February to August 2019. Inclusion criteria were patients with chronic HE and age ≥18 years. Exclusion criteria were antibiotic treatment within the past 2 weeks from baseline, pregnancy, or breastfeeding. Topical and systemic treatment, as well as normal hand hygiene, were allowed.

In the same period, healthy controls were recruited through advertisement, with identical eligibility criteria as patients in addition to no medical history of HE or any currently active skin disease.

Patients and controls were scheduled for clinical evaluation and bacteria sampling at 5 visits over a 3-week period, on days 1, 3, 5, 8 and 21. Current or past medical history of AD ("Have you had childhood eczema" (17) or "Have you had atopic dermatitis diagnosed by a dermatologist") was noted. For patients, the dermatologist-diagnosed subtype of HE (irritant, allergic, atopic, hyperkeratotic or vesicular) (18) was registered. Disease severity was assessed by the HE Severity Index (HECSI) (19) at the first and last visit, and categorized as mild, moderate, and severe-to-very severe, defined as HECSI < 17, 17–37, and > 37, respectively (20).

Following oral and written information, all participants provided written informed consent. The Danish Ethics Committee of the Capital Region (H-18049625) and the Data Protection Agency (VD-2019-15) approved the study.

Search strategy (PubMed, 2 August 2021)

The following search strategy was used: ("hand eczema" OR "hand dermatitis" OR "contact dermatitis") AND (microbiome OR microbe OR microorganism* OR microbial OR microbiota). This resulted in 225 results, of which 1 study partly assessed the hand microbiome in HE, i.e. a pilot study including 7 healthcare workers (HCWs) with HE compared with 7 HCWs without HE.

Collection of skin and nasal swabs

Bacterial swabs (E-Swab, Copan, Brescia, Italy) were collected from the dominant hand (the most severe eczematous lesional skin (LS) and the dorsal non-lesional skin (NLS)) and the anterior nares of each participant at each visit. Samples were stored at –80°C until DNA extraction.

DNA extraction and targeted amplicon sequencing

DNA extraction and amplicon sequencing were performed as described previously (21). In short, bacterial DNA was extracted from swabs on a MagNa-Pure 96 instrument using the DNA and Viral NA small volume kit (Roche, Mannheim, Germany) with an enzymatic pre-lysis. A viable bacterial mock community (ZymoBIOMICS Microbial Community Standard D6300, Zymo Research, Irvine, CA, USA) was used as a positive control, and ultrapure nuclease free H_2O as a negative control for each DNA extraction batch (96 samples).

Amplicon libraries were prepared using 2-step PCR amplification of the 16S rRNA V3–V4 regions (22) and sequenced using an Illumina v3 reagent kit on a MiSeq instrument (Illumina Inc., San Diego, CA, USA).

Sequence pre-processing

For amplicon sequence pre-processing raw reads were demultiplexed with the bcl2fastq Conversion Software (Illumina Inc.). Next, heterogeneity spacers and primers were trimmed off using Cutadapt (v. 2.3) (23) at an 8% error rate (corresponding to 1 mismatch per primer) in paired-end mode. Trimmed reads were quality filtered and amplicon sequence variants (ASVs) were inferred with DADA2 (v. 1.12.1) (24). The DADA2 pipeline was utilized run-wise with default settings, except for truncation length. 16S rRNA gene reads were truncated at 270 bp (forward reads) and 210 bp (reverse reads). Consensus chimera removal was performed. In case a sample had a read count <5,000 after quality filtering, it was re-sequenced. Taxonomic assignment of 16S rRNA gene sequence-derived ASVs was performed with DADA2's *assignTaxonomy* and *addSpecies* functions, using the Silva reference database (25) and species-level training set (v. 138) formatted for DADA2, respectively. Based on the 16S rRNA gene V3-V4 DADA2 taxonomy assignments, *S. epidermidis* is indistinguishable from *S. caprae*, as is *S. aureus* from *S. schweitzeri* and *S. argenteus*. Therefore, the top-3 *Staphylococcus* classified ASVs were blasted (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to deduce species classification. The current study found that ASV1 most likely corresponded to *S. epidermidis*, ASV2 to *S. aureus* and ASV12 to *S. hominis*. Moreover, species classification of *S. aureus* as ASV2 was supported by our previous culture-based results on *S. aureus* carrier state in this population (Fig. S1[1]) (6).

Contaminants were removed using the Decontam package (v. 1.6.0). ASVs identified as contaminants by the frequency method (threshold 0.05) using post PCR2 concentrations and the prevalence method (threshold 0.1) using negative controls were removed, except for ASVs from the genera *Staphylococcus* and *Veillonella*. Unclassified ASVs at order level and ASVs belonging to Archeae, Chloroplasts and mitochondria were also removed. In cases where samples had been re-sequenced, these were merged with the original sample. Samples with <4,500 reads were excluded from further analysis. In addition, ASV18, *Brevibacterium* genus, was removed manually, due to a high over-representation in several healthy control samples, and was considered to be a contaminant.

Statistical analysis

Statistical analyses were conducted in *R*, (v. 3.6.2), including the package phyloseq (v. 1.30.0) (26) and visualized with ggplot2 (v. 3.3.2) (27).

Bacterial α-diversity, or complexity within samples, was calculated using Shannon's diversity index. Shannon indices and relative ASV abundance were compared using Wilcoxon signed-rank tests for paired samples (LS vs NLS) and Mann–Whitney tests for unpaired samples. Dissimilarity in overall bacterial community structure between test groups (β-diversity) was examined using principal coordinates analysis (PCoA) plots based on Bray-Curtis distances of Hellinger-transformed data (sqrt(x/sum(x)). Significance of group differences was assessed using permutational multivariable analysis of variance (PERMANOVA), implemented in the vegan package (v. 2.5-6). Homogeneity of group dispersions were investigated with an analysis of variance (ANOVA) test on dispersions calculated by the betadisper function. Correlations were calculated and visualized using Spearman's rank correlation through the ggscatter function from the ggpubr package (v. 0.4.0) (28). The significance level was set to $p < 0.05$.

RESULTS

Fifty patients with HE (mean ± standard deviation (SD) age 40.1 ± 11.7 years, female 56%, AD history 34%) and 50 controls (42.5 ± 12.6, female 82%, AD history 8%)

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Table I. Demographics and clinical data for patients with hand eczema (HE) and healthy controls

	Patients with HE ($n=50$)	Healthy controls ($n=50$)
Age (years), mean (SD) [range]	40.1 (11.7) [22–62]	42.5 (12.6) [24–64]
Sex (women), *n* (%)	28 (56)	41 (82)
Medical history of AD, *n* (%)	17 (34)	4 (8)[a]
Hand Eczema Severity index visit 1		
Median [range]	23 [2–145]	–
Mild (<17), n (%)	19 (38)	–
Moderate (17–37), *n* (%)	15 (30)	–
Severe/very severe (>37), *n* (%)	16 (32)	–
Hand Eczema Severity index visit 5		
Median [range]	14.5 [0–113]	–
Mild (<17), *n* (%)	27 (54)	–
Moderate (17–37), *n* (%)	13 (26)	–
Severe/very severe (>37), *n* (%)	10 (20)	–
HE sub-type		
Atopic, *n* (%)	13 (26)	–
Non-atopic, *n* (%)	37 (74)	–
Ventral/dorsal sample site (LS), *n* (%)	17/33 (34/66)	–

[a]Childhood atopic dermatitis.
AD: atopic dermatitis: LS: lesional skin.

were included in the study with no missing visits (**Table I**). The HE subtypes included irritant (30%), allergic (22%), atopic (26%), hyperkeratotic (16%), vesicular (4%) and unknown (2%).

From baseline (visit 1) to the 3-week follow-up timepoint (visit 5), 21 patients had a new medical treatment prescribed; initiation of topical corticosteroids ($n=12$); change of topical corticosteroids ($n=7$); initiation of methotrexate ($n=2$); additional systemic antibiotics ($n=2$); and potassium permanganate bath ($n=2$).

Baseline: hand microbiome on lesional skin differs from non-lesional skin and healthy control skin

Bacterial α-diversity was lower for LS compared with NLS (effect size=–0.46; 95% confidence interval (95% CI) –0.67;–0.21; $p<0.001$) and with controls (effect size=–0.31; 95% CI –0.50;–0.11; $p=0.003$), respectively (**Fig. 1**a). No significant difference in bacterial α-diversity within the nose was observed between patients and controls (Fig. 1b). The bacterial community structure (β-diversity) was different between LS and controls (r=0.03; $p=0.001$) and appeared more inhomogeneous for LS (Fig. 1c). Likewise, the nose samples from patients were different in community structure compared with controls (r=0.02; $p=0.039$) (Fig. 1d).

The most abundant genera found on the hands were *Staphylococcus* followed by *Corynebacterium*, *Streptococcus* and *Micrococcus*, which were all found in all patients and controls (Fig. S2[1]).

Overall, the relative abundance of *S. aureus* on LS was higher compared with NLS (r=0.52, $p<0.001$) and control skin (r=0.46, $p<0.001$), respectively. No significant differences were observed for the relative abundances of *S. epidermidis* or *S. hominis* on LS compared with NLS and control skin (Fig. S3[1]). Patients with relative abundance of *S. aureus* on LS seemed to be colonized with *S. aureus* on NLS, and partly in the nose, to a higher degree than those with low *S. aureus* abundance on LS. Furthermore, the inter-individual differences in the relative abundance of *S. aureus* in patients were high (**Fig. 2**a–c). *S. epidermidis* was the most abundant staphylococcal species on the hands of the controls (Fig. S4[1]). Combined, the reduced alpha diversity and increased abundance of *S. aureus* on LS compared with control skin indicate that the bacterial community on LS was dysbiotic.

No sex-differences (Fig. S5[1]), differences between ventral vs dorsal sampling sites, or between atopic HE vs other HE sub-types were observed concerning α- and β-diversity (LS). High relative abundance of *S. aureus*

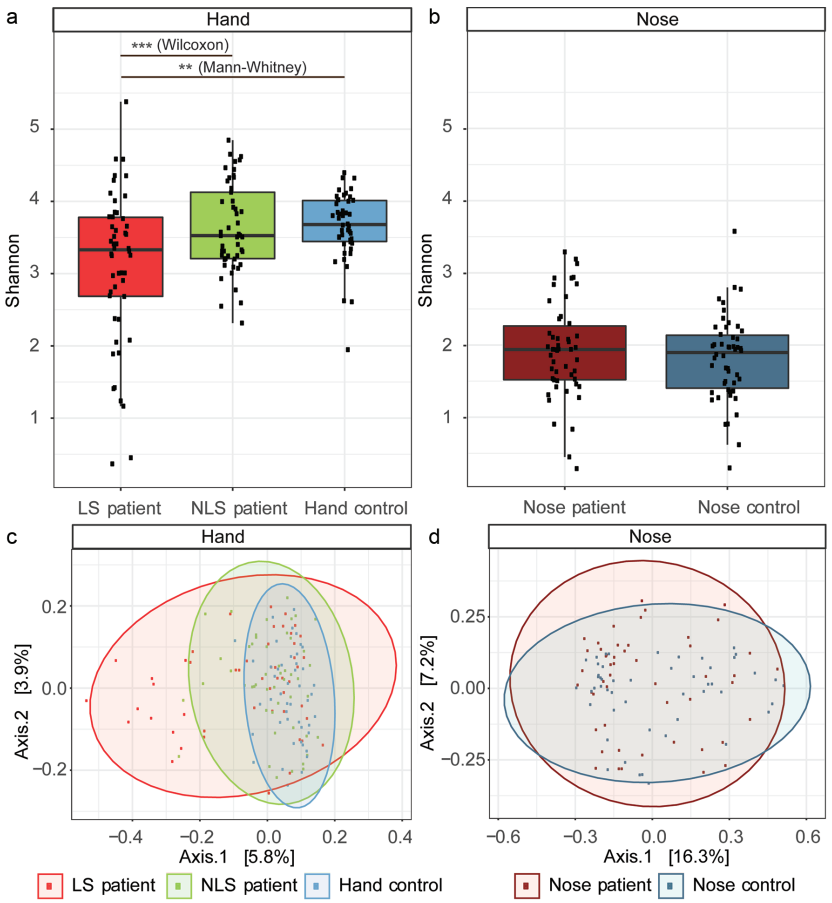

Fig. 1. Bacterial diversity stratified by sample site. The α-diversity, measured using the Shannon index: (a) on the hands and (b) in the nose of patients with hand eczema and healthy controls. Skin bacterial community structures (β-diversity) (c) on the hands of patients and controls, and (d) in the nose shown in principal coordinates analysis (PCoA) plots. Statistical significance: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

appeared more frequently in atopic HE (Fig. 2), although this was not statistically significant (atopic HE vs all other HE subtypes, $p=0.10$).

Disease severity is linked with skin dysbiosis

The HECSI score at baseline (median 23 (range 2–145)) correlated positively with relative abundance of *S. aureus* (r=0.56; $p<0.001$). Furthermore, the group of patients with severe HE ($n=16$) had a significantly lower α-diversity on LS compared with the patients with mild HE ($n=19$) (effect size=0.44; 95% CI 0.13; 0.69; $p=0.008$) (**Fig. 3**a). Concerning β-diversity, severe HE was also significantly different from both mild and moderate HE (r=0.06; $p<0.001$ and r=0.05; $p=0.028$, respectively) (Fig. 3b).

Temporal variations in the hand eczema microbiome

Neither the α- (Fig. S6[1]) nor β-diversities for LS samples differed significantly from baseline to follow-up. The median HECSI score decreased from baseline to visit 5 (median 14.5 [range 0–113]) ($p<0.01$), though this decrease neither correlated significantly with a reduction in relative abundance of *S. aureus* (r=0.20; $p=0.17$) nor an increase in α-diversity (r=0.12; $p=0.42$). However, for patients with *S. aureus* colonization on LS (defined as ≥50 counts ASV2 at 1 visit or more) and an improvement in HECSI score (≥1) from baseline to visit 5 ($n=29$), a significant reduction was observed in the relative abundance of *S. aureus* (r=0.63, $p<0.001$) (**Fig. 4**), whereas patients with no improvement in disease severity (HECSI ≤0) ($n=9$) experienced no difference in *S. aureus* relative abundance after 3 weeks (r=0.23, $p=0.53$).

The hand microbiome of healthy controls was not significantly different from baseline to 3 weeks later concerning α- and β-diversity.

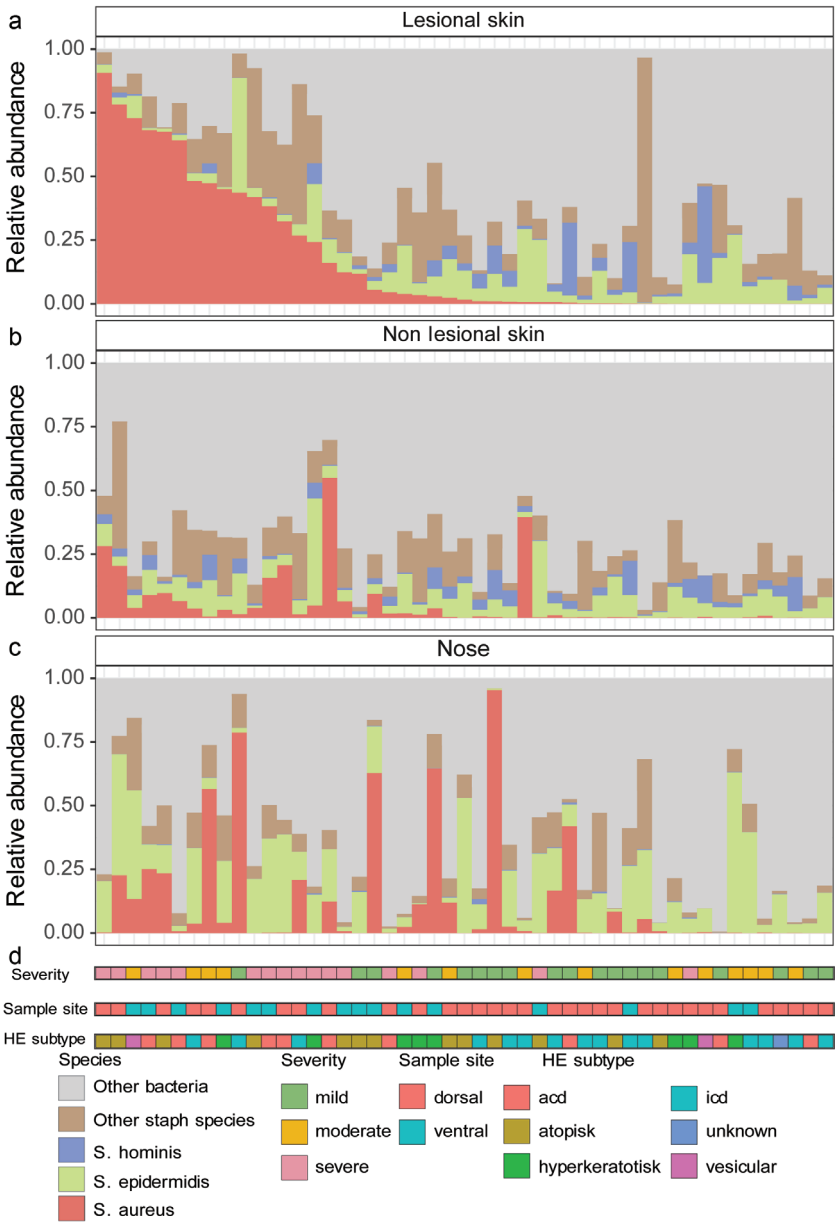

Fig. 2. Staphylococcal communities on the hands and in the nose of patients with hand eczema (HE). Barplots showing the relative abundance per patient of bacteria on (a) lesional skin, (b) non-lesional skin, and (c) in nose with a specific focus on *S. aureus*, *S. epidermidis* and *S. hominis*. Samples collected from the same patients are shown in the same order in all 3 panels and sorted by *S. aureus* abundance on lesional skin. Disease severity (HECSI), sample site, and hand eczema subtype are illustrated. acd: allergic contact dermatitis; icd: irritant contact dermatitis.

DISCUSSION

These results reveal a bacterial dysbiosis in the eczematous lesions of patients with HE. Distinct bacterial communities were observed on the hands for HE and healthy controls, and although the hands are constantly exposed to extrinsic factors the temporal stability of the skin microbiome persisted in both patients and controls during a period of 3 weeks. Consistent with the study hypotheses, and previous findings in AD (29), the patients with HE had lower α-diversity compared with controls and an increased relative abundance of *S. aureus*, and these characteristics were also significantly associated with high disease severity. The findings were less pronounced in NLS, suggesting that there is a potential for patients with HE to achieve a balanced skin microbiome. Importantly, the altered bacterial

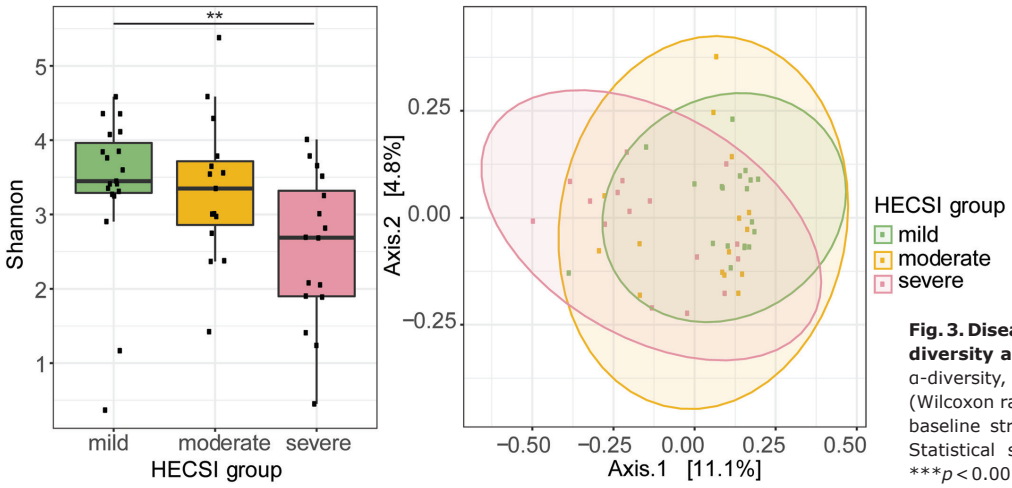

Fig. 3. Disease severity in relation to bacterial diversity and community structure. (a) The α-diversity, measured by the Shannon index (Wilcoxon rank sum test), and (b) β-diversity at baseline stratified by disease severity groups. Statistical significance: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

diversities were observed in all subtypes of HE, and not significantly different for atopic HE.

Despite thorough efforts to reduce the prevalence of HE through educational programmes (30), the 1-year prevalence of approximately 10% has remained stable for decades (1, 31). Therefore, a different approach to reduce the prevalence and severity is needed. Understanding the microorganisms on the hands of patients with HE might lead to new treatment and prevention strategies. Culture-based studies have identified high colonization rates of *S. aureus* in patients with HE (3–5). Reduced costs and increased availability of next-generation sequencing methods now allow a global investigation of the skin microbiome. However, pitfalls in the study of the skin microbiome must be considered (32), since the hands are highly exposed to microorganisms from the surroundings (33), thus potentially complicating the study of the skin microbiome of HE. A study investigating the effects of hand washing found unaffected bacterial α-diversity, whereas the community composition was influenced immediately, but re-established within a few hours (34). Furthermore, stability of the skin microbiome in healthy individuals has been reported for dry skin sites including the palms (35) as well as individual *S. aureus* strain-specificity (6). Therefore, bearing in mind that hands are vectors of microbial transmission and thereby at risk of contamination, the skin microbiome on the hands does appear to exhibit stability, supported by our observed temporal stability of α- and β-diversity on the hands of patients with HE and healthy controls.

In this study, assessment of the nose samples in patients and controls revealed dissimilar bacterial community structures, whereas no distinct difference was found in α-diversity, in contrast to a study on AD (10). This could

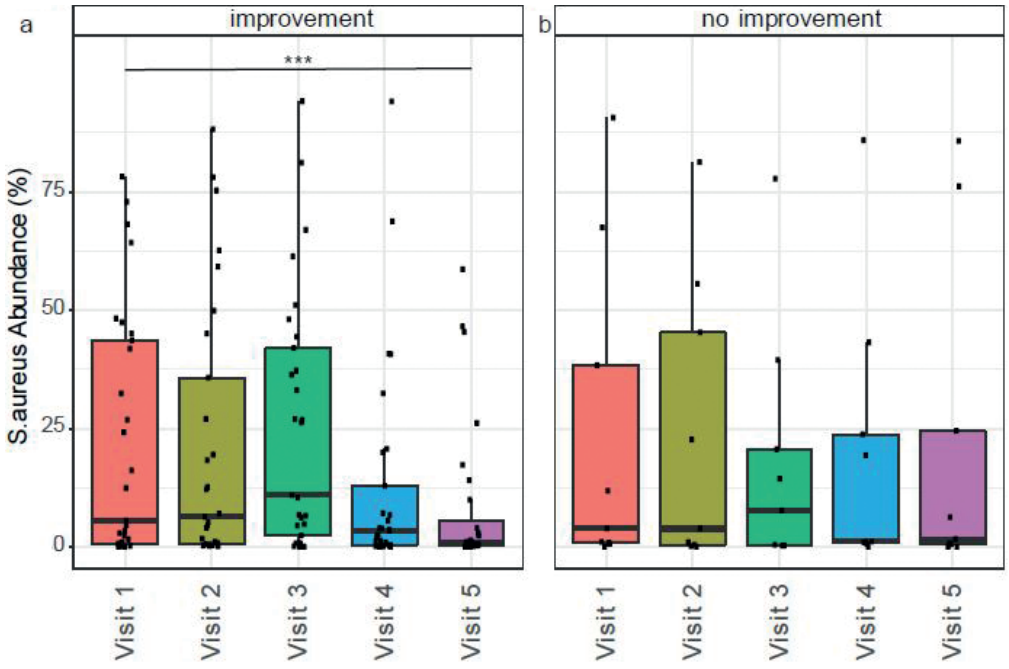

Fig. 4. Temporal variations in S. aureus relative abundance. Boxplot of *S. aureus* relative abundance for patients with *S. aureus* at 1 or more visits. (a) Patients with improvement in disease severity from baseline to 3 weeks later ($n = 29$), and (b) patients with no improvement in severity ($n = 9$). Improvement in disease severity was defined as a decrease in hand eczema severity score, HECSI ≥ 1. Wilcoxon signed-rank test (paired) were used to compare *S. aureus* relative abundance at visit 1 with visit 5 for each group ("improvement" and "no improvement"), respectively.

indicate that the nasal microbiome in patients with HE is not as influenced as in AD; however, rather serving as a reservoir for certain bacteria such as *S. aureus*, as documented in our previous culture-based study on *S. aureus* strain-specific CC-types in this population (6).

Strengths and limitations

To our knowledge, this study is the first to thoroughly examine the skin and nasal microbiome in a HE-population and may serve as a reference for future studies further examining the skin microbiome of HE. A recent Japanese pilot study using 16S rRNA gene sequencing found no difference in the skin microbiome considering α- or β-diversity in 7 HCWs with and without HE (36). The current real-life longitudinal study design with a 100% follow-up rate including information on disease severity strengthens the study, allowing comparison as well as characterization within and between the groups without attrition bias.

Limitations include the controls, which were unmatched concerning age, sex and sampling area (dorsal hand for controls, mixed dorsal/ventral hand for patients), although subgroup analyses on LS samples from the ventral vs dorsal hand, and female vs male, respectively, presented no significant differences in either α- nor β-diversity. Four individuals among our control population had a childhood medical history of atopic dermatitis. Non-eczematous skin among adults with active eczema express skin dysbiosis, and therefore, the α- and β-diversity, respectively, among our controls could theoretically have been higher than we observed. Methodologically, the V3–V4 region of the 16S rRNA gene is widely used for bacterial classification (29), although certain bacteria at species level are indifferent in this region. Consequently, the *S. aureus* ASV assignment could not exclude the rarer, closely related *S. argenteus* or *S. schweitzeri* that have an identical V3–V4 region, and the *S. epidermidis* ASV assignment could also include *S. caprae*. Nevertheless, previous cultivation of our samples identified similar *S. aureus* colonization patterns of this population (Fig. S1[1]) (5, 6), supporting our present findings. Furthermore, DNA-based analyses cannot distinguish metabolically active bacteria from inactive, dead bacteria.

Concluding that high relative abundance of *S. aureus* is positively associated with disease severity calls for *S. aureus* decolonization treatment strategies. Antibiotics have several disadvantages, including potential insufficiency towards *S. aureus* reduction in AD, eradication of beneficial bacteria, and promotion of antibiotic resistance (37–39). An improved understanding of the skin microbiome might be essential and contribute to future targeted treatment of HE. In particular, elaboration of inhibitory mechanisms by skin commensals selectively eliminating *S. aureus* might prevent exacerbation and the chronic course of HE. Promising results on such anti-*S. aureus* interventions and skin microbiome manipulation in AD (40) might also be applicable in HE. Future studies including shotgun sequencing, that allow characterization of the metabolic pathways and identification of relevant and useful biomarkers might improve our understanding.

Conclusion

This study suggests that chronic HE and its disease severity are associated with skin microbiome dysbiosis, characterized by reduced bacterial α-diversity and dissimilar community structure compared with the hands of healthy individuals. The skin microbiome of HE manifests temporal stability, as no significant differences were observed in bacterial diversity or in community structure after 3 weeks. The presence and abundance of *S. aureus* may increase the severity of the condition during the chronic course of HE and might be present at the expense of other typically commensal bacteria.

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Conflicts of interest: TA: Involved in advisory boards, consultant, lectures and clinical trials with Leo Pharma, Sanofi, Pfizer, Eli-Lilly and Abbvie. All other authors have no conflicts of interest to declare.

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