Subtype Specific Alterations in the Skin Microbiome of Patients with Cutaneous T-Cell Lymphoma

Journal of Investigative Dermatology (2025) ■, ■–■; doi:10.1016/j.jid.2025.01.009

TO THE EDITOR

Cutaneous T-cell lymphoma (CTCL) comprises a group of skin-homing lymphomas, including mycosis fungoides (MF) and Sézary syndrome (SS). Folliculotropic MF (FMF) is a subtype of MF characterized by folliculotropic infiltration with neoplastic T cells (Willemze et al, 2019). Patients with CTCL are more frequently colonized by pathogenic bacteria, including *Staphylococcus aureus*. Accordingly, changes in the skin microbiome have been suggested to play a key role in disease development and progression by providing antigenic stimulation that promotes the proliferation of malignant T cells (Willerslev-Olsen et al, 2021). We included 40 patients with CTCL (29 with MF, 7 with FMF, and 4 with SS) (Supplementary Table S1) and collected samples (ESwabs, Copan) from the anterior nares and lesional and contralateral nonlesional skin. For patients with SS, nonlesional skin was collected from skin areas with no visible redness. DNA was extracted, and 16S RNA (version 3v4) sequencing was performed (Supplementary Materials and Methods). Overall, our data showed significant alterations in the skin microbiome related to CTCL subtype, whereas there was no difference between lesional and nonlesional skin (all subtypes pooled) (Supplementary Figure S1) or between the patch and plaque morphology (Supplementary Figure S2).

Lesional and nonlesional skin of patients with FMF showed lower Shannon diversity ($P = .02$ for nonlesional and $P = .07$ for lesional skin) and higher abundance of *Staphylococcus* ($P = .02$ for nonlesional and $P = .08$ for lesional skin) than that of patients with classical MF (Figure 1a and b). Principal component analysis also indicated differences in the bacterial diversity of patients with FMF from that in patients with MF (Figure 2). *Staphylococcus* contributed the most to the detected variation, indicating increased abundance within the FMF. The 25 amplicon sequence variants (ASVs) with the highest relative abundance were identified (Supplementary Figure S3). The lesional and nonlesional skin of patients with FMF displayed a significantly higher relative abundance of ASV1 (*S epidermidis*) than that of patients with MF ($P = .01$ and $P = .005$, respectively) (Figure 1c). ASV6 (*S aureus*) was detected more often on the skin of patients with FMF than on that of patients with MF, although the difference was not statistically significant (Figure 1d).

Patients with SS exhibited lower Shannon diversity on lesional and nonlesional skin than patients with MF, although no statistical difference was observed (Figure 1a). Patients with SS also displayed a lower relative abundance of ASV1 (*S epidermidis*) in both lesional ($P = .007$) and nonlesional skin ($P = .003$) than patients with FMF (Figure 1b). All patients with SS carried ASV6 (*S aureus*) in the lesional and nonlesional skin, which was significantly higher than that in patients with MF ($P = .003$ and $P = .02$, respectively) (Figure 1d). The relative abundance of ASV6 on the skin of patients with SS ranged from 0.5 to 20% (Supplementary Figure S3).

Differential abundance analysis was performed to further compare taxa abundance between CTCL subtypes. Although no significant differences were observed after adjusting for multiple testing, 5 taxa showed differential abundances in the crude analysis. *Staphylococcus* and *Streptococcus* increased in FMF compared with those in MF, whereas *Staphylococcus* and *Finegoidia* increased, and *Cutibacterium*, *Kocuria*, and *Paracoccus* decreased in SS compared with those in MF. However, the *Streptococcus* finding was probably driven by a single outlier in the FMF group.

For the nasal microbiome, Shannon diversity was higher in patients with SS than in those with MF ($P = .05$) and FMF ($P = .03$) (Figure 1a), and patients with SS differed from those with MF in having a higher presence of ASV6 (*S aureus*) ($P = .005$) and reduced abundance of ASV1 (*S epidermidis*) ($P = .02$) (Figure 1c and d).

To date, only a few studies have investigated the skin microbiome in CTCL (Dehner et al, 2022; Harkins et al, 2021; Hooper et al, 2022; Salava et al, 2020; Zhang et al, 2022). Although these studies did not assess the skin microbiome in relation to subtype, our results align with those of these studies by showing no significant difference in Shannon diversity and community structure between lesional and contralateral nonlesional skin.

Overall, our results suggest that the bacterial community structure and abundance of staphylococcal species are related to disease subtype, supporting the association between the skin microbiome and CTCL pathophysiology. Colonization with *S aureus* has been demonstrated to promote CTCL disease progression by expression of superantigens that promote malignant T-cell proliferation (Willerslev-Olsen et al, 2021). FMF is associated with a more aggressive disease course (Farabi et al, 2022); hence, it can be speculated that the observed increased abundance of *Staphylococcus* in FMF may contribute to poor prognosis. Although the role of *S epidermidis* in CTCL is largely unknown, it is possible that strains of *S epidermidis* and other commensal staphylococcal species inhibit *S aureus* colonization in a manner similar to that observed in other inflammatory skin diseases (Nakatsuji et al, 2017). In addition, the anatomical predilection sites of FMF may also have contributed to the observed microbiome

Abbreviations: ASV, amplicon sequence variant; CTCL, cutaneous T-cell lymphoma; FMF, folliculotropic mycosis fungoides; MF, mycosis fungoides; SS, Sézary syndrome

Accepted manuscript published online XXX; corrected proof published online XXX

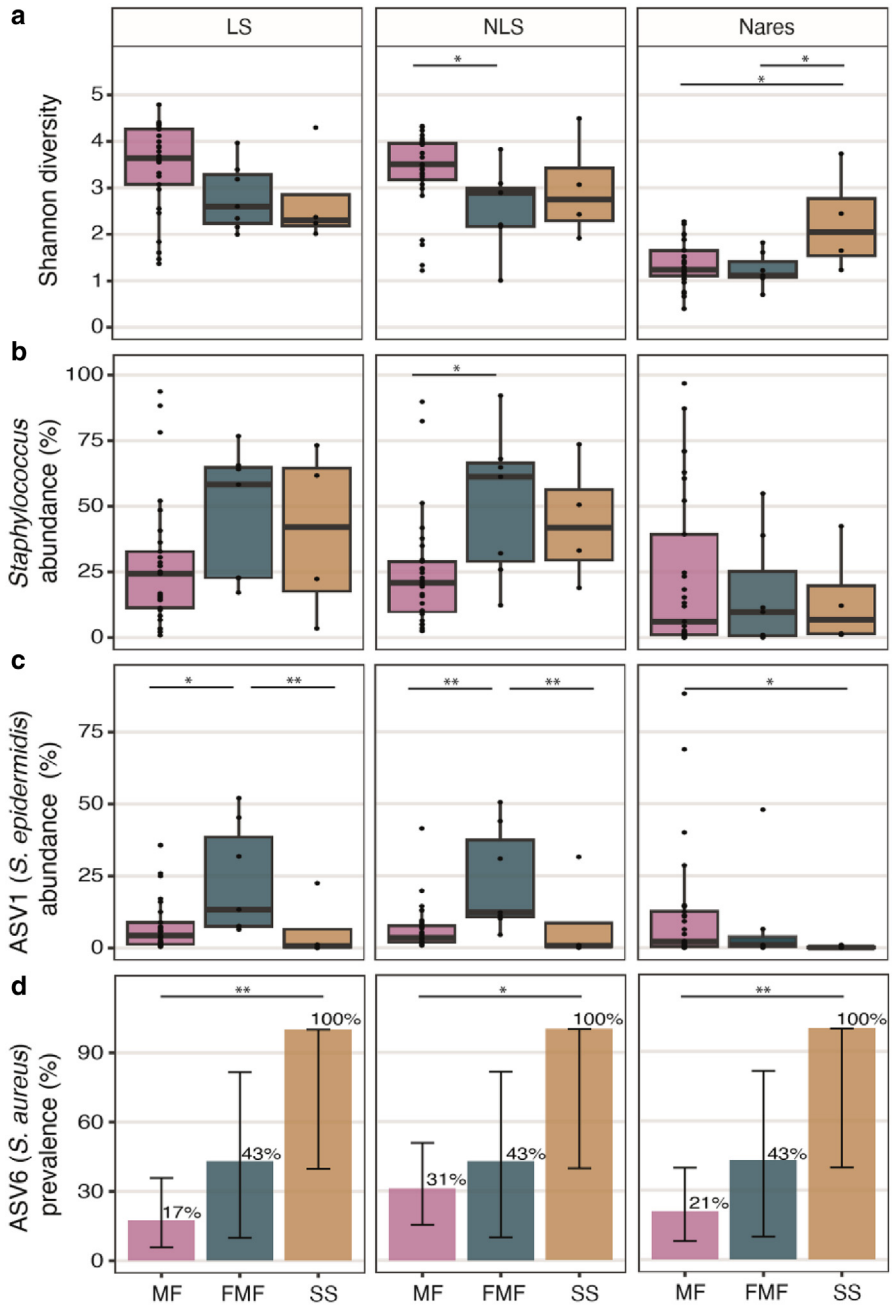


Figure 1. Shannon diversity and staphylococcal colonization in relation to CTCL subtypes. Box plots showing (**a**) Shannon diversity, (**b**) the relative abundance (%) of *Staphylococcus*, and (**c**) the relative abundance of ASV1 (*S epidermidis*). (**d**) Bar plots showing the prevalence (%) of ASV6 (*S aureus*) with 95% confidence levels in LS, NLS, and nose in relation to subtype. *$P < .05$ and **$P < .01$. ASV, amplicon sequence variant; CTCL, cutaneous T-cell lymphoma; FMF, folliculotropic mycosis fungoides; LS, lesional skin; MF, mycosis fungoides; NLS, nonlesional skin; SS, Sézary syndrome.

differences. To the best of our knowledge, no previous study has investigated the skin microbiome in patients with FMF. Further research is needed to explore the potential clinical significance of skin microbiome alterations in FMF.

The finding that patients with SS displayed increased cutaneous and nasal prevalence of *S aureus* and decreased relative abundance of *S epidermidis* supports the hypothesis that skin microbiome alterations are related to the disease stage. Similarly, Zhang et al (2022) demonstrated a correlation between skin microbiome alterations and CTCL symptom severity, with increased *Staphylococcus* in patients with marked erythema. Likewise, a culture-based study found the highest prevalence of nasal and cutaneous *S aureus* colonization in advanced disease stages (Talpur et al, 2008), suggesting an association between *Staphylococcus* and the disease stage.

The limitations of this study include the small number of patients in the SS and FMF groups. Moreover, the interactions between the CTCL subtype, demographics, disease stage, treatment, morphological lesion phenotype, and lesion predilection site complicate the determination of factors that drive alterations in the skin microbiome.

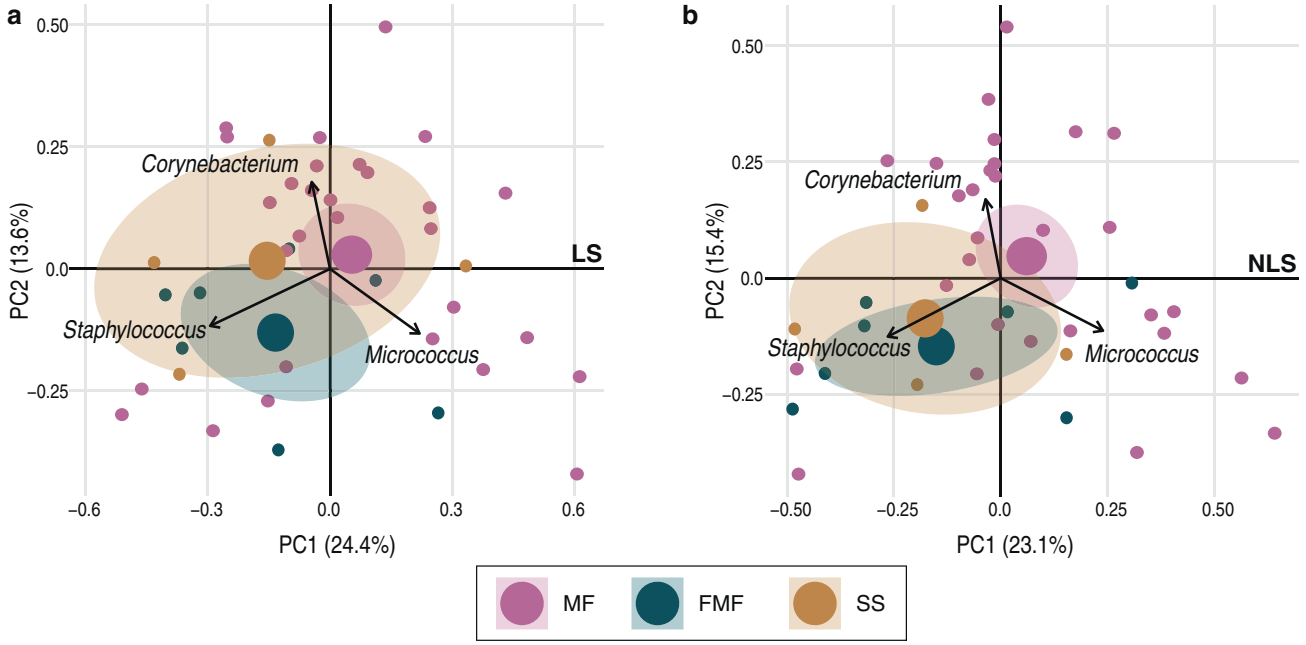


Figure 2. Community composition in relation to subtype. PCA biplot showing the variation in community composition in (**a**) LS and (**b**) NLS in relation to subtype. FMF, folliculotropic mycosis fungoides; LS, lesional skin; MF, mycosis fungoides; NLS, nonlesional skin; PC1, principal component 1; PC2, principal component 2; PCA, principal component analysis; SS, Sézary syndrome.

In conclusion, our data indicate that patients with SS and FMF have an altered skin microbiome compared with patients with MF. Whether these alterations play a role in worsening disease remains to be elucidated in larger studies. However, our data raise the question of whether the skin microbiome is a prognostic marker as well as a target for therapeutic intervention.

ETHICS STATEMENT

This study was approved by the local ethics committee (project number H-20006928) and the Danish Data Protection Agency. Oral and written information was provided by the investigator, and written informed consent was obtained from all patients.

DATA AVAILABILITY STATEMENT

Datasets related to this study can be shared after a Data Protection Agreement is approved by the Danish Data Protection Agency.

KEYWORDS

Cutaneous T-cell lymphoma; Skin microbiome; Staphylococcus; Subtypes

ORCIDs

Caroline Meyer Olesen: http://orcid.org/0000-0002-3938-7121
Sofie Marie Edslev: http://orcid.org/0000-0002-8379-0145
Yasemin Topal Yüksel: http://orcid.org/0000-0003-1289-1827
Tove Agner: http://orcid.org/0000-0002-7543-8299
Maria Rørbæk Kamstrup: http://orcid.org/0000-0002-2900-3155

CONFLICT OF INTEREST

CMO states industrial postdoc at Leo Pharma and Bispebjerg Hospital. MRK is on advisory board for Kyowa Kirin. The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS

This research was funded by the Aage Bangs foundation.

AUTHOR CONTRIBUTIONS

Conceptualization: CMO, SME, TA, MRK; Data Curation: CMO, SME, GTA, YTY, MRK; Formal Analysis: GTA, SME, CMO; Methodology: CMO, SME, TA, MRK; Project Administration: TA, MRK; Resources: TA, MRK; Software: GTA, SME; Validation: GTA, SME; Visualization: SME; Writing - Original Draft Preparation: CMO; Writing - Review and Editing: CMO, SME, GTA, YTY, TA, MRK

***Caroline Meyer Olesen*[1,*], *Sofie Marie Edslev*[2], *Gitte Nygaard Aasbjerg*[2], *Yasemin Topal Yüksel*[1], *Tove Agner*[1] *and Maria Rørbæk Kamstrup*[1]**

[1]*Department of Dermato-venereology, Bispebjerg Hospital, University of Copenhagen, Denmark; and* [2]*Department of Bioinformatics, Statens Serum Institute, Copenhagen, Denmark*

[*]*Corresponding author e-mail: caroline.meyer.olesen@regionh.dk*

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2025.01.009.

REFERENCES

Dehner CA, Ruff WE, Greiling T, Pereira MS, Redanz S, McNiff J, et al. Malignant T cell activation by a Bacillus species isolated from cutaneous T-cell lymphoma lesions. JID Innov 2022;2:100084.

Farabi B, Seminario-Vidal L, Jamgochian M, Akay BN, Atak MF, Rao BK, et al. Updated review on prognostic factors in mycosis fungoides and new skin lymphoma trials. J Cosmet Dermatol 2022;21:2742–8.

Harkins CP, MacGibeny MA, Thompson K, Bubic B, Huang X, Brown I, et al. Cutaneous T-cell lymphoma skin microbiome is characterized by shifts in certain commensal bacteria but not viruses when compared with healthy controls. J Invest Dermatol 2021;141:1604–8.

Hooper MJ, Enriquez GL, Veon FL, LeWitt TM, Sweeney D, Green SJ, et al. Narrowband ultraviolet B response in cutaneous T-cell lymphoma is characterized by increased bacterial diversity and reduced Staphylococcus aureus and Staphylococcus lugdunensis. Front Immunol 2022;13:1022093.

Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, et al. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci Transl Med 2017;9:eaah4680.

Salava A, Deptula P, Lyyski A, Laine P, Paulin L, Väkevä L, et al. Skin microbiome in cutaneous T-cell lymphoma by 16S and whole-genome

shotgun sequencing. J Invest Dermatol 2020;140:2304–2308.e7.

Talpur R, Bassett R, Duvic M. Prevalence and treatment of Staphylococcus aureus colonization in patients with mycosis fungoides and Sézary syndrome. Br J Dermatol 2008;159:105–12.

Willemze R, Cerroni L, Kempf W, Berti E, Facchetti F, Swerdlow SH, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. Blood 2019;133:1703–14.

Willerslev-Olsen A, Gjerdrum LMR, Lindahl LM, Buus TB, Pallesen EMH, Gluud M, et al. Staphylococcus aureus induces signal transducer and activator of transcription 5–dependent miR-155 expression in cutaneous T-cell lymphoma. J Invest Dermatol 2021;141:2449–58.

Zhang Y, Seminario-Vidal L, Cohen L, Hussaini M, Yao J, Rutenberg D, et al. Alterations in the skin microbiota are associated with symptom severity in mycosis fungoides. Front Cell Infect Microbiol 2022;12:850509.

SUPPLEMENTARY MATERIALS AND METHODS

Study design and patients

Patients diagnosed with cutaneous T-cell lymphoma were recruited from the Department of Dermatology at Bispebjerg Hospital (Copenhagen, Denmark) between May 2020 and December 2021. ESwabs (Copan) were collected from the anterior nares and lesional and nonlesional skin of the patients. For each patient, nonlesional skin was sampled from a matched skin area corresponding to the same anatomical location as the skin lesion. Data on topical and systemic treatments, disease subtypes, morphological phenotypes, and disease stages were also collected.

DNA extraction, 16S ribosomal RNA gene sequencing, and sequence preprocessing

DNA was extracted from swabs using an enzymatic prelysis step. Subsequently, DNA extraction was performed on a MagNa-Pure 96 robot using a DNA and Viral NA Small Volume Kit (Roche). The V3−V4 region of the 16S ribosomal RNA gene was amplified by 2-step PCR, and amplicon libraries were sequenced on a MiSeq instrument using the 600-cycles reagent kit, version 3 (Illumina). The sequences were subjected to quality filtering and grouped into amplicon sequence variants (ASVs) using the DADA2 (version 1.22.0) pipeline (Callahan et al, 2016). The taxonomic classification of the ASVs was performed using the SILVA reference database (version 138.1) (https://www.arb-silva.de) (McLaren et al, 2021). ASVs flagged as contaminants by the is.decontam function (using the prevalence method with a 0.5 threshold) in R package Decontam (version 1.16.0) (Davis et al, 2018) were excluded. In addition, ASVs belonging to phyla not associated with human colonization (Abditibacteriota, Acidobacteriota, Aquificota, Armatimonadota, Chloroflexi, Cyanobacteria, Deinococcus, Desulfobacterota, Nitrospirota, Patescibacteria, and Planctomycetota) as well as those not classified at the taxonomic order level were removed. After ASV filtering, the sequence depth within the samples exceeded 6000 reads for all samples.

Statistical analysis

Statistical analyses and visualizations were performed using the R statistical software (version 4.2.1). The R packages phyloseq (version 1.4) (McMurdie and Holmes, 2013), vegan (version 2.6.4) (Oksanen, 2019), FactoMineR (version 2.6), factoextra (version 1.0.7), FactoMineR (version 2.6), factoextra (version 1.0.7) (Kassambara and Mundt, 2020), and ggplot2 (version 3.4.1) and ggplot2 (version 3.4.1) were used for analysis and visualization. Principal component analysis was conducted to visualize the differences in the overall bacterial community composition between sample sites and cutaneous T-cell lymphoma subtypes. In addition, pairwise Bray−Curtis dissimilarity values were calculated for the samples, and PERMANOVA (permutational multivariate ANOVA) test was used to determine whether there were significant differences in community composition between groups by comparing the variance of dissimilarity values within groups with the variance between groups. Univariate PERMANOVA was conducted to test the effects of sex, age, and treatment. Principal component analysis and PERMANOVA were performed on Hellinger-transformed sequence counts agglomerated at the genus level. The bacterial composition was further assessed by plotting the relative abundance of the 25 most abundant ASVs within the samples. BLAST (Basic Local Alignment Search Tool) on the National Center for Biotechnology Information platform was used to conduct a BLAST search for the 5 most abundant ASVs classified as *Staphylococcus* to assess the species classification of these ASVs. Kruskal−Wallis test followed by Dunn's posthoc test was used to test for differences in the relative abundances of *Staphylococcus* and ASV1 (*S epidermidis*) in patients with mycosis fungoides (MF), folliculotropic MF, and Sézary syndrome in lesional skin, nonlesional skin, and nares, respectively. Fisher's exact test was used to test for differences in the prevalence of ASV6 (*S aureus*) within the cutaneous T-cell lymphoma subtypes (MF vs folliculotropic MF, Sézary syndrome vs MF, and Sézary syndrome vs folliculotropic MF) across sample sites.

Taxa differential abundance analyses were performed using the maaslin2 function (maaslin2, version 1.10.0) (Mallick et al, 2021) and negative binomial (ZINB) models. Taxonomic counts were agglomerated at the genus level and transformed using cumulative sum scaling. Taxa that were present in a minimum of 15% of the sample set and with a relative abundance >1% within a minimum of 15% of the samples were included, and *P*-values were adjusted for multiple testing using the Benjamini−Hochberg method.

Alpha diversity was measured on raw ASV counts using the Shannon index, which is a measure of the richness and evenness of bacterial ASVs within samples. The Mann−Whitney *U* test was used to test for differences between sex, treatment groups (topical corticosteroid treatment: yes/no; systemic treatment: yes/no), and skin sites (lesional vs nonlesional). Kruskal−Wallis test followed by Dunn's posthoc test was used to test for differences between age groups (<60, 60−70, >70 years) and cutaneous T-cell lymphoma subtypes (MF vs folliculotropic MF and Sézary syndrome vs MF).

SUPPLEMENTARY REFERENCES

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581−3.

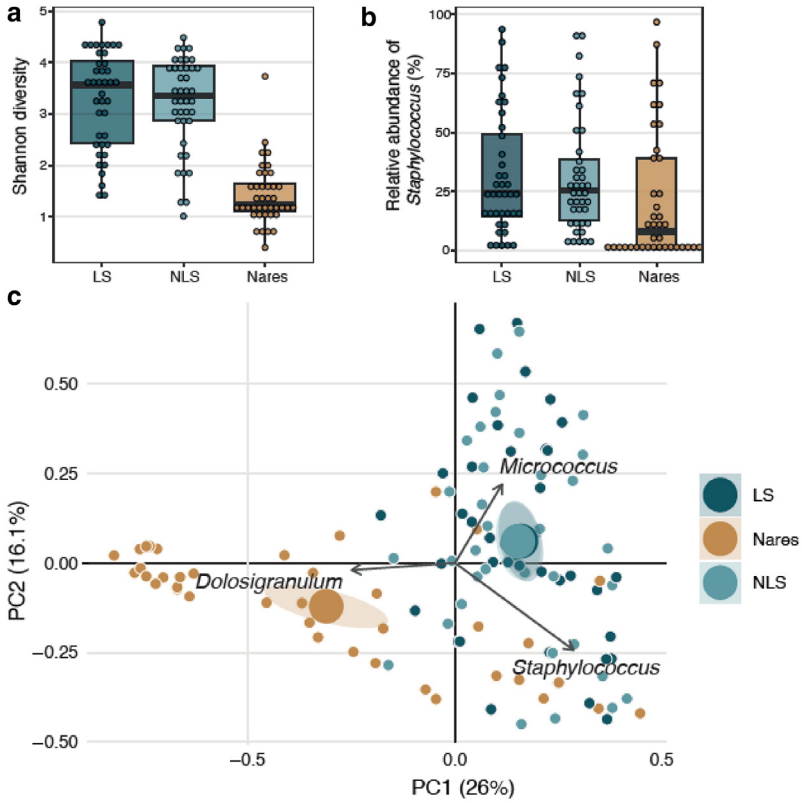
Davis NM, Proctor DM, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminating sequences from marker genes and metagenomic data. Microbiome 2018;6:226.

Kassambara AM, Mundt F. factoextra: Extract and visualize the results of multivariate data analyses. https://CRAN.R-project.org/package=factoextra; 2020. (accessed June 2, 2024).

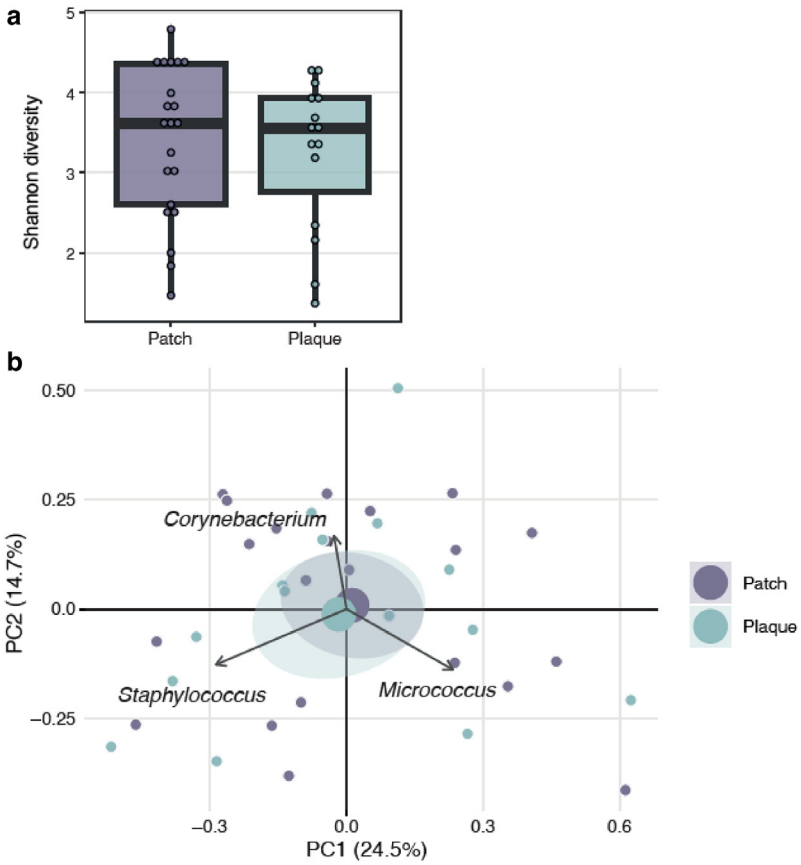
Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH, et al. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput Biol 2021;17:e1009442.

McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013;8:e61217.

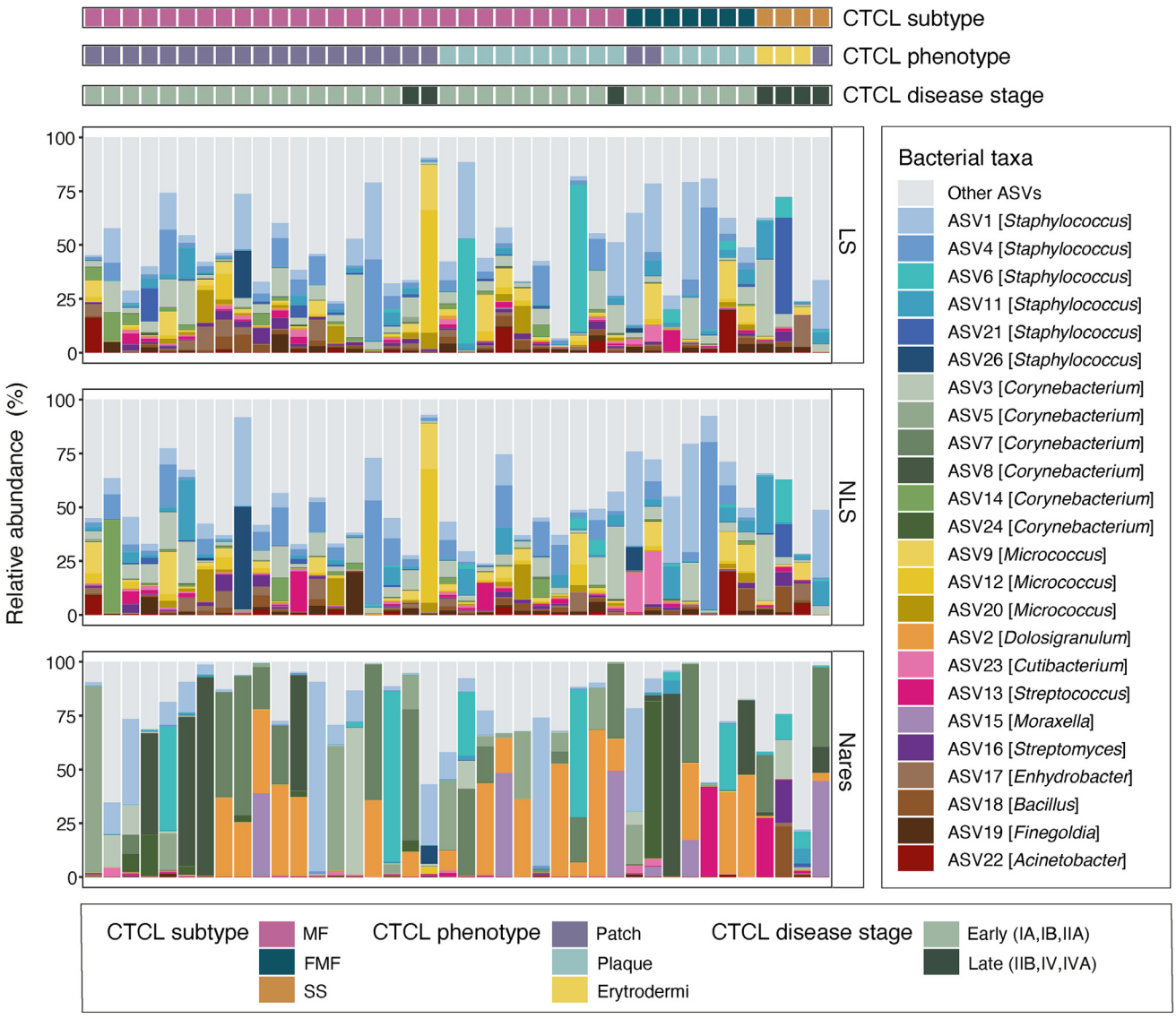
Oksanen JB,FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Solymos, P. . R package vegan: Community Ecology Package. https://cran.r-project.org/web/packages/vegan/vegan.pdf.



Supplementary Figure S1. Bacterial diversity across sample sites. **(a)** Shannon diversity, (**b**) relative abundance of *Staphylococcus*, and (**c**) community composition in in lesional skin, nonlesional skin, and nose. LS, lesional skin; NLS, nonlesional skin; PC, principal component.



Supplementary Figure S2. Bacterial diversity in relation to disease phenotypes in lesional skin of patients with MF and FMF. (**a**) Shannon diversity and (**b**) community composition in relation to lesion morphology. FMF, folliculotropic mycosis fungoides; MF, mycosis fungoides; PC, principal component.



Supplementary Figure S3. Community composition within sample sites. Bar plots show the relative abundance of the most abundant ASVs in lesional skin, nonlesional skin, and nose. The genera are shown in brackets. Samples are ordered on the basis of CTCL subtype, phenotype, and disease stage. ASV, amplicon sequence variant; CTCL, cutaneous T-cell lymphoma; FMF, folliculotropic mycosis fungoides; LS, lesional skin; MF, mycosis fungoides; NLS, nonlesional skin; SS, Sézary syndrome.

Supplementary Table S1. Demographics and Disease Characteristics Stratified by Disease Subtype

	Subtypes			
Characteristic	**Mycosis Fungoides**	**Folliculotropic Mycosis Fungoides**	**Sézary Syndrome**	**All Subtypes**
Number of patients	29	7	4	40
Sex, n (%)				
Female	14 (48.3)	1 (14.3)	1 (25.0)	16 (40.0)
Male	15 (51.7)	6 (85.7)	3 (75.0)	24 (60.0)
Age, y				
Median	69.0	61.0	83.0	69.0
Phenotype, n (%)				
Patch	19 (65.5)	2 (28.6)	1 (25.0)	22 (55.0)
Plaque	10 (34.5)	5 (71.4)	0 (0)	15 (37.5)
Tumor	0 (0)	0 (0)	0 (0)	0 (0)
Erythrodermic	0 (0)	0 (0)	3 (75.0)	3 (7.5)
Skin type of sample site, n (%)				
Dry	27 (93.1)	4 (57.1)	4 (100.0)	35 (87.5)
Moist	2 (6.9)	0 (0)	0 (0)	2 (5.0)
Sebaceous	0 (0)	3 (42.9)	0 (0)	3 (7.5)
Treatment, n (%)				
Systemic	7 (24.1)	5 (71.4)	4 (100.0)	16 (40)
Topical	19 (65.5)	3 (42.9)	3 (75,0)	25 (62.5)
Disease stage, n (%)				
IA	20 (72.4)	4 (57.1)	0 (0)	24 (60)
IB	5 (17.2)	3 (42.9)	0 (0)	8 (20)
IIA	1 (3.4)	0 (0)	0 (0)	1 (2.5)
IIB	3 (6.9)	0 (0)	0 (0)	3 (7.5)
IVA	0 (0)	0 (0)	2 (50)	2 (5)
IVB	0 (0)	0 (0)	2 (50)	2 (5)