Association of Skin Microbiome Dynamics With Radiodermatitis in Patients With Breast Cancer

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IMPORTANCE The interindividual differences in severity of acute radiation dermatitis are not well understood. To date, the pathomechanism and interplay of microbiome and radiodermatitis before and during treatment remain largely unknown.

OBJECTIVE To assess the association of skin microbiome baseline composition and dynamics with severity of radiodermatitis in patients undergoing adjuvant radiotherapy for breast cancer.

DESIGN, SETTING, AND PARTICIPANTS A longitudinal prospective pilot observational study was conducted between January 2017 and January 2019. Sequencing results were received in March 2021, and the data were analyzed from August 2021 to March 2023. This study was performed at an urban academic university cancer center. A total of 21 female patients with breast cancer after surgery were consecutively approached, of which 1 patient withdrew consent before the study started.

EXPOSURE Adjuvant radiotherapy for breast cancer for 7 weeks.

MAIN OUTCOMES AND MEASURES The main outcome was the association of baseline skin microbiome composition and its dynamics with the severity of radiodermatitis. A total of 360 skin microbiome samples from patients were analyzed, taken before, during, and after radiotherapy, from both the treated and contralateral healthy sides. The skin microbiome samples were analyzed using 16S (V1-V3) amplicon sequencing and quantitative polymerase chain reaction bacterial enumeration.

RESULTS Twenty female patients with breast cancer after surgery who underwent radiotherapy enrolled in the study had a median (range) age of 61 (37-81) years. The median (range) body mass index of the patients was 24.2 (17.6-38.4). The 16S sequencing revealed that low (<5%) relative abundance of commensal skin bacteria (*Staphylococcus epidermidis, Staphylococcus hominis, Cutibacterium acnes*) at baseline composition was associated with the development of severe radiodermatitis with an accuracy of 100% (sensitivity and specificity of 100%, $P < .001$). Furthermore, in patients with severe radiodermatitis, quantitative polymerase chain reaction bacterial enumeration revealed a general non–species-specific overgrowth of skin bacterial load before the onset of severe symptoms. Subsequently, the abundance of commensal bacteria increased in severe radiodermatitis, coinciding with a decline in total bacterial load.

CONCLUSIONS AND RELEVANCE The findings of this observational study indicated a potential mechanism associated with the skin microbiome for the pathogenesis of severe radiodermatitis, which may be a useful biomarker for personalized prevention of radiodermatitis in patients undergoing adjuvant radiotherapy for breast cancer.

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Radiation-induced skin injury (hereafter, radiodermatitis) is among the most frequent adverse effects of radiotherapy. Radiodermatitis substantially impairs the quality of life of patients undergoing radiation therapy and poses a risk for clinical complications.[1] To date, the interindividual differences regarding the severity of radiodermatitis are not sufficiently understood.[2,3] This lack of information impedes individualized prophylactic treatment approaches and leads to unsatisfying management of radiodermatitis for many patients.

Recently published studies have suggested an association between nasal microbiome colonization and severity of radiodermatitis, as well as radiodermatitis recovery outcomes based on skin microbiome.[4,5] Furthermore, a randomized clinical trial showed that skin decolonization may be effective for acute radiodermatitis prophylaxis.[6] However, to our knowledge, research has yet to unravel the pathomechanism and association of the skin microbiome before and during radiotherapy with radiodermatitis severity.

Key Points

Question Is the composition and dynamics of the skin microbiome associated with severity of acute radiation dermatitis in patients with breast cancer undergoing radiotherapy?

Findings In this observational prospective study investigating the skin microbiome of 20 patients with breast cancer undergoing radiotherapy, low colonization with commensal skin bacteria (*Staphylococcus epidermidis, Staphylococcus hominis, Cutibacterium acnes*) at baseline was associated with the development of severe radiodermatitis. In patients with severe radiodermatitis, overgrowth of non–species-specific skin bacterial load was observed before the onset of severe symptoms.

Meaning The study results suggest that the skin microbiome may be used as a biomarker for personalized prevention strategies of radiodermatitis.

Methods

This observational prospective longitudinal pilot study aims to investigate the association between skin physiology, the skin microbiome, and radiodermatitis severity. To accomplish this objective, 20 female patients with breast cancer after surgery undergoing radiotherapy were included in the analysis. The study was conducted between January 2017 and January 2019 at the Department of RadioOncology, Technical University Munich. Data analysis was performed at the Institute of Environmental Medicine, Faculty of Medicine, University of Augsburg, from August 2021 to March 2023. All patients provided informed written consent, and the study was approved by the Technical University Munich ethics committee. R statistical software, version 4.1 (R Project for Statistical Computing), and GraphPad Prism, version 9.3.1 for Windows (GraphPad Software), were used for data analysis and presentation. Tests of significance were 2-tailed with $P < .05$ considered significant.

Patients were treated with 3-dimensional adjuvant whole breast conventional radiotherapy and a prescribed dose of 50.4 Gray (Gy) (single dose: 1.8 Gy) for 7 weeks. Computed tomography scans were used to plan the radiotherapy (eMethods in Supplement 1). In total, 9 visits were performed before radiotherapy (baseline), weekly during radiotherapy (weeks 1-7), and after radiotherapy (week 12). At each visit, the skin pH and transepidermal water loss were measured, and samples for skin microbiome analysis were taken from the irradiated affected breast and the contralateral unaffected breast. Additionally, radiodermatitis severity was assessed according to Common Terminology Criteria for Adverse Events (CTCAE) grade, version 4.03 (grade 1 indicates mild radiodermatitis; grade 2, moderate radiodermatitis; grade 3, severe radiodermatitis) (**Figure 1**A).

In total, 360 swabs were collected by rubbing a 2 × 2–cm area a total of 20 times with a premoistened swab centrally at the lower skin crease (at the 6-o'clock position). A TaqMan assay (Thermo Fisher Scientific) was used to perform 16S rRNA absolute quantification, and the MiSeq platform (Illumina) was used for sequencing (eMethods in Supplement 1).

Results

Demographics

Twenty female patients with breast cancer were enrolled after surgery. Demographics were assessed at baseline. Patients had a median (range) age of 61 (37-81) years. The median (range) body mass index of the patients, calculated as weight in kilograms divided by height in meters squared, was 24.2 (17.6-38.4). The median (range) patient breast volume was 678 (170-3147) cm^3. Overall, 13 patients were classified as type 2 on the Fitzpatrick skin scale (type 1: 1 patient; type 3: 4 patients; type 4: 2 patients) (eTable in Supplement 1).

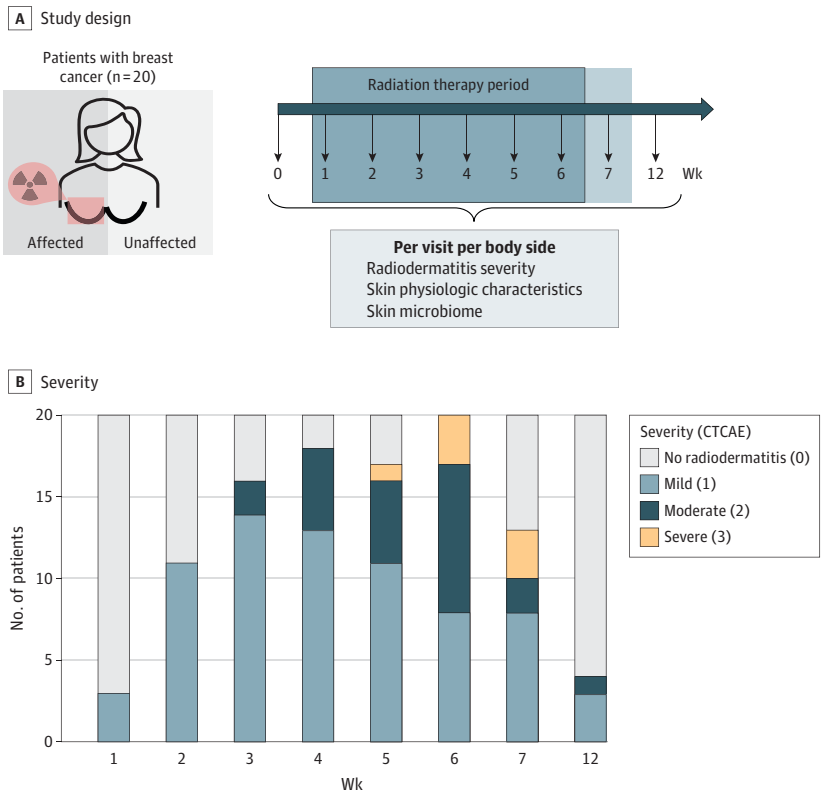
Radiodermatitis Severity

A total of 7 patients developed mild skin symptoms (CTCAE grade 1), and 9 patients developed moderate skin symptoms (CTCAE grade 2). However, 4 patients (20%) experienced severe radiodermatitis (CTCAE grade 3), marked by confluent moist desquamation. Severe radiodermatitis was observed near the end of radiotherapy, between weeks 5 and 7 (Figure 1B).

Association of Radiodermatitis Severity With Baseline Microbiome Composition

Before radiotherapy, similar microbial compositional patterns were observed intraindividually between the affected and unaffected body sides (eFigure 1 in Supplement 1). However, large differences in the interindividual microbial composition occurred at baseline. Low baseline levels (<5%) of the combined relative abundance of the 3 commensals (*Staphylococcus epidermidis*, *S hominis,* and *Cutibacterium acnes*) in the affected body side were associated with the development of severe radiodermatitis with 100% accuracy (specificity = 100%, sensitivity = 100%; $P < .001$). This association was still significant after multiple testing correction (**Figure 2**A, 2B,

Figure 1. Study Design and Radiodermatitis Severity



A, To investigate the association of skin physiology and microbiome with radiodermatitis severity, a longitudinal study with 9 visits throughout 12 weeks before, during, and after radiotherapy was conducted. B, At each visit, skin physiology and radiodermatitis severity were measured, and samples for skin microbiome analysis were taken from the affected, radiated breast and the unaffected, nonradiated breast side. All patients developed either a mild (light blue, CTCAE grade 1), moderate (dark blue, CTCAE grade 2), or severe (yellow, CTCAE grade 3) form of radiodermatitis. Severe symptoms were observed in 4 patients between visits at weeks 5 to 7. CTCAE indicates Common Terminology Criteria for Adverse Events.

and 2D; eFigure 1 in Supplement 1). Low baseline commensals relative abundance was also observed in the unaffected side (eFigures 1 and 5 in Supplement 1).

None of the baseline factors, including breast volume, patient body mass index, skin pH, and transepidermal water loss, were significantly associated with the development of severe radiodermatitis (eFigure 2 in Supplement 1).

Longitudinal Development of Skin Microbiome by Radiodermatitis Severity

While the total bacterial load in patients with mild and moderate radiodermatitis remained stable throughout the study period, a large increase of bacterial load relative to baseline occurred in the affected body side of patients who developed severe radiodermatitis (**Figure 3**A, 3B, and 3D; eFigures 3 and 4 in Supplement 1). The increase in bacterial load, only in the affected body side of patients with severe radiodermatitis, was already observed at weeks 1 to 3 of the study, hence before the development of severe symptoms at weeks 5 to 7. During this early phase, total bacterial load increased in patients with severe radiodermatitis. Neither the aforementioned commensal bacteria frequency, the frequency of the *Corynebacteriaceae* species, nor any of the top 20 most common bacterial species increased in the early phase (eFigure 3 in Supplement 1).

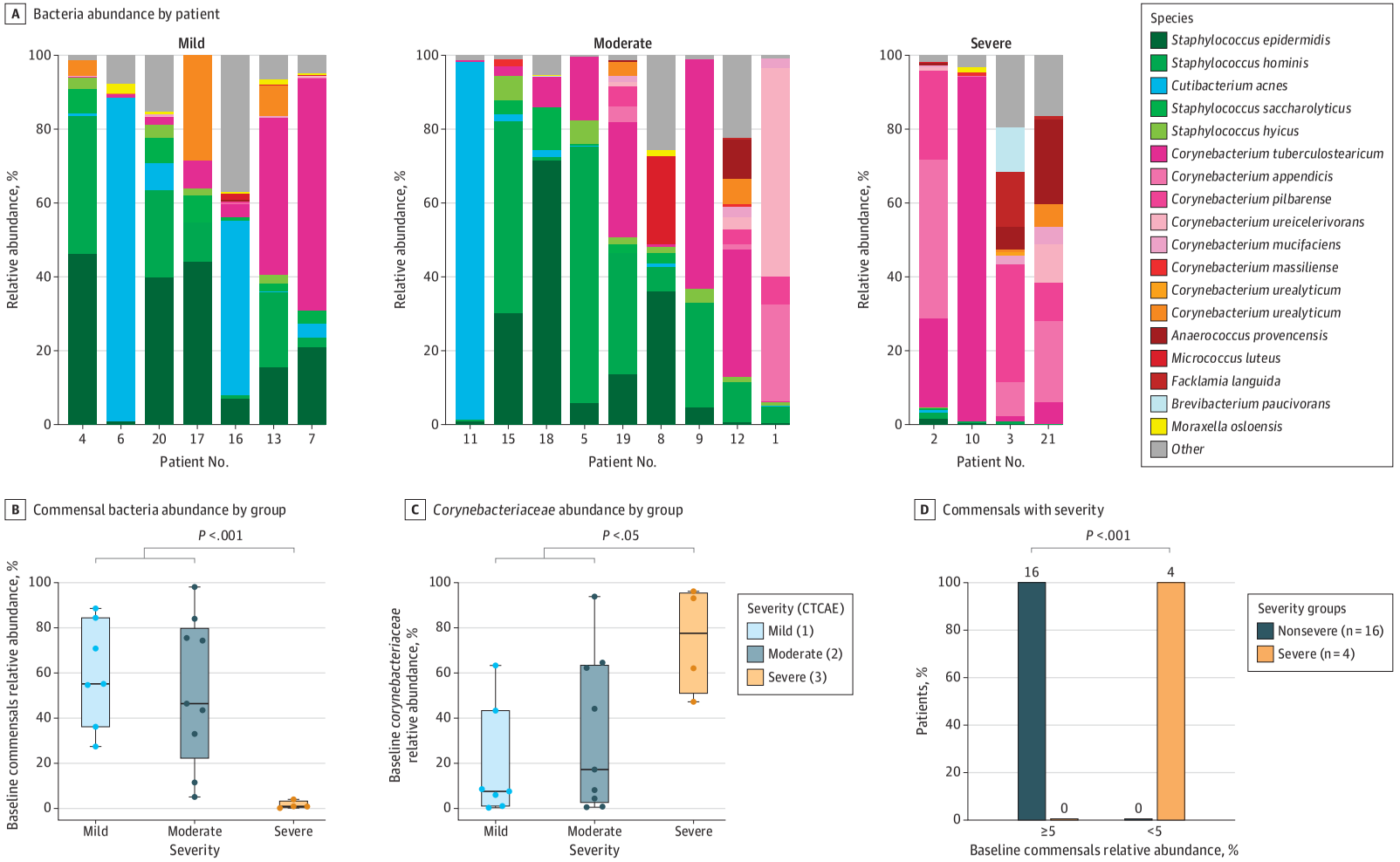
At weeks 5-7, concomitant to the appearance of severe symptoms, total bacterial load declined, while absolute and relative commensals increased, compared with low baseline levels in patients with severe radiodermatitis. Microbial composition remained stable in patients without severe radiodermatitis (Figure 3C and 3D; eFigure 3 in Supplement 1). The combination of an early-phase increase in total bacterial load with a late-phase increase in commensals (Figure 3D) was observed only in the affected side of patients with severe radiodermatitis (Fisher exact test, 4 severe vs 16 nonsevere; $P < .001$). This association was found to be significant, even after multiple testing correction.

Discussion

In the current study, we observed 3 skin microbiome phenomena found exclusively in patients with severe radiodermatitis: a low baseline level of commensals, an early increase in total bacterial load, and a subsequent increase in commensals concomitant with a decline in total bacterial load. Commensal coagulase-negative staphylococci, such as *S epidermidis* and *S hominis,* are among the most common bacterial species found in analysis of healthy skin samples. Generally, these bacteria are regarded as beneficial to host health because they inhibit the growth of pathobionts, such as *S aureus,*[7,8] and balance the host's immune response.

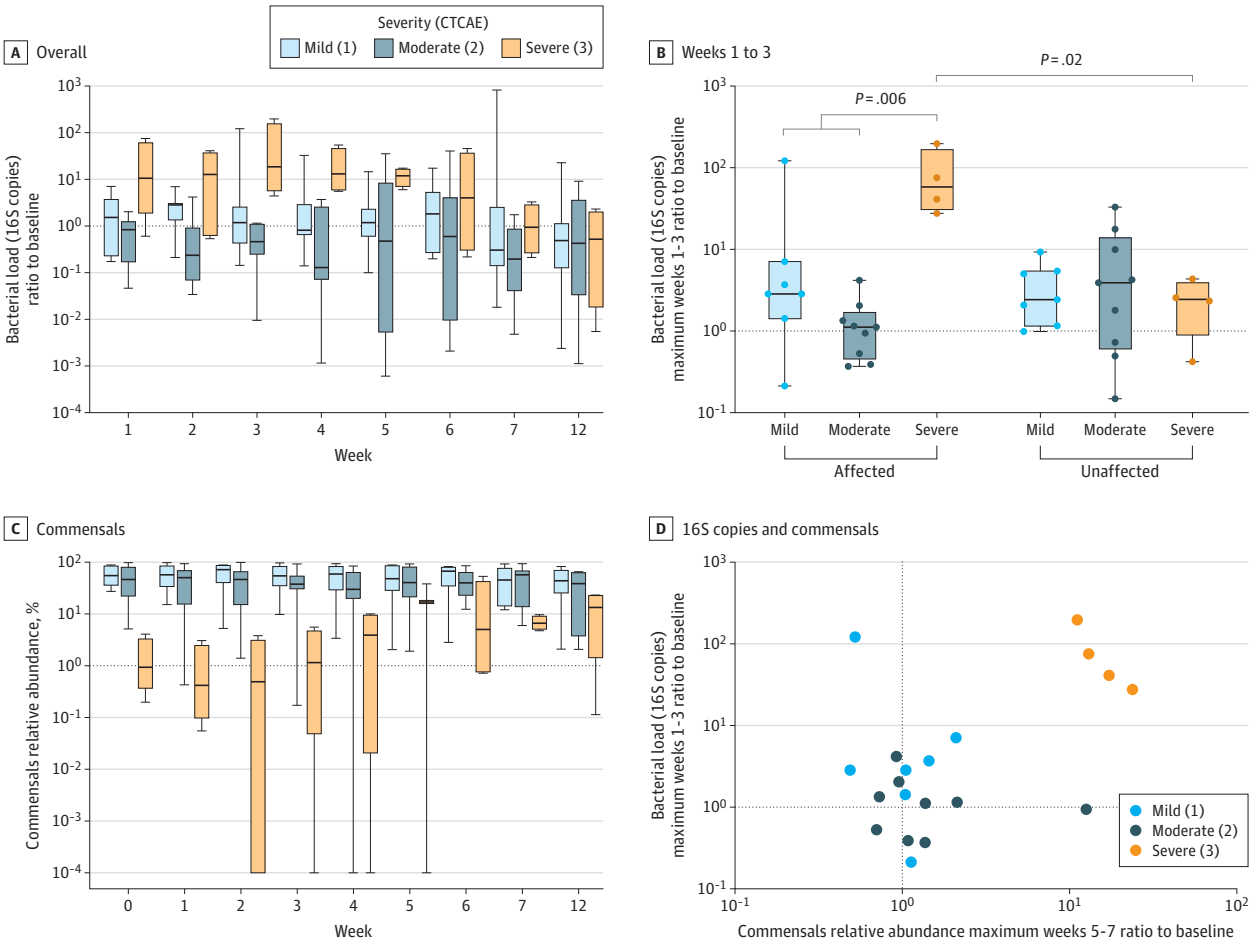
Patients with severe radiodermatitis, who all started with low baseline commensal frequency, exhibited the combination of early-phase increase in total bacterial load in the affected radiated area (weeks 1-3) and late-phase increase in

Figure 2. Low Level of Commensal Skin Bacteria at Baseline and Severe Radiodermatitis Development



Patients who develop severe radiodermatitis have a significantly lower baseline relative abundance of commensals at the affected body side ($P < .001$). Commensals are defined as the sum of *Cutibacterium acnes*, *Staphylococcus epidermidis*, and *S hominis* relative abundances in this study. Patients with severe radiodermatitis had higher relative abundances of *Corynebacteriaceae* species, compared with patients with mild and moderate radiodermatitis, as shown per individual (A) and group (B and C). No significant difference was found in commensals relative abundance at baseline between patients with mild and moderate radiodermatitis. The maximum Common Terminology Criteria for Adverse Events (CTCAE) severity score reached in each patient during the study period was used to group patients with mild (grade 1), moderate (grade 2), and severe (grade 3) radiodermatitis. Green and blue colors represent skin commensal species; pink, red, and orange colors represent different *Corynebacteriaceae* species. A sum of relative abundances of these commensals below 5% at baseline in the affected side (D) was significantly associated with the development of severe radiodermatitis with an accuracy of 100% ($P < .001$). The number of patients is indicated on top of the bars.

Figure 3. Increase of Total Bacterial Load and Recovery of Commensal Skin Bacteria During Radiotherapy in Patients With Severe Radiodermatitis



A, Patients with severe radiodermatitis (yellow, Common Terminology Criteria for Adverse Events [CTCAE] grade 3) had an increase in bacterial load (measured as 16S copy numbers) as compared with their baseline values in the affected side, prior to the period with severe symptoms. B, The maximum total bacterial load increased during the early phase of the study (weeks 1-3) and was significantly higher in the affected side of patients with severe radiodermatitis, compared with mild to moderate radiodermatitis. Only the affected body side in patients with severe radiodermatitis showed an increase in bacterial load and not the unaffected side. C, The commensal frequency (defined as the sum of *Cutibacterium acnes, Staphylococcus epidermidis,* and *Staphylococcus hominis* frequencies) in patients with severe radiodermatitis recovered over time, and the median crossed 5% frequency at week 5. D, Only patients with severe dermatitis (yellow) exhibited both an increase of 16S copy numbers during weeks 1 to 3 and an increase of commensals frequency during weeks 5 to 7, compared with patients with mild (light blue) and moderate (dark blue) radiodermatitis ($P < .001$).

commensals frequency (weeks 5-7). The bacterial overgrowth, as compared with baseline and the healthy contralateral side, occurred before the appearance of clinical symptoms. Therefore, to our knowledge, this study showed for the first time that the pathogenesis of severe radiodermatitis may be mechanistically driven by bacterial overgrowth due to patient-specific predispositions. We hypothesize that the bacterial overgrowth during radiotherapy is a consequence of the pretherapeutic conditions that we observed (high pH and lack of commensals at baseline), as well as additional risk factors, such as immune status, together with the effect of radiation, giving rise to a threshold effect that causes inflammation and/or skin barrier impairment. Consequently, these effects may lead to severe radiodermatitis.

Kost et al[6] showed that decolonization of the skin before and during radiation treatment prevents severe radiodermatitis. On the basis of our results, a possible explanation for the positive effect of chlorhexidine body cleanser could be the prevention of bacterial overgrowth in these patients, who due to a low baseline level of commensals are at higher risk for severe radiodermatitis.

Limitations

This pilot study was performed at a single institution with a limited number of patients. Moreover, the study focused only on patients with breast cancer. Furthermore, patients receiving hypofractionated radiation regimens were not studied.

Conclusions

In this observational study, a low level of commensal skin bacteria in patients before radiotherapy was associated with

the development of severe radiodermatitis in 20 female patients with breast cancer after surgery. To our knowledge, this study is the first to show that the skin microbiome composition before, during, and after radiotherapy is associated with severe radiodermatitis. This information may have important clinical implications for personalized prevention therapy for radiodermatitis in patients treated with radiotherapy.

ARTICLE INFORMATION

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