The skin microbiome as a clinical biomarker in atopic eczema: Promises, navigation, and pitfalls

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Key words: *Atopic dermatitis, atopic eczema, skin microbiome,* Staphylococcus aureus*, clinical biomarker, personalized medicine, standardized methods, next-generation sequencing, deep sequencing, quantitative PCR*

Atopic eczema (atopic dermatitis [AD]) is a disease with severe quality-of-life impairment that affects 40% of children and 5% of the adult population.[1] The underlying pathomechanisms of AD are predominantly a skin barrier defect together with microbiome dysbiosis and significant immune dysregulation.[1,2]

A number of new therapeutic options are being investigated, mostly antibodies directed against relevant cytokine pathways (eg, binding IL-4 receptor) and kinase inhibitors (eg, Janus kinase inhibitors).[3] The involvement of *Staphylococcus aureus* in AD has been known for more than 40 years,[4] although many issues related to the ensuing skin microbiome dysbiosis are still open, notably the chicken and egg question.[1,5] Nevertheless, with advancements in deep next-generation sequencing (NGS) and bioinformatics analysis,[6] we see promises, but also missing navigation information and technological pitfalls to overcome, for the possibility of using the skin microbiome as a biomarker in AD clinical management. Here we focus on the bacterial microbiome composition, rather than on fungal and viral microbiome domains or bacterial metabolomics, proteomics, and transcriptomics, because it is only the skin bacterial microbiome composition that currently shows evidence as a promising clinical biomarker.

PROMISES: BIOMARKER FOR AD SEVERITY AND PERSONALIZED TREATMENT

AD severity is currently estimated by using a semiquantitative clinical score largely based on subjective information from patients and assessment by physicians.[3] Skin microbiome dysbiosis, measured either as microbiome diversity or more reliably as abundance of *S aureus*, was clearly shown to correlate with AD severity[7,8] and might turn out to have a better prognostic value (Fig 1). Moreover, recent studies have shown *S aureus* levels to decrease during treatment of AD and to rebound after the end of treatment in some but not all patients.[8,9] Furthermore, we now have a better understanding of the mechanism behind the interaction between *S aureus* and other bacterial species, the skin barrier, and immune dysregulation in patients with AD.[10] Thus skin microbiome measurement of *S aureus*, notably a noninvasive procedure, might become a clinical biomarker for treatment success, most importantly indicating a sustained microbial response (SMR) that will allow determination of optimal treatment duration (Fig 1).

MISSING NAVIGATION INFORMATION

However, before the skin microbiome can be used as a clinical biomarker, there is additional important information that needs to be obtained to prove which bacterial measurement method, most probably by using *S aureus* quantification, is the most suitable.

Validation in clinical trials

Results from large registries, optimally with a longitudinal design, to validate the prognostic value of the skin microbiome in both children and adults are still missing. Critically, because various drugs for AD are currently being tested, it is important to include skin microbiome measurements in all clinical trials to validate their significance in assessing treatment success. In particular, unless we accept lifelong therapy with antibodies directed against key cytokines or with kinase inhibitors, prospective clinical trials need to be performed to find the optimal treatment duration, possibly using the skin microbiome as a personalized medicine biomarker.

Skin microbiome frequency or bacterial load

An important question to answer in such studies is whether the relative frequency of various bacteria (eg, *S aureus* frequency, as obtained from 16S-based NGS) is a good enough biomarker or whether the absolute microbial load (eg, as obtained by using quantitative PCR [qPCR]) is better. Furthermore, is it enough to quantify the DNA abundance from a nonstandardized amount of skin samples, or is the absolute microbial load of standardized skin samples needed?

Taxonomic level of the skin microbiome

An additional question to answer is which taxonomic level to use as a skin microbiome biomarker: family/genus, species, or strain. Because we know that *S aureus* (in contrast to other *Staphylococcus* species) is important for AD pathogenesis, measurement at least on the species level is important, but going down to the strain level might be needed.[10] This highlights a

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Disclosure of potential conflict of interest: M. Reiger, C. Traidl-Hoffmann, and A. U. Neumann are principal investigators on projects funded by research grants from Asana Biosciences, Sanofi, Novartis, Sebapharma, and La Roche-Posay.

Received for publication October 14, 2019; revised November 1, 2019; accepted for publication November 4, 2019.

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J Allergy Clin Immunol 2020;145:93-6.

0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2019.11.004

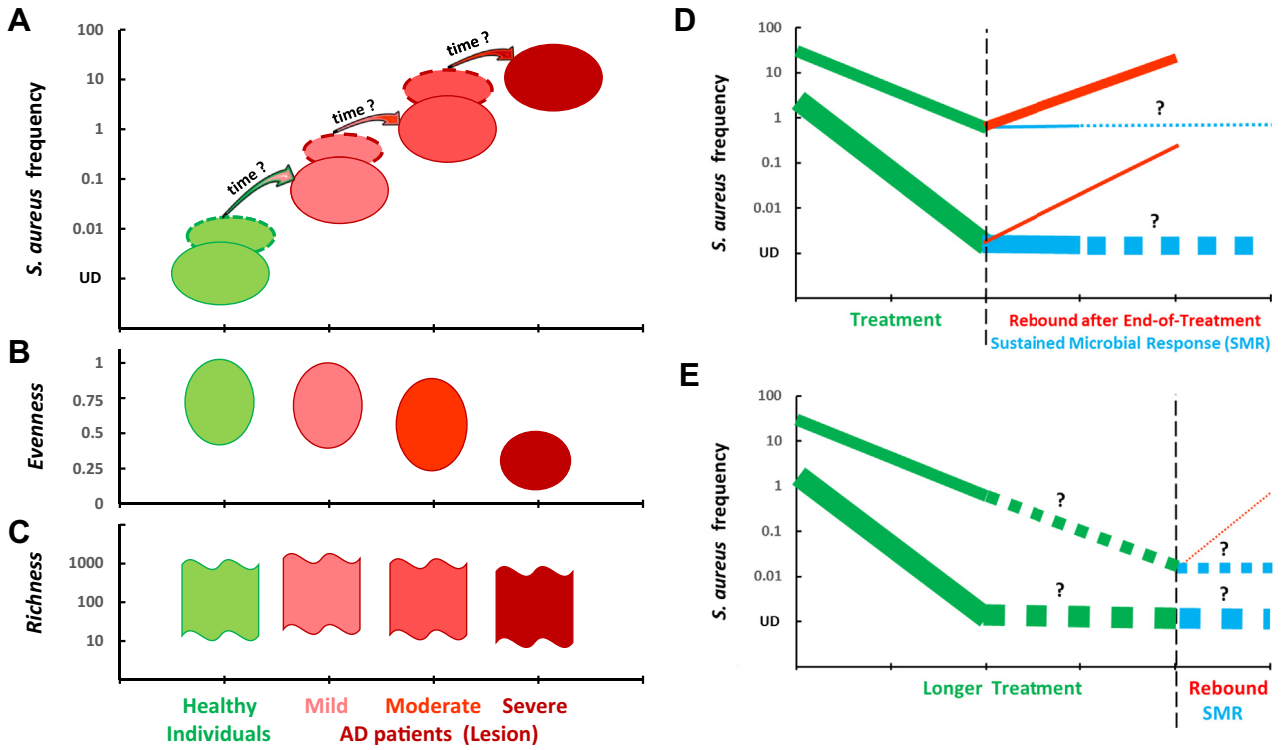

FIG 1. The skin microbiome as a biomarker for AD severity and personalized treatment optimization: schematic representation. **A,** *S aureus* frequency (and load) is higher in lesional (and also nonlesional) skin of AD patients compared with that in healthy subjects and is positively correlated with AD disease severity. However, it still needs to be determined (*dashed ovals* in Fig 1, *A*) whether higher than normal *S aureus* frequency in healthy subjects predicts possible development of AD and whether higher frequencies in patients with milder AD indicate worse prognosis. **B,** Microbiome α-diversity indexes, such as evenness (or Shannon and Simpson), are negatively correlated with AD severity but do not differentiate as well between healthy subjects and patients with mild or moderate AD. **C,** Microbiome richness is not correlated with AD severity and is highly variable and methodology dependent. **D,** During treatment with anti–IL-4 receptor[9] or Janus kinase inhibitor,[8] lesional skin *S aureus* frequency (and load) decreases in most patients, and the probability of achieving a sustained microbial response *(SMR)*, rather than a rebound after the end of treatment, is greater in those reaching very low or undetectable *S aureus* frequencies.[8] It will be important to verify whether SMR is sustained for a long period of time without treatment (*dashed lines* in Fig 1, *D*). **E,** More importantly, clinical trials need to test whether longer treatment duration gives rise to further *S aureus* decrease and greater probability for SMR (*dashed lines* in Fig 1, *E*), and thus skin *S aureus* levels could be used as a biomarker for optimal treatment duration, leading to a sustained microbial and clinical response. *UD,* Undetectable.

methodological pitfall because current tools for annotation at the species level are not reliable, and new robust bioinformatics tools are needed.

Species frequencies or 16S frequencies

Furthermore, the most used microbiome sequencing methods currently rely on measuring the 16S gene, with varying copy numbers in different bacterial families or even species. Thus what is measured is the frequency of the 16S gene copies and not species frequency. Therefore new methods for measuring the real microbiome distribution need to be developed.

Need for standardized quantitative skin microbiome methodology

Last but not least, the current methods for skin microbiome measurement are not standardized; testing the same material in different laboratories is prone to produce different results. For the skin microbiome to be used clinically as a biomarker, standardized methodology needs to be developed and validated so it can be reliably used across different laboratories.

TECHNOLOGICAL PITFALLS

In association with the above questions, current skin microbiome analysis methodologies suffer from a number of technological pitfalls[6] that need to be solved for use of the skin microbiome as a clinical biomarker.

Skin sampling

The advantage of sampling the skin is that it is easily accessible for sample collection and in general can be noninvasive, with minimal discomfort for patients. Various techniques range (by order of invasiveness) from skin swabs and tape stripping through shaving and scraping to biopsy. However, the great disadvantage of skin sampling is the low amount of input material, in particular the low amount of bacterial DNA.[5] Furthermore, in contrast to blood or stool samples, skin sampling lacks standardization of

TABLE I. Technologies for bacterial microbiome analysis, sequencing, and quantification: Benefits and drawbacks

Platform	Technology	Accuracy	Read length	Taxonomic level	Quantification	Benefits	Drawbacks
Culture of isolates	Bacterial cultivation and functional analysis*	—	—	Down to strain level	Not quantitative	Allows detailed characterization and functional analysis	Restricted to culturable bacteria, assay dependent, low coverage
qPCR (dPCR)	Target-based quantitative amplification	Highly accurate (target/assay dependent)	—	Any level (depends on primers)	Absolute quantification	Best quantification, fast, relatively cheap	Low microbiome coverage caused by limited number of targets per assay
16S amplicon sequencing	Amplicon-based NGS	99.9%	Up to 2× 300 bp	Down to species level	Relative frequencies only	Wide microbiome coverage, moderate cost	16S copy number bias, amplification artifacts
Whole-genome shotgun sequencing	Metagenomics NGS	>99%	Assembly of <500-bp reads	Down to strain level	Not quantitative	Taxonomic and functional analysis, cross-domain microbiota analysis	High cost, bias for high abundancies
PacBio	SMRT sequencing genomics and metagenomics	85% to 90%; with CCS, >99%	10-15 Kbp; with CCS, 0.5-4 Kbp	Down to strain level (mostly used for isolates)	Possibly semi quantitative (depending on deepness)	Longer reads, improved scaffolding	High cost, high error rates, large amount of input material needed
Nanopore	SMRT sequencing genomics and metagenomics	70% to 90%	5-10 Kbp; possibly 2-3 Mbp	Potentially down to strain level	Potentially absolute quantification	Ultralong reads, cheap, low amount of input material needed	High error rates, technology still under development

CCS, Circular consensus sequencing; *dPCR*, digital PCR; *SMRT*, single-molecule real-time.
*Functional analysis methods, such as Biolog, molecular identification, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

the input material; for example, skin swab yield is highly dependent on the examiner, the material used for the swab, the applied pressure during sampling, and the size of the area sampled. If the only information needed is the relative frequency of certain bacteria, then this poses less of a problem. However, if actual absolute bacterial load is a better biomarker, then new methods and tools for standardization of skin sampling are needed urgently.

DNA extraction

Various kits are used for extraction of DNA from the skin sample. In addition to the fact that this step is not standardized, all extraction methods suffer from a taxonomic selectivity bias in the yield of DNA extraction. For example, bacteria from certain families are more easily disrupted to provide DNA through each method. Thus the obtained microbiome distribution is distorted through any extraction method used. Correction of this DNA extraction–introduced bias must be performed for the skin microbiome to be used as a standardized clinical biomarker.

Skin microbiome sequencing

Although 16S amplicon–based NGS is the most widely used sequencing technology for microbiome analysis and produces relative quantitative information on microbiome distribution, it is limited by a relatively short sequencing length that necessitates amplification of only a part of the 16S gene. Additional studies are needed to select the optimal variable 16S region for the skin microbiome to be used as a clinical biomarker for AD. Current technologies for whole-genome sequencing (eg, shotgun or single-molecule real-time sequencing) do not allow (for practical and financial reasons) to obtain quantitative and accurate results and thus so far are not useful to analyze the microbiome as a clinical biomarker. Therefore improved technologies using a longer sequencing span need to be developed and validated. See Table I for a summary of sequencing technologies, their drawbacks, and their benefits.

Contaminations

Because of the low amount of input bacterial material from the skin, skin microbiome analysis can be particularly sensitive to even slight secondary contaminations.[5] Contaminant bacterial DNA can come from the sampling tool, the extraction kit, or the amplification reagents, as well as from cross-contamination during amplification and sequencing.[5,6] In addition, human DNA contamination in skin samples dictates an increase in the sequencing depth and thus an increased cost. Special care is needed to minimize the contamination, and further correction methods using appropriate negative controls and advanced bioinformatics pipelines need to be developed.

Skin microbiome quantification

Although 16S amplicon–based NGS produces relative frequencies of the different taxa, it does not allow absolute quantification. On the other hand, more quantitative methods, such as qPCR, can only target a small number of genes and thus do not quantify the whole microbiome distribution. Targeted gene qPCR would suffice if we knew the target we wanted to quantify (eg, *S aureus*), but a smart combination of both techniques could allow for more broad absolute quantification of the microbiome. Furthermore, to correct for biases introduced by the various steps discussed above, it is advisable to introduce spike-in controls[6] for better quantification and standardization.

Skin microbiome functional analysis

Neither qPCR nor 16S-based deep sequencing can provide functional information on the skin bacterial microbiome; for that, cultivation of bacterial isolates, metagenomics sequencing, or metatranscriptomics, followed by functional analysis, is needed. Although these latter methods are not appropriate as a clinical biomarker because of their lack of quantitative information, they can provide important information on the mechanism behind the biomarker to be used.

ROADMAP AHEAD

In conclusion, the skin microbiome, in particular measurement of *S aureus*, shows great promise to be used as a clinically important biomarker for atopic eczema. However, prospective clinical trials and large longitudinal registries that include skin microbiome testing still need to be performed. In parallel, it is critical to develop standardized quantitative methodologies for skin bacterial microbiome analysis, which can then be tested, compared, and validated in these studies. Collaboration between large academic consortia and pharmaceutical companies is essential for such endeavors.

In addition, new promising technologies, such as single-molecule real-time sequencing, which could improve skin microbiome analysis with greater accuracy and/or longer sequencing length, and more advanced bioinformatic tools need to be further developed and tested.

The road ahead for the skin microbiome as a clinical biomarker in patients with AD is still long and winding, and thus the sooner we start the journey, the earlier we will reach this important target.

We thank Madhumita Bhattacharyya, Amedeo de Tomassi, Claudia Huelpuesch, Luise Rauer, and Katherine Wald for comments on the manuscript.

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