ORIGINAL ARTICLE

Suction blistering the lesional skin of vitiligo patients reveals useful biomarkers of disease activity

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Background: Vitiligo is an autoimmune disease of the skin with limited treatment options; there is an urgent need to identify and validate biomarkers of disease activity to support vitiligo clinical studies.

Objective: To investigate potential biomarkers of disease activity directly in the skin of vitiligo subjects and healthy subjects.

Methods: Patient skin was sampled via a modified suction-blister technique, allowing for minimally invasive, objective assessment of cytokines and T-cell infiltrates in the interstitial skin fluid. Potential biomarkers were first defined and later validated in separate study groups.

Results: In screening and validation, $CD8^+$ T-cell number and C-X-C motif chemokine ligand (CXCL) 9 protein concentration were significantly elevated in active lesional compared to nonlesional skin. CXCL9 protein concentration achieved greater sensitivity and specificity by receiver operating characteristic analysis. Suction blistering also allowed for phenotyping of the T-cell infiltrate, which overwhelmingly expresses C-X-C motif chemokine receptor 3.

Limitations: A small number of patients were enrolled for the study, and only a single patient was used to define the treatment response.

Conclusion: Measuring CXCL9 directly in the skin might be effective in clinical trials as an early marker of treatment response. Additionally, use of the modified suction-blister technique supports investigation of inflammatory skin diseases using powerful tools like flow cytometry and protein quantification. (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2016.12.021.)

Key words: autoimmunity; biopsy; blisters; biomarkers; CD8; CXCL9; CXCL10; inflammatory skin disease; suction blister; vitiligo.

Vitiligo is a common autoimmune disease of the skin that causes disfiguring depigmented macules and patches.[1] Depigmentation is the result of $CD8^+$ T-cell–mediated killing of melanocytes, the pigment producing cells in the skin.[2] Phototherapy and topical immunosuppressants are used off-label with moderate results that require long

Abbreviations used:

CXCL:	C-X-C motif chemokine ligand
CXCR:	C-X-C motif chemokine receptor
ELISA:	enzyme-linked immunosorbent assay
IFN-γ:	interferon-γ
ROC:	receiver operating characteristic

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treatment courses. Based on studies in our mouse model and human tissues,[3,4] we hypothesized that targeting the interferon-γ (IFN-γ)/C-X-C motif chemokine ligand (CXCL) 10 axis could be an effective new treatment strategy for vitiligo.[5] Recent case reports revealed that Janus kinase inhibitors, which block IFN-γ signaling, are effective at reversing vitiligo,[6,7] supporting our hypothesis. Clinical trials to test new, targeted treatments for vitiligo are on the horizon.

When conducting clinical trials on vitiligo, monitoring disease activity will be critical for measuring the treatment response. A common misconception is that biomarkers of treatment response are unnecessary, since the skin can be observed clinically; however, even the earliest clinical changes in vitiligo take 2-3 months to become apparent because of the time required for melanocyte precursors to proliferate, migrate, differentiate, and produce pigment. Measurable responses require 6 months or more, and current outcome measures are largely subjective and insensitive to small changes.[8,9] Thus, relying only on observable changes in skin pigmentation is problematic.

Recent studies reported that serum levels of IFN-γ–induced chemokines are elevated in patients with active vitiligo[4,10] and correlate with treatment responses.[10] Serum chemokine levels, while reflecting general trends of disease activity in large cohorts, are likely too variable to serve as sensitive and specific markers of disease activity in a single patient. Moreover, patients with other autoimmune diseases exhibit increased inflammatory markers in the serum as well,[11,12] precluding their use as specific markers in vitiligo. Measuring markers directly in the skin could be a more sensitive and specific approach; however, conventional analysis of skin through immunohistochemistry is problematic because lesions have low numbers of infiltrating T cells that are difficult to detect and quantify, unlike more inflammatory diseases. Thus, a more efficient method to sample vitiligo lesional skin and reliably measure treatment responses is needed.

We adapted a minimally invasive, nonscarring skin biopsy technique[13-17] to reliably and accurately sample vitiligo lesions by inducing suction blisters, which contain interstitial skin fluid[18] that can be used for analysis. Lesional blister fluid revealed an infiltrate of $CD8^+$ T cells and CXCL9 protein that was both sensitive and specific for disease activity, in contrast to analysis of patient blood and serum. Suction blistering provides an opportunity to sample lesional skin in patients in a minimally invasive way, while yielding highly useful material to study markers of disease activity for translational studies and treatment responses in clinical trials.

CAPSULE SUMMARY

- Suction blistering is a minimally invasive method to measure immune biomarkers directly in skin.
- C-X-C motif chemokine ligand (CXCL) 9 protein concentration and $CD8^+$ T-cell number in blister fluid are sensitive and specific biomarkers of active disease in vitiligo.
- These markers might support clinical trial investigators in identifying active patients for enrollment and detecting early treatment responses.

METHODS

Study design

Subjects were recruited into 2 sequential groups for this study: the first to define markers of disease activity in vitiligo, and the second to independently validate these markers in a separate, nonoverlapping group of subjects (Table I and Supplemental Table I [available at http://www.jaad.org]). Inclusion/exclusion criteria were as follows: subjects with a diagnosis of vitiligo by clinical exam performed by a dermatologist and off treatment for at least 6 weeks were included in screening and validation groups. Subjects with recent onset of new lesions and objective clinical signs of activity, specifically confetti-like macules or trichrome lesions, were recruited to represent the active subgroup. Subjects with stable disease or no recent change in lesions and no objective clinical signs of disease activity (no confetti, trichrome, or inflammatory lesions and no Koebner phenomenon) were selected on the basis of patient self-reports. Those on treatment, with other inflammatory skin diseases or presenting with the segmental subtype of vitiligo, were excluded from the study. One subject was blistered repeatedly before and after starting treatment to define the treatment response.

Suction-blister skin biopsy and fluid aspiration was performed with internal review board–approved protocols at the University of Massachusetts Medical School and all samples were de-identified before use in experiments. Active subjects with a 1-month history of new or spreading lesions were blistered at sites featuring confetti-like depigmentation or trichrome lesions.[19,20] In subjects with stable disease, sites along the lesional edge consisting of normal and depigmented skin were selected for suction blistering. The nonlesional sites selected were normal-appearing, nondepigmented