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# High frequency ultrasound: a novel instrument to quantify granuloma burden in cutaneous sarcoidosis

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**Abstract.** *Background:* As is the case for many skin diseases, cutaneous sarcoidosis does not currently have an objective measure of disease burden to establish disease severity and response to treatment. The disease has traditionally been assessed by visual skin changes, including induration and erythema; however, such assessments may fail to quantify the total skin granuloma burden, as the majority of the granulomatous inflammation may lie deep within the dermis and not be reliably detected by sight or palpation. *Objectives:* The purpose of this pilot study is to evaluate the feasibility of high frequency ultrasound as an objective measure of granuloma burden in cutaneous sarcoidosis and to compare high frequency ultrasound to a previously validated clinical instrument Cutaneous Sarcoidosis Activity and Morphology Instrument (CSAMI) and histopathology evaluation. *Results:* A strong correlation was observed between the mean brightness of high frequency ultrasound images and both the lesional CSAMI score (Spearman's rho: 0.9710, p=0.0012) and percent of dermis with granulomas histopathology (Spearman's rho: 0.8407 p=0.0361). *Conclusions:* These results confirm high frequency ultrasound is a valid, objective measure of granuloma burden in cutaneous sarcoidosis and represents a novel, non-invasive measure of disease severity that correlates to the previously validated CSAMI clinical severity score and histopathology evaluation. *(Sarcoidosis Vasc Diffuse Lung Dis 2017; 34: 136-141)*

**Key words:** sarcoidosis, non-caesating granuloma, high frequency ultrasound, disease severity instrument

## Introduction

Sarcoidosis is a multi-system, chronic inflammatory disease characterized by the presence of non-caseating granulomas. Cutaneous involvement occurs in 25-30% of patients, with skin findings presenting prior to, or at the same time as systemic disease, in the majority of patients (1). For patients with disfiguring or ulcerating cutaneous disease, effective therapy can minimize the psychological impact of the disease; however high quality evidence for effective treatment does not currently exist (2, 3). A major impediment to conducting rigorous cutaneous sarcoidosis trials is the lack of valid, reproducible, and clinically relevant measures of disease severity.

Two previously validated, subjective instruments for measuring disease severity in cutaneous sarcoidosis have been reported. The Sarcoidosis

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Activity and Severity Index (SASI) was developed in 2008 to measure the severity of chronic facial lesions (4). The SASI scores erythema, induration and desquamation in each of four quadrants of the face, in addition to the nose. In 2013, we developed the Cutaneous Sarcoidosis Activity and Morphology Instrument (CSAMI), a disease activity score representative of total body cutaneous disease (5). The CSAMI reports both activity and damage scores in 11 areas of the body. Its use has been previously validated by dermatologists, rheumatologists and pulmonologists (6). Both the SASI and the CSAMI are visual measures of disease severity and therefore are associated with some subjectivity. While objective measurements of skin disease are rare, their development is important to reduce the variability associated with subjective measures.

Also, the SASI and the CSAMI both rely upon visual skin changes and/or the degree of palpable induration. These measures may fail to reliably reflect the total skin granuloma burden, as the majority of the granulomatous inflammation may lie deep within the dermis and not be reliably detected by sight or palpation. As most sarcoidosis therapies are antigranulomatous, it is ideal to use sarcoidosis outcome measures that appropriately reflect the total granuloma burden.

The purpose of this pilot study is to evaluate the feasibility of high frequency ultrasound (HFU) as a measure of granuloma burden and disease severity in sarcoidosis, and compare HFU the previously validated CSAMI instrument and histopathology evaluation of granuloma burden. The potential advantage of HFU is, unlike the CSAMI and SASI, it can assess cutaneous sarcoidosis in an objective and quantitative fashion and does not require subjective assessment.

## Methods

### Study Population

Six patients were recruited from the cutaneous sarcoidosis clinic at the University of Pennsylvania between June 1st, 2015 and July 31st, 2015. All patients with cutaneous sarcoidosis on a site appropriate for skin biopsy were approached about participation in the pilot study. A diagnosis of sarcoidosis was based on both clinical presentation and a skin biopsy consistent with the diagnosis. This study was developed in accordance with the STROBE guidelines and was approved by the Institutional Review Board at the University of Pennsylvania. Written, informed consent was obtained from all participants prior to participation.

### Study Protocol

*Clinical Assessment*

A global CSAMI and physician's global assessment (PGA) scores were calculated for each patient by a single dermatologist (MR). A target lesion was identified, and photographs of the target lesion were obtained. The target lesion was then scored using a "lesional CSAMI". Since the HFU and pathology assessments are for a single lesion, we documented a "lesional CSAMI" by rating the single target lesion with the standard CSAMI activity parameters: inflammation, induration, surface change. Size was not included because all were single lesions.

*Ultrasound Assessment*

A 4 mm region within the target lesion was selected and marked for ultrasound assessment and skin biopsy. The ultrasound images were obtained by a single, experienced ultrasonographer (SS), using the Visualsonics 2100 High-Resolution In Vivo Micro-Imaging System (Fujufilm: Toronto, Canada). The same 40 MHz ultrasound transducer was used for all subjects. Gray scale images, color doppler images and 3D volumes were obtained in a sagittal, transverse and, if necessary, in an oblique orientation. The images were exported and downloaded into MaZda, version 4.6 (Technical University of Lodz: Lodz, Poland) for analysis (7). All grayscale images were normalized to a standardized background. Image optimization was performed for depth, focal zone placement and gain distribution in gray scale and with pulse repetition frequency, box size and angle for color Doppler.

For each subject, one transverse and one sagittal image were selected for quantitative analysis. A region of interest within each image was selected that included the granuloma, including both epidermis and dermis. Image brightness and image texture of

the region of interest were calculated using MaZda, version 4.6. Because collagen appears bright on ultrasound and granulomatous inflammation appears darker, the inverse of brightness was used to more appropriately correspond to the percentage of granuloma present.

*Histology Assessment*

A 4 mm punch biopsy was performed down to subcutaneous fat. The biopsy specimen was processed with standard H&E staining. Each slide was separately reviewed by two investigators (MR, LT). The glass slides were scanned using an Aperio CSO Slide Scanner (Leica Biosystems Inc, Buffalo Grove, IL). Measurements were taken using virtual slide imagery using Imagescope™ software (Leica Biosystems Inc: Buffalo Grove, IL). To compute the area of the granulomatous infiltrate, the maximum length and width of the infiltrate were recorded and the product calculated. To determine the percent of the dermis occupied by granulomas, a grid was overlaid on the digital image for each biopsy, with a cell area of ¼ $mm^2$. The numbers of gridcells containing disease was divided by the numbers of gridcells containing dermis. Percentages for partial gridcell involvement was estimated and rounded to the nearest quarter.

**Statistical Analysis**

Statistical analysis was performed using STATA 14.0 (College Station, TX). Descriptive statistics including medians and interquartile ranges were calculated for the clinical, histologic and ultrasound assessment measures. Spearman's rank correlation coefficients were calculated between all assessment measures, using a significance level of 0.05.

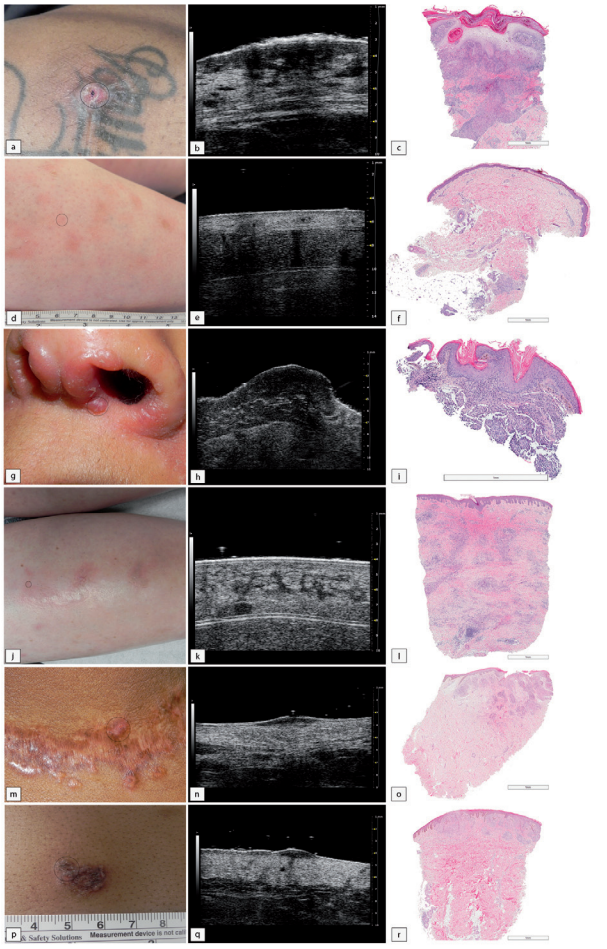

**Fig. 1. Clinical images with corresponding high frequency ultrasound and histopathology.** Clinical images (a, d, g, j, m, p) show erythematous to violaceous papules and plaques corresponding to cutaneous sarcoidosis on various body sites. The circle in the clinical photograph corresponds to the area where the biopsy was performed. High frequency ultrasound images from the biopsied portion are shown (b, e, h, k, n, q). The corresponding histopathology is shown in low power (hematoxylin and eosin, 4x) demonstrating the granulomatous infiltration of the tissue specimens (c, f, i, l, o, r)

## Results

Six patients with cutaneous sarcoidosis were included in this pilot study: 2 males and 4 females (figure 1). The average age was 54 yrs. Four of the six patients were African American. A summary of patient characteristics is presented in table 1. Participants had a median global CSAMI activity score of 14 (IQR: 11-21) and a median lesional CSAMI activity score of 6 (IQR: 5-11). Since the HFU and pathology assessments are for a single lesion, we documented a "lesional CSAMI" by rating the single target lesion with the standard CSAMI activity parameters: inflammation, induration, surface change. Size was not included because all were single lesions. On histopathology assessment of the skin biopsies, the median percent dermis involved was 44%. On HFU, the median mean brightness value was 1.34 (IQR: 1.17-1.67) and the median mean texture value was 0.74 (IQR: 0.64-0.84).

**Table 1.** Baseline characteristics of patients

| Sex | Age | Race | Organs Involved | Current Treatments |
|---|---|---|---|---|
| M | 39 | AA | skin, lungs | clobetasol ointment |
| F | 45 | C | skin, lungs, LN | methotrexate 12.5 mg weekly |
| F | 53 | AA | skin, lungs, sinuses, heart | adalimumab 40 mg weekly<br>methotrexate 10 mg weekly |
| F | 65 | C | skin, lungs, eyes, heart | mycophenolate mofetil 1500 mg BID<br>hydroxycholorquine 400 mg QD<br>prednisone 5 mg every other day |
| F | 51 | AA | skin, lungs, sinuses | prednisone 5 mg QD<br>minocycline 100 mg BID<br>hydroxychloroquine 200 mg QD<br>quinacrine 100 mg QD |
| F | 58 | AA | skin, lungs, sinuses, eyes | None |

Abbreviations: AA – African American, C - Caucasian, LN – lymph nodes

A very strong, statistically significant correlation was found between the lesional CSAMI score and inverse of mean brightness of the ultrasound image (Spearman's rho: 0.9710, p=0.0012) (figure 2a). There was a strong, statistically significant correlation between the percent dermis involved by granulomas on histopathology and inverse mean brightness on ultrasound. (Spearman's rho: 0.8407 p=0.0361) (figure 2b). A strong positive correlation was also found between the lesional CSAMI score and the percent dermis involved by granulomas on histopathology (Spearman's rho: 0.8359, p=0.0382) (figure 2c). Mean texture, another ultrasound measure of tissue composition, was not significantly correlated with any clinical or histopathology measurement.

## Discussion

This pilot study confirms that HFU is a valid measure for quantifying granuloma burden in cutaneous sarcoidosis and represents a novel, non-invasive measure of disease severity. HFU strongly correlates to the previously validated CSAMI clinical severity score and also to histopathology evaluation. Unlike the CSAMI, HFU is a purely quantitative and objective assessment, whereas the CSAMI requires subjective visual assessment. In addition, our results also show that CSAMI correlates to percent dermis involved by granulomas on histological evaluation, which has not been previously reported.

Other imaging modalities have been previously investigated as possible non-invasive measure of disease burden in sarcoid. Case reports have shown that positron emission tomography/ computed tomography (PET/CT) scanning can characterize sarcoidosis lesions in an effort to identify active inflammation and overall disease burden, however a procedure for quantifying disease burden has not been established or validated for PET/CT (8). Also, PET/CT is not a practical method of evaluation for most patients. It is expensive, exposes the patient to radiation and as stated previously, a gold standard procedure for measuring granuloma burden has not been established.

Previous reports have attempted to describe the ultrasonographic characteristics of sarcoidosis using traditional sonography (maximum 17 MHz) (9-12). Unfortunately, traditional sonographic evaluation of the skin has a limited capability to distinguish granulomatous inflammation from the surrounding dermis. HFU utilizes a 20-100 MHz transducer, which greatly improves the resolution of structures in the skin compared to conventional ultrasound (13). HFU has previously been used successfully in other chronic inflammatory skin diseases to measure response to treatment (14). A benefit of ultrasound, over PET/CT, is that it can be performed in an office setting, allowing the measurement of granuloma burden and skin lesion activity in real-time. Importantly, HFU does not involve any radiation or additional risk of harm to the patient like PET/CT.

The results of this pilot study suggest a potential unique solution to the challenge of treating and monitoring response to treatment in cutaneous sarcoidosis. Not only is HFU an ideal alternative to histopathology analysis because it is non-invasive, but our results demonstrate that HFU correlates to both the CSAMI and histopathology analysis. HFU can provide an objective and quantitative assessment of pre- and post-treatment lesion characteristics. Thus, HFU may be a useful outcome measure for clinical care as well as clinical trials of cutaneous sarcoidosis.

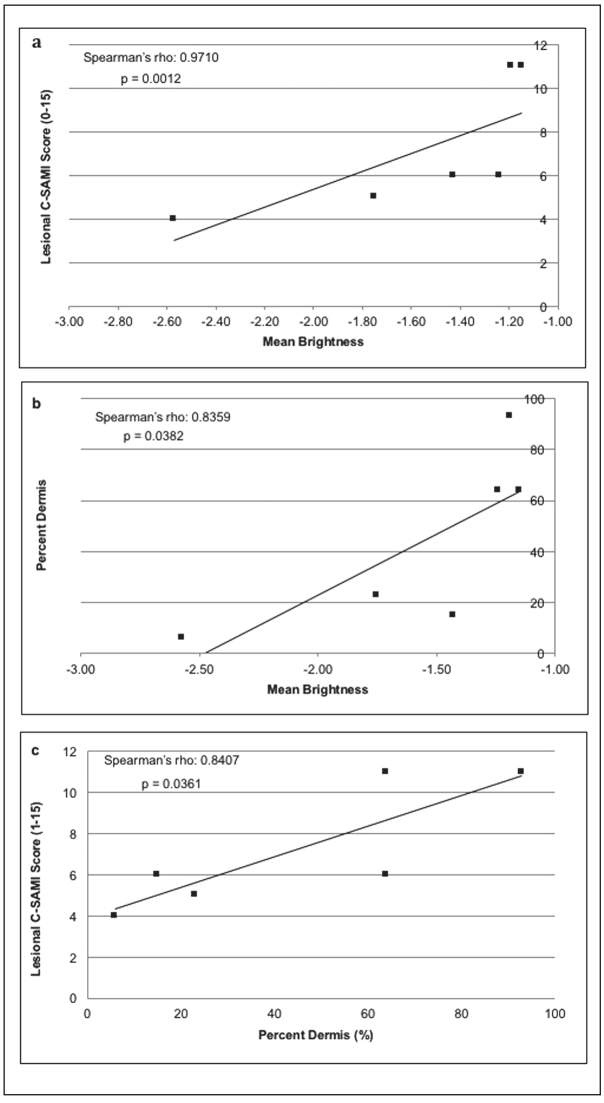


**Fig. 2. A strong correlation exists between various measures of granuloma burden.** The inverse of the mean brightness on high frequency ultrasound strongly correlates with both lesional CSAMI (a) and percent dermis on histopathology (b). Lesional CSAMI also strongly correlates with percent dermis on histopathology (c)

While the results are encouraging, there are some limitations to this analysis. This was a pilot study performed in a small number of patients at a single institution. A larger study is necessary to confirm these results and validate the findings in a more diverse patient population. Further study of HFU will be necessary to develop an imaging protocol that will be reliable and valid on all body sites and measure the response to treatment. Also, while HFU can be performed in a clinic setting, it is not currently widely available in dermatology clinics.

In summary, this study demonstrates the feasibility of HFU to objectively measure granuloma burden in cutaneous sarcodosis. HFU strongly correlated with the validated, physician-reported clinical instrument (CSAMI). Both HFU and CSAMI score independently correlated with the measured granuloma burden on histopathology sections, further strengthening the results. HFU appears to be an excellent technique for objectively and reliably assessing the granuloma burden in cutaneous sarcoidosis, potentially obviating the need for invasive biopsies or subjective clinical assessments.

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