

Research Article

Morphological Analysis of Dermatoporosis by in vivo Reflectance Confocal Microscopy and Ultrasonography

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Keywords

Dermatoporosis · In vivo reflectance confocal microscopy · Ultrasound

Abstract

Background: Dermatoporosis is defined as a chronic cutaneous fragility and insufficiency syndrome. It results from chronological aging, long-term and unprotected sun exposure, genetic factors, or the chronic use of topical and systemic corticosteroids. There is currently a lack of noninvasive tools for the evaluation and quantification of dermatoporosis. **Objectives:** The aim of this study was to define the dermal-epidermal modifications which characterize dermatoporosis using noninvasive methods such as in vivo reflectance confocal microscopy (RCM) and ultrasound (US). **Subjects and Methods:** Seventeen patients with stage I dermatoporosis and 14 healthy volunteers were included in the study. The posterior surface of the right forearm was analyzed in all subjects, and stellate pseudoscars and senile purpura in patients with dermatoporosis were analyzed when possible. We used a commercially available reflectance confocal microscope and measured different histometric parameters (thickness of the epidermis and its different layers, cellular architecture, aspect of the dermal-epidermal junction and the dermis). We also used a commercially available US skin system to define the dermal-epidermal thickness (DET) in all subjects. **Results:** The DET measured with the US skin system was significantly different between the two groups: mean value 1.19 mm (volunteers group) versus 0.81 mm (patient group). The significant differences measured with RCM were (1) epidermal thickness, (2) number of dermal papillae, and (3) thickness of solar elastosis. Stellate pseudoscars are also characterized by a modified dermis, with a linear organization of the collagen bundles. **Conclusion:** US and in vivo RCM are useful tools for the diagnosis of

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dermatoporosis. Dermal-epidermal atrophy, reduction of dermal papillae/area, and the thickness of dermal elastosis seem to be the major histometric parameters which characterize dermatoporosis.

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Introduction

Dermatoporosis [1] is defined as a chronic cutaneous syndrome characterized by a cutaneous fragility and insufficiency. There are two types of dermatoporosis, according to their etiological mechanisms. Primary dermatoporosis is the most commonly seen type and results from chronological aging, long-term and unprotected sun exposure, and probably genetic factors, which are not yet identified. The secondary type is due to the chronic use of topical and systemic corticosteroids. The clinical manifestations of dermatoporosis include skin atrophy, senile purpura, stellate pseudoscars, skin lacerations, delayed wound healing, and dissecting hematomas. There are no significant differences between primary and secondary iatrogenic dermatoporosis [2–4].

The molecular mechanisms leading to this condition are not fully understood, but we know that hyaluronate, a component of the dermal extracellular matrix, and its homeostasis play an important role in the pathogenesis of dermatoporosis [4–6].

Dermatoporosis has already been characterized using a skin ultrasound (US) system and histology. Histological sections show dermal-epidermal thinning, loss of epidermal ridges (and dermal papillae), and a significant degree of elastosis. The content of collagen, elastic fibers, and mucin is decreased. Sonographically, dermatoporotic skin is characterized by a significant dermal-epidermal atrophy (around 0.7–0.8 mm; normal skin: >1 mm) [4]. Many studies describe a subepidermal low-echogenic band linked with skin aging. It seems that the presence and thickness of the subepidermal low-echogenic band increase significantly with age [7].

High-frequency US (HFUS) allows the visualization of skin atrophy and quantification in real time, but its resolution is very poor. We currently lack tools for the evaluation and quantification of dermatoporosis in particular. The aim of this study was to develop *in vivo* reflectance confocal microscopy (RCM) in this indication.

In vivo RCM is a noninvasive technique that allows optical *en face* sectioning of the skin with high, almost histological resolution and good contrast. Tissue processing and staining is not necessary because imaging is based on the detection of backscattered light. A single point within the skin is illuminated via a point light source of a few micrometers. Backscattered light from the specimen is focused by the objective lens through a pinhole aperture. Only the light emanating from the point of focus can pass through the pinhole aperture to reach the detector. The light source, the illuminated spot, and the detection aperture are in optically conjugated focal locations, and this arrangement is called confocal. The images are based on the presence of endogenous contrast, which is provided by microstructures such as melanin, hemoglobin, or cellular organelles [8]. The penetration depth of imaging allows visualization of the epidermis and the upper dermis. The main current application is for diagnostic purposes, but it might also be used for monitoring therapy response as the method allows repeated visualization of the same site over time to evaluate dynamic changes [9, 10].

Longo et al. [11] described age-related dermal-epidermal modifications using *in vivo* RCM on a pool of 75 volunteers distributed in five age groups. The imaged site was the left cheek for all patients. Significant epidermal modifications were observed: decreased thickness, linear rather than rhomboidal aspect of furrows, irregular honeycomb pattern, alteration of pigment distribution, as well as dermal modifications (width of the sebaceous glands, papillae aspect, architecture of the extracellular matrix).

Table 1. Characteristics of subjects included in this study

	Patients (<i>n</i> = 17)	Healthy volunteers (<i>n</i> = 14)
Age, years	62–96	28–82
Median age, years	76	68
Male:female ratio	2:15	8:6
Fitzpatrick skin type	I–III	I–III
Sites	posterior surface of the right forearm senile purpura stellate pseudoscars	posterior surface of the right forearm

In 2002, Sauermann et al. [12] aimed to develop histometric parameters to investigate and quantify aging processes on human skin using *in vivo* RCM. Sites in the middle of the volar forearm of two groups of healthy volunteers (aged 18–25 years and >65 years) were analyzed. The measures included thickness of the cornified layer (no differences between groups), the granular layer, the basal layer, the entire epidermis, and the number of papillae per area (significant differences).

In another *in vivo* RCM study, Wurm et al. [13] tried to define and identify the features of chronological aging and photoaging with measures on the volar and dorsal skin of the forearm, aiming to identify microscopic changes in relation with each phenomenon. This study assessed some changes aggravated by UV exposure.

All these studies characterized the changes associated with skin aging. Primary dermatoporosis is linked to photoaging processes, but its clinical consequences lead us to consider it as a syndrome. The aim of this study was to define the dermal-epidermal modifications that characterize dermatoporosis using *in vivo* RCM.

Subjects and Methods

Subjects

Seventeen patients with stage I dermatoporosis and 14 healthy volunteers were included in the study. Sites in the middle of the posterior surface of the forearm were analyzed in all subjects, and stellate pseudoscars and senile purpura in patients with dermatoporosis were analyzed when possible. Table 1 describes the main characteristics of the subjects.

Instruments

We used a commercially available reflectance confocal microscope (VivaScope 1500; Lucid Inc., Rochester, NY, USA). It uses a laser with a wavelength of 830 nm and a 30× water immersion objective lens with a numerical aperture of 0.9. The laser power is typically 5–10 mW on the skin and causes no tissue damage. A metal ring with a glass window is attached to the skin with an adhesive patch. The ring is then magnetically connected to the objective lens housing to stabilize the site of imaging. A small drop of oil is applied to the skin lesion. The water immersion lens requires US gel placed between the window and objective lens. The RCM acquires horizontal tissue images at a 500 × 500 μm field of view with a resolution of 1,024 × 1,024 pixels. The objective lens may be translated parallel to the skin surface and a two-dimensional sequence of images captured and placed in the software to create a mosaic. This mosaic, called VivaBlock, displays a larger field of view. The VivaScope 1500 creates a mosaic of 8 × 8 images to display a 4 × 4 mm field of view. A digital camera (VivaCam; Lucid Inc.) connected to the RCM computer enables direct viewing of the dermoscopic structures on the RCM monitor.

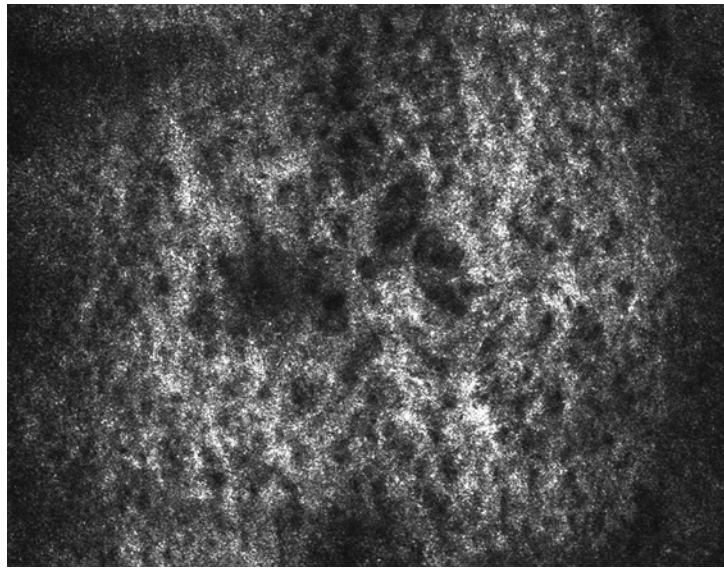


Fig. 1. Superficial dermis: SE in a patient (in vivo RCM). RCM, reflectance confocal microscopy; SE, solar elastosis.

We also used a commercially available US skin system (Episcan; Longport Inc., USA). It is a HFUS imaging system that utilizes US at frequencies as high as 50 MHz to image the skin and underlying soft tissue. The transducer was mounted in a water chamber. The chamber window was covered with a disposable plastic membrane. A conductive gel was used to ensure satisfactory contact between the membrane and the skin.

Parameters and Image Analysis

We assessed the thickness of the cornified layer, the granular layer, the spinous layer, and the basal layer, measuring the difference between the surface and the depth of the first granular cell, between the first granular cell and the first spinous cell, etc., with the micron screw. The cornified layer appeared as a variably refractile surface with large polygonal-shaped anucleated keratinocytes, with an approximate dimension of 25–50 μm . The granular cells appeared as bright circles that correspond to keratohyalin granules, surrounding large oval dark nuclei. They measured approximately 25–35 μm . The spinous cells appeared as polygonal cells with an oval to round dark central area, corresponding to its nucleus. The spinous cells were smaller than granular cells, measuring around 15–25 μm , and were arranged in a honeycomb pattern. The basal cells appeared brighter than spinous cells, containing a lot of melanin, were uniform in size and shape, and measured approximately 7–12 μm . The minimal thickness of the epidermis was measured as the distance between the surface and the level of the first visualized dermal papilla. Dermal papillae appeared as round to oval dark areas surrounded by epidermal cells and centered by a capillary. The maximal thickness of the epidermis was measured as the distance between the surface and the level of the last basal cell. Images of the upper dermis were also taken by descending as deep as possible into the tissue. The thickness of elastosis was measured in the superficial dermis when this was present. Elastotic fibers were recognizable by their curly appearance (Fig. 1). These measurements were repeated at least three times at different skin sites. All results were calculated by taking the mean of the three measures. Stellate pseudoscars and senile purpura were investigated in the same manner when possible. We performed at least one VivaBlock (4 \times 4 mm) at the level of the dermal-epidermal junction, with the aim of assessing the number of papillae per area (500 \times 500 μm).

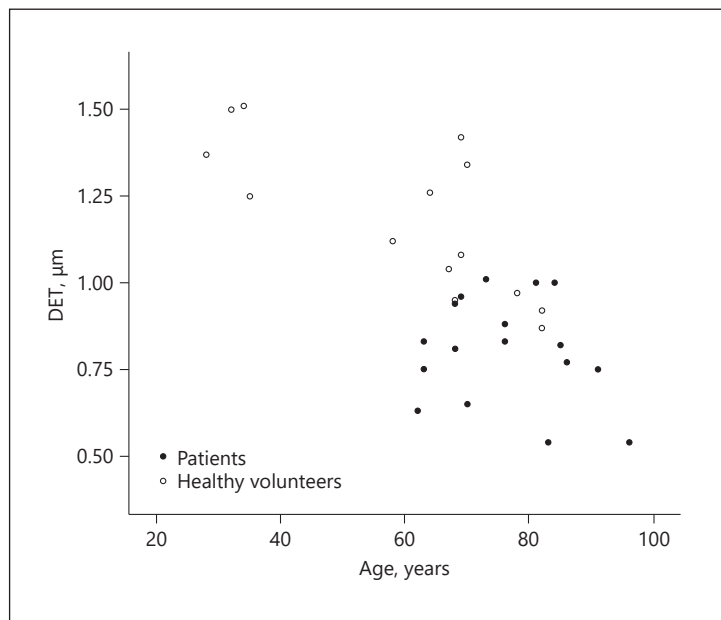


Fig. 2. Scatterplot. Linearity in the decrease of the DET with age can be seen. DET, dermal-epidermal thickness.

Statistical Analysis

Statistical analysis was performed by using two programs: Excel (Microsoft Office) and SPSS (IBM). We first calculated the means, quartiles, medians, and standard deviations for the different measurements and then applied the Fischer and the Student *t* tests as well as a nonparametric test (Mann-Whitney). We also applied a linear regression model to our data when appropriate. Analyses with a *p* value <0.05 were considered statistically significant.

Results

We found a strong female predominance in patients with dermatoporosis in our study. The male:female ratio was 2:15 (Table 1). On the other hand, in the control group, the ratio was more balanced (8:6).

We assessed dermal-epidermal thickness (DET) with HFUS. The mean thickness in the volunteer group was 1.19 mm, the mean thickness in the patient group was 0.81 mm. This difference was statistically significant with parametric and nonparametric tests. We then analyzed our data with a linear regression model with adjustment for age (i.e., the effect of age was neutralized in both groups), with DET as a dependent variable. We found a significant difference between the two groups, which implies an effect of the disease on this result. Conversely, by neutralizing this “disease effect,” we could identify a significant effect of age on DET, with a decrease in the latter. By imposing a linearity on our data via this model, we could estimate a decrease in DET of 0.007 mm per year of life (Fig. 2).

We then used *in vivo* RCM to measure and visualize the epidermal layers and the superficial dermis with a higher definition. The first step was to evaluate minimal and maximal epidermal thickness (ET) in patients and healthy volunteers. The minimal and maximal thicknesses were identical in patients due to the “horizontalization” of the dermal-epidermal junction. We therefore compared these data with the maximal ET in healthy volunteers. The difference between the groups was statistically significant – 68 µm (healthy volunteers) versus 56 µm (patients) – with parametric and nonparametric tests. As before, we analyzed

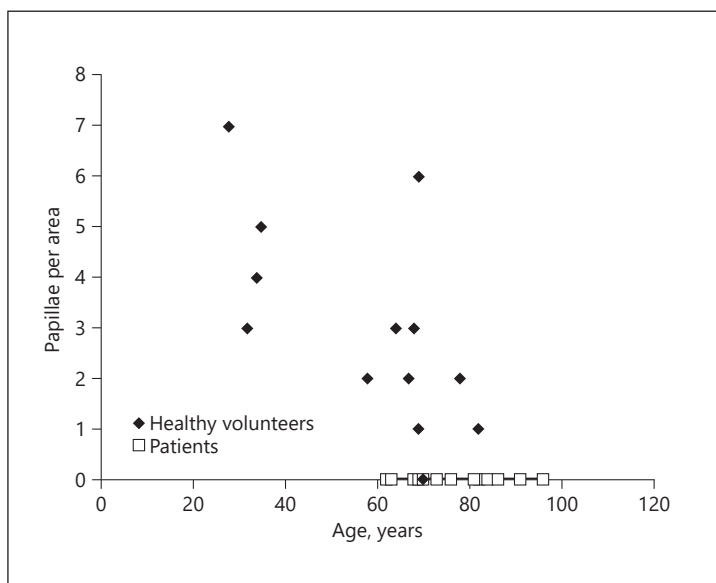


Fig. 3. Scatterplot: papillae per area.

the data with linear regression models, with adjustment for age, and with maximal ET as a dependent variable. The difference observed was not significant ($p = 0.065$). After adjustment for age, the difference in thickness was estimated to be $9 \mu\text{m}$ between the two groups instead of $12 \mu\text{m}$ before adjustment.

The measurement of the different layers of the epidermis (cornified, granular, spinous, and basal layers) did not reveal differences between the groups.

We then measured the number of dermal papillae per area at the dermal-epidermal junction. All patients were characterized by total absence of dermal papillae, whereas only one healthy volunteer did not have any papilla detected during the analysis (Fig. 3). The difference between the groups was obviously statistically significant with the parametric and nonparametric tests.

In the superficial dermis, the most significant modification was the presence of a thicker band of solar elastosis (SE) in the patient group. Indeed, the difference was statistically significant between the two groups – $21 \mu\text{m}$ (healthy volunteers) versus $44 \mu\text{m}$ (patients) – with parametric and nonparametric tests. We could observe in the scatterplot an almost perfect separation of the groups (Fig. 4). In the linear regression model, with adjustment for age and using elastosis thickness as a dependent variable, the difference remained significant ($20 \mu\text{m}$ instead of $23 \mu\text{m}$ without adjustment). By neutralizing the “disease effect” we did not identify any effect of age on this variable.

We performed the same analysis on stellate pseudoscars when possible. The only difference was morphological. Stellate pseudoscars were characterized by a largely modified dermis, with a linear organization of the collagen bundles.

Analysis of senile purpura in patients with dermatoporosis did not reveal a particular microscopic characteristic in confocal microscopy.

Discussion

We identified differences in histometric parameters between dermatoporotic and healthy skin with HFUS and in vivo RCM. Indeed, in this study, several parameters revealed highly significant differences: DET (HFUS), ET, number of papillae per area, and thickness of SE (RCM).

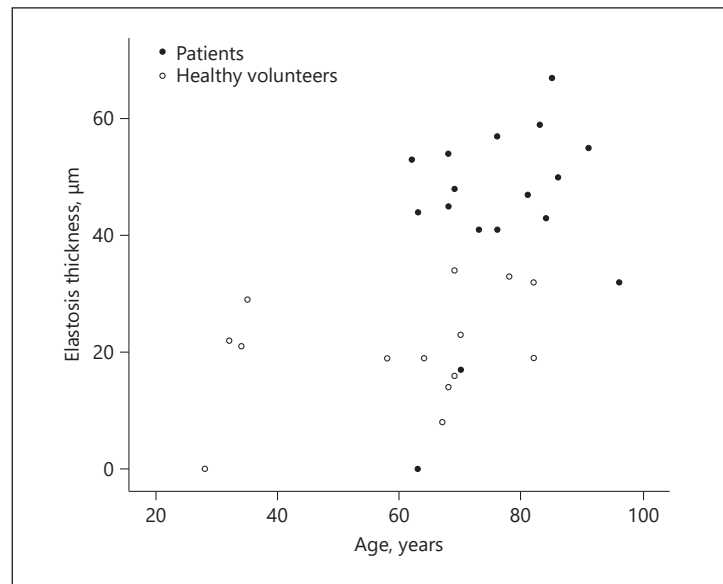


Fig. 4. Scatterplot: elastosis thickness. An “almost-perfect” separation between the groups can be seen.

ET and DET appear to decrease almost linearly with age (Fig. 2). For DET, the linear regression model validated this visual impression because an “age effect” was identified which was statistically significant. We could not demonstrate it for ET. It is likely that with a larger sample of patients and controls, these values could be significant. We could hypothesize that this decrease is physiological and thus related to the normal aging process. In patients with dermatoporosis, these two parameters showed a much greater and statistically significant decrease, which we could define as a “disease effect.” This was demonstrated by adjusting the age for DET because the difference remained significant.

We demonstrated a “horizontalization” of the dermal-epidermal junction reflected by the disappearance of the dermal papillae and the epidermal ridges. We also observed a decrease in the number of papillae per area in relation to age, but we could not demonstrate it statistically. All patients were characterized by the absence of dermal papillae, whereas a single healthy volunteer had no papilla measured in this analysis (Fig. 3).

The thickness of SE was much higher in patients than in healthy volunteers. This factor appears to be strongly correlated with dermatoporosis (Fig. 4). Moreover, an “age effect” was not demonstrated with the linear regression model. It is known that SE is related to sun exposure, but a dose-dependent effect has never been demonstrated. We do not know whether there is any effect of topical or systemic corticosteroids or genetic factors on its thickness. However, these data should be carefully interpreted since the measurement of the depth margin of elastosis is difficult because of the decrease of resolution quality of the images with the confocal microscope.

The thickness of SE and the number of papillae per area represent qualitative modifications of the skin. Indeed, the thickness of SE and the flattening of the dermal-epidermal junction decrease its quality and resistance. DET and ET parameters represent quantitative modifications. These observations highlight the fact that dermatoporosis is a state of cutaneous insufficiency, showing qualitative and quantitative modifications of the dermis and the dermal-epidermal junction that impair the protective mechanical properties of the skin.

We identified a “disease effect” which is probably linked to both intrinsic (genetic predispositions) and extrinsic factors, as cited above. The extrinsic factors probably have a cumulative effect over the years, and this is why patients with dermatoporosis are elderly subjects.

The weaknesses of this study are (1) the relatively small sample size and (2) the discrepancy between the age of the patients and the healthy volunteers. However, it was also

important to have volunteers of different ages to assess an “age effect” on the parameters. The female predominance in patients with dermatoporosis in our study is important. Mengeaud et al. [14] reported in 2012 a 32% prevalence of dermatoporosis in 202 hospitalized patients ≥ 60 years, but did not report a male or female predominance. Using a specific dermatoporosis questionnaire/self diagnosis tool, Saurat et al. [15] estimated that the overall prevalence of dermatoporosis was 37.5% in French subjects aged ≥ 65 years and also demonstrated a female predominance (27.5% males vs. 43.9% females; $p < 0.05$).

Conclusion

In vivo RCM and HFUS are useful tools in the analysis and diagnosis of dermatoporosis and could also be useful for therapeutic follow-up. This study revealed dermal-epidermal atrophy, epidermal atrophy, loss of dermal papillae, and significant SE as parameters characterizing dermatoporosis.

Statement of Ethics

We received the approval of the ethics committee of the University Hospital of Geneva to conduct this study. Informed consent was obtained before investigating patients and healthy volunteers.

Disclosure Statement

The authors have no conflicts of interest to declare.

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No funding was received.

Author Contributions

S. Menzinger contributed to the acquisition, analysis, and interpretation of data for the work, participated in drafting the work, approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved. J.-H. Saurat made substantial contributions to the conception and design of the work, participated in revising it critically for important intellectual content, approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved. G. Kaya made substantial contributions to the conception and design of the work and to the acquisition, analysis, and interpretation of data for the work, participated in drafting the work, revising it critically for important intellectual content, approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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