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A nerve-wrecking event?

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3D Characterization Of Nerve Fiber Morphology In Psoriatic Skin And It's Relation To Clinical Evaluation

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ABSTRACT

Recent evidence suggests that psoriasis is a neurogenic inflammatory skin disease. Understanding nerve morphology in psoriatic skin is essential, but previous studies have shown inconsistencies due to variations in skin section thickness and layers analyzed. Additionally, most studies don't consider nerve proximity to other psoriasis-related features.

This study imaged 69 sections from 23 patients in 3D at 70 μm thickness, covering both the epidermis and dermis to a depth of 1.6 mm. High-resolution confocal imaging enabled detailed nerve quantification by location and type, including epidermal, papillary perivascular, and reticular nerves. Nerves comprised about 0.1% of total skin volume, forming complex networks around capillaries in the papillary dermis, with most located near vasculature except in the epidermis.

Correlation analysis showed a significant positive correlation between papillary and epidermal nerve volumes and a negative correlation between epidermal nerve volume and epidermal thickness, age, and erythema ($p < 0.05$). No correlation was found between other nerve types and vascular volume or other clinical parameters.

This 3D dataset provides a foundational resource for future studies on neurogenic pathways and offers a reference for comparing treatment effects on nerve and vascular morphology. Additionally, it supports modeling the thermal effects of light and laser therapies on psoriatic lesions.

INTRODUCTION

Psoriasis is a dermatological condition that is distinguished by aberrant vasculature and elevated levels of inflammation. Although numerous studies have been conducted with the objective of elucidating the underlying inflammatory pathways, there is mounting evidence to suggest that the nervous system plays a pivotal role in psoriasis. The involvement of nerves in psoriasis is perhaps most evident in the well-documented observation that symptoms worsen in response to stress. The connection between nerves and psoriasis was first recognized by Szodoray in 1955 in his publication, "Nervale Faktoren im Pathomechanismus der Psoriasis". Later, Farber et al. further documented this relationship, highlighting how psoriasis symptoms can worsen in response to stress and that neuropeptides mediate neurogenic inflammation in psoriasis¹⁻³. More specifically, previous research has shown that the local release of neuropeptides in the skin can cause hyperproliferation of keratinocytes, vasodilation, leukocyte infiltration, and stimulate mast-cell degranulation⁴⁻⁷. Despite the explanations of potential mechanisms of neurogenic contribution to psoriasis and studies on these mechanisms, there is no definitive understanding of the morphology of these nerves in the skin. There are three unresolved issues within the current base of literature on nerve morphology in psoriasis: 1) there is a difference in approach as to what depth nerve fibers are quantified contributing to discrepancies of nerve fiber counts in outcomes between papers, which in part contributes to potential differences in outcomes. Indeed, some studies have shown that nerve fiber density is increased in psoriasis^{8,9}, while others have suggested a decrease^{10,11}, 2) the majority of the studies use (too thin) sections that do not allow the full imaging of the tortuous course of thin nerve fibers, and 3) the present collection of studies concentrate on nerve fiber imaging without taking into account their proximity to their interactive compounds to psoriasis, such as vasculature, keratinocytes, and immune cells.

A comprehensive re-evaluation of the distribution of nerve fibers in psoriatic skin is required with consideration of all skin layers and the use of thicker tissue sections to accurately trace nerve trajectories. Additionally, better understanding of nerve fiber networks in psoriasis can be useful to verify whether any characteristics of nerves are associated with clinical evaluations. Lastly, if nerve damage is indeed a factor in relieving psoriasis symptoms, it is essential to establish a baseline and to identify any variations among patients, which can later be useful to assess treatment efficacy.

In the present study, we quantified the volume of nerve fibers within the epidermis, papillary dermis, and reticular dermis in sixty-nine 70 μm thick 3D skin sections from 23 patients (three per patient). Given the significant role that nerves play in psoriasis, our objective was to correlate nerve density in the skin with clinical parameters, including psoriasis severity (assessed by the Physician Global Assessment), itch perception, redness, scaling, biopsy location, BMI, age, and other relevant clinical data. Additionally, we considered the location of the nerves in proximity to the vasculature and immune cells.

MATERIALS AND METHODS

Study design and participants

We performed a biopsy study to evaluate the correlation between clinical assessment and histopathology of skin tissue, with a special focus on the neurovascular anatomy of the skin. The study was conducted between December 2022 and April 2024. Eligible patients were adults (18-69 y/o) with psoriasis and Fitzpatrick skin type I-III. Exclusion criteria were pregnancy and known neurological conditions. In total 23 patients were included. This study was conducted in accordance with the protocol and consensus ethical principles derived from international guidelines including the declaration of Helsinki and the ICH guidelines for Good Clinical Practice (GCP), approved by the Ethics committee of the Medical Ethical Committee of MEC-U, Nieuwegein, The Netherlands. All study participants signed the informed consent form prior to participation in the study.

Clinical assessment and sampling

Patients were asked for weight in kg, length in cm, smoking status (smoker, stopped smoking, non-smoker), and itch. The clinicians scored the study plaques for erythema (0-4), scaling (0-4), and induration (0-4). An external expert scored the photographs for the Physician Global Assessment (PGA), which is a commonly accepted score for the severity of psoriasis. Scores were classified with 1 being non-existent and 7 being a severe psoriasis lesion. Three-millimeter punch biopsy specimens were obtained from psoriatic skin lesions under local anesthesia of 1% Xylocaine.

Immunohistochemistry

Skin sections were labeled for a junctional protein of endothelial cells (CD31), nerve fibers (PGP9.5), and cell nuclei (Dapi, Hoechst). To do so, biopsies were fixed upon arrival for 24 h in Zamboni Fixative at 4 °C, washed twice in Sorrenson's buffer, and

stored at 4 °C in a 20% glycerol buffer for 24 to 72h. Then, tissues were embedded in OCT TissueTek (Sakura Finetek Europe, Alphen aan den Rijn, The Netherlands) and sectioned at 80 µm with a Cryostat Cryostar NX70 (Thermo Scientific). Free-floating 80µm thick skin sections were submerged in antifreeze buffer and stored at -20 °C in round bottom 96-well plates, until used. The staining protocol consisted of a 10 min wash in dPBS, followed by a 2h blocking step in 4% normal goat serum (Agilent Technologies Singapore, X0907) and 0.1% triton X-100 in dPBS to permeabilize the tissue. Sections were then incubated with 1:500 mouse anti-human CD31 conjugated to AF647 (BD pharmingen, 561654) and 1:300 rabbit anti-human PGP9.5 (Abcam, ab108986) in a 1:1 ratio of dPBS and blocking solution at 4°C overnight. Sections were rinsed twice in dPBS for 5 min and then incubated with 1:500 goat anti-rabbit Cy3 (Jackson Lab, 115-165-044) and 1:1000 Hoechst (0.1 mg/ml, 33342) diluted in dPBS for 2h on a mild shaker at RT. To prevent photobleaching the container lid was covered with aluminum foil. Then, slides were rinsed in MilliQ to remove salt crystal formation and mounted in Dapi-containing Vectashield (Vector Laboratories, H-1200-10).

Imaging & Histology

Three non-consecutive sections from each patient were imaged using an SP8-X DLS Lightsheet confocal microscope (Leica) with an HC PL APO 40x/1.30 OIL CS2 objective. Each section was imaged with dimensions of 1.1 mm (x) x 1.6 mm (y) and a total depth of 70 µm (z), using a 0.5 µm z-step size in-between images. Laser power was optimized for each patient and maintained consistently across sections for that individual. Nerve fiber volume was calculated using IMARIS software (Oxford Instruments, version 10.1) as the relative volume (%) of fluorescently stained nerve fibers (green) compared to the total skin volume (video 1). Nerves were classified based on their location in the epidermis, papillary dermis or reticular dermis. Similarly, blood vessel volume (red) was measured in the same manner. Blood vessels were classified as either papillary vasculature or reticular vasculature. Total skin volume was determined by overexposing the blue channel.

To evaluate scaling, we measured the thickness (µm) of the hyperparakeratotic layer, and epidermal thickness was determined from the skin surface to the base of the rete ridges (epidermal extensions reaching from the surface to the dermo-epidermal junction). Five measurements were taken per section, across three sections (yielding 15 measurements per biopsy), and averages with standard deviations were calculated.

Statistics

The data are presented as mean \pm standard deviation (SD). To compare two groups we used an unpaired t-test. For comparison amongst three or more groups, One-Way ANOVA testing was used with a Tukey test for multiple comparisons. For correlation calculations, we used Pearson r statistics. Correlation graphs show the Pearson r value unless otherwise indicated. P-values were considered significant at a value of 0.05. All statistical analyzes were performed with GraphPad Prism software (Graphpad, Boston, USA, version 9.5.1).

RESULTS

General study population

A total of 23 participants were included in this study. Biopsies were taken from the elbows (n=10), trunk (n=8), and the extremities (n=5) (Table 1). Images of the corresponding plaques are shown in Figure 1, sometimes the location of the biopsy is also demarcated. The average age was 44 ± 13 years (Table 1). The mean BMI of the participants was 25.9 ± 4.0 , of which 43% (10/23) had a normal BMI equal to or less than 24.9, 39% (9/23) were overweight (BMI between 25.0 and 29.9), and 17% were obese (BMI > 30). The Physician Global Assessment (PGA) score indicated that the mean severity of psoriasis was 3.7 ± 1.38 . PGA did not correlate with BMI or age, but age correlated significantly with BMI. PGA score was significantly higher in smokers than people who did not smoke at the moment of inclusion. Epidermal thickness was significantly lower in patients that had family with psoriasis ($p > 0.05$). Correlation plots of the parameters are shown in Supplemental Figure 1.

Table 1 Patient demographics and clinical features of study participants

Patient	Skin Type	Location	Genetic	Gender	Age	Smoking	BMI	Itch (0-4)	PGA(1-7)
1	3	Elbow	Yes	Male	43	Yes	23	1	6
2	2	Upper Leg	Yes	Female	60	Stopped	26	2	3
3	3	Elbow	No	Male	35	No	27	4	4
4	2	Lower Arm	Yes	Male	29	Stopped	24	3	3
5	2	Hip	No	Male	53	Yes	33	1	3
6	2	Elbow	No	Male	38	Yes	21	2	4
7	2	Elbow	Yes	Male	63	No	34	3	2
8	2	Hip	No	Male	39	Stopped	29	3	4
9	2	Trunk	Yes	Male	41	Yes	25	1	4
10	2	Hip	Yes	Male	60	Stopped	29	2	2
11	2	Hip	Yes	Male	45	Stopped	31	2	3
12	2	Hip	No	Male	68	Stopped	26	1	3
13	2	Back	No	Male	31	Yes	21	2	3
14	2	Upper Legs	No	Male	54	N.A.	29	4	5
15	2	Elbow	No	Male	35	Stopped	24	0	4
16	2	Upper Legs	Yes	Male	40	No	24	2	2
17	3	Elbow	No	Female	19	Yes	20	4	6
18	3	Elbow	No	Female	40	Stopped	22	0	5
19	2	Elbow	Yes	Male	46	Stopped	24	2	4
20	1	Elbow	Yes	Male	54	N.A.	30	1	5
21	2	Elbow	No	Male	61	Yes	29	2	7
22	2	Back	Yes	Female	25	Stopped	21	2	2
23	2	Upper Arm	No	Male	33	Stopped	26	0	3
Mean± SD					44 ± 13		26 ± 4	1.9 ± 1.2	3.8 ± 1.4



Figure 2. images of psoriatic lesions from 23 patients. Each image shows the severity and extent of erythema, scaling, and plaque thickness across different patients. Standardized measurement scales are placed next to each lesion for reference. Variations in plaque morphology are evident, highlighting the heterogeneity in the clinical presentation of psoriasis across individuals.

Histological evaluation of the neurovascular anatomy in psoriatic skin

Nerve fibers were present in the epidermis, papillary dermis, and reticular dermis where they tended to have distinct morphologies. Figure 2 illustrates the most common characteristics of the innervation and its relation to the vasculature, and all microscopy images from all patients can be seen in Figure 3. The distribution of nerve fiber percentages in the epidermal, papillary, reticular, and total nerve fiber layers was assessed using descriptive statistics. Since the data were not normally distributed, median and interquartile ranges (IQR) are reported. The median percentage of epidermal nerve fibers was 0.0007% (IQR: 0.0002–0.0026), while the papillary nerve fibers had a median of 0.0768% (IQR: 0.0514–0.1343). The median percentage for reticular nerve fibers was 0.0964% (IQR: 0.0469–0.1225), and for total nerve fibers, it was 0.0872% (IQR: 0.0638–0.1172). The 95% confidence intervals for these medians are presented in Supplementary Table 1.

Morphologically, epidermal nerve fibers varied in branching complexity and tortuous course (Figure 2A). We observed that some epidermal nerve fibers branched from the perivascular nerves surrounding the capillaries (Figure 2A, B). Statistical testing confirmed the observation that epidermal nerves branched from papillary nerves and showed a significant relationship between papillary nerve fibers and epidermal nerve fibers ($R^2=0.24$, $p=0.02$, Figure 4a). Epidermal nerve fiber volume also correlated significantly with epidermal thickness ($R^2=0.18$, $p=0.04$, Figure 4b). Perivascular nerves were found abundantly surrounding the capillaries in the papillary epidermis (Figure 2B). The majority of perivascular nerves were observed to run parallel with the capillaries. However, there was a notable variation in the network density of the fibers among patients, with some exhibiting a single nerve fiber running through the capillaries and others displaying a full mesh network of fibers. The capillaries observed in the papillary dermis exhibited the typical tortuosity and elongation extending to a considerable depth within the epidermis. However, it should be noted that the degree of tortuosity observed among these capillaries varied between patients. There was no relationship between the papillary blood vessel volume (%) and the perivascular nerve fibers in the papillae (%) ($R^2=0.03$, $p=0.46$, Figure 4c), indicating that more perivascular nerves do not infer more blood vessels, or *vice versa*. Directly beneath the papillary dermis, we frequently observed perivascular leukocyte infiltrates surrounding the supplying blood vessels (Figure 2C, Supplementary Figure 2) which were often accompanied by (perivascular) nerve fibers. We also observed fibroblast-shaped cells positive for

nerve staining in the upper and lower reticular layer, although not in all patients (Figure 2C). Toward the lower reticular dermis, we observed larger supplying blood vessels that were sometimes wrapped in unmyelinated nerve fibers, crossing thicker nerve bundles, or wavy patterns of nerve fibers that are characteristic of the innervation of erector pili muscles (Figure 2D). There was no significant correlation between nerve density in the reticular dermis and blood vessel volume in the reticular dermis ($R^2=0.01$, $p=0.58$). The papillary nerve volume did not correlate to the reticular nerve fiber volume ($R^2=0.04$, $p=0.39$), suggesting that the density of nerve fibers in the papillary dermis is not because of a high density of reticular ‘supplying’ nerve fibers. In blood vessels, we did see a correlation between papillary blood vessel volume and the ‘supplying’ reticular blood vessel volume of $r=0.5$ ($p=0.017$). Total nerve fiber volume did also not correlate with total vascular volume (Figure 4f).

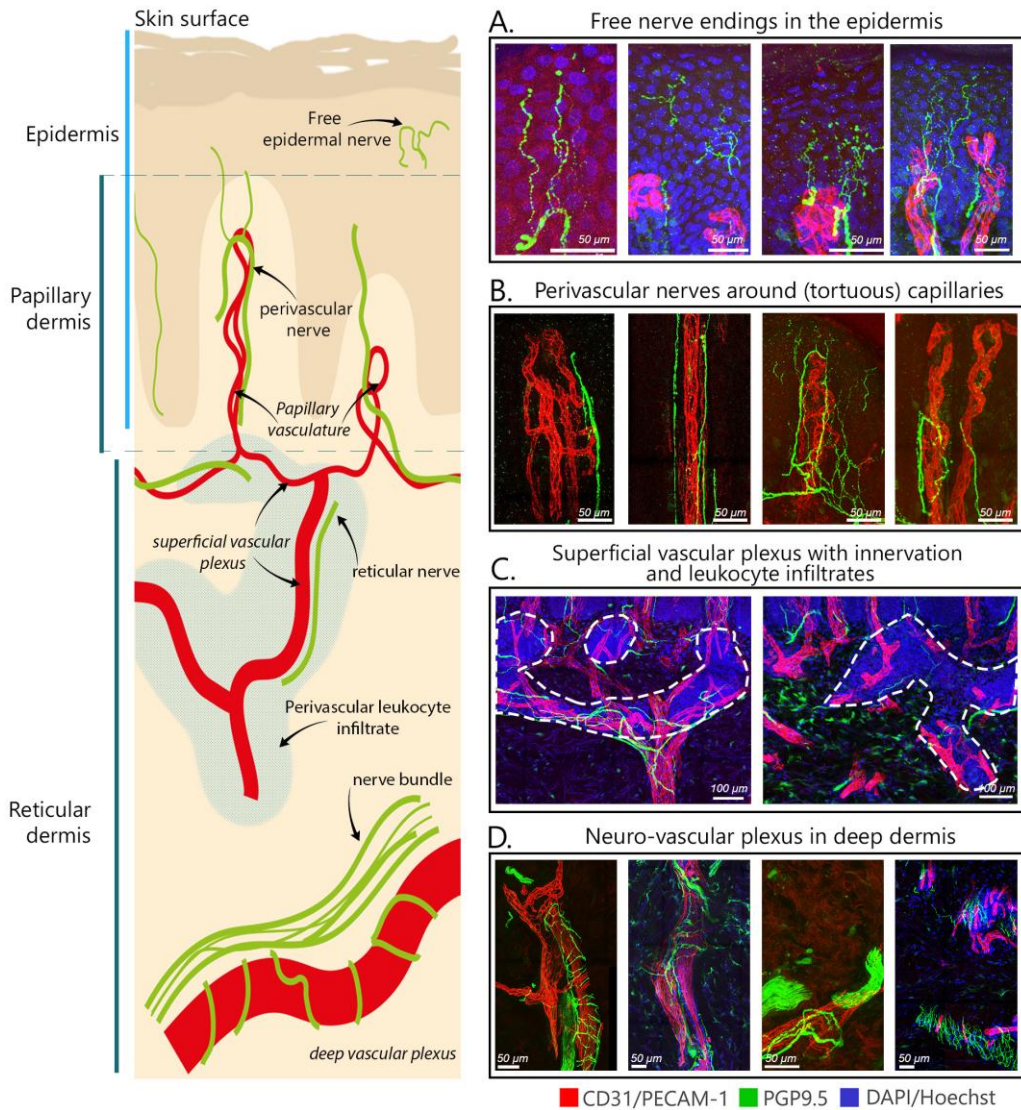


Figure 3. Neurovascular architecture in psoriatic skin, highlighting nerve and vascular interactions across epidermal, papillary, and reticular dermal layers. **Left panel:** Schematic representation of psoriatic skin layers, showing the distribution of free nerve endings (green) and vascular structures (red) in the epidermis, papillary dermis, and reticular dermis. **A:** Free nerve endings (green) in the epidermis that split off from perivascular nerves in the papillae (blue arrow). **B:** Papillary perivascular nerves surrounding capillaries in the papillae (blue arrow). **C:** Superficial reticular vascular plexus with accompanying nerve fibers (yellow arrows) and leukocyte infiltrates (line drawing), and ppg9.5+ fibroblasts (orange arrows). **D:** Neurovascular plexus in the deep reticular dermis, showing thick nerve bundles (grey arrow) running parallel to deep vascular plexus and fine nerve fibers surrounding the vasculature (arteries) (white arrows). Orange arrows show ppg9.5+ fibroblasts in some patients but not in all. Brown arrow shows innervation typically seen at the erector pili near hair follicles.

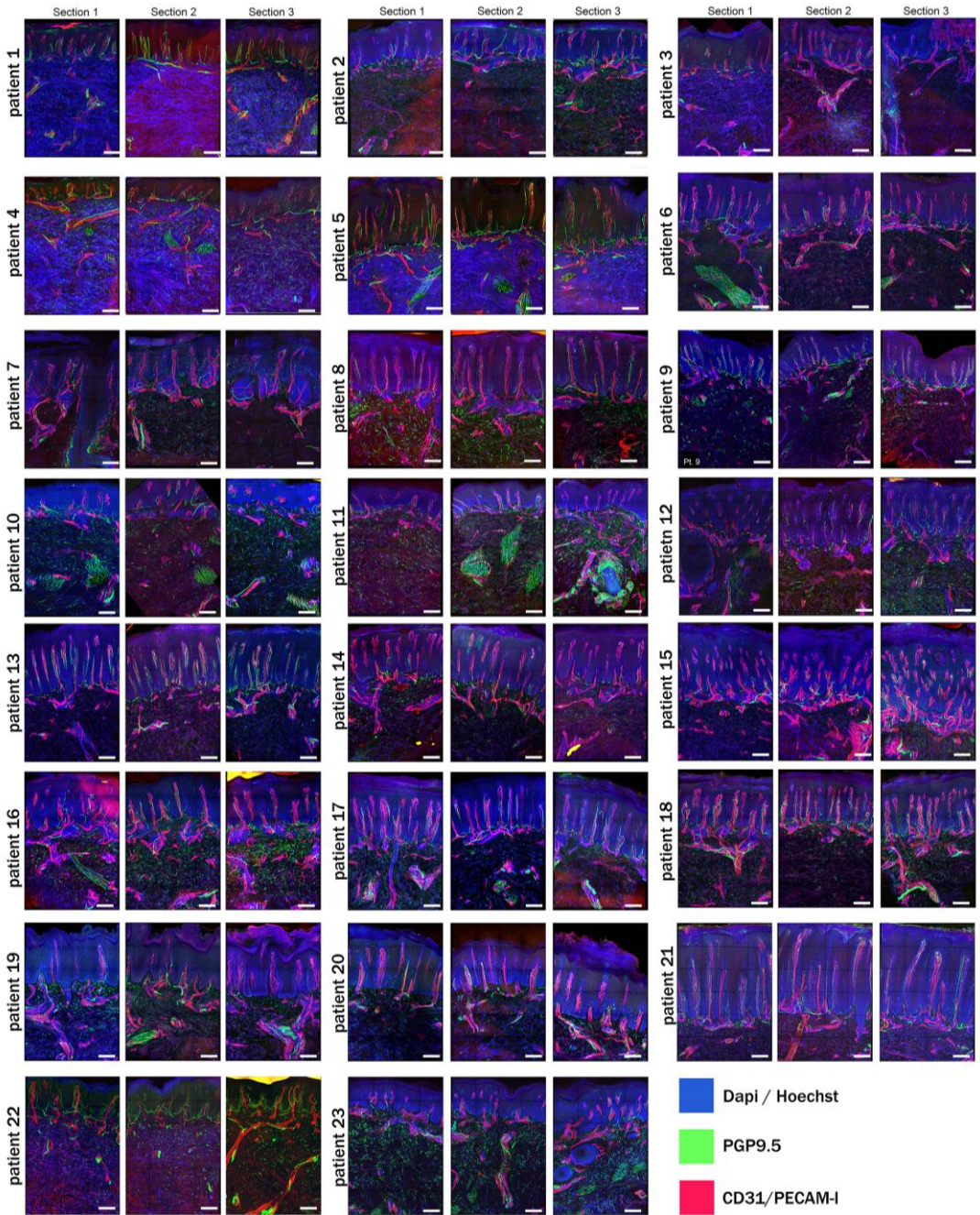


Figure 4. Immunofluorescent histological images of skin sections, oriented with the epidermis at the top and the reticular dermis at the bottom. The psoriatic vasculature, characterized by tortuous and elongated vessels, is visible in red. Thin green fibers indicate (peri)vascular nerves, including those innervating the erector pili muscles (patient 6 S1, patient 11 S2) and the hair follicle (patient 11 S3). Variations in epidermal thickness are also evident, with vertical vasculature marking the transition between the epidermis and the horizontal vasculature of the reticular dermis.

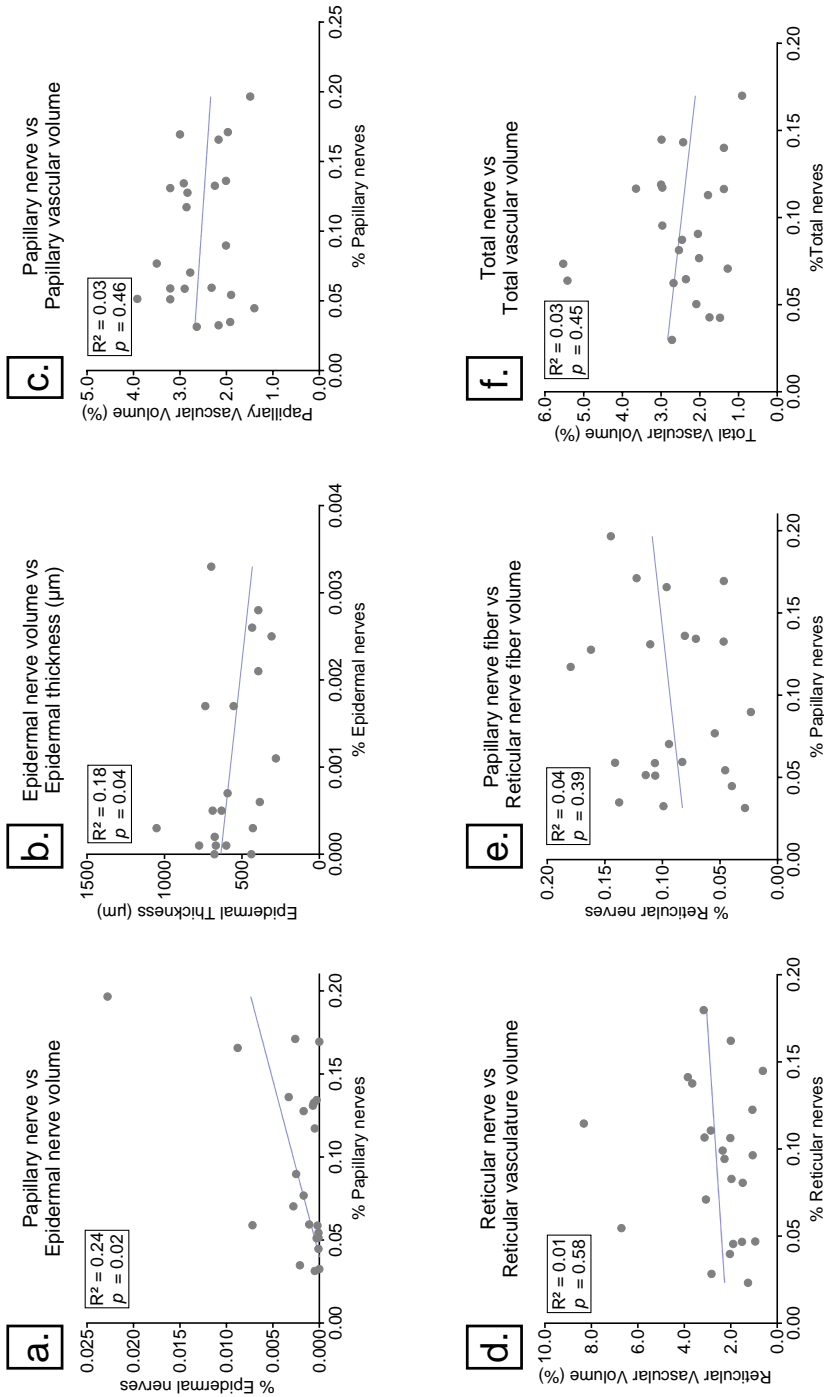


Figure 5. Correlation analyses of nerve fiber and vascular volume percentages across different skin layers in psoriatic tissue sections. (a) Comparison of papillary nerve fiber volume with reticular nerve fiber volume shows no significant correlation ($R^2 = 0.04$, $p = 0.39$). (b) Papillary nerve fiber volume positively correlates with epidermal nerve fiber volume ($R^2 = 0.24$, $p = 0.02$). (c) A significant inverse relationship is observed between epidermal nerve fiber volume and epidermal thickness ($R^2 = 0.18$, $p = 0.04$). (d) No significant correlation is found between papillary nerve fiber volume and papillary vascular volume ($R^2 = 0.03$, $p = 0.46$). (e) Reticular nerve fiber volume shows no significant correlation with reticular vascular volume ($R^2 = 0.01$, $p = 0.58$). (f) The total nerve volume does not correlate significantly with total vascular volume ($R^2 = 0.03$, $p = 0.45$).

Intra- and Interpatient variability of nerve fiber density

As previously mentioned in the introduction, variations in nerve fiber density may hold clinical significance, especially if they become therapeutic targets. To investigate the validity of our histologically derived data and variations in the dataset, we quantified both the intra-biopsy (within a single biopsy of which we quantified three different sections) and the inter-patient (between patients) variation. Our results show that the majority of the variation in nerve fiber density is attributable to differences between patients, with minimal and statistically non-significant variation observed between sections of the same biopsy (see Table 2). These findings suggest that, despite some visual heterogeneity in histological images, histology remains a reliable method for assessing nerve fiber density differences between patients. Additionally, the coefficient of variation (CV) was calculated to represent the relative variability by expressing the standard deviation as a percentage of the mean (Table 2). This metric allows for the comparison of variability across nerve fiber types (epidermal, papillary, reticular, and total), regardless of their differing percentages. The results show that the highest degree of variability was observed in the dataset for epidermal nerve fibers, where the standard deviation was 190% of the mean (Table 2). This substantial variation aligns with our histology observations, where epidermal nerve fibers were absent in some patients and, when present, occupied a very small volume. For the other nerve fiber types, the coefficient of variation was approximately 50%. While still relatively high, this variability reflects the considerable differences in nerve fiber volume observed between patients in our dataset.

Table 2. Contribution of variation between nerve fiber volume for patients and biopsy section

Nerve fiber type	Variability explained by Patient (%)			Variability explained by Section nr (%)			Coefficient of variation (CV)
	F	<i>p</i>		F	<i>p</i>		
Epidermal	76.9	6.9	<0.0001	0.8	0.8	0.45	190.4%
Papillary	70.1	8.2	<0.0001	0.5	0.5	0.61	54.1%
Reticular	50.8	2.2	0.015	2.1	1.0	0.39	47.1%
Total	60.2	3.1	0.0007	0.6	0.4	0.71	40.8%

Nerve density and clinical parameters

Given that we established in the previous paragraph that there is a significant discrepancy in nerve fiber density between patients, we proceeded to investigate whether variation in nerve fiber density could be explained by a correlation with clinical parameters. These parameters included itch, erythema, scaling, thickness, Physician Global Assessment, BMI, age, smoking status, and location of the biopsy.

No significant differences in nerve fiber density were identified based on the biopsy location, which was divided into trunk, extremities, and elbows ($p > 0.05$) (Figure 5 a-d). Thus, the location of the biopsy does not explain the observed variation between patients. We also found that patients with family members who are also affected by psoriasis had a significantly thinner epidermis than patients without family members affected by psoriasis ($p > 0.01$, Supplemental Figure 3).

A correlation matrix was generated to explore potential correlations between nerve fiber density and (continuous) clinical parameters (see Supplemental Figure 1). Although clinical severity (PGA) appeared to exhibit a slight correlation with epidermal nerve fiber volume, no statistically significant Pearson's r or R^2 values were observed ($r = -0.38$, $R^2 = 0.15$, $p = 0.071$) (Figure 5e). The relationship between PGA and papillary nerves was even less strong ($r = 0.1$, $R^2 = 0.01$, $p = 0.66$), as was the relationship between PGA and reticular nerves ($r = 0.07$, $R^2 = 0.005$, $p = 0.74$), as well as the relationship between PGA and total nerve fiber volume ($r = 0.1$, $R^2 = 0.01$, $p = 0.64$) (Figure 5f-h, Supplemental Figure 1). Thus

nerve fiber density has no relationship with the observed clinical severity of psoriatic plaques.

Significant correlations were observed between age and total nerve fibers ($r = -0.46$, $p = 0.04$), as well as between BMI and age ($r = 0.68$, $p > 0.001$) (Supplemental Figure 1). Although BMI did not correlate significantly with total nerve fibers ($r = -0.35$, $p = 0.1$), weight and height individually did correlate significantly with total nerve fibers. Significant correlations were also observed between epidermal nerve volume and erythema score ($r = -0.62$, $R^2 = 0.38$, $p = 0.002$) (Figure 5-I, Supplemental Figure 1). There was no significant correlation between itch, induration, smoking status, and scaling with any of the nerve fiber volumes ($p > 0.05$).


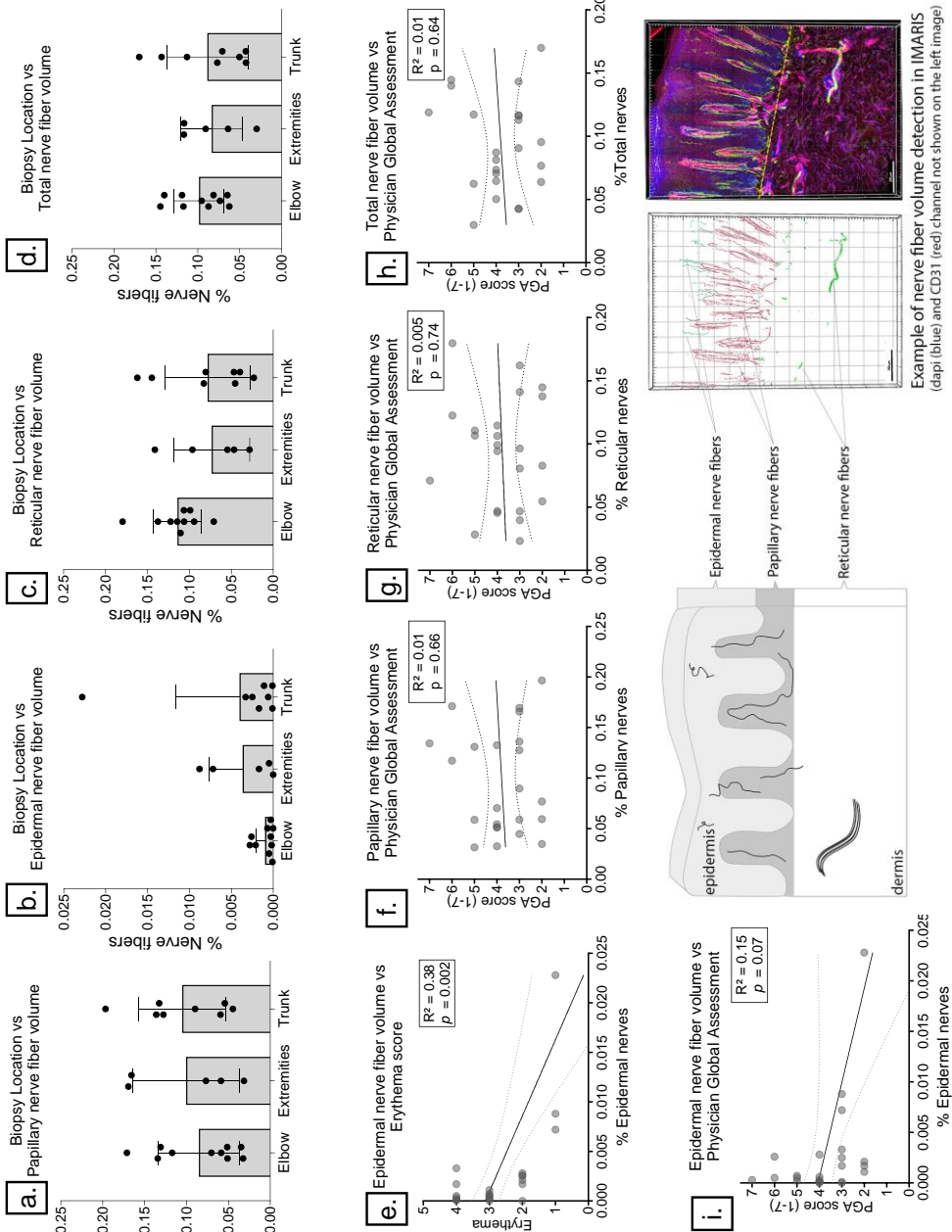


Figure 6 Nerve fiber volumes in different skin layers compared by biopsy location (elbow, extremities, trunk) and correlations with Physician Global Assessment (PGA). (a-d) Nerve fiber volume (%) in the epidermis (a), papillary dermis (b), reticular dermis (c), and total nerve fiber volume (d) are shown for different biopsy locations. No significant differences in nerve volume were observed between these locations. (e-h) Linear regression plots between nerve fiber volumes in the epidermis (e), papillary dermis (f), reticular dermis (g), and total nerve volume (h) with clinical severity as indicated by the Physician Global Assessment (PGA). Despite the trends, none of the correlations reached statistical significance ($p > 0.05$).



DISCUSSION

The objective of this study was to quantify the volumes of nerve fibers within the epidermis, papillary dermis, and reticular dermis, providing novel data on the percentage of nerve fibers in each skin layer. The total percentage of nerves in psoriatic skin samples was approximately 0.1%. The greatest variability among patients with psoriasis was observed for the papillary and epidermal nerve fibers, while the reticular nerve fibers showed less variation. Our use of 70 μm skin sections allowed for a clearer distinction between the rete ridges and the papillary dermis, enabling more accurate differentiation between epidermal and papillary nerve fibers. This methodology improves reliability over previous studies where perivascular nerves in the papillary dermis may have been falsely identified as penetrating epidermal nerve fibers.

We found some significant correlations between clinical parameters and nerve densities. Epidermal nerve fiber density correlated significantly with erythema, epidermal thickness, and age. The link between epidermal nerve fibers and erythema may be driven by the release of vasodilatory neuropeptides like CGRP¹² for example in pressure-driven erythema¹³ and/or the interaction between epidermal nerve fibers and mast-cell regulated production of histamine and other inflammatory mediators, contributing to redness and swelling¹⁴.

Inverse correlation between epidermal nerve fiber density and age has been previously documented^{15,16}, and our data suggests that this phenomenon is possibly constrained to epidermal nerve fibers alone, with no such correlation observed for papillary or reticular nerve fibers. The distribution of nerve fibers has often been described or illustrated as free epidermal nerves coming straight from the bottom of the reticular dermis and trespassing the dermo-epidermal junction. However, we observed that most epidermal nerve fibers branched from the papillary perivascular nerve networks surrounding the tortuous capillaries of the papillary dermis in the rete ridges. We saw a similar branching from papillary nerves into the epidermis in the microscopy images of Aschenbeck *et al.* in a study on the effect of Botox on psoriasis¹⁷.

Based on our results we did not see a relationship between reported itch scores and the amount of nerve fibers. Literature has reported both negative¹⁸, and positive^{5,19,20} relationships between innervation and itchy psoriatic lesions. Possible explanations for the difference in the outcome of the relationship between nerve fibers and itch in

previous studies and the current are differences in assessment of itch (which range from a single-moment score to a very extensive 3-day assessment of itch) and the longevity of the plaque, maturation (lasting over 4 weeks) to long-established plaques (lasting over 6 months)¹⁰. Additionally, we found no significant correlation between papillary, reticular, or total nerve volume and clinical parameters such as the Physician Global Assessment, redness, scaling, biopsy location, and BMI.

The lack of correlation between clinical severity and nerve fiber density suggests that the presence of nerves alone is insufficient to explain the clinical symptoms of psoriasis, suggesting that it is not the presence of the nerve fibers, but rather the activity, i.e. the release of neuropeptides and frequency thereof. Supporting this notion, Kennedy et al. (1993)²¹ performed nerve staining on thick sections of healthy human skin, revealing an innervation pattern (as shown in Figure 1 of their publication) that closely resembles the pattern we observed in the affected skin of psoriasis patients. This further indicates that nerve fiber activity, rather than their presence, may be the key factor in the pathogenesis of psoriasis.

In addition to the discrepancy between the physiology and morphology of nerves in psoriasis, another potential explanation for the absence of a correlation between clinical parameters and nerve fiber volume is that the clinical symptoms of psoriasis are not solely the result of nerve activity. Rather, they are the product of a more intricate interaction between keratinocytes, immune cells, and the vasculature. Even when not in close proximity to blood vessels, nerves were observed to be in close association with leukocyte infiltrate clusters in the vicinity of the vasculature (supplemental Fig 3). The proximity of nerves to both the vasculature and immune cell infiltrates suggests a functional role in the pathophysiology of psoriasis. This role likely includes the release of neuropeptides such as substance P (SP), Calcitonin Gene-Related Peptide (CGRP), Neuropeptide Y (NPY), neurokinin A (NKA), and Vasoactive Intestinal Peptide (VIP), all of which are known to mediate vasodilation in the skin²². Vasodilation, in turn, increases vascular permeability, allowing plasma proteins and other inflammatory markers to leak into surrounding skin tissue. As a result, a chemotactic gradient forms, drawing leukocytes to the site of inflammation. Additionally, some neuropeptides are known to enhance the expression of adhesion molecules involved in leukocyte rolling and adhesion, processes that facilitate immune cell transmigration from the endothelial barrier into the skin tissue. Therefore, the close contact between nerve fibers and blood vessels is critical, and

dysregulation of these nerves and their neuropeptide release may contribute to the persistent inflammatory state characteristic of psoriasis.

Apart from nerves in proximity to blood vessels, we also observed nerve fibers around immune cell clusters, which suggests involvement of nerves in the inflammatory processes associated with psoriasis. Neuropeptide release from nerves can activate dendritic cells and macrophages, leading to the production of pro-inflammatory cytokines such as IL-23. IL-23, in turn, promotes the differentiation and maintenance of Th17 cells, a subset of T-helper cells. Th17 cells produce IL-17, which stimulates keratinocytes, driving hyperproliferation and the release of additional pro-inflammatory cytokines and chemokines, including Nerve Growth Factor (NGF)²³. NGF then promotes further nerve growth, creating a positive feedback loop that amplifies inflammation and sustains psoriatic lesions²⁴⁻²⁶. A recent study on 3D *in vitro* cell models, have shown that the neuropeptide calcitonin gene-related peptide (CGRP), released by sensory nerve fibers, increases keratinocyte proliferation²⁷. Thus, our finding that nerve fibers are located near keratinocytes, vasculature, and immune cell infiltrates aligns with the existing evidence supporting the concept of neurogenic inflammation in psoriasis.

The developmental stage of the plaque adds an additional layer of complexity that must be considered when attempting to understand the role of nerves in psoriasis. Pergolizzi *et al* (1998) observed that in long-standing plaques nerve fibers were decreased^{4,10} whereas Naukarinen *et al* (1989) found that nerve fibers were highest in one-week old lesions²⁸. This might indicate that there is a negative feedback loop to shush the over-active nerves in newly formatted plaques. However, if down-regulating nerve fiber density in skin is a mechanism of the skin to self-protection then why does a decrease of nerve fibers in mature plaques not lead to a remission of the psoriasis? On the other hand, the decrease of nerve fibers in mature lesions may be biased by age as we and others have seen that age also decreases (epidermal) nerve fiber density¹⁶. A limitation of the present study apart from the limited group size, is that we did not collect data on the longevity of the plaques and we did not include uninvolved skin biopsies and could thus not compare the nerve fiber densities in involved skin, with uninvolved skin. Since the lack of correlation between clinical parameters and nerve fiber densities suggests that psoriatic nerves have a physiological rather than a morphological abnormality, it would make sense for future studies to not only stain for the presence of nerve fibers through PGP9.5 but additionally stain for presence of neuropeptides by using CGRP, SP, or other

neuropeptide markers or even include functional nerve testing. Although we did not stain for neuropeptides, there is a general distribution in which SP and CGRP-containing nerves are mainly portrayed in the dermal papillae, whereas VIP and peptidylglycine alpha-hydroxylating monooxygenase (PHM) neuropeptides were found in nerves in the reticular dermis surrounding blood vessels and eccrine sweat glands^{4,29}.

In conclusion, we showed that nerve fiber density does not relate to clinical severity of psoriasis and we showed that nerve fibers are mostly present around the vasculature, or in close proximity to keratinocytes or immune cells which suggests that their contribution to psoriasis is most likely a complex interaction with the environment. We also showed that from the nerve fibers we distinguished, free epidermal nerve fibers showed the highest variation (76%) between patients, and also a significant correlation with erythema, epidermal thickness, and age. It would be beneficial for future studies to consider a more comprehensive approach, incorporating not only the role of nerves but also the interactions occurring within the psoriatic skin environment, which encompasses inflammatory cells, aberrant vasculature, and hyperproliferation of keratinocytes. Functional testing of nerve fibers and physiology-based experiments might therefore be more appropriate than histological stainings. Nevertheless, the current dataset on the 3D morphology of a wide range of psoriatic patients enables future studies to model the thermal effects of light treatment, as demonstrated in our recent work on pulsed dye laser treatment³⁰. Lastly, this data provides an excellent baseline histology for comparing the effects of various treatments on psoriatic lesions.

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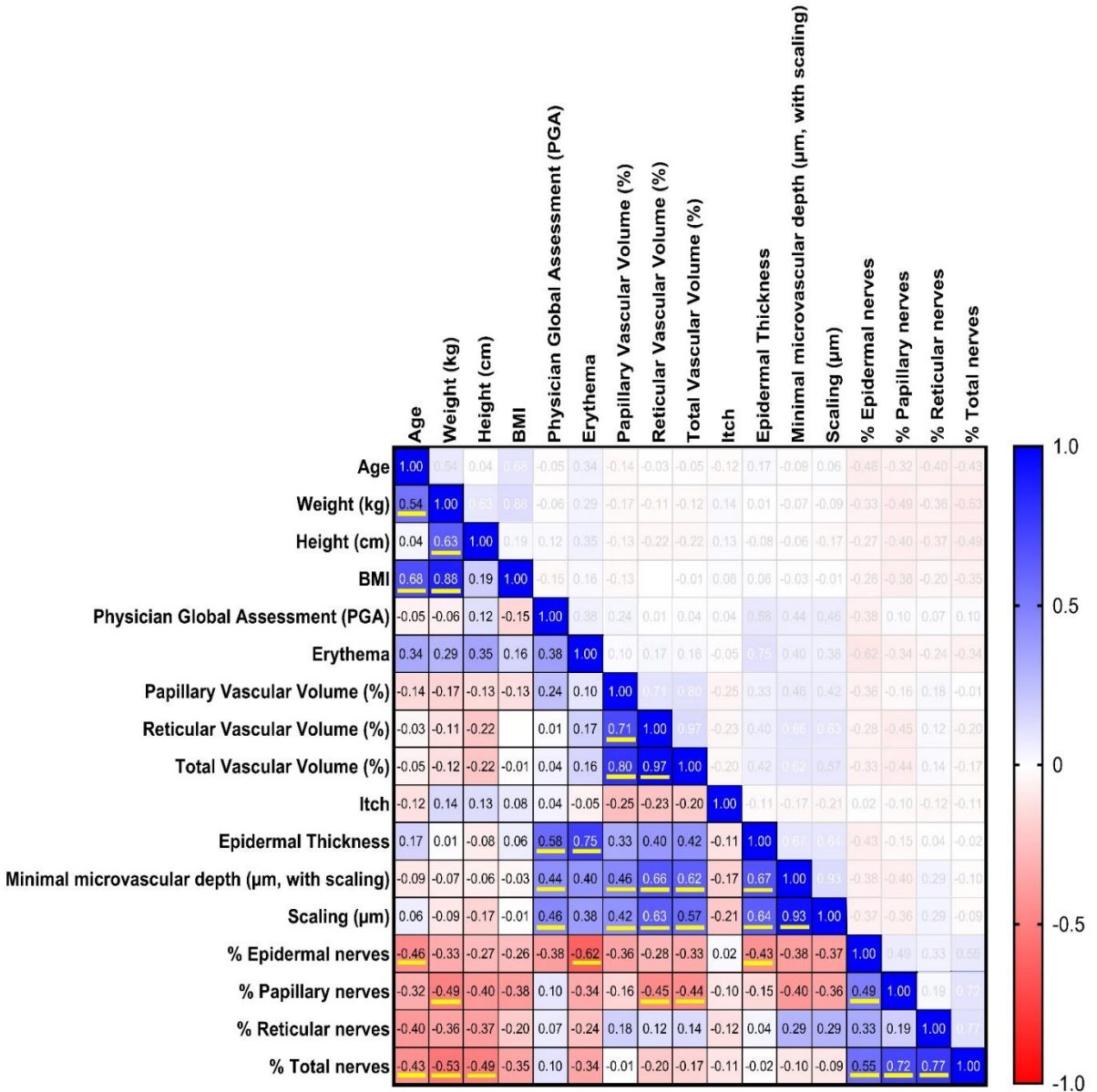
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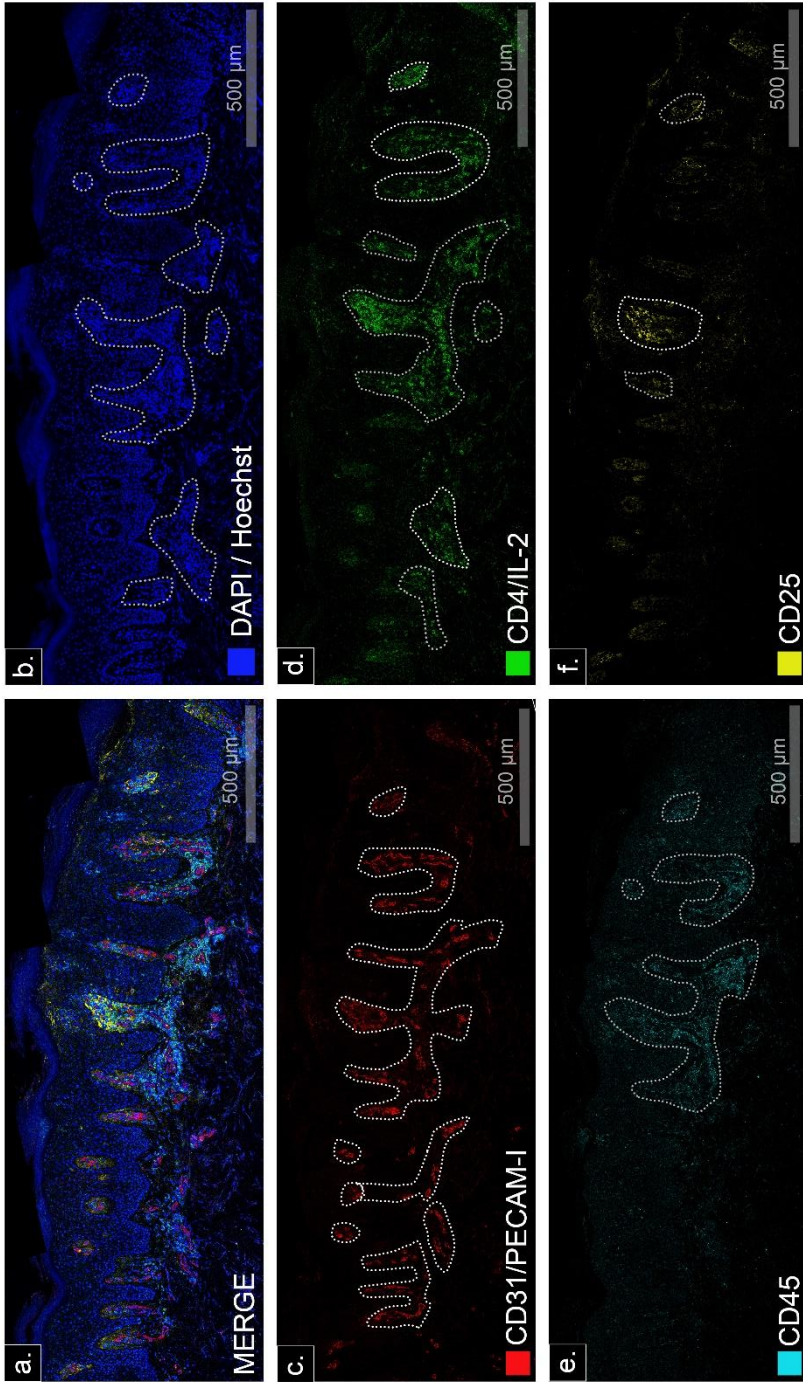
SUPPLEMENTS

Supplementary Table 1. Nerve Fiber Distribution Across Skin Layers

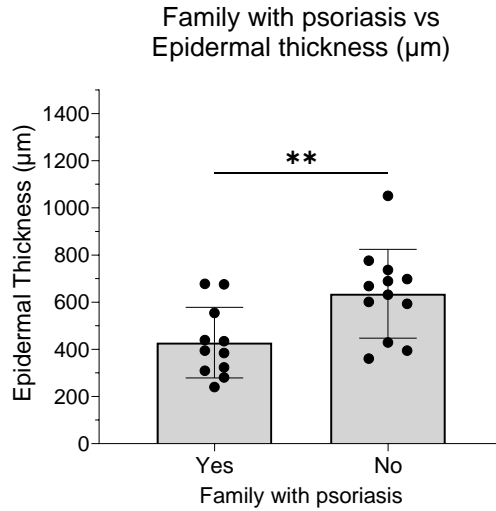
Nerve Fiber Type	Median (%)	IQR (%)	Minimum (%)	Maximum (%)	95% CI of Median (%)
Epidermal	0.0007	0.0002–0.0026	0.0000	0.0228	0.0003–0.0025
Papillary	0.0768	0.0514–0.1343	0.0314	0.1966	0.0543–0.1326
Reticular	0.0964	0.0469–0.1225	0.0232	0.1797	0.0546–0.1146
Total	0.0872	0.0638–0.1172	0.0298	0.1699	0.0646–0.1166



Supplemental Figure 1. Correlation matrix depicting relationships between clinical and histological parameters in psoriasis. The heatmap illustrates the strength of correlations between various parameters, including age, weight, height, BMI, Physician Global Assessment (PGA), erythema, vascular volume, nerve fiber densities, epidermal thickness, and scaling. Blue shades represent positive correlations, while red shades indicate negative correlations. Stronger correlations are indicated by darker shades. Values shown are Pearson r values. Significant correlations ($P < 0.05$) are highlighted with yellow underscore.



Supplemental Figure 2. Immunofluorescent staining of psoriatic skin highlighting various immune markers: (a) MERGE: Composite image showing the overlay of all stained markers in the psoriatic lesion. (b) DAPI/Hoechst: Blue staining indicates cell nuclei. (c) CD31/PECAM-1: Red staining highlights endothelial cells associated with blood vessels. (d) CD4/IL-2: Green staining denotes CD4⁺ T cells suggesting immune cell activity. (e) CD45: Cyan staining represents leukocyte common antigen, marking immune cells. (f) CD25: Yellow staining shows the expression of the IL-2 receptor, associated with T cell activation. Dashed lines indicate regions of interest, including the dermal and epidermal layers. Scale bar: 500 µm.



Supplemental Figure 3. Comparison of epidermal thickness (μm) between individuals with and without a family history of psoriasis. The epidermal thickness was significantly lower in individuals with a family history of psoriasis (mean \pm SD: [insert value]) compared to those without a family history (mean \pm SD: [insert value]). Statistical significance was assessed using a [insert test], with a p-value of $p < 0.01$ (denoted by **).



Supplemental video 1: [3D Neurovascular anatomy in psoriatic skin](#)