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# Impact of inflammation on iron stores in involved and non-involved psoriatic skin

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ABSTRACT

Accumulating evidence supports a role for cellular Fe in cell proliferation, inflammation, and disease tolerance. Psoriasis is a severe inflammatory and hyper proliferative condition of human skin whose aetiology remains poorly understood. Herein, we performed nuclear microscopy techniques to quantify with cellular resolution and high sensitivity the concentration of Fe in lesional (psoriatic plaques) and non-lesional adjacent skin of psoriatic patients. Fe contents were measured across skin depth and along epidermal strata either by quantitatively imaging Fe distribution in regions of interest, or by determining Fe profiles through analysis of sequential points along selected transepts. Both procedures require deconvolution of spectra to project quantitative elemental data through the application of different software codes. Using these approaches a detailed quantitative distribution of Fe was resolved. We show that in both lesional and non-lesional skin, the epidermal profiles of Fe contents showed a peak at the basal layer and that Fe concentration along the basal layer was not uniformly distributed. Typically, Fe levels were significantly higher in epidermal ridges relative to regions above dermal papillae. Lesional skin displayed excess Fe over extended regions above basal layer.

In conclusion, we found significantly increased Fe deposits in the epidermis of psoriatic patients, particularly in areas of epidermal hyper proliferation. These findings suggest an important role for Fe in the pathogenesis of psoriasis. They also raise the possibility that manipulation of Fe levels in the skin may become relevant for the clinical management of psoriasis.



## 1. Introduction

Psoriasis is a chronic inflammatory condition of skin, characterized by an increased proliferation of keratinocytes [1]. The etiology of the disease still remains poorly understood. The latent capacity of uninvolved skin of patients to boost psoriatic plaques has become an important area of investigation [2]. Despite the role of Fe in cellular proliferation and cellular damage, both of which are of relevance in psoriasis, the specific distribution of Fe in the skin of psoriatic patients has not been previously addressed. This may allow further correlations with the localization and function of other components involved in iron metabolism, *eg* iron transporters, and thus contribute to a better understanding of iron metabolism in the psoriatic epidermis.

Accumulating evidence supports a role for elevated Fe stores in disease susceptibility and in response to infection and inflammation [3–5]. Fe is physiologically essential in many cell processes but the $Fe^{2+}$ species is extremely toxic in the free form or in complexes with small molecules as it can catalyze the conversion of reactive oxygen species (ROS) into harmful hydroxyl radicals [6,7]. These radicals are responsible for oxidation of unsaturated lipids, proteins and nucleic acids thus damaging cells, tissues and organs [6–9]. The Fe is stored intracellularly bound to ferritin in its ferric state Fe(3+). The oxidation of residues of ferritin sub-units may expose the protein core and the Fe reduction sites favoring Fe(2+) release [7,8]. As a result, Fe overload may amplify damage due to ROS in a broad spectrum of disorders and inflammatory conditions. Previous studies demonstrated that Fe was mainly deposited in the lower epidermal layers [10,11] in the skin of patients with Fe overload disorders. On the other hand, UV irradiation

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induces the expression of ferritin in vitro [12] and in skin, and this expression was most evident in the lower keratinocyte layer of human normal epidermis [13].

The main objective of this study was to prospectively study the Fe distribution pattern in lesional skin (psoriatic plaque) and in adjacent, apparently not involved skin (non-lesional skin). Nuclear microprobe techniques are excellent to image the elemental distribution and density variations in biological tissue [14,15]. In the present study, different elemental quantitative analytical methods and the detailed localization of Fe in skin was assessed using quantitative mapping of selected regions, and quantitative profiles across skin depth and along epidermal strata. Fe contents were measured either by quantitatively imaging Fe distribution in regions of interest, or by determining Fe profiles through analysis of sequential points along selected transepts. Both procedures require deconvolution of spectra to project quantitative elemental data through the application of different software codes, such as Dan32 [16] and GeoPIXE [17]. Using these approaches a detailed quantitative distribution of Fe in skin was resolved.

## 2. Material and methods

Six patients older than 18 years with moderate to severe chronic plaque-type psoriasis (psoriasis area severity index score >10.0) followed in Psoriasis outpatient's clinic at the Dermatology Service, Hospital de Santa Maria (Lisboa, Portugal) were enrolled in the study. Informed consent has been obtained for all subjects in accordance with the Declaration of Helsinki.

For each patient two punch biopsies of 4 mm were obtained: (i) from plaque lesion (lesional skin); (ii) from macroscopically normal skin at a distance <1 cm from plaque (region without plaque defined by clinical observation – non-lesional skin). Biopsies were immediately frozen in liquid nitrogen and kept at −80 °C until processing. Sections of 14 μm thickness were cut in a cryostat at −25 °C (Cryotome 620E, Thermo Shandon, UK), dried and mounted in specific frames as described elsewhere [18,19]. At least two sections from each sample were analyzed.

Distribution of Fe in transversal sections of cryopreserved skin samples were carried out with nuclear microscopy using a combination of three techniques [18,20]: (1) PIXE (Particle Induced X-ray Emission), (2) RBS (Rutherford Backscattering Spectroscopy) and (3) STIM (Scanning Transmission Ion Microscopy). Experiments have been carried out at CTN/IST [18] and CMAM/UAM [21,22] facilities. Briefly, a focused 2.0 MeV proton beam 2–3 μm has been used to scan across a selected area of interest of the skin section. Concentration profiles were obtained by analyzing a sequential number of points (corresponding roughly to the focused beam area) along a transept of the scanned skin section. The identification of skin layers were achieved using STIM technique, as previously described [e.g., 18]. Quantitative analyses of Fe along skin profiles were performed with Dan32 software [16,23] combining PIXE and RBS data. RBS provides information on matrix (major elements of the sample) composition and incident charge, and is used in conjunction with PIXE to quantify the trace element concentration results.

Full quantitative maps of scanned areas were also produced using GeoPIXE software [17]. This software has the ability to accumulate on-line PIXE elemental maps using dynamic analysis that are inherently overlap-resolved and background-subtracted [24,25]. Concentrations are expressed in mg/kg dry weight.

For immunofluorescent detection of ferritin, frozen sections were fixed in 2% formaldehyde in PBS, and incubated with a mouse monoclonal antibody specific for the heavy chain of human ferritin (ab134276; Abcam) and a secondary DyLight 488-coupled antibody (Jackson ImmunoResearch Laboratories). Sections were counterstained with DAPI (0.5 μg/mL) to visualize DNA and mounted in Vectashield (Vector Laboratories) before confocal microscopy. Samples were then examined with a Zeiss LSM 510 META laser scanning confocal microscope (Carl Zeiss).

Exploratory data analysis considered medians and parametric tests to estimate differences between groups of samples (lesional skin vs non-lesional skin) and skin regions (e.g., different epidermal layers). Differences were estimated using non-parametric Mann–Whitney $U$ test. Differences were considered significant when $p < 0.05$. The calculations were performed using SPSS (version 22).

## 3. Results

A characteristic distribution of Fe across the depth of the epidermis was observed in non-lesional skin at marginal areas of a psoriatic plaque. Epidermal layers were identified by comparing different types of images obtained for the scanned area. Reflection microscopy of the skin section analyzed provided information on major skin regions (most external layer, the *stratum corneum*, the underlaid epidermis and dermis). The STIM images were especially useful to identify cell layers of epidermis whereas PIXE images provided precise information on strata interface as described in previous studies [10,11,18,19]. In Fig. 1 the correlation of Fe distribution (Fig. 1C and D) with features of the human skin (Fig. 1A and B) are depicted. Fe concentrations were relatively constant across the epidermis but increased abruptly in the basal cell layer. Although Fe peaks at the basal cell region, its concentration was not uniformly distributed along this layer (Fig. 1C). Indeed, Fe deposits were preferentially localized at the epidermal ridges (R), where keratinocyte proliferation is amplified, when compared to the regions above dermal papillae (supra papillary, SP), where the epidermis is thinner (Fig. 1D). The histopathology of the psoriatic plaque is characterized by extended regions of excessive production and exfoliation of epidermal cells, among other features. The distribution of Fe across the epidermis of lesional skin was similar to the marginal skin. Accordingly, higher Fe concentrations were observed in the deeper, basal epidermal layers particularly in the epidermal ridges of hyper-proliferative regions (Fig. 2C). However, high Fe levels were not confined to the basal cell layer, extending to the upper epidermal layers. Again, as for non-lesional skin, the distribution of Fe along the basal cell layer was heterogeneous with areas of higher concentration (mostly at rete ridges/R) alternating with areas of lower Fe concentration (mostly in supra-papillary regions/SP). Importantly, this distribution pattern closely mimicked that of the iron binding protein ferritin assessed by immunofluorescence microscopy with a ferritin-specific antibody (Figs. 1A, 2A).

Whereas quantitative maps provide a quick evaluation of Fe distribution, localization and level range, as illustrated in Figs. 1 and 2, concentration profiles will enable assessing Fe changes with cell resolution detail. Fe profiles across skin depth and along epidermal strata, were done based on (i) sequential individual spectra taken along transepts of interest; (ii) data extracted from regions of interest selected from maps. Data from the two epidermal regions, R and SP, were grouped according to depth, i.e., the deepest layer – the basal cell layer (BL) – corresponding to the cells attached to the basement membrane in the epidermis/dermis interface, the low supra basal region (SB-L), which include immediate layers of keratinocytes above BL, and a third region typically 10 μm above SB-L, the upper basal region (SB-U).

The average concentrations of Fe measured in these sequential layers of the epidermis, for both R and SP regions of lesional and non-lesional skin are plotted in Fig. 3. In BL areas of non-lesional skin the Fe contents vary significantly between epidermal ridges