Skin Iron Deposition Characterises Lipodermatosclerosis and Leg Ulcer

A. Caggiati,*, C. Rosi, A. Casini, M. Cirenza, V. Petrozza, M.C. Acconcia, P. Zamboni

Department of Anatomy, Sapienza University of Rome, Italy
Department of Pathology, Sapienza University of Rome, Italy
Department of Cardiology, Sapienza University of Rome, Italy
Department of Surgery, University of Ferrara, Ferrara, Italy

Submitted 31 May 2010; accepted 1 August 2010
Available online 28 September 2010

KEYWORDS
Chronic venous disorders;
Venous ulcer;
Lipodermatosclerosis;
Iron;
Haemosiderin

Abstract
Background: It is commonly reported that chronic venous disease (CVD) increases the skin iron content in which the excess is stored as haemosiderin. Despite increasing interest in the role of haemosiderin in venous ulceration, no study has systematically evaluated the occurrence of iron overload in the limbs of patients with CVD.

Purpose: To evaluate skin haemosiderin deposition in relation to the presence and severity of skin changes in CVD legs designated according to the clinical, etiologic, anatomic and pathophysiologic (CEAP) classification.

Methods: A total of 85 skin biopsies were taken from the medial aspect of 49 limbs with CVD of CEAP clinical stages C2, C3, C4 and C6. The content of ferric ions was assessed by Perl’s Prussian Blue (PPB) stain.

Results: No haemosiderin deposition was found in normal skin of C2, C3 and C4a legs, in less severe regions of pigmentation and in some parts of more severely affected limbs. Haemosiderin was always present in lipodermatosclerotic skin and ulcers. Occasionally, haemosiderin was found in the apparently normal perilesional skin of C4b and C6 legs. The regenerating dermis at the base of healing ulcers showed none or light haemosiderin deposition.

Conclusion: Iron overload is not present in the less severe stages of skin damage due to CVD but lipodermatosclerosis and leg ulcers are always accompanied by haemosiderin deposition. In fact, no severe skin changes occur in CVD legs until iron overload occurs. Our results are in agreement with previous reports suggesting that a genetic inability to counteract skin iron overload is common in CVD legs.

* Corresponding author. A. Caggiati, M.D., Department of Anatomy, University ‘La Sapienza’, Via Borelli 50, 00161, Rome, Italy. Tel.: +39 06 4991 8114; fax: +39 0 4991 8081.
E-mail address: alberto.caggiati@uniroma1.it (A. Caggiati).
Legs afflicted with chronic venous disease (CVD) may undergo skin changes ranging from small spot-like pigmentation to large ulcers. According to the clinical, etiologic, anatomic and pathophysiologic (CEAP) classification, the presence of skin lesions characterizes the severity of CVD. Although a number of cellular and molecular mediators, biochemical mechanisms and structural changes have been described in detail, the exact pathogenesis of stasis dermatitis is still not completely understood. However, all current studies agree that skin changes are due to an inflammatory process triggered by venular hypertension.

An increased content of iron in the skin of CVD legs was first reported in 1965 by Myers and is currently explained by extravasation and disruption of erythrocytes, followed by decomposition of haemoglobin. The resulting excessive tissue iron (Fe$$^{++}$$, ferric ions) is stored as haemosiderin. The abnormal presence of this compound in the skin of CVD legs has been demonstrated by Perl’s Prussian Blue stain (PPB).

Haemosiderin is currently considered to be the cause of the brownish skin pigmentation typical of CVD. An increasing interest in the role of haemosiderin in the pathogenesis of venous disease followed the demonstration of either the pro-inflammatory properties of ferric ions and the link between venous ulcer and genetical disorders of iron metabolism. Despite these important data, no study has systematically evaluated haemosiderin accumulation in relation to the presence and severity of skin changes.

To contribute to an understanding of the effective role of ferric ions in the pathogenesis of CVD-related skin changes and ulcer development, we have morphometrically evaluated haemosiderin deposition in skin samples from legs afflicted with CVD at different clinical stages of the CEAP classification.

Patients, Materials and Methods

A total of 44 Caucasian patients in whom clinical investigation and duplex ultrasonography demonstrated the presence of CVD were included in this study. The severity of CVD in each leg was assigned to the appropriate CEAP clinical class by a physician skilled in the management of venous disease. The severity of pigmentation was scored according to the Venous Clinical Severity Score (VCSS).

As many as 85 skin biopsies were taken from the medial aspect of 49 legs with CVD (C2: 16 legs; C3: four legs; C4A: 12 legs; C4B: 10 legs; C6: seven legs) in 44 patients (20 men, 24 women; mean age 62 years). A total of 49 biopsies were from apparently normal skin, 12 were from pigmented lesions (seven scored 1–2 by VCSS; five scored 3), 10 were taken from skin areas with lipodermatosclerosis (LDS) and 14 were from open ulcers. No samples were obtained from C5 legs.

In C2, C3 and C4 legs, punch biopsies (2–4 mm) were obtained mostly during surgery for varicose veins (the resulting holes were then used to perform avulsion of varicosities). Specimens from C6 legs were also obtained during ulcer debridement or skin grafting. All biopsies included the whole dermis and at least a thin layer of subcutaneous tissue.

All legs with skin changes (pigmentation, LDS or ulcer) underwent at least two biopsies, one from the affected skin and one from the adjacent apparently normal skin. In C6 legs, ulcer tissues were taken from both the wound edge and ulcer bed. Finally, four skin biopsies were taken from the medial aspect of the leg in four subjects (two male, two female; mean age 57.7 years) in whom there was no clinical or duplex evidence of CVD. These patients were undergoing orthopaedic surgery. Skin biopsies were fixed in 10% buffered formalin solution and routinely processed for haematoxylin/eosin (HE) and PPB stains. This technique is able to demonstrate the presence of haemosiderin in the dermis as has been previously demonstrated.

Quantitative measurements of haemosiderin deposition were performed on digital images captured by the D-Sight Fluo microscope (Menarini, Italy). PPB stain positive areas were objectively quantified using the D-Sight Viewer package (Menarini, Italy) where the margin of the tissue was traced and the area containing haemosiderin was defined as the Area of Interest (AoI). This resulted in colour segmentation expressed...
as percentage of AoI. Data were finally transferred into an Excel (Microsoft) spreadsheet for further analysis.

The mean value of AoI and standard deviation (SD) were calculated in PPB + specimens grouped according to the CEAP-Class of the donor leg. Due to the small sample size, these values were compared by using Mann–Whitney test. A \( p \)-value < 0.05 was regarded as significant. Bonferroni’s correction was applied to control the possibility of a type I error.

Results

Haemosiderin was visible as granules (siderosomes), which appeared brownish or black in HE sections and blue in PPB sections (Fig. 1). Siderosomes were found within dermal macrophages (siderophages) (Fig. 1(A)) as well as in the interstitium between the collagen bundles of the dermis (Fig. 1(B)). In case of more intense haemosiderin deposition, PPB stained diffusely the dermis (Fig. 1(C)) and even the stroma of the subcutaneous layer (Fig. 2(D)). The prevalence of PPB + specimens and the mean values of AoI are reported in Table 3.

Control legs: All samples from control legs were negative for haemosiderin deposition (AoI = 0%).

Apparently normal skin from CVD legs: No haemosiderin was found in most (46/49) biopsies taken from apparently normal skin. The only exceptions were two samples taken from C4B legs in proximity of a lipodermatosclerotic plaque (AoI = 1.6 and 9.6, mean 5.6, SD 5.7) and one from a C6 leg taken close to the border of an active ulcer (AoI = 2.8%).

Pigmented skin: Haemosiderin was absent in all biopsies involving mild-to-moderate pigmentation (scored 1–2 by VCSS). It was found in only three out of five biopsies taken from severe pigmentation that scored 3 by VCSS (mean AoI = 10.3%, SD 11.4).

Lipodermatosclerotic skin: All these biopsies showed severe haemosiderin deposition in the dermis (Fig. 2(C)) and even in the subcutaneous layer (Fig. 2(D)) (mean AoI = 45%, SD 9.0).

Specimens from ulcerated skin: Specimens from ulcer tissues showed haemosiderin deposition (mean AoI of 28, SD 9.5) (Fig. 1(C)). No significant differences were seen between specimens from wound edge and bed (mean AoI: 28, SD 9.3 and 25 ± 17, respectively). In two ulcers, which showed reparative phenomena (Fig. 3), the regenerating dermis at the wound bed was nearly completely devoid of haemosiderin (AoI: 0% and 7.4%, respectively).

Statistical evaluation

A Mann–Whitney \( U \) test with Bonferroni’s correction demonstrated that haemosiderin deposition was
significantly higher in lipodermatosclerotic skin if compared with samples from heavily pigmented skin \((p < 0.01)\) and ulcer tissues \((p < 0.01)\).

**Discussion**

Iron is normally present in the skin because about 20–25% of absorbed iron is daily eliminated by exfoliation of epidermal cells. As a protection against its toxicity, iron is sequestered in iron-binding or haeme-containing proteins (ferritin and haemosiderin, respectively). Haemosiderin is currently considered the histological marker of tissue iron excess.

In the past, skin iron overload was demonstrated in CVD legs by PPB, X-ray spectrometry and atomic absorption spectrum analysis. However, most of these investigations considered ulcerated legs. The present study is thus the first to have systematically evaluated skin haemosiderin deposition in legs at any stage of severity of CVD. The main findings can be summarised as follows:

No haemosiderin deposition was found in the normal skin of C2, C3 and C4A legs, in less severe pigmentation and in part of more severe ones. By contrast, haemosiderin was always present in lipodermatosclerotic skin and active ulcers. Occasionally, haemosiderin was found in the apparently normal perilesional skin of C4b and C6 legs.

These results indicate that haemosiderin deposition does not intervene in the initial phases of stasis dermatitis. In turn, PPB-positive ferric overload accompanies the worsening of skin damage and, from the histological point of view, it characterises LDS and ulcers. Iron loss due to ulcer secretion could explain why iron content in ulcer samples is lower than in LDS skin.

The regenerating dermis at the base of two healing ulcers showed none or very light haemosiderin deposition. This agrees with previous studies, which demonstrated that iron levels decrease when venous ulcer heals.

Unfortunately, biopsies of healing or healed ulcers are difficult to obtain due to ethical implications. We could only hypothesise that reduction of PPB staining might correlate with ulcer healing.

Iron is a well-known mediator of skin toxicity in a variety of pathological conditions including sunburn, porphyria cutanea tarda, inflammation and even skin cancer. In CVD legs, it has been demonstrated that ferric ions contained in haemosiderin intervene in the pathogenic chain leading to matrix disruption and ulcer development by generation of free radicals, activation of metalloproteinase or, finally, down-regulation of tissue inhibitors of metalloproteinase. Our findings do not explain the exact iron-derived mechanism of skin damage but clearly demonstrate that severe skin changes and tissue iron overload are highly correlated.

<table>
<thead>
<tr>
<th>C Class</th>
<th>C2</th>
<th>C3</th>
<th>C4A</th>
<th>C4B</th>
<th>C6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparently normal skin</td>
<td>0/16 (0%)</td>
<td>0/4 (0%)</td>
<td>0/12 (0%)</td>
<td>2/10 (5.6%)</td>
<td>1/7 (2.8%)</td>
<td>49</td>
</tr>
<tr>
<td>Pigmentations ++/++</td>
<td>–</td>
<td>–</td>
<td>0/7 (0%)</td>
<td>–</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>Pigmentations ++++</td>
<td>–</td>
<td>–</td>
<td>3/5 (10.3%)</td>
<td>–</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Lipodermatosclerotic</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>10/10 (45.0%)</td>
<td>–</td>
<td>10</td>
</tr>
<tr>
<td>Ulcer edge</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6/7 (28.0%)</td>
<td>7</td>
</tr>
<tr>
<td>Ulcer bed</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>7/7 (25.4%)</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 3 Prevalence of PPB-positive samples and intensity of haemosiderin deposition. Samples are designated according to both C-class of the donor leg and clinical appearance of the skin where biopsies were taken. Mean AoI between brackets.
In 1868, John Gay noted that "varicose veins can be present for many years without any ulcer or bronzing of the skin...". In medical practice, it is a common finding to see legs with severe and long-lasting CVD and normal skin or, by contrast, young subjects with heavy pigmentation and even LDS or ulcer (Fig. 3). Similarly, skin iron overload correlates with the entity of skin changes but not with the duration and severity of CVD. It is evident that impairment of venous haemodynamic is "an essential but insufficient factor in explaining subsequent skin changes".

Our findings suggest that patients with LDS or ulcer are unable to counteract CVD-induced skin iron overload. It has been hypothesised that such inability could be genetically determined. This has been demonstrated in other cutaneous pigmentation diseases such as porphyria cutanea tarda and explains why cutaneous siderosis occurs in only about one-half of patients with haemochromatosis.

Recently, a strong genetic component in the pathogenesis of ulcer has been reported. In particular, a positive correlation has been demonstrated between susceptibility, healing time and size of venous ulceration with mutations of genes involved in iron metabolism. If these mutations are currently considered asymptomatic for systemic diseases, in combination with an acquired condition (venular hypertension) they could be responsible of LDS and ulcer.

The weaknesses of this study include the fact that few biopsies were taken from healing ulcers and none from C5 legs due to ethics committee restrictions. These limited the extent of this research. We have so far not performed genetic investigation to assess iron metabolisms disorders in these patients.

In conclusion, our findings indicate that haemosiderin does not intervene in the initial phases of skin changes in venous disease and that its deposition appears to be obligatory when severe skin changes and ulceration develop. Our results are in agreement with previous reports suggesting a genetic inability to counteract skin iron overload. Considering that at least 10% of Caucasians are carriers of iron metabolism disorders, it is desirable that genetic investigation should be included in the clinical evaluation of patients with CVD. This could help clinicians to identify leg ulcers that require advanced wound-healing treatment or surgery, in conjunction with compression therapy.

Conflict of Interest

None.

Acknowledgements

This study was supported by funds from Italian MURST and by a grant from Servier Company.
References


