Skin erythrodiapedesis during chronic venous disorders

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Background: **Extravasation of erythrocytes (erythrodiapedesis [ED]) is currently included among causes of skin damage in legs with chronic venous disorders (CVD) and ascribed to venular hypertension. ED is followed by erythrocyte disruption, degradation of hemoglobin, and storing of ferric iron into hemosiderin. The aim of this study was to evaluate the occurrence of ED in the skin of legs with different clinical stages of CVD.**

Methods: **One hundred eighteen skin biopsies from legs with CVD underwent histologic evaluation for ED and hemosiderin deposition (HD).**

Results: **ED was found in only 21/118 specimens. In particular, it was found in ulcer samples, in tissues surrounding varicophlebitis and, finally, in acute eczematous skin. ED was found in only 15/30 samples showing HD.**

Conclusion: **Our findings confirm the occurrence of ED during CVD. However, it was found only in concomitance of severe dermal inflammation. Hemosiderin deposition in the absence of actual ED could be explained with previous healed episodes of skin inflammation. However, ED is not likely the only cause of skin iron overload, which could also occur by a molecular mechanism. Further studies are needed to define the mechanism of iron deposition in the skin of legs afflicted with CVD. (J Vasc Surg 2011;53:1649-53.)**

In 1980, an elegant electron microscopic study demonstrated extravasation of erythrocytes (erythrodiapedesis [ED]) in the skin surrounding venous ulceration.[1] These findings were reproposed in 1981 by the same group.[2] Since then, an important role is commonly attributed to ED in the pathophysiology of skin changes and ulceration associated to chronic venous disorders (CVDs).[3-5]

ED is currently attributed to venular hypertension, which would impel erythrocytes to migrate across the microvascular wall into the dermis.[6] Here, erythrocytes would be disrupted and hemoglobin decomposed. The resulting excessive tissue ferric ions are finally stored in hemosiderin, the role of which in the pathogenesis of venous ulceration has been widely demonstrated.[7,8]

Despite the relevant pathophysiologic role currently attributed to ED, no study has to date systematically evaluated its occurrence in relation to the severity of CVD, presence of skin changes, and iron deposition. Therefore, in order to better define the pathophysiologic role of ED, skin biopsies from legs presenting different clinical stages of CVD were microscopically and histochemically evaluated.

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METHODS

One hundred eighteen skin biopsies were taken from the medial surface of 67 legs of 58 patients (23 males, 35 females; mean age, 58.8 years) (Table I) undergoing surgery for varicose veins and venous ulcers debridements were considered. History or evidence of morbidities or treatments possibly influencing skin trophism and metabolism were considered exclusionary (Table II). All patients gave their informed consent to be enrolled into the study that was previously approved by the local ethic committee. The gravity of CVD was determined in each leg according to the Clinical-Etiology-Anatomy-Pathophysiology classification.[3] The severity of symptoms was scored according to the Venous Clinical Severity Score (VCSS).[5]

Skin biopsies were obtained mostly by punches (2-4 mm) during surgery for varicose veins or superficial thrombectomy. 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 pigmentation, lipodermatosclerosis, or ulcer underwent at least two biopsies, from both lesional skin and adjacent apparently normal skin. Finally, four biopsies were taken from the medial face of the leg of four subjects undergoing orthopedic surgery (two males, two females; mean age, 57.7 years), which did not show clinical or duplex evidence of CVD.[9]

Skin biopsies were fixed in 10% buffered formalin solution, embedded in paraffin wax and sectioned at 5 μm for hematoxylin and eosin (HE) and Perl's stainings.[10] Samples were considered positive for ED when erythrocytes were seen crossing the microvascular wall (Fig 1, *A*) or lying in the surrounding interstitial tissue (Fig 1, *B-D*). The quantity of extravasated erythrocytes varied greatly between ED-positive samples. As a consequence, the entity of ED has been calculated in a semiquantitative manner as

Table I. Designation of samples according to the C-class of the donor leg

CEAP class	*C2*	*C3*	*C4a*	*C4b*	*C6*
Number of legs	31	5	14	10	7
Number of biopsies	34	13	28	20	23

Table II. Exclusion criteria

(1) Arterial diseases, lymphatic disorders, microangiopathies (diabetes, rheumatoid arthritis, vasculitis, collagen vascular disease)
(2) Dermatologic disorders
(3) Hematologic diseases
(4) Cardiac, pulmonary, renal, or hepatic failure
(5) Previous surgical treatment, trauma, or irradiation of the leg
(6) History or current administration of drugs known to affect skin trophism and iron metabolism

follows: Each specimen was observed at low magnification (×10) and 12 areas were delimited. Then, each area was observed at a higher magnification (×20-×40) and ED quantified according to a nonparametric score (Table III). The mean score was finally calculated for each ED-positive specimen and designated as ED index (EDi).

Samples were considered positive for hemosiderin deposition when Perl's positive material was present within macrophages (Fig 2, *A* and *B*) or scattered within the interstitial spaces (Fig 2, *C*).

RESULTS

Control specimens showed no ED or hemosiderin deposition. Only 21/118 skin biopsies from CVD legs showed ED (Table IV). Extravasated erythrocytes were found in all biopsies from ulcer tissues (mean EDi, 2.19) and in two samples from periulcerative lipodermatosclerotic skin (EDi, 1.7 and 1.9, respectively). In turn, seven biopsies taken from apparently normal skin of the same C6 legs did not show ED. In addition, ED was found in two specimens from skin with acute eczema (mean EDi, 1.15) and three samples of erythematous skin overlying saphenous tributaries with varicophlebitis (mean EDi, 1.45).

ED was not found in the remaining 97 biopsies. In particular, ED was absent in samples from C2 legs with long-lasting extensive varicose veins (graded 3 by VCSS, mean duration >20 years), biopsies from C3 legs with severe edema (graded 3 by VCSS) (Fig 2, *D*), and specimens from normal, pigmented, or lipodermatosclerotic skin of C4 legs.

ED and hemosiderin deposition. Perl's staining demonstrated iron overload in 30/118 specimens, but ED was found in only 15 samples of this series, all belonging to C6 legs. No ED was found in the remaining Perl's positive samples even in the presence of a heavy iron deposition (Fig 2, *A* and *B*).

DISCUSSION

ED is currently described as a common phenomenon in the skin of CVD legs and attributed to venular hypertension.[1-6] Moreover, ED is considered the cause of the iron overload responsible of severe skin changes in CVD legs.[8]

The present findings, obtained after an accurate stratification of samples according to the CEAP classification of the donor leg and the appearance of the skin where biopsies were taken, do not fully support these statements. In fact, ED was found in only a minority of legs and did not correlate to severity of CVD nor to skin iron deposition.

In particular, (1) ED was found only in the presence of acute skin inflammation, independently from the C-class of the donor leg, with greater EDi in ulcerated skin and surrounding tissues (mean EDi, 2.19 and 1.8, respectively); (2) all samples from apparently normal skin did not show ED, even if belonging to C4b and C6 legs; (3) ED was not found in samples from edematous, pigmented, or lipodermatosclerotic skin of C3 and C4 legs; and (4) the occurrence of ED and its intensity were not associated with the presence and severity of skin iron overload.

The apparent disagreement between our findings and current knowledge about ED raise important questions, which can be explained by an accurate re-evaluation of the literature.

Does ED really occur in CVD legs? ED was demonstrated in 1980 ". . .in six cases of severe chronic venous insufficiency. . .by biopsies from skin surrounding crural ulcers."[1] These data were republished in 1981.[2] In the latter article, it was omitted that all biopsies belonged to ulcerated legs and it was lapidarily stated that ED is "a regular finding in chronic venous stasis syndrome." Unfortunately, both articles appeared in poorly diffused journals and only their abstracts were available on the Web. Unavailability of the full texts has probably induced to believe that ED is really diffused to all legs with venous insufficiency.

The hypothesis of an erroneous interpretation of literature is strengthened by the interesting article of Pappas et al.[11] They clearly demonstrated by electron microscopy normal microvascular walls even in the presence of severe skin lesions (lipodermatosclerosis) and no differences in width of interendothelial junctions between the C classes. Accordingly, Pappas et al criticized the theories of Wenner and Leu[1,2] affirming that ". . .*widened gap junctions observed in previous reports may be a result of biopsies taken from ulcer edges or granulating ulcer bases,*" as it was indeed the case. The conditional used by Pappas et al demonstrates that they knew the theories of Wenner and Leu but could not read the whole articles. This erroneous interpretation possibly recurred all times the articles of Wenner and Leu were quoted to include ED between microvascular changes typical of CVD-legs.

Surprisingly, the strong criticism from Pappas et al passed unnoticed and the theories on ED continued to diffuse. This is even more surprising if considering that: (1) no images of extravasated erythrocytes appeared in all articles that mentioned ED and its pathophysiologic role;[3-

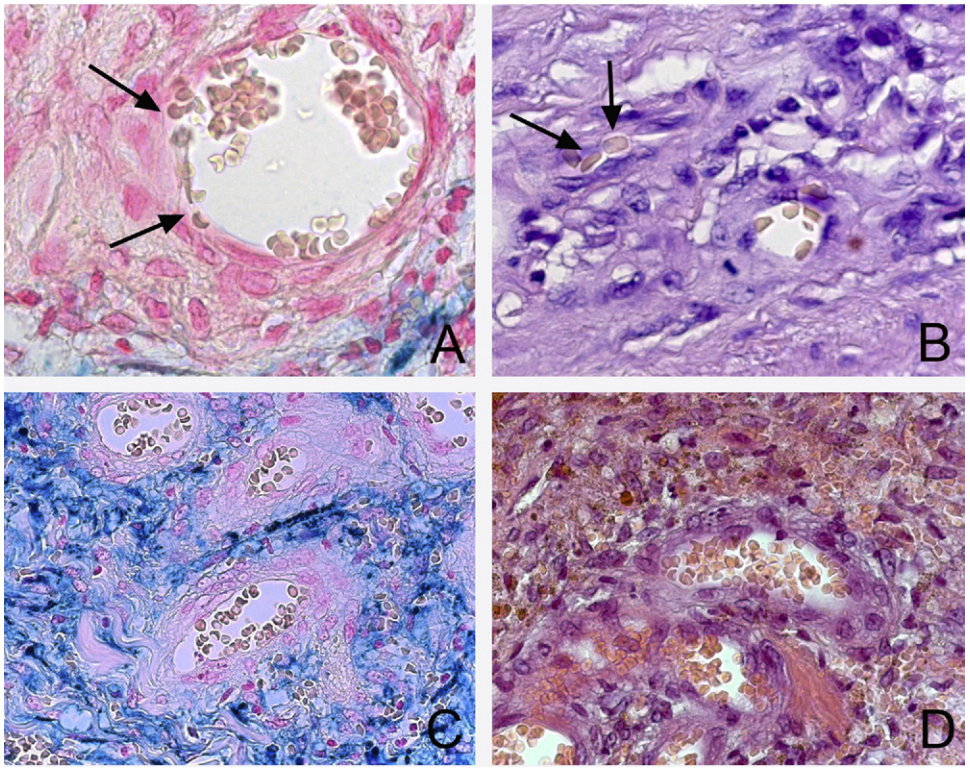

Fig 1. Erythrodiapedesis (ED) in the skin of chronic venous disorder (CVD) legs. **A,** ED grade 1: Erythrocytes crossing the venular wall (*arrows*). Hematoxylin and eosin (HE), original magnification: ×40. **B,** ED grade 2: Scattered erythrocytes in the interstitial tissue (*arrows*). HE, original magnification: ×40. **C,** ED grade 3: Cluster of erythrocytes in the interstitial tissue. Perl's, original magnification: ×20. **D,** ED grade 4: Microhemorrhage in the skin surrounding an active ulcer. HE, original magnification ×20.

Table III. Erythrodiapedesis (ED) score

Grade 0: No extravasated erythrocytes
Grade 1: Erythrocytes crossing the microvascular wall (Fig 1, *A*)
Grade 2: Scattered erythrocytes in the interstitial space (Fig 1, *B*)
Grade 3: Clusters of extravasated erythrocytes (Fig 1, *C*)
Grade 4: Microhemorrhage (Fig 1, *D*)

[8,12-14] (2) ED was not mentioned in the subsequent articles of Leu on skin changes related to CVD;[15,16] and (3) none of the important studies, which investigated microvascular changes and cellular infiltration in CVD legs, demonstrated ED, even if light microscopy,[12,17-23] transmission electron microscopy,[11,12,24] histochemistry,[25,26] immunohistochemistry,[12,19,24] or immunocytochemistry[14] were used.

Our results confirm that ED occurs in ulcer tissues[1,2,7,8,13] and in lipodermatosclerotic skin with acute inflammation.[27,28] Moreover, our findings demonstrate that ED may occur also in the skin of legs with less severe grading of CVD but only in the presence of acute dermal inflammation.

What causes ED? It is currently reported that ED is due to venular hypertension.[6] This pathogenesis was hypothesized by Wenner et al in 1980 (". . .alterations are supposed to be due to the increased intravenous pressure."),[1] but it became an absolute certainty 1 year later, even if no pressure measurement was performed: ". . .under the influence of an elevated intravenous pressure, erythrocytes are passively squeezed through the endothelial lining."[2] Nevertheless, no study has up to now correlated skin changes to pressure measurements to possibly confirm or exclude such hypothesis.[7,11-28] We did not evaluate venular pressure, but ED was not found in the normal skin of C4 and C6 legs, nor in legs with objective signs of severe impairment of blood return (edema or varicose veins graded 3 by VCSS). In turn, ED was always found in samples from skin with acute inflammation. These evidences led us to suppose that, more than to venular hypertension, ED is likely to be due to inflammatory mediators, which possibly determine the opening of endothelial cells' junctions. Such a hypothesis is corroborated by the knowledge that inflammation has a key role in skin damaging during CVD[29] and that a large number of mediators are able to increase endothelial permeability[30-31] and to induce microhemorrhage.[32]

Is ED the cause of skin iron overload? According to our data, ED was not associated with skin iron overload. The absence of ED in Perl's positive specimens from C4

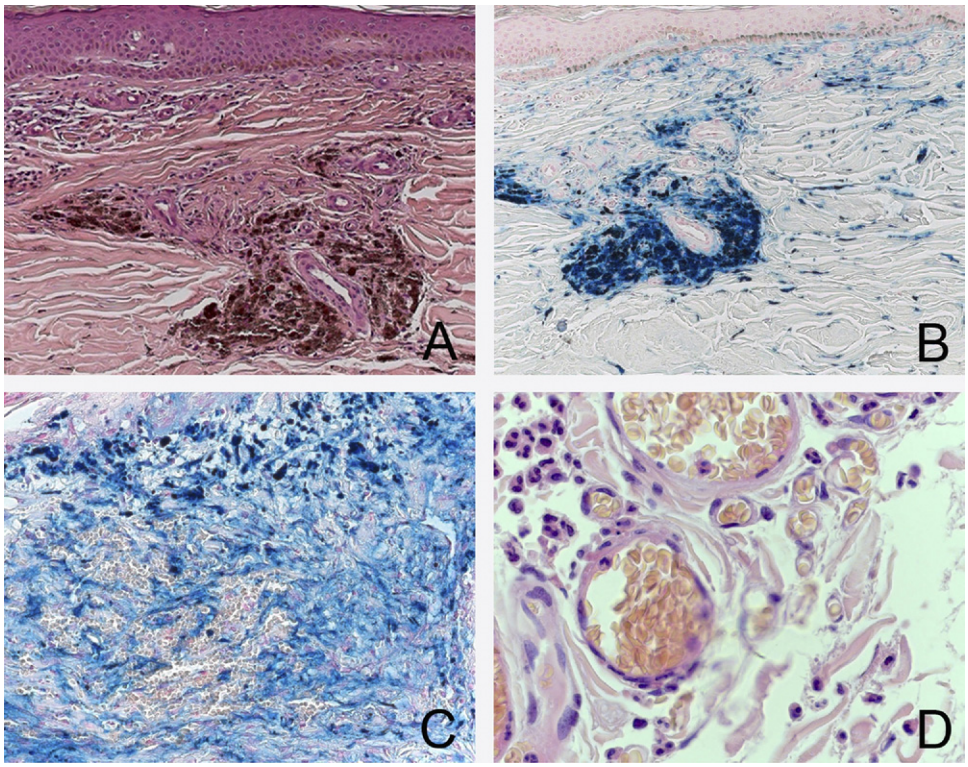

Fig 2. Erythrodiapedesis and iron deposition and in chronic venous disorder (CVD) legs. **A,** Hemosiderin granules are visible within macrophages. No extravasated erythrocytes are visible. Hematoxylin and eosin (HE), original magnification: ×40. **B,** The corresponding Perl's stained section ×40. **C,** Perl's diffusely stains interstitial spaces where clusters of extravasated erythrocytes are present (original magnification ×20). **D,** Skin biopsy from a C3 leg. Despite the severe dermal edema, no extravasated erythrocytes are visible. HE, original magnification: ×40.

Table IV. Occurrence of erythrodiapedesis (*ED*) and hemosiderin deposition (*HD*)

C-class skin appearance	*C2*		*C3*		*C4a*		*C4b*		*C6*	
	ED	*HD*	*ED*	*HD*	*ED*	*HD*	*ED*	*HD*	*ED*	*HD*
Apparently normal	0/31	0/31	0/5	0/5	0/14	0/14	0/10	2/10	0/7	1/7
Edematous			0/8	0/8						
Acute inflammation	3/3 (1.45)	0/3			2/2 (1.15)	0/2				
Pigmented					0/12	2/12				
Lipodermatosclerotic							0/10	10/10	2/2 (1.8)	2/2
Ulcerated									14/14 (2.19)	13/14

Samples are designated according to the C-class of the donor leg and clinical appearance of the skin where biopsies were taken. Mean ED index (Edi) is reported between brackets.

legs suggests to us two hypotheses. First, hemosiderin deposition could be related to previous healed episode(s) of skin inflammation. Second, iron transportation across the microvascular wall should occur by a molecular mechanism. This hypothesis is supported by the knowledge that iron is physiologically able to cross the microvascular wall and to migrate across the dermis if conjugated to specific proteins.[33] Such a molecular mechanism allows the physiologic elimination of about one-fourth of excessive body iron.[34,35]

Based on the present findings, we cannot identify which protein causes skin iron accumulation in CVD legs. However, previous experiences are suggestive for a possible role of ferritin in iron transcapillary transportation and hemosiderin accumulation in CVD legs.[36-38] In fact, an increased skin content of ferritin was demonstrated in legs with severe impairment of venous return,[36,37] and it is well known that excessive ferritin is converted to hemosiderin by degradation of its protein shell.[38]

CONCLUSION

The present study is the first one to have systematically evaluated the occurrence of ED in skin specimens from CVD legs after an accurate stratification of samples. Our

findings demonstrate that ED occurs during acute skin inflammation. Further studies are needed to demonstrate the molecular mechanisms allowing erythrocytes' extravasation as well as those responsible for iron transcapillary transportation and hemosiderin accumulation in the skin.

AUTHOR CONTRIBUTIONS

Conception and design: AC
Analysis and interpretation: AC, CR
Data collection: MF, CR
Writing the article: AC, RH
Critical revision of the article: AC
Final approval of the article: AC
Statistical analysis: AC
Obtained funding: AC
Overall responsibility: AC

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