Abnormal venous function in patients with homozygous sickle cell (SS) disease and chronic leg ulcers

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ABSTRACT

Chronic leg ulceration is a major cause of morbidity in homozygous sickle cell (SS) disease in Jamaica. These ulcers have features in common with venous ulcers in patients with a normal haemoglobin genotype (AA). Thus we sought to determine whether there is abnormal venous function in the legs of patients with SS disease who have ulcers. Experiments were performed on 15 SS patients with ulcers, and on 15 SS patients and 15 AA subjects with no history of leg ulcers. Changes in venous blood volume of the bottom one-third of the leg induced by venous occlusion and release were studied by air plethysmography, providing indices of segmental venous capacitance (SVC), maximal venous outflow (MVO) and venous emptying time (VET). The changes in volume (ambulatory volume change; AVC) induced by a period of leg exercise were also measured at the ankle (AVCa) and calf (AVCc); venous refilling times at these sites (RTa and RTc respectively) were also measured. Finally, cutaneous red blood cell flux recovery time (FRT) after ankle exercise was assessed by laser Doppler flowmetry. Measurements were also made of haematological variables. SVC, MVO and VET did not differ between the groups, indicating no deep venous obstruction in the SS patients with ulcers. AVCa, AVCa and RTc did not differ among the three subject groups. However, compared with AA subjects, SS patients with ulcers had reduced RTa and FRT. Moreover, RTa and FRT were further shortened in SS patients with ulcers relative to SS patients without ulcers. Since the levels of anaemia were similar in SS patients with and without ulcers, these differences cannot be attributed to differences in arterial flow secondary to anaemia. These results suggest abnormal venous function in SS patients with ulcers, relative to both AA subjects and SS patients without ulcers. We propose that there is incompetence of venous valves draining the ankle region of SS patients with ulcers: the consequent raised venous pressure contributes to the slow healing and, possibly, to the onset of leg ulceration in SS disease.

INTRODUCTION

Chronic leg ulceration is a major cause of morbidity in homozygous sickle cell (SS) disease, affecting up to 75% of Jamaican adults aged over 30 years [1]. Rapid healing of the ulcers on complete bed rest and deterioration on prolonged standing suggests a role of high venous pressure in their maintenance. This might be due to an impaired cutaneous postural vasoconstrictor response to dependency, as observed in diabetes mellitus [2]. How-
ever, our previous study indicated that this is unlikely, as postural vasoconstriction in normal skin did not differ between SS patients with and without ulcers, and was comparable in absolute terms with that observed in subjects with normal haemoglobin genotype (AA) [3].

An alternative possibility, i.e. that the ulcers are associated with abnormal venous function, was examined in the present study. This is particularly attractive, for, in AA subjects, abnormal venous function and chronic venous hypertension of the legs may be accompanied by pre-ulcerative skin changes in the legs, including hyperpigmentation and mild ankle oedema with thickening and hardening of subcutaneous tissue [4]. Such skin changes are similar to those that precede spontaneous leg ulcers in SS disease (J. S. Mohan, unpublished work). Other similarities of SS leg ulcers and venous ulcers include their location on the distal third of the leg above the ankle, the pain and persistence of the ulcers, and the relief of pain by rest and elevation of the leg [1,5].

Limited data are available on venous function of the legs in SS disease. A study on a mixed group of 16 patients with active ulcers who had either SS disease or Sβthalassaemia showed rapid refilling times after venous occlusion. This was attributed to the high peripheral blood flow that is typical of sickle cell disease, and not to venous insufficiency [6]. However, these authors based their conclusions on the observations that venous pressures were normal when measured invasively in just four of 16 of this mixed group of patients and that venography performed in another four patients indicated no venous insufficiency in the area of the ulcer. No quantitative data were provided for the ‘rapid’ refilling times or ‘normal’ venous pressures in patients with ulcers to support the conclusions, and no observations at all were made on SS patients without ulcers.

Thus, in the present study, we have performed a series of tests of venous function in SS patients with and without ulcers and in AA subjects without ulcers to test the hypothesis that venous function is abnormal in SS patients with ulcers.

**MATERIALS AND METHODS**

**Subjects**
The SS patients attended the Sickle Cell Clinic of the University Hospital of the West Indies, Kingston, Jamaica, and the study was confined to males with SS disease to avoid possible confounding effects of the menstrual cycle on peripheral blood flow [7,8]. Most patients were participating in a Cohort Study which also provided the group of 15 AA control subjects [9]. The study groups comprised 15 SS patients with active ulcers, 15 SS patients with no history of leg ulcers, and 15 AA subjects, the age ranges being 19–24, 17–24 and 21–24 years respectively. The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association [10], and was approved by the Ethics Committee of the University Hospital, University of West Indies. Each subject gave informed consent to participate in the study.

**Exclusion criteria**
Since the measured venous variables may be influenced by arterial inflow, all subjects were screened for an ankle/brachial systolic pressure index (ABPI; see below) to ensure an index greater than 1 and therefore to exclude subjects with occlusive arterial disease [11] from the study. Subjects with swelling of the lower limb, or a history of cardiac failure, renal, liver, endocrine or lymphatic disease, were not recruited to the study.

**Methods**
All experiments were performed between 10.00 and 12.00 hours, since baseline peripheral blood flow falls in the early afternoon compared with morning values in both SS patients and AA subjects [12].

**Measurement of ankle and arm systolic blood pressure**
Arterial blood pressure was determined by the oscillometric method with an air plethysmograph (VascuMAP model AP 102V; Carolina Medical Inc., King, NC, U.S.A.). With the subject resting quietly in the supine position, ankle and arm systolic blood pressures were measured by using a standard-width air cuff (9 cm or 12 cm, according to the circumference of the limb); the ABPI was calculated for each subject.

**Measurement of the change in leg volume during venous occlusion**
With the subject in a supine position, the change in volume of the bottom one-third of the leg during venous occlusion was measured by using the VascuMAP air plethysmograph cuff. This was fitted on to the lower leg above the malleolus, and a sphygmomanometer cuff was placed on the leg, proximal to the air cuff. The sphygmanometer cuff was inflated to 60 mmHg to temporarily occlude venous drainage, and the increase in volume of the leg distal to the occluding cuff was measured using the VascuMAP air cuff (see Figure 1). After approx. 2 min a plateau was reached, indicating an equilibrium between arterial inflow and venous outflow. The maximal increase in volume was measured [13]; this was termed the segmental venous capacitance (SVC) [14]. After deflation of the sphygmanometer cuff, measurements were made of the fall in volume within the first 1 s, or the maximal venous outflow (MVO) [13], and the interval between release of the occlusion and the restoration of leg volume to pre-occlusion levels, or venous emptying time (VET; see Figure 1).
Venous insufficiency in sickle cell disease

Figure 1 Diagrammatic representation of leg volume changes during temporary arrest of venous return by inflation of a sphygmomanometer cuff to 60 mmHg

Figure 2 Diagrammatic representation of leg volume changes during leg exercise

Measurement of changes in leg volume during and following leg exercise

The air plethysmograph cuff was fitted on to the bottom one-third of the leg above the malleolus while the subject sat in a chair with his legs placed firmly on the floor in front of him. The subject then plantar-flexed the foot 10 times in 15 s, so as to empty the venous system of the lower leg [13]. The change in volume of the lower leg (ankle) was measured during exercise (ambulatory volume change; AVCa) and the time required for the ankle volume to return to pre-exercise levels (refilling time; RTa) were recorded (Figure 2) [13]. This same procedure was performed while the cuff was fitted around the calf region, so that AVC and RT for the calf could be measured (AVCc and RTc respectively).

Measurement of post-exercise cutaneous red cell flux recovery time (FRT)

Cutaneous red cell flux (RCF) was monitored continuously and simultaneously in both legs using laser Doppler flowmetry, as described previously [3]. The subjects sat comfortably with the feet in a non-weight-bearing position, to eliminate possible interference from muscle contraction [15]. The laser Doppler probes were fixed by double-sided sticky tape to the ankle region, 10 cm above the medial malleolus of each leg. After monitoring baseline values for 2 min, the subject was asked to dorsiflex the feet maximally 10 times within 15 s to empty the venous system of the leg; measurements were made of the time taken for the post-exercise cutaneous RCF to return to the pre-exercise level (FRT).

In a pilot study on five SS patients with ulcers and five AA subjects without ulcers, FRT was found to be similar to the post-exercise cutaneous refilling time measured by photoplethysmography: 14.2 ± 6.0 and 12 ± 6.5 s respectively in the SS patients with ulcers, and 52.8 ± 7.6 and 48 ± 22 s respectively in AA subjects without ulcers (J. S. Mohan, unpublished work). Therefore the value of FRT measured by laser Doppler flowmetry was taken to be comparable with the post-exercise cutaneous refilling time measured by photoplethysmography.

Protocol

All studies were conducted in a temperature-controlled (23–25 °C), quiet environment, after the subject had rested comfortably for 30 min in a supine position on a couch. The systolic blood pressure at the ankle and arm was measured twice at 2 min intervals. This was followed by two periods of venous occlusion at 2 min intervals, during which the change in the volume of the leg was recorded and the SVC, MVO and VET were determined.

The subject then sat in a chair with legs placed firmly on the ground, and four periods of foot plantar flexion were performed (see above). During the first two periods AVCa and RTa were measured, and during the latter two periods AVCc and RTc were measured.

After a 10 min rest, the subject moved back to the couch and dorsiflexion of both feet was performed while they were in a non-weight-bearing position (see above), so that FRT could be measured. This manoeuvre was repeated twice more at 2 min intervals.

Haematological analyses

Blood samples were taken from both groups of SS patients for measurement of haemoglobin concentration and red blood cell count using a Coulter Counter (Coulter Electronics, Hialeah, FL, U.S.A.). Packed cell volume was measured as the microhaematocrit.

Statistical methods

The average of the two measurements of ABPI, SVC, MVO, VET, AVCa, RTa, AVCc and RTc was calculated for each subject. Similarly, the average of the three measurements of FRT was calculated for each subject. The FRT was measured in the ulcerated leg in patients with unilateral ulcers and in a randomly chosen leg in patients with bilateral ulceration or no ulceration.

The distribution of each measurement was examined using informal graphical methods with formal inference provided by the Shapiro–Wilk test for normality [16].
Table 1 Variables used in the assessment of venous function in the legs of SS patients with and without a history of leg ulcers and of AA subjects

Values are given as median, interquartile range (IQR) and range for AVCa, RTa, AVCc, RTc, post-exercise FRT, SVC and MVO, and as mean (S. D.) for VET, haemoglobin concentration (Hb), red blood cell concentration (RBC) and packed cell volume (PCV). P values are for ANOVA after adjustment for correlation between variables (see text).

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<tr>
<th>Variable</th>
<th>SS patients</th>
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<td>IQR</td>
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<td>IQR</td>
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<td>AVCa (ml)</td>
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<td>-0.2 to</td>
<td>-0.1 to</td>
<td>-0.3</td>
<td>-0.2 to</td>
<td>-0.1 to</td>
<td>-0.4</td>
<td>-0.3 to</td>
<td>-0.1 to</td>
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<td>RTa (s)</td>
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<td>2.5–6.4</td>
<td>1.5–9.8</td>
<td>6.0</td>
<td>4.5–6.2</td>
<td>3.8–17.0</td>
<td>17.2</td>
<td>12.5–19</td>
<td>5.2–45.8 &lt; 0.001</td>
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<tr>
<td>AVCc (ml)</td>
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<td>-0.3 to</td>
<td>-0.2 to</td>
<td>-0.6</td>
<td>-0.4 to</td>
<td>-0.3 to</td>
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<td>RTc (s)</td>
<td>4.5</td>
<td>2.8–5.2</td>
<td>2.5–13.0</td>
<td>5.5</td>
<td>4.6–8.8</td>
<td>2.8–15.0</td>
<td>8.6</td>
<td>5.5–11.5</td>
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<td>Post-exercise FRT (s)</td>
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<td>5.4–11.8</td>
<td>3.7–18.7</td>
<td>22.3</td>
<td>18–27.3</td>
<td>14.7–31.3</td>
<td>43.3</td>
<td>37.7–51.3</td>
<td>22.0–55.0 &lt; 0.001</td>
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<td>SVC (ml/100 ml)</td>
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<td>0.2–0.4</td>
<td>0.1–1.2</td>
<td>0.3</td>
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<td>MVO (ml ⋅ min⁻¹ ⋅ 100 ml⁻¹)</td>
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<td>14.4–22.2</td>
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<td>11.4</td>
<td>7.8–19.2</td>
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<th>Variable</th>
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<td>No ulcers (n = 15)</td>
<td>AA subjects (n = 15)</td>
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<td>Mean (S. D.)</td>
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<td>VET (s)</td>
<td>2.2</td>
<td>(0.7)</td>
<td>2.6</td>
<td>(0.5)</td>
<td>2.6</td>
<td>(0.7)</td>
<td>0.17</td>
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<td>Hb (g/dl)</td>
<td>8.0</td>
<td>(1.2)</td>
<td>7.5</td>
<td>(1.6)</td>
<td>–</td>
<td>0.56</td>
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<td>10⁻¹² ⋅ RBC (cells/l)</td>
<td>2.8</td>
<td>(0.6)</td>
<td>2.7</td>
<td>(0.8)</td>
<td>–</td>
<td>0.2</td>
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<td>PCV (%)</td>
<td>23.2</td>
<td>(3.9)</td>
<td>21.5</td>
<td>(4.4)</td>
<td>–</td>
<td>0.52</td>
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*Tested between SS patients with and without ulcers only.

Summary measures, robust to deviation from normality (median, interquartile range, range), are presented for non-normal variables, and mean (S. D.) values are presented otherwise. Logarithmic transformations towards normality were performed where necessary.

One-way analysis of variance (ANOVA) on transformed variables was used to compare mean differences among the three subject groups independently for each variable. Since such analysis assumes that the variables are independent, ANOVA was then performed after adjusting for correlation between the variables by using multiple linear regression, with the aim of showing the strongest effects. Variables that showed significant differences across the three subject groups were examined in more detail, comparing means across the groups by using unpaired t tests. Significance was assumed at the 5% level, and analyses were conducted using Stata statistical software [17].

RESULTS

Table 1 shows the variables recorded and the P values for differences among the groups after adjustments had been made for correlation between variables. Other results of the statistical analyses are described below.

The measurements of venous volume during and after venous occlusion showed no significant differences among the three groups for SVC, MVO or VET (Table 1) before or after adjustment.

Measurement of the changes in ankle and calf volume during and after leg exercise showed significant differences among the three groups for RTa and RTc (P < 0.001 and P < 0.01 respectively). Significant correlations were also found between RTa and RTc, RTa and AVCa, and RTc and AVCc. Before adjustment was made for correlation between variables, the difference in RTc among the three groups reflected a significant difference between the SS patients with ulcers and the AA subjects (P < 0.05). When adjustment was made for correlation between variables, only RTa remained significantly different among the three groups (P < 0.001; see Table 1). This difference reflected a shorter RTa in both groups of SS patients than in AA subjects (P < 0.001 in each case), and a further shortened RTa in SS patients with ulcers compared with SS patients without ulcers (P < 0.05).
When RCF was monitored during and after dorsiflexion of the foot, FRT was significantly different among the three subject groups (Table 1). This was attributable to a shorter FRT in both groups of SS patients than in AA subjects, and also to a further shortened FRT in SS patients with ulcers compared with SS patients without ulcers (P < 0.001 in each case).

Haemoglobin concentration, red blood cell count and packed cell volume did not differ between SS patients with and without ulcers (Table 1).

**DISCUSSION**

The present study indicates that there are differences in venous function between AA subjects and SS patients without ulcers and, notably, between SS patients with and without ulcers. SS patients with ulcers had shortened RTa and shortened FRT relative to SS patients without ulcers. These differences appear to be consistent with venous insufficiency.

All of the subjects in the present study had an APBI greater than 1, so that significant arterial obstruction was unlikely. It is therefore reasonable to assume that the venous variables we measured were not altered by abnormally low arterial inflow. In fact, patients with SS disease have increased cardiac output at rest [19] and increased forearm blood flow [20], so that venous flow from the leg might reasonably be expected to be increased as a consequence of haemodynamic adaptations to the chronic anaemia of SS disease (see [20]). Since increased venous flow in SS disease is likely to influence the results of the venous function tests, any comparisons of the results of venous function tests in different groups of SS patients can only be reliable if the subjects have similar levels of anaemia. In the present study the SS patients with and without ulcers did indeed have similar levels of anaemia.

In studies of venous function, direct measurement of ambulatory venous pressure provides an accurate, quantitative method of assessment, but this is invasive and liable to complication, especially in oedematous limbs. However, measurements of the changes in leg volume induced during and after a period of venous occlusion or exercise can provide simple non-invasive methods for the assessment of venous function that correlate well with simultaneous direct measurements of venous pressure [13,18]. In the present study, the SVC was used as a measure of the venous capacity to accumulate blood in the segment of the leg enclosed by the VascuMAP air cuff during venous occlusion. It seems reasonable to assume that SVC is directly related to the venous capacitance of the whole calf, as measured in previous studies (see [13,14]). As in those studies, we used MVO to represent the functional capacity of the leg to drain blood collected during the period of occlusion [13]. When there is deep venous obstruction, both venous capacitance and MVO are reduced and, because the ability to empty accumulated blood is decreased, the VET is prolonged [13]. Since the values of SVC, MVO and VET for the two groups of SS patients in the present study did not differ from those of the AA subjects, it is reasonable to deduce that neither the SS patients without ulcers nor those with ulcers had deep venous obstruction. Thus failure of the calf muscle pump, either primary or secondary to venous incompetence, is a more likely mechanism for abnormal venous function in SS patients.

In normal limbs, contraction of calf muscles during leg exercise compresses the intramuscular and surrounding veins, forcing blood from superficial to deep veins and thence centrally towards the heart. Consequently, the volume change recorded on leg exercise (AVC) is substantial. By contrast, AVC tends to be reduced if there is incompetence of valves in superficial or deep veins, because blood may pass retrogradely into the superficial system, while AVC is substantially lower than normal if there is deep venous obstruction [5,13,21]. On this basis, the present observations that AVCc and AVCa were similar in the three groups of subjects reinforces the view that there was no deep venous obstruction in the SS patients with ulcers, and might seem to suggest that there was no venous incompetence either. However, it should be noted that, in previous studies using air plethysmography of the whole calf, which allows calibration of the absolute venous volume, the values of AVC overlapped in normal limbs and in those with superficial or deep venous incompetence [13,21]. Nevertheless, the limbs with incompetence could be distinguished from the normal limbs because they had a larger absolute venous volume and a smaller ejection fraction on muscle contraction [21]. Thus, in fact, the present observations allow the possibility of venous incompetence in the SS patients with ulcers. Supporting evidence for venous incompetence comes from our measurements of RT and FRT following exercise.

In the normal limb after exercise, the veins fill from the arteries in approximately 15 s, but in venous insufficiency they fill more quickly by retrograde flow or venous reflux [13]. Our finding that RTc and RTa differed significantly among the AA subjects and the two groups of SS patients, being shorter in the SS patients, could well be explained by the higher arterial inflow in SS patients as a consequence of their anaemia (see above). The significant correlation of RTc and RTa is consistent with that view. The fact that there was no significant difference between the RTc values of SS patients with and SS patients without ulcers, irrespective of whether adjustment was made for a correlation between RTc and other variables, suggests that there was no venous incompetence in the drainage of the calf region in SS patients with ulcers. However, the highly significant difference between the three groups for RTa values that persisted...
after adjustments were made for correlations and which reflected a difference between the SS patients with and without ulcers, as well as between each SS group and the AA subjects, raises the possibility of venous incompetence in the drainage of the ankle region of the SS patients with ulcers.

Similarly, if we assume that FRT measured by laser Doppler flowmetry in the superficial cutaneous microcirculation is comparable with recovery time measured by photoplethysmography in previous studies (see Introduction and Material and methods sections), then the present finding that post-exercise FRT in the ankle region was greater in AA subjects than in both groups of SS patients may be explained by higher peripheral blood flow in SS disease (see above). However, the fact that FRT was shorter in SS patients with ulcers than in SS patients without ulcers, despite their similar levels of anaemia, is consistent with incompetence in the venous drainage of the ankle region in those with ulcers.

In summary, on the basis of our comparative analyses, we conclude that SS patients develop ulcers in the supra malleolar region in the absence of deep venous obstruction. However, we propose that they do have incompetence of the venous system of the lower leg, and that this most probably affects drainage of the ankle region. We suggest that chronically raised venous pressure resulting from venous incompetence plays a role in the onset and slow healing of leg ulcers in SS patients, as well as between each SS group and the AA subjects, raises the possibility of venous incompetence in the drainage of the ankle region of the SS patients with ulcers.

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