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# Variations in white blood count, thromboxane B<sub>2</sub> levels and hematocrit in chronic venous hypertension

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**Objective:** To analyze variations in leukocyte count and thromboxane B<sub>2</sub> production in the femoral vein of patients with chronic venous hypertension (CVH). **Design:** Prospective clinical study, controlled, non randomized and open. **Location:** Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, referral center, university hospital. **Participants:** 15 patients with recurring stasis ulcer were analyzed, selected randomly from the venous diseases outpatient center, and 4 without lower limb venous alterations were also analyzed. **Intervention:** Blood samples from the femoral and brachial veins were drawn following supine and 45° reverse Trendelenburg. **Main outcomes measures:** Direct leukocyte count and analysis of the thromboxane B<sub>2</sub> with enzyme linked immunosorbent assay test. **Results:** After 30 minutes in reverse Trendelenburg, patients with CVH showed a leukocyte count reduced by  $\pm 27\%$  ( $p=0.02$ ) and thromboxane B<sub>2</sub> levels increased by  $\pm 158\%$  ( $p=0.02$ ). **Conclusions:** We suggest that future studies of medications for stasis ulcers include their effects on leukocyte entrapment and thromboxane B<sub>2</sub> production in the lower limb venous system.

**UNITERMS:** Venous ulcers. Venous insufficiency. White blood count. Thromboxane B<sub>2</sub>.

## INTRODUCTION

Venous stasis ulcer is a chronic disease with a high rate of recurrence. Coon<sup>(1)</sup> and Wright<sup>(2)</sup> found an incidence of 0.5% in the general population. In Brazil, similar data has been recorded, finding ulcers in 2.3% of men and 4% of women.<sup>(3)</sup>

The main pathophysiological mechanism responsible for chronic venous stasis is the high venous pressure maintained in the lower extremities while walking.<sup>(4-9)</sup> The logical treatment for stasis ulcer has always been based

on the pathophysiology of the disease, i.e. medical and surgical means to decrease the chronic venous hypertension (CVH) in the lower limbs.

Several hypotheses have attempted to explain the mechanism by which CVH leads to tissue destruction. Initially, Homanns<sup>(10)</sup> reported a reduced blood flow to the limbs, a theory which was soon discarded since studies demonstrated a greater flow in the femoral arteries of patients with CVH<sup>(11-13)</sup> than in normal individuals. Recently, the most popular theory in the literature<sup>(14,15)</sup> has been that the leukocytes are sequestered at the level of capillaries of the lower extremities, occluding them and causing regional hypoflow leading to ischemia and tissue destruction.

Several authors have confirmed the relationship between the production of thromboxane A<sub>2</sub> and the regional reduction of flow in different tissues, such as the splenic region,<sup>(16,17)</sup> the kidneys<sup>(18)</sup> and pulmonary

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hypertension induced by endotoxins.<sup>(19)</sup> In some cases the production of thromboxane A<sub>2</sub> is very high which might lead to ischemia and tissue injury, i.e. in coronary<sup>(20)</sup> and cerebral vasoconstriction,<sup>(21)</sup> in decubitus ulcers<sup>(22)</sup> and in skin burns.<sup>(23)</sup> Thromboxane A<sub>2</sub> is decomposed to thromboxane B<sub>2</sub>, a stable compound, and Dernell<sup>(24)</sup> found a relationship between a high level of thromboxane B<sub>2</sub> and the histological degree of skin injury.

The aim of this study was to determine whether these phenomena are of sufficient intensity to alter the white blood count (WBC) and thromboxane B<sub>2</sub> values at the femoral level, with different postural positions in chronic venous hypertension patients.

## METHODS

The study was performed on nineteen patients separated into two groups. The first group consisted of fifteen patients with CVH in at least one of the lower extremities and with a previous history of recurring stasis ulcer, selected randomly from the venous diseases outpatient center of the vascular surgery service of "Hospital das Clínicas", Medical School of São Paulo University, between January 1993 and July 1996, in accordance with the following inclusion criteria: 1) healed venous stasis ulcer; 2) venous refill time less than 20 seconds, confirmed by photoplethysmography; 3) ankle/brachial Doppler ratio over 0.9; 4) valvular incompetence of the deep venous system of the limb and absence of concomitant venous occlusion, either by extrinsic compression or deep non-rechanneled venous thrombosis demonstrated by duplex scanning; 5) insufficiency of the deep venous system as far as below the knee joint confirmed by descending phlebography; 6) not in drug therapy within the previous 10 days. This group was composed of 8 males and 7 females, mean age 36.33 years (range 18 to 53, standard deviation (SD) =12). Among the women, four had delivered three children, two had four and one had five. None was on hormonal contraceptives at the time of the analysis. All patients had had at least two episodes of stasis ulcer in the analyzed leg, with five of them reporting three or more recurrences. Four patients admitted having had a previous ulcer in the contralateral limb. In nine, the left lower extremity was analyzed and in six, the right one.

The second (control) group consisted of four patients (2 males and 2 females) without varicose veins in the lower extremities or prior history of deep venous thrombosis and with venous refill time >20 seconds. They had arterial

hypertension (they were not on medication) and were to be submitted to a renin test on blood taken from the renal vein, via the right femoral vein. The mean age was 39.50 years (range 18 to 58, SD=18.12).

After selection based on the inclusion criteria, the two groups underwent the following procedures:

1. a 60 minute rest period in the supine position.
2. placement of a cannula in the common femoral vein at the inguinal level for collection of blood samples (the patient was allowed to perform spontaneous dorsal flexion of the foot during the examination, although without flexing the thigh or knee joint).
3. collection of two samples, one for the hematocrit and WBC (in EDTA) and another for thromboxane B<sub>2</sub> analysis (in sodium citrate). The samples were immediately refrigerated at 5°C.
4. Patient accommodated in 45° reverse Trendelenburg position.
5. Blood samples collected after 30 minutes.
6. Patient returned to the supine position for collection of blood samples after 1, 5, 30 and 90 minutes (a total of 6 pairs of blood samples).
7. Collection of blood samples from a left arm vein, only for the first five patients in group I, following the same aforementioned steps.

The method used for analysis of the thromboxane B<sub>2</sub> was the ELISA (enzyme linked immunosorbent assay) test (International Immuno-Diagnostics®).

The statistical methods utilized were: Friedman's non-parametric test for analyzing the WBC and Thromboxane B<sub>2</sub> levels in group 1, the Matal-Hanzel contrast test to identify statistically different samples in group 1 and the Wilcoxon test for comparative analysis of the first and second samples of groups 1 (arm vein) and 2. Statistical significance was set at p<0.05.

This study was approved by the Committee for Rules and Ethics, University of Sao Paulo Medical School, and written informed consent was obtained from all patients.

## RESULTS

The WBC of the CVH group are displayed in Table 1, and the levels of thromboxane B<sub>2</sub> in the CVH group are shown in Table 2.

The results of hematocrits in the patients with CVH did not demonstrate a significant statistical difference with a change in position nor did the WBC, thromboxane B<sub>2</sub> or hematocrit of the arm vein samples. There was no variation

of the WBC (Table 3), thromboxane B<sub>2</sub> (Table 4) or hematocrit in patients without CVH with this change in position.

## DISCUSSION

In spite of the association of ambulatory venous hypertension with the establishment and continuation of a venous ulcer, the mechanism leading to tissue destruction is still controversial. The control group in the present study consisted of subjects who were about to undergo a renin analysis, for diagnosis of arterial hypertension, but with normal venous circulation of the right lower extremity. The contra lateral limb of the CVH patients was not used because there is a high CVH incidence rate in this limb, even when there are no symptoms.<sup>(25-27)</sup> In the CVH group

four patients had had a previous ulcer in the contra lateral limb, so there was a high CVH rate in the "normal" limb.

The initial observation<sup>(14)</sup> of the WBC reduction in blood from the superficial veins was soon confirmed by other authors, but whether this phenomenon is restricted to these veins or not remains unknown.

This study showed a reduction of 27.57% in WBC after 30 minutes in the reverse Trendelenburg position, compared to the sample drawn after the patient had remained supine for the initial 60 minutes (first sample). Thomas<sup>(14)</sup> noticed a reduction of 30% in the WBC in blood from the superficial venous system in patients with venous hypertension when going through the same steps.

The samples collected one and five minutes after the patient resumed the supine position attempted to show whether the WBC quickly returned to normal. A statistically significant difference was observed in the WBC in these samples compared to the sample collected 60 minutes after the initial supine position (first sample).

**Table 1**

**Average and standard deviation of the WBC in samples from the femoral vein of the 15 patients in the group with chronic venous hypertension and its relationship to position. Changes in percentage compared to the initial sample and standard deviation. Matael-Hanzel contrast test: 2#(3=4)#(1=5=6).**

Condition before blood testing			Leukocytes x 10 <sup>3</sup> /mm <sup>3</sup>		Change (%)	
Phase	Time (min)	Decubitus	Mean	SD	Mean	SD
1	60	supine	7.17	2.60		
2	30	reverse	5.12	1.87	-27.57	11.15
3	1	supine	5.67	2.06	-19.65	14.08
4	5	supine	6.07	2.05	-12.99	15.63
5	30	supine	6.55	2.04	-5.76	16.54
6	90	supine	6.92	2.08	-0.13	17.68
			p<0.001*			

\*Analysis using the Friedman test.

**Table 2**

**Average and standard deviation of thromboxane B<sub>2</sub> in samples from the femoral vein of the 15 patients in the group with chronic venous hypertension and its relationship to position. Changes in percentage compared to the initial sample and standard deviation. Matael-Hanzel contrast: (1=3=4=5)#2#6.**

Condition before blood testing			Thromboxane B <sub>2</sub> (pg/mm <sup>3</sup> )		Change (%)	
Phase	Time (min)	Decubitus	Mean	SD	Mean	SD
2	30	reverse	288.66	175.23	158.28	117.02
3	1	supine	166.80	118.75	57.30	106.77
4	5	supine	138.00	101.34	29.71	75.97
5	30	supine	109.60	126.66	-6.39	63.59
6	90	supine	65.60	53.54	-34.74	46.81
			p<0.001*			

\*Analyses of the group with Friedman test.

Table 3

Average and standard deviation of the WBC in samples from the femoral vein of the 4 patients in the control group and its relationship to position. Changes in percentage compared to the initial sample and standard deviation (SD).

Condition before blood testing			Leukocytes/mm <sup>3</sup> x 10 <sup>3</sup>		Change (%)	
Phase	Time (min)	Decubitus	Mean	SD	Mean	SD
1	60	supine	8.64	4.15		
2	30	reverse	8.67	4.29	-0.23	1.52
3	1	supine	8.64	4.04	0.45	2.70
4	5	supine	8.46	3.74	-0.71	4.33
5	30	supine	8.62	4.09	0	1.21
6	90	supine	8.65	4.06	0.46	1.44

p=1.000\*

\*Analyses of the group with Friedman test.

Table 4

Average and standard deviation of thromboxane B<sub>2</sub> in samples from the femoral vein of the 4 patients in the control group and its relationship to position. Changes in percentage compared to the initial sample and standard deviation (SD).

Condition before blood testing			Thromboxane B <sub>2</sub> (pg/mm <sup>3</sup> )		Change (%)	
Phase	Time (min)	Decubitus	Mean	SD	Mean	SD
1	60	supine	49.25	48.00		
2	30	reverse	55.75	55.50	30.96	40.64
3	1	supine	53.75	59.00	40.32	68.98
4	5	supine	71.00	68.50	125.11	180.03
5	30	supine	67.25	59.00	124.70	205.33
6	90	supine	62.25	73.00	43.62	34.83

p=0.3613\*

\*Analyses of the group with Friedman test.

In all published studies, samples were only collected 30 minutes after the return to the supine position.<sup>(14,15)</sup> No significant statistical difference was apparent in the samples drawn 30 minutes after the return to the horizontal position compared to the first sample. This point suggests margination or adhesiveness of the leukocytes to the capillary endothelium without migration to the tissue, or simply a cessation of leukocyte sequestration with a return to initial values.

The blood samples from the femoral vein in the control group also failed to show a statistically significant difference, either in the WBC or the hematocrit. This data is quite different from that of Moyses,<sup>(28)</sup> who observed a 25% rise in the hematocrit and a relative reduction of 30% in the WBC. These findings are probably related to the different positions of the patients in that study. Moyses<sup>(28)</sup> analyzed them sitting up for 30 minutes with their feet immobilized (the subjects analyzed showed venous hypertension because they remained erect and immobile

despite not suffering from CVH) whereas in the present study they were maintained in a 45° reverse Trendelenburg position and allowed to actively move their feet.

The biphasic pattern mentioned by Edwards<sup>(29)</sup> was not found, but rather a rise with sequential reduction in thromboxane B<sub>2</sub> value compared to the assay after 30 minutes in reversed Trendelenburg. A decrease of 34.74% in the value of thromboxane B<sub>2</sub> at the end of the period of return to the supine position was observed (statistically significant). This fact may be explained as the result of platelet aggregation in the test tubes in spite of their having been maintained only a short time at 5°C. The platelets in the initially collected sampling tubes would produce more thromboxane B<sub>2</sub> since they were kept longer at 5°C with a smaller production in the test tubes collected at the end (drawn 30 and 90 minutes after return to the supine position). The basal serum values of thromboxane B<sub>2</sub> could not be analyzed due to the above findings, as we know they are at most 1-2 pg/ml.<sup>(30)</sup> Nevertheless, the variations

in the value of thromboxane  $B_2$  reflect changes in the endogenous production of thromboxane  $A_2$ .<sup>(31)</sup> These variations are broadened by "ex-vivo" platelet activation with consequent formation of thromboxane  $A_2$  and its metabolization to thromboxane  $B_2$ .

Supplementing this study, thromboxane  $B_2$  and WBC were analyzed in the blood from the upper limb in order to confirm whether the change in blood from the lower limb with the change in position was associated with some change at a systemic level. No statistically significant difference was observed.

A reduction in WBC and a rise in thromboxane  $B_2$  from the femoral vein of the affected limb was revealed in the patients with CVH after taking up the reverse Trendelenburg position. This factor did not occur with the arm vein nor with subjects in the control group. We suggest that future studies of medications for the treatment of stasis ulcers should include their effects on leukocyte entrapment in the lower limb venous system and the correlation of these effects with the ulcer healing rate, in order to elucidate some important aspects of venous ulcer etiology and treatment.

## RESUMO

**Objetivos:** Analisar a variação do número de leucócitos e produção de tromboxane  $B_2$  em coleta na veia femoral de pacientes com hipertensão venosa crônica. **Tipo de estudo:** Estudo clínico, prospectivo, controlado, não randomizado e aberto **Local:** Disciplina de Cirurgia Vascular, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. **Participantes:** Quinze doentes, com história pregressa de úlcera de estase venosa recidivada, selecionados aleatoriamente no Ambulatório de Doenças Venosas. Quatro pacientes sem alterações venosas dos membros inferiores. **Intervenção:** Amostras de sangue colhidas na veia femoral e veia braquial após decúbito horizontal e proclive a 45°. **Variáveis:** Contagem dos leucócitos com câmara de contagem, e dosagem de tromboxane  $B_2$  com teste ELISA (Enzyme Linked Immunosorbent Assay). **Resultados:** Após 30 minutos de proclive, houve redução na contagem de leucócitos de  $\pm 27\%$  ( $p=0,02$ ) e elevação na dosagem de tromboxane  $B_2$  de  $\pm 158\%$  ( $p=0,02$ ). **Conclusões:** Sugerimos que futuros estudos com medicações para tratamento de úlceras de estase avaliem a ação destes no seqüestro leucocitário e produção de tromboxane  $B_2$  e a correlação desta ação com o índice de cicatrização, no intuito de elucidar alguns aspectos importantes na etiologia das úlceras de estase.

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