Could Gut-Liver Function Derangements Cause Chronic Venous Insufficiency?

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Upregulation of adhesion molecules and neutrophil infiltration of venous valve cusps may be risk factors for chronic venous insufficiency. But studies that focus on the target organ (vein) fail to consider the influence of systemic inflammation on WBC behavior in the microcirculation. This study probes the gut-liver axis as a potential source of gut-derived oxidative stress and free radical production leading to white blood cell activation in chronic venous insufficiency.

Venous hemodynamics (ambulatory venous pressure, air plethysmography, duplex) and gut-derived oxidative stress markers were studied in nine patients with chronic venous insufficiency (group I) and nine age- and sex-matched control subjects with no venous disease (group II). Group I had healed venous ulcers (class 5, CEAP) but near-normal ambulatory venous pressure, to eliminate high ambulatory venous pressure as a chronic venous insufficiency risk factor. Markers of gut-derived oxidative stress included: stool analysis; intestinal permeability; hepatic detoxification challenges with caffeine, salicylate, and acetaminophen; and urine lipid peroxides.

Ambulatory venous pressure did not significantly differ (group I, 42.5 ±5.3 mm Hg; group II, 35.5 ±5.5 mm Hg; $p = NS$). Candida overgrowth in stool distinguished group I from group II (7/9 pts vs 1/9 pts, respectively; $p = 0.015$). Increased intestinal permeability (lactulose/mannitol ratio) was prevalent in both groups (group I 0.07 ±0.02, group II 0.17 ±0.08, $p = NS$; normal range, 0.01–0.03). Both groups showed similar incidence of elevated urine lipid peroxides (5/9 pts vs 6/9 pts, respectively; $p = NS$), yet group I exhibited underfunction of both sulfation (group I 16.8 ±2.9%, group II 43.3 ±11%, $p < 0.03$; normal acetaminophen recovery 16–36%) and glucuronidation (group I 30.4 ±4.1%, group II 64.1 ±14.4%, $p < 0.04$; normal acetaminophen recovery 27%–56%) relative to oxidative stress, perhaps an indicator of diminished antioxidant capacity in patients with chronic venous insufficiency.

Gut dysbiosis (as indicated by stool yeast) and hepatic detoxification challenge pathway exhaustion may lead to subclinical, systemic inflammation and peripheral white blood cell adhesion in chronic venous insufficiency. Further exploration of the relationship between oxidative stress and venous disease is needed.

Vascular Surgery 35:107–114, 2001

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Resource support for this project was provided by the Straub Foundation, Honolulu; Great Smokies Diagnostic Laboratory, Asheville, NC, provided free testing kits

Presented at the Eleventh Annual Meeting of The American Venous Forum, Dana Point, California, February 21–24, 1999

The views expressed in this manuscript are those of the authors and do not reflect the views or opinions of the Department of the Army, Department of Defense or the US Government

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Introduction

Theories regarding the pathogenesis of venous stasis ulceration have evolved gradually in the 1900s, although treatment remains largely unchanged. The association between deep venous thrombosis and venous ulceration has been recognized since the early nineteenth century and, in addition, a substantial cohort of patients may have primary deep venous valvular incompetence. Proposed mechanisms for venous ulcer formation include tissue hypoxia due to stasis, arteriovenous communications, the fibrin cuff theory of Browse and Burnand, and the white blood cell trapping theory as put forward by Coleridge-Smith. Indeed, much attention has focused on neutrophil behavior in the microcirculation. Experimental data suggest that endothelial activation is also a feature of chronic venous disease by mechanisms that are not entirely clear.[1] Data suggest that venous hypertension, either experimentally induced or in patients with chronic venous insufficiency (CVI), enhances neutrophil-endothelial interaction, leading to neutrophil activation.[1] Hence, a venous ulcer may become a self-perpetuating phenomenon.

Despite the appeal of this local activation theory, neutrophil behavior in response to any source of inflammation must be considered. Smoking, diabetes mellitus, diets high in saturated fat, and clinical or subclinical infection all likely influence systemic neutrophil behavior. Trauma surgeons have recently focused on factors in mesenteric lymph that may be responsible for multisystem organ failure associated with hemorrhagic shock.[2] Nonallopathic medicine practitioners have long implicated the gut in a variety of etiologically obscure diseases such as inflammatory bowel disease, rheumatoid arthritis, chronic fatigue syndrome, and various food allergies.[3] For these reasons, we performed a global exploration of the gut and gut-liver axis as a potential source of subclinical, systemic inflammation that might influence neutrophil behavior in the lower extremity of patients with venous disease.

Patients and Methods

Patients

This study was conducted under a protocol approved by the Institutional Review Board of the Straub Foundation, Honolulu; all subjects gave informed consent. Eligible patients with CVI were age > 18 years, male or female, non-smoking, non-diabetic, and had palpable pedal pulses or ankle-brachial indices > 0.9. Each exhibited CEAP clinical class 4 or 5 and normal or near-normal ambulatory venous pressure (AVP).[4] None had active venous ulceration or active cellulitis associated with lipodermatosclerosis.

Age- and sex-matched control subjects were randomly chosen from operative case logs. Women had undergone breast biopsy for benign disease within the previous 2 years; men had undergone open or laparoscopic inguinal hernia repair. Control subjects met similar eligibility criteria to patients with CVI except no control subject had clinical or laboratory evidence of venous disease. No control subjects had a history of deep vein thrombosis or a current clinically overt infection and no women were pregnant.

Methods

Each patient with CVI and each control subject underwent standard vascular laboratory testing including duplex scanning (ATL Ultramark 9, Bothell, WA) and direct AVP measurement (n = 7) or air plethysmography (APG) (n = 11; ACI Medical, San Marcos, CA). The residual volume fraction by APG was used to estimate AVP in patients with CVI not undergoing direct AVP measurement and for control subjects.

All study subjects underwent three laboratory tests: a comprehensive digestive stool analysis (SA), intestinal permeability (IP), and a hepatic detoxification challenge (HDC) with oxidative stress analysis. Testing kits were provided and results interpreted blindly by Great Smokies Diagnostic Laboratory, Asheville, NC.

The patient provided a single random stool sample for SA. Antibiotics, antifungals, mineral oil, and other products were stopped 3 days prior to stool collection. Aspirin, digestive enzymes, and vitamin C in excess of 250 mg per day were stopped 2 days prior to collection. The SA battery of tests evaluates markers of digestion, absorption, microbiology, mycology, metabolic markers, immunology, macroscopic parameters, and a global score of the "bacterial dysbiosis index." Digestion tests included triglycerides, chymotrypsin, valerate/iso-butyrate ratio, and light microscopy for meat and vegetable fibers. Absorption tests included long-chain fatty acids, cholesterol, total fecal fat, and total short-chain fatty

acids. Microbiology tests included cultures for beneficial bacteria such as *Lactobacillus, Bifidobacterium, Escherichia coli,* and other, perhaps less beneficial, bacteria. Culture results were reported as 1+ to 4+. Culture and light microscopy for stool yeast was performed. Metabolic markers included: n-butyrate, B-glucuronidase, pH, and short chain fatty acid distribution. Fecal secretory IgA was performed. A macroscopic examination for color, mucus, and occult blood was performed as well as additional testing for *Campylobacter jejuni* and enterohemorrhagic *E. coli* cytotoxin. A global bacterial dysbiosis index was calculated based on gut microbiology, pH, short-chain fatty acids, and B-glucuronidase.

The IP test requires an overnight fast and elimination of fructose (fruit sugar) from the diet the day before the test. Two urine samples are collected the following morning. The first is a random pretest sample. The second sample is drawn from a 6-hour urine collection after the patient ingests a challenge drink. The challenge drink is a 100 mL solution containing 5 g lactulose, 1 g mannitol, and 10 g glycerol. The IP test directly measures the ability of two nonmetabolized sugar molecules, mannitol and lactulose, to permeate the intestinal mucosa. Mannitol (a monomer) is readily absorbed and serves as a marker of transcellular uptake. Lactulose (a dimer) is only slightly absorbed in the normal gut and serves as a marker of mucosal integrity. Lactulose, mannitol, and the urine lactulose/mannitol ratio were reported.

For HDC, one caffeine caplet (200 mg NoDoz®) was taken in the morning and clearance of caffeine assessed from two saliva specimens collected 2 and 8 hours after ingestion. Caffeine tests the cytochrome P-450 mixed function oxidase system (known as phase I). Results are expressed as salivary caffeine clearance (mL/min/kg). Two tablets of aspirin (Bayer®, 650 mg total) and two tablets of acetaminophen (Tylenol®, 650 mg total) were ingested that evening. The patient provided a 10-hour overnight urine specimen. Salicylate and acetaminophen ingestion tests four phase II hepatic detoxification pathways, including glutathione conjugation, sulfation, glucuronidation, and glycine conjugation. Results were expressed as percent recovery of acetaminophen mercapturate, salicyluric acid, acetaminophen sulfate, and acetaminophen glucuronide, respectively. Plasma cysteine and sulfate were also measured. Calculated ratios included phase I/sulfation, phase I/glycination, phase I/glucuronidation, and plasma cysteine/sulfate. Oxidative stress analysis included measurement of blood reduced glutathione, urine or serum lipid peroxides, superoxide dismutase, and three derivatives of salicylate expressed as percent recovery: catechol, 2,3-dihydroxybenzoate, and 2,5-dihydroxybenzoate.

This study was designed with an 'n' of 9 in each group to detect a difference in mean values of ≥ 20% with ±15% variability in measurements and power of 0.80. A p value of less that 0.05 was used as the criteria to consider the values in the two groups significantly different. Statistical analyses comparing group I and II were: student's t test for continuous variables expressed as means ± SEM and Fisher's Exact Test for proportional data. The Mann-Whitney U test was used in one instance (unequal variances).

Results

Table I provides the CEAP classification for each patient in group I. Table II summarizes the demographic and AVP data, and Table III shows the data from each of three laboratory tests. There was one diabetic smoker in group I. The diabetes mellitus was well-controlled. Stool overgrowth of *Candida* (*Candida albicans* or *Candida* species) distinguished group I from group II (7/9 pts vs 1/9 pts, respectively; $p = 0.015$, FET). The bacterial dysbiosis index, a global measure of gut health, was moderate to severely elevated in both groups, but not different. Moderately increased intestinal permeability as manifest by an increased lactulose/mannitol ratio was shown in both groups, but did not differ (group I 0.07 ±0.02, group II 0.17 ±0.08; $p = 0.22$ tt). One disproportionately elevated value occurred in group II (unknown reason). With respect to HDC, there was no difference in caffeine clearance (phase I detoxification) between groups (group I 1.18 ±0.60 mL/min/kg, group II 0.7 ±0.11 mL/min/kg; $p = 0.45$ tt). The phase II pathways of sulfation and glucuronidation did distinguish group I from group II with mean values in group I significantly lower than group II, although remaining within a normal range (group I 16.8 ±2.9% recovery, group II 43.3 ±11.1% recovery, $p = 0.03$ tt for sulfation; group I 30.4 ±4.1% recovery, group II 64.1 ±14.4% recovery, $p = 0.04$ tt for glucuronidation). Mean urine lipid peroxide values did not differ between groups (group I 9.01 ±0.49 nmol/mg, group II 9.29 ±1.28 nmol/mg, $p = 0.84$ tt).

Table I. CEAP classification for group I.

Patient	Classification			
	Clinical (C_{0-6})	Etiologic ($E_{C,P,S}$)	Anatomic ($A_{S,D,P}$)	Pathophysiologic ($P_{R,O}$)
1	4	S	S, D	R, O
2	4	P	D	R
3	4	P	S, D	R
4	5	P	S, D, P	R
5	5	P	S, D, P	R
6	4	S	D	R
7	5	P	S, P	R
8	5	P	S, D, P	R
9	5	S	S, D, P	R

Table II. Demographic and AVP data by group.

Variable	Normal	Group I	Group II	p Value
Age (yr)	—	51.9 ±5.7	52.0 ±5.5	0.99, tt
Diabetes mellitus	—	1/9 pts	0/9 pts	0.99, FET
Smoker	—	1/9 pts	0/9 pts	0.99, FET
AVP (mm Hg)	<35	42.5 ±5.3	35.5 ±5.5	0.38, tt

Discussion

The major findings of this study are that stool *Candida* overgrowth and underfunction of two important phase II detoxification pathways (sulfation and glucuronidation) relative to OS are associated with the development of CVI. Neither IP nor urine lipid peroxides, a marker of cell membrane damage, could distinguish patients with CVI from control subjects. No attempt was made to correlate the derangements identified in this study with either systemic markers of inflammation or direct measurements of neutrophil or endothelial activation.

Candida albicans (CA) is a one-celled opportunistic organism that reproduces by budding and lives by ingesting dead tissue, a saprophyte. It is universally found both on and in the human body and, under normal circumstances, controlled by surveillance immune defenses and symbiotic bacterial flora. But overcolonization by CA can occur in response to lowered host defenses, nutritional deficiencies, or various environmental insults. Large and/or repetitive doses of antibiotics (eg, treatment of venous ulceration) may substantially reduce total populations of indigenous bacterial gut flora, flora that usually out-compete CA for mucosal adhesion.

Chronic inflammation associated with CA overgrowth may lead to immune suppression and increased intestinal permeability to noxious macromolecules, overwhelming hepatic detoxification mechanisms such as phase II pathways. Nonallopathic practitioners implicate CA overgrowth in a myriad of diseases and describe the yeast syndrome purportedly associated with this problem.[5] Whether or not this phenomenon could impact systemic neutrophil behavior in the microcirculation of the lower extremity is a speculative but intriguing concept at this time.

The liver is the body's primary detoxifying organ and two related processes (phase I and II) carry out biotransformation reactions. Phase I detoxification chemically alters substances through oxidation, reduction, or hydrolysis using the inducible cytochrome P-450 mixed-function oxidase enzymes. This process increases the solubility of various molecules for excretion. We could demonstrate no difference between groups I and II in phase I detoxification using caffeine as a challenge substance. In phase II hepatic detoxification, conjugation reactions add a polar hydrophilic molecule to the metabolite or toxin, converting lipophilic substances to water-soluble forms for excretion and elimination. Major phase II pathways include glutathione, sulfate, glycine, and glucuronide conjugations. Both sulfation and glucuronidation pathways were significantly less efficient in group I when compared to group II (as measured by acetaminophen challenge) in the context of elevated urine lipid peroxides in each group. Although glucuronidation may be a supplemental pathway in most individuals, many substances (neurotransmitter, steroid hormones, certain drugs, xenobiotic and phenolic compounds) use sulfation as a primary route of detoxification.

These data support our previously published hypothesis.[6] Overgrowth of enteric pathogens (eg, yeast) results in activation of mucosal monocytes and macrophages, leading to cytokine production and induction of free radicals (FR). Adhesion of enteric pathogens also loosens tight junctions between mucosal cells leading to leaky gut. Leaky gut leads to translocation of noxious molecules, which may overwhelm phase I and II hepatic detoxification pathways again, resulting in excess production of FR. Excess FR results in cell membrane, mitochondrial, and nuclear membrane damage, potentially altering endothelial gene expression (for example). Ultimately, development of CVI skin changes may be determined by three phenomena: AVP (higher AVP enhancing the opportunity for neutrophil-endothelial interaction), inflammation from any source that influences systemic neutrophil behavior and an individual's anti-inflammatory capacity, or ability to effectively respond to oxidative stress by quenching FR. Experimental evidence with neutrophil degranulation products such as lactoferrin and elastase and rise in various endothelial adhesion molecules during experimental venous hypertension does appear to support the concept of self-perpetuation.[7,8] However, these data do not exclude alternative explanations for systemic neutrophil activation, including and perhaps significantly, the role of the gut.

In conclusion, AVP likely influences neutrophil behavior by reducing shear force and encouraging adhesion to the endothelium of postcapillary venules. Factors that influence the outcome of this interaction include the activation state of neutrophils entering the leg, expression of endothelial adhesion molecules as a result of neutrophil adhesion, and further neutrophil activation as a result of this endothelial-neutrophil interaction. This preliminary study investigates the role of the gut and gut-liver connection as a potential source of cytokine production and free radical release leading to systemic neutrophil upregulation. Stool yeast overgrowth and underfunctioning phase II hepatic detoxification pathways are treatable sources of ongoing subclinical (but important) systemic inflammation. Future approaches to CVI treatment may include identification and eradication of sources of inflammation (perhaps originating in the gut), bolstering of anti-oxidant defense mechanisms (such as phase II pathways), and surgery to lower AVP and limit deleterious neutrophil-endothelial interactions.

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Table III. Stool analysis, intestinal permeability, hepatic detoxification challenge, and oxidative stress test results.

	Normal	Group I	Group II	p Value
*Stool Analysis (SA) Test**				
Triglyceride (%)	0–0.3	0.26 ±0.06	0.13 ±0.04	0.12, tt
Chymotrypsin (IU/g)	6.2–41	15.7 ±4.3	20.7 ±6.8	0.55, tt
Valerate, iso-butyrate (umoles/g)	0–10	6.0 ±1.5	4.4 ±0.5	0.31, tt
Meat fibers present	0	4/9 pts	3/9 pts	0.99, FET
Vegetable fibers present	0	4.0 ±0.2	3.7 ±0.2	0.33, tt
Long chain fatty acids (%)	0–1.1	0.97 ±0.31	0.78 ±0.21	0.62, tt
Cholesterol (%)	0–0.3	0.20 ±0.02	0.20 ±0.04	1.0, tt
Total fecal fat (%)	0–1.6	1.44 ±0.36	1.10 ±0.23	0.44, tt
Total short chain fatty acids (umoles/g)	56–156	77.6 ±21.3	55.2 ±9.5	0.35, tt
n-Butyrate (umoles/g)	10–30	13.1 ±3.7	9.2 ±1.5	0.34, tt
B-glucuronidase (IU/g)	0–300	16.1 ±1.5	17.4 ±1.8	0.58, tt
pH	6–7.2	6.9 ±0.2	6.7 ±0.21	0.61, tt
Short-chain fatty acid distribution:				
% acetate	54–67	65.7 ±1.7	59.6 ±2.8	0.08, tt
% proprionate†	16–24	18.3 ±0.9	23.0 ±1.3	0.01, MW
% n-butyrate	14–23	16.1 ±1.5	17.4 ±1.8	0.58, tt
Fecal sIgA (ug/g)	44–183	56.9 ±13.9	98.9 ±43.6	0.37, tt
Occult blood	None	0/9 pts	2/9 pts	0.47, FET
Bacterial dysbiosis index	0–4	12.3 ±1.4	11.4 ±1.4	0.67, tt
Intestinal permeability				
Lactulose (% recovery)	0.1–0.8	0.64 ±0.16	0.69 ±0.12	0.83, tt
Mannitol (% recovery)	5–25	11.9 ±2.1	12.4 ±4.4	0.91, tt
Lactulose/mannitol ratio	0.01–0.03	0.07 ±0.02	0.17 ±0.08	0.22, tt
HDC and oxidative stress analysis				
Caffeine clearance (mL/min/kg)	0.5–1.6	1.18 ±0.60	0.7 ±0.11	0.45, tt
Plasma cysteine (mg/dL)	3.1–3.9	4.50 ±0.66	3.82 ±0.18	0.20, tt

(Table III continued)

	Normal	Group I	Group II	p Value
Plasma sulfate (mg/dL)	4.8–5.3	4.45 ±0.67	4.66 ±0.26	0.73, tt
Acetaminophen mercapturate (% recovery)	5.6–11.4	6.6 ±1.1	13.7 ±4.1	0.11, tt
Salicyluric acid (% recovery)	30–53	27.9 ±5.2	38.4 ±10.1	0.37, tt
Acetaminophen sulfate (% recovery)	16–36	16.8 ±2.9	43.3 ±11.1	0.03, tt
Acetaminophen glucuronide (% recovery)	27–56	30.4 ±4.1	64.1 ±14.4	0.04, tt
Phase I/sulfation ratio	3.5–13	19.6 ±15.7	2.1 ±0.5	0.28, tt
Phase I/glycination ratio	1.3–3.5	8.4 ±6.0	2.9 ±0.8	0.37, tt
Phase I/glucuronidation ratio	1.9–4.2	4.9 ±2.8	1.6 ±0.4	0.26, tt
Plasma cysteine/sulfate ratio	0.6–0.78	0.84 ±0.13	0.83 ±0.03	0.96, tt
Catechol (% recovery)	0–0.024	0.066 ±0.021	0.099 ±0.065	0.65, tt
2,3 DHBA (% recovery)	0–0.0072	0.003 ±0.003	0.022 ±0.016	0.30, tt
Urine lipid peroxides (nmol/mg)	3–9	9.01 ±0.49	9.29 ±1.28	0.84, tt
Reduced glutathione (mg/dL)	32–64	32.77 ±3.13	34.67 ±1.79	0.61, tt
Glutathione peroxidase (U/gHgb)	20.3–38.1	30.47 ±5.47	33.46 ±3.45	0.64, tt
Superoxide dismutase (U/gHgb)	1610–2162	1987 ±87	2092 ±262	0.80, tt

* Lactobacilli, bifidobacterium, *E. coli, Bacillus* species, g-streptococcus, *Klebsiella pneumoniae,* mucoid *E. coli,* hemolytic *E. coli, Morganella morgagni, Pseudomonas aeruginosa, Proteus mirabilis, Bacteroides fragilis* group, *Enterobacter, Candida albicans* or species, *Candida tropicalis, Geotrichum, Enterobacter cloacae* were cultured from subjects in both groups and graded as 1–4+. Only overgrowth of stool *Candida* > 1+ distinguished group I from group II (7/9 pts vs 1/9 pts, respectively; p = 0.015 FET).

† Short chain fatty acid (% proprionate) also differed (uncertain significance).

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DISCUSSION

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This presentation correctly emphasizes leukocyte activation and its relationship to chronic venous insufficiency (CVI). In defining the purpose of this study, the authors separate leukocyte activation and its effect on valve dysfunction from leukocyte activation in the target organ of venous insufficiency—the skin.

Coleridge Smith's group has emphasized the importance of activated leukocytes in the etiopathogenesis of skin damage in CVI. They

documented the accumulation of macrophages and T-cells as an event associated with development of lipodermatosclerotic skin changes in CVI.[1] Pappas' group studied activation of leukocytes in the systemic circulation using a variety of markers and found that circulating neutrophils showed no evidence of activation. This could be explained by the fact that activated leukocytes are trapped in the periphery and are therefore not circulating.[2]

This study measured markers of gut-derived oxidative stress (GDOS) in patients with CVI compared with healthy control subjects, both groups having near-normal ambulatory venous pressures. An increased lactulose/mannitol ratio was seen in patients and in the healthy control subjects. In other studies, it has been found that intestinal permeability, as measured by the lactulose/mannitol test, is significantly increased in patients with intermittent claudication and that this ratio decreases after arterial reconstruction.[3] However, the chief difference between the healthy subjects and the patients with CVI in the present study was stool overgrowth by *Candida* and underfunction of the sulfation and glucuronidation pathways to oxidative stress. The authors find the possibility that this phenomenon could impact neutrophil activation but that proof is lacking.

As the Phase II hepatic detoxification pathways were deficient in the patients with CVI, the authors believe that this supports the previous hypothesis that enteric pathogen overgrowth results in activation of mucosal monocytes and macrophages, which leads to cytokine production and induction of free radicals. However, Pappas' group in New Jersey has found that patients with CVI do not exhibit the same degree of proliferation in response to a mitogenic challenge as healthy subjects. Also, they demonstrated a trend of diminished mononuclear cell function with progression of CVI from CEAP classes 3 through 6. Their conclusion was that deterioration of mononuclear cell function is associated with CVI, a finding that conflicts with the data presented in the present study.[4]

The differences in findings are unimportant. The fact is that basic studies are being carried out that will eventually elucidate the fundamental mechanisms in chronic venous insufficiency. It appears as though study of the association of leukocytes, adhesion molecules, and the target organs of either skin or valves and venous wall will produce the most revealing results. The Middlesex group has shown that venous hypertension results in endothelial activation and that patients with lipodermatosclerosis exhibit an increased VCAM-1.[5] This has been confirmed in Freiberg where both ICAM-1 and VCAM-1 expression was high during stasis dermatitis and was not downregulated to baseline levels after progression of the pathologic process from stasis dermatitis through to skin ulceration.[6]

Our studies have focused on leukocyte and adhesion molecules in relationship to valve dysfunction[7] as well as the effects of venous hypertension on leukocyte rolling adhesion and migration.[8]

All of these studies taken as a whole suggest that pharmacologic intervention in primary venous insufficiency as well as in chronic venous insufficiency might prove to be possible.[9]

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