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Oscillatory flow suppression improves inflammation in chronic venous disease

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ABSTRACT

Background: To assess if suppression of the oscillatory component of reflux may improve the inflammatory phenotype in chronic venous disease (CVD).

Materials and methods: From 193 CVD patients, we selected 54 (13 males, 41 females, CEAP C2-4EpAsPr) for a blinded, case-control prospective investigation. All of them underwent echo-color-Doppler assessment of reflux parameters. In the same patients a blood systemic assessment of 19 inflammatory cytokines was obtained. Follow-up lasted 6 months. The control group (C) was constituted by 21 homogenous CVD patients, unselected and not operated.

Results: Forty-one of 54 patients were excluded from post-operative evaluation in consequence of reported new other inflammatory episodes. Twenty-three (23) completed the follow up, showing the suppression of the oscillatory component of venous reflux; 4 of the 19 cytokines decreased significantly after the procedure: Tumor Necrosis Factor-α (TNFα), Granulocyte Colony Stimulating Factor (G-CSF), Interferon gamma-induced Protein 10 (IP-10), Interleukin-15 (IL-15). Particularly, TNFα and IP-10 even returned inside a physiological range: 5.3 ± 2.7 to 4.2 ± 2.2 pg/mL ($P < 0.003$) and from 303.7 ± 168.4 to 254.0 ± 151.6 pg/mL ($P < 0.024$), respectively. Both cytokines showed a weak but significant correlation with parameters of oscillatory flow correction. Finally, three cytokines implicated in repair and remodeling of tissue, Epidermal Growth Factor, Monocyte Chemoattractant Protein-1 and Platelet Derived Growth Factor-BB (PDGF-BB), significantly increased. Our findings are further reinforced by the significant changes of the same cytokines when compared to C group.

Conclusions: The surgical suppression of the oscillatory component of reflux modulates the inflammatory phenotype, suggesting a pivotal role of flow among factors concurring to inflammation in CVD.

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Introduction

Ethiopathogenesis of chronic venous disease (CVD) is almost completely obscure. The pathophysiology is dominated by lower limbs venous hypertension. In most cases, venous hypertension is caused by reflux through incompetent valves, disregarding the incompetence origin from a primary valvular failure or secondary to parietal dilation.[1-3]

It is well known how in course of CVD, the inflammatory process is dominated by the so-called white cell trapping phenomenon.[4,5] On the endothelium side, inflammation is characterized by a cytokine cascade with activation of matrix metallo proteinases and sustained remodeling of the valves and venous wall.[6-9] However, the effective contribution of hemodynamics to the inflammatory phenotype of the endothelium is unknown. *In vitro* investigations, aimed to understand the contribution of flow to atherosclerosis, have already demonstrated the direct relationship between hemodynamic forces and endothelial expression,[10] whereas a laminar flow is associated with low-inflamed vessel walls,[11] an oscillatory flow is linked to a pro-inflammatory endothelial lining.[12]

Reflux in the veins of the lower limbs is a perfect example of oscillatory flow.[1,13,14] There is an upward component at muscular systole followed by a reverse flow wave at muscular diastole (Fig. 1).

Varicose veins ablation permitted *ex vivo* assessment of inflammatory molecules released by the endothelial cells[9] in the same segments where reflux hemodynamics has been preoperatively measured. Preliminary experience in such ex-vivo setting showed an interesting correlation between reflux as an oscillatory flow and release of endothelial cytokines from varicose veins.[15] This result suggests the role of flow as an underestimated factor in modulating cytokines release also in CVD. Moreover, most of the cytokines released *ex vivo* by the venous endothelium were also found increased in the blood, becoming potential biomarkers of the CVD inflammatory process.[16]

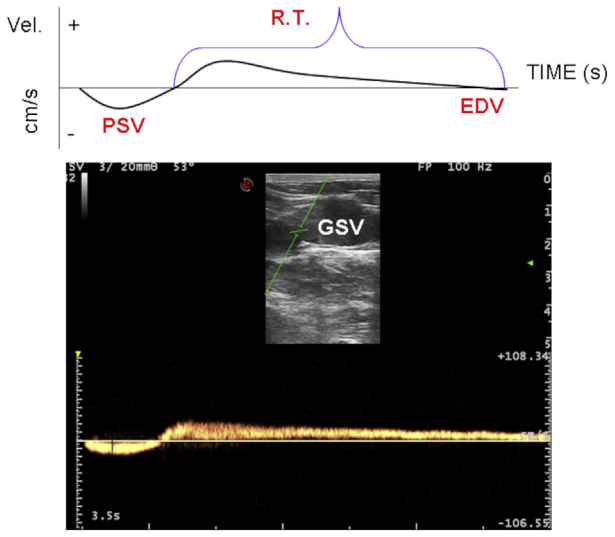

Fig. 1 – The classic oscillatory flow of venous reflux, with bi-directional positive and negative components is depicted. Top: exemplification of the parameter assessed, peak systolic velocity (PSV), end diastolic velocity (EDV), and reflux time (RT). Bottom: duplex scanning of the great saphenous vein (GSV) 15 cm below the junction, longitudinal access, where the parameters were assessed. (Color version of figure is available online.)

The aim of the present work was to verify how the surgical suppression of the oscillatory component of reflux may modulate the inflammatory phenotype assessed by measurement of circulating endothelial cytokines.

Materials and methods

Patients population and samples collection

From a cohort of 193 patients affected by primary CVD, we selected patients for the present study according to the following inclusion criteria:

- Primary CVD
- CEAP Clinical class ranging from 2 to 4
- Reflux confined to the GSV territory
- Type I shunt[17,18]
- Type III shunt with competent terminal valve[19]
- Age 18-65 y
- BMI $\leq$28
- Willing to participate to the study

Exclusion criteria were the following:

- Absence of concomitant acute and chronic inflammatory diseases
- Active and healed venous ulceration
- Smoking
- Absence of significant comorbidities affecting the cardiovascular, hepatic, renal, and nervous apparatus
- Concomitant reflux in the SSV, deep venous system, pelvic veins, and controlateral limb
- Type III shunt with incompetent GSV terminal valve[17,18]

Fifty-four patients (13 male and 39 female, mean age 52.25 $\pm$ 13.73) fulfilled the inclusion and exclusion criteria and entered the study. The study was approved by the Ferrara University—Hospital Ethical Committee.

All the patients underwent an echo-color-Doppler (ECD) investigation (Esaote My-Lab 70, Esaote Genoa, Italy) in standing position with complete scanning of the great saphenous vein (GSV) and small saphenous vein (SSV) systems, including junctions and tributaries. In addition, the main trunk of the deep venous system and the perforators were completely examined. Calf muscular pump was elicited by manual squeezing, considering as reflux the detection of a reverse flow lasting more than 0.5 s in all the examined segments. At the junction level, competence of the valve was also tested, as previously described, by means of a combination of squeezing and Valsalva maneuver, with the Doppler sample volume placed on the femoral side of the terminal GSV valve.[13,14,19]

Reflux elimination test was used to differentiate between type I and Type III shunt.[19]

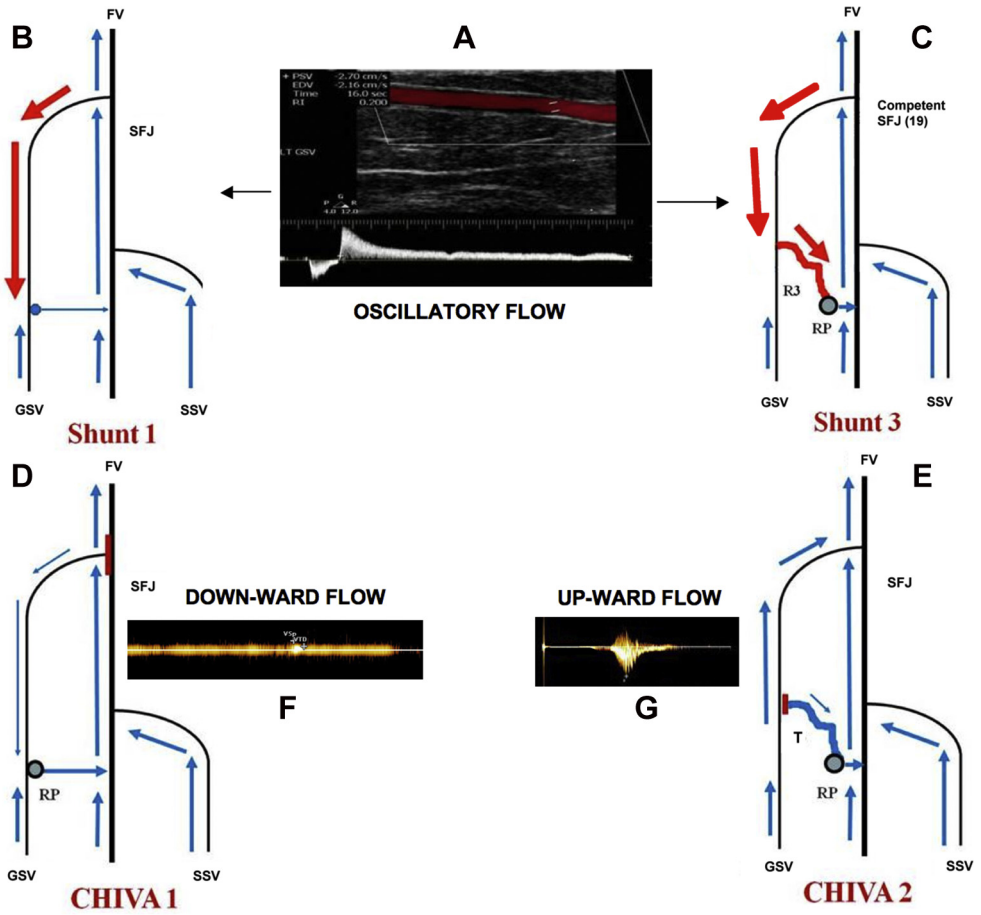

Fig. 2 – (A) Echo-color-Doppler of preoperative reflux in the great saphenous vein. This pattern of reflux was registered either in type 1 or in type 3 shunt. (B) Exemplification of type 1 shunt, characterized by a re-entry perforator on the GSV trunk. (C) Exemplification of type 3 shunt with competent SFJ characterized by a re-entry perforator on a varicose tributary. (D) Procedure for type 1 shunt, consisting in high ligation and phlebectomy (CHIVA 1). (E) Procedure for type 3 shunt, consisting in flush ligation of the varicose tributary on the GSV wall (CHIVA 2). (F) Elimination of the oscillatory flow in the GSV after CHIVA 1 procedure. The results in terms of flow are a low-velocity downward flow. (G) Elimination of the oscillatory flow in the GSV after CHIVA 2 procedure. The result in terms of flow is an upward flow. FV = femoral vein; SFJ = sapheno-femoral junction; GSV = great saphenous vein; SSV = small saphenous vein; RP = re-entry perforator; T = tributary. (Color version of figure is available online.)

Moreover, at 15-cm distal to the sapheno-femoral junction following hemodynamic parameters were assessed into the GSV: peak systolic velocity (PSV), end diastolic velocity (EDV), and reflux time (RT; Fig. 1).

Blood samples were collected from the patients arm before the surgical treatment before entering the operating room. The samples were immediately sent to the biological laboratory for centrifugation and plasma isolation. After surgery aimed in correcting the oscillatory component of the flow without any vein ablation, both hemodynamics and cytokines were re-assessed 6 months after the surgical procedure.

Control group

The control group was constituted by 21 patients matched for age, gender (7 male, 14 female, mean age 54 ± 10, 80), and CEAP clinical class. They also presented at ECD investigation reflux in the main GSV trunk and along the tributaries but were not operated.

Surgical procedure

After clinical assessment, patients underwent a saphenous-sparing surgical treatment in accordance with the CHIVA strategy. When both squeezing and Valsalva and/or one of the two maneuvers, above the GSV terminal valve, were negative for reflux, we simply disconnected the incompetent tributaries from the GSV (the so called CHIVA 2 procedure; Fig. 2).[17-20]

To the contrary, when both maneuvers were positive with negative reflux elimination test, we applied the so-called CHIVA 1 procedure consisting in high-tie and incompetent tributaries disconnection from the trunk (Fig. 2).[17,18]

Analysis of cytokines and chemokines in plasma samples

Plasma samples were frozen and thawed only once before performing the MILLIPLEX MAP Human Cytokine/Chemokine Panel (Merck Millipore, Billerica, MA), a bead-based multiplex immunoassay, which allows the simultaneous quantification of the following 29 human cytokines: Interlukin-1α (IL-1α), Interlukin-1 β (IL-1β), Interlukin-1 receptor antagonist (IL-1 ra), Interlukin-2 (IL-2), Interlukin-3 (IL-3), Interlukin-4 (IL-4), Interlukin-5 (IL-5), Interlukin-6 (IL-6), Interlukin-7 (IL-7), Interlukin-8 (IL-8), Interlukin-10 (IL-10), Interlukin-12 protein 40 (IL-12(p40)), Interlukin-12 protein 70 (IL-12(p70)), Interlukin-13 (IL-13), Interlukin-15 (IL-15), Interlukin-17A (IL-17A), Epidermal Growth Factor (EGF), Eotaxin, Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Interferon-α2 (IFN-α2), Interferon-γ (IFN-γ), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1α), Macrophage Inflammatory Protein-1β (MIP-1β), Tumor Necrosis Factor-α (TNFα), Tumor Necrosis Factor-β (TNF-β), and Vascular Endothelial Growth Factor (VEGF). Moreover, a custom made MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel (Merck Millipore) was used to quantify the cytokines Platelet Derived Growth Factor- AB/BB (PDGF-AB/BB) and Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES). Samples were processed in duplicate following the manufacturer's recommended protocols and read on a MAGPIX instrument equipped with the MILLIPLEX-Analyst Software using a five-parameter nonlinear regression formula to compute sample concentrations from the standard curves.

Statistical analysis

The data were reported as mean ± standard deviation. The results were compared by using Student t test for paired data and Wilcoxon test for unpaired data, when appropriate. Spearman correlation coefficient was calculated to identify correlation between hemodynamic and laboratory parameters. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed with GraphPad Instat software (San Diego, CA).

Results

Clinical assessment

We excluded from the final evaluation 41 patients who in course of follow-up reported the onset of an acute and/or chronic inflammatory disorder, changed the BMI, the lifestyle, and used Vitamin supplement or developed someone of the initial exclusion criteria. Therefore, 23 of 54 patients completed the 6 mo follow-up.

The CEAP class decreases from 2.87 ± 0.75 measured preoperatively to 0.56 ± 0.76 postoperatively ($P < 0.0001$).

The control group was not significantly different for age, gender, CEAP clinical class. We did not exclude any patient based on presence/absence of new or coexistent inflammatory condition.

Surgical and hemodynamic outcomes

In 11 cases, CHIVA 1 procedure was performed, so determining a reverse but monodirectional flow during muscular diastole.[17,18] To the contrary, CHIVA 2 was performed in the remaining 12 cases, in which reflux was eliminated, and an upward flow was restored (Fig. 2).[19]

In both post-operative scenarios, the oscillatory flow was substituted by a monodirectional drainage flow, respectively downward for CHIVA 1 and upward for CHIVA 2. Neither major nor minor postoperative complications were recorded.

The postprocedural oscillatory flow elimination led to significant changes of all hemodynamic parameters shown in Table 1. The RT was significantly decreased from 3.04 ± 0.73 s at baseline to 1.27 ± 0.51 s at follow-up ($P < 0.0001$). Also, the PSV decreased significantly at follow-up time moving from 41.78 ± 13.99 cm/s to 21.53 ± 6.75 cm/s ($P < 0.0001$). To the contrary, EDV significantly increases after the surgical procedure, passing from a pre-operative negative values −16.39 ± 6.87 cm/s to post-operative positive values + 11.34 ± 4.22 cm/s ($P < 0.0001$). The main reason was oscillatory flow disappearance in all patients who underwent procedures.[17,19]

Correlation between circulating levels of cytokines/chemokines after surgical hemodynamic correction

In Table 2, the change of post-operative levels of cytokines respect to pre-operative one is given, together the normal range and the level of significance as well. The table also reports the bottom line cytokines level of the control group. Respect to the initial 31 planned cytokines, 12 cytokines were found constantly below the detection range and so excluded from the final evaluation. The list of the 19 evaluated molecules is given in Table 2. Interestingly, 4 of 19 cytokines significantly improved at 6-month follow-up. Among the panel of studied cytokines, TNFα, IP-10, IL-15, and G-CSF showed a significant decrease 6 months after the procedure and re-entered into the normal range. TNFα from 5.3 ± 2.7 to

Table 1 – Clinical assessment.

Clinical and hemodynamics	CEAP	PSV (cm/s)	EDV (cm/s)	RT (sec)
Preoperative mean ± SD	2.87 ± 0.75	41.78 ± 13.99	−16.39 ± 6.87	3.04 ± 0.73
Postoperative mean ± SD	0.56 ± 0.76	21.53 ± 6.75	11.34 ± 4.22	1.27 ± 0.51
P value ($P < 0.05$)	$P < 0.0001^*$	$P < 0.0001^*$	$P < 0.0001^*$	$P < 0.0001^*$

Statistically different values from pre-operative to post-operative are marked by *.

Table 2 – Cytokines-chemokines assessment.

Cytokines/ chemokines (pg/mL)	Levels of normality, mean ± SD (pg/mL)	Control group, mean ± SD (pg/mL)	Preoperative, mean ± SD (pg/mL)	Post-operative, mean ± SD (pg/mL)	*P* value, Preop *versus* postop ($P < 0.05$)	*P* value, control *versus* postop ($P < 0.05$)
EGF	40 ± 23.3	38.1 ± 38.8	70.73 ± 130.77	87.42 ± 88.41	0.034 *	0.032 *
Eotaxin	54.90 ± 26.00	139.1 ± 69.4	121.4 ± 152.04	279.43 ± 137.30	0.290	0.005 *
G-CSF	17.9 ± 6.8	7.6 ± 8.7	22.09 ± 12.9	16.37 ± 8.7	0.023 *	0.003 *
GM-CSF	4.9 ± 1.9	5.1 ± 7.5	3.98 ± 2.14	4.33 ± 1.74	0.186	0.684
IFN-α2	12.00 ± 8.3	9.5 ± 15.5	11.24 ± 8.01	11.48 ± 5.09	0.285	0.265
IFN-γ	3.7 ± 1.9	4.3 ± 5.6	7.09 ± 12.34	5.17 ± 7.91	0.459	0.707
IL-12 (p70)	3.8 ± 2.00	3.6 ± 3.7	3.40 ± 5.44	2.47 ± 1.12	0.424	0.160
IL-15	<OOR	2.4	0.72 ± 1.41	0.48 ± 1.54	0.027 *	0.044 *
IL-17a	<OOR	2.5	1.28 ± 3.1	0.97 ± 1.98	0.455	<0.001 *
IL-1ra	20.3 ± 10.00	9.7 ± 11.8	14.99 ± 13.1	17.65 ± 7.39	0.078	0.008 *
IL-7	2.9 ± 1.3	2.9 ± 1.3	2.31 ± 2.17	2.34 ± 1.36	0.323	0.628
IL-8	2.8 ± 1.8	2.8 ± 1.8	6.65 ± 8.23	6.2 ± 2.75	0.458	0.404
IP-10	203.5 ± 67.8	203.5 ± 67.8	303.69 ± 168.41	254.03 ± 151.69	0.024 *	0.002 *
MCP-1	162.50 ± 56.00	162.50 ± 56.00	212.78 ± 123.74	279.43 ± 137.30	0.016 *	0.001 *
MIP-1b	20.20 ± 6.80	8.2 ± 5.5	26.84 ± 22.34	20.93 ± 12.45	0.101	<0.001 *
TNFα	3.8 ± 1.6	8.0 ± 3.3	5.32 ± 2.75	4.24 ± 2.21	0.003 *	<0.001 *
VEGF	34.80 ± 17.00	43.5 ± 83.6	57.49 ± 58.77	54.75 ± 58.77	0.494	0.677
PDGF—BB	5476.00 ± 5027.00	5476.00 ± 5027.00	5628.21 ± 8686.30	10,397.10 ± 10,706.00	0.011 *	0.104
RANTES	16,126.00 ± 15,567.00	16,126.00 ± 15,567.00	27,799.60 ± 58,799.00	23,910.00 ± 29,680.00	0.105	0.118

<OOR = out of range.
Statistically different values from preoperative to postoperative and the values statistically different from the control (CVI patients with comorbidities) to the postoperative are marked by *.

4.2 ± 2.2 pg/mL ($P = 0.003$), IP-10 from 303.7 ± 168.4 to 254.0 ± 151.6 pg/mL ($P = 0.024$), IL-15 from 0.72 ± 1.41 to 0.48 ± 1.54 pg/mL ($P = 0.027$), and G-CSF from 22.09 ± 12.9 to 16.37 ± 8.7 pg/mL ($P = 0.023$), respectively.

At the same time, three cytokines, EGF, MCP-1, and PDGF-BB, showed a significantly higher level in the follow-up. Specifically, EGF from 70.73 ± 130.77 to 87.42 ± 88.41 pg/mL ($P = 0.034$), MCP-1 from 212.78 ± 123.74 to 279.43 ± 137.30 pg/mL ($P = 0.016$), and PDGF-BB from 5628.21 ± 8686.30 to 10,397.10 ± 10,706.00 pg/mL ($P = 0.011$).

Moreover, all the cytokines pre and post-operative levels were plotted with the hemodynamic parameters. Again, either IP10 or TNFα showed a weak but significant inverted correlation with the EDV value (TNFα: $r = -0.34$; $P = 0.02$; IP10: $r = -0.32$, $P = 0.03$).

Cytokines changes according to the surgical procedure

We subset the 23 patients into two groups according to the surgical procedure CHIVA 1 or CHIVA 2. We performed a statistical analysis comparing the cytokines changed significantly in the follow-up. No statistical difference has been demonstrated: TNFα from 5.32 ± 2.75 preoperatively to 4.16 ± 1.79 after CHIVA 2 and 4.33 ± 2.69 after CHIVA 1 ($P = 0.86$); IP-10 from 303.69 ± 168.4 preoperatively to 242.82 ± 161.44 after CHIVA 2 and 266.27 ± 147.09 after CHIVA 1 ($P = 0.72$); G-CSF from 22.09 ± 13 preoperatively to 15.23 ± 7.12 after CHIVA 2 and 17.62 ± 10.37 after CHIVA 1 ($P = 0.5$); IL-15 from 0.94 ± 1.58 preoperatively to 0.83 ± 2.1 after CHIVA 2; and 0.11 ± 0.2 after CHIVA 1 ($P = 0.20$).

No statistical difference has been also demonstrated by comparing levels of cytokines with higher level after flow correction: EGF from 70.73 ± 130.76 preoperatively to 109.72 ± 99.3 after CHIVA 2 and 63.09 ± 71.4 after CHIVA 1 ($P = 0.21$); MCP-1 from 212.78 ± 123.74 preoperatively to 279.41 ± 109.78 after CHIVA 2 and 279.45 ± 167.94 after CHIVA 1 ($P = 0.99$); PDGF-BB from 5628.22 ± 8688.27 preoperatively to 13,574.41 ± 10,777.39 after CHIVA 2 and 6931 ± 9951.94 after CHIVA 1 ($P = 0.14$).

Cytokines level in the control group

The control group did not undergo to any treatment also including conservative treatment like compression or medical treatment of CVD, both influencing the cytokines cascade. The levels of circulating cytokines are given in Table 2, in comparison with the postoperative levels measured in the operated group.

They were significantly different from the operated group in EGF, EOTAXIN, G-CSF, IL-15, IL-17, IL-1 ra, IP-10, MCP-1, MIP-1b, TNFα. Again, interestingly, the cytokines with postoperative recovery below the normal range were significantly different as compared to controls. Details of cytokines concentrations and levels of significance are reported in Table 2.

Discussion

To the best of our knowledge, this is the first study which demonstrates, *in vivo*, that surgical elimination of the oscillatory flow component of venous reflux improves the inflammatory phenotype of the endothelial cells. The reassessment of the cytokines levels was set 6 months post-operatively to give time to the endothelial cells to eventually modify the inflammatory phenotype related to the surgical suppression of the oscillatory flow. Moreover, the reduced levels of inflammatory cytokines cannot be a product of the extent of varicosity ablation, because we did not perform any vein excision but simply minimally invasive ligations aimed to restore the flow direction by maintaining the drainage.[21,22]

We found four cytokines significantly improved after the surgical procedure. Interestingly, IP10 and TNFα improved until the range of normality. This suggests a possible role modulated by flow correction of both molecules, apparently confirmed in the present study by an inverted correlation between flow parameters and both cytokines.

IP10 is a crucial cytokine expressed by lymphocytes, monocytes, and endothelial cells, that is activated by INF-γ; therefore, it is even called interferon gamma-induced protein 10.[23]

More specifically though IP10 has a pivotal role in the inflammation of endothelium, regarding the angiostatic mechanism, induced by the inhibition of proliferation of the endothelial cells.[24,25]

In addition, IP-10 was found associated to the increased thickness of the arterial walls and recruitment of smooth muscle cells in the atheromatous complex.[26]

In CVD, proliferation index of endothelial cells was found to be significantly increased confirming a possible role of IP10 in inducing such a pathological behavior of the endothelium.[9]

The other cytokine, TNFα, is one of the main actors of the inflammation cascade. It is very much present in activated macrophages and lymphocytes T, inducing chemotaxis of monocytes, neutrophils, and lymphocytes adhesion to the endothelium. This action of reclamation is principally done though the endothelial cells, releasing the factor itself and increasing their permeability, finally representing the key mechanism of any local inflammation compound.[27,28]

In addition, the release of TNFα in the inflammatory field,[29] is the apical mechanism of the elicitation of the inflammation symptoms, such as heaviness or tension, swelling, aching, and itching, more frequently reported by patients in the Edinburgh Study.[30] Moreover, symptoms were subsequently reported more prevalent in the presence of reflux, speculatively confirming the correlation between oscillatory flow, molecular mediators, and inflammatory symptomatology.[31]

It is worth of note, the dramatic reduction of postoperative edema in our cohort passing from class 3 to class 1, representing a clinical correlation with the return of TNFα at normal range.

Finally, the significant reduction of IL-15 and G-CSF further confirms the change of phenotype in the endothelial cells after correction of reflux.

However, the concomitant significant increased levels of PDGF-BB, MCP-1, and EGF seem to contradict the modulation of the inflammatory phenotype induced by surgery. Alternatively, we may interpret the increased trend of the above cytokines as release of protective factors and/or healing phase from the previous inflammatory state.

For instance, PDGF-BB is a required element in cellular division, growth, proliferation, and differentiation[32] and plays a major role in the healing process.[33] In addition, it enhances proliferation of fibroblasts and production of extracellular matrix by these cells in wound healing.[34,35]

MCP-1 is a cytokine implicated in complex and multiple pathways. However, both in the bone[36] and in the wound,[35] it has been assessed increased during processes of skeletal remodeling and wound repair as well. So, mirroring the role, we assigned to PDGF-BB.

Our vision is further confirmed by the EGF increasing, because in wound healing EGF and its receptors are a cornerstone in re-epithelisation and dermal maturation.[37] EGF increased level might express a remodeling also in the subcutaneous distal limb, in consequence of the better drainage of the tissue.

Our findings are further reinforced by the comparison of postoperative cytokines level with those of unselected CVD patients, with still the reflux in the GSV system. Of course, the possible concomitant presence of inflammation is visible for the significant differences detected in the interleukin system (IL 15, IL-17, IL-1ra). However, again, the same cytokines above described were significantly different in this cohort of CVD patients, suggesting that reflux is a mechanical stimulus in the production of endothelial inflammation (Table 2).

The major limitation of our study is represented by the systemic measurement of circulating cytokine levels. It cannot of course be completely attributed to an exclusive effect on the endothelial cell of the saphenous system. On the other hand, the moderate level of *r* correlation suggests us how the levels of circulating cytokines are only partially explained by the hemodynamic changes induced by surgery. In perspective, we plan a broader study to evaluate all factors capable to contribute to the change of cytokines plasma concentration, out of reflux flow, to analyze on a proper statistical sample with multivariate regression analysis the role of smoking, BMI, exercise, and so forth in producing venous endothelial inflammation.[38-40]

Conclusions

The present *in vivo* study seems to confirm previous *in vitro* and *in vivo* studies all pointing on the oscillatory flow as a potent physical signaling of endothelial inflammation.

Someone can argue that other independent factors might contribute to a change of cytokines plasma concentrations, such as acute and chronic inflammatory diseases, smoking, diet, vitamin supplement, exercise, jobs requiring standing long time, seasonality, and so forth.[38-43]

However, in our cohort, we excluded all patients with significant comorbidity. In addition, of patients spontaneously lost at follow-up, we also excluded patients who changed

significantly BMI, exercise, and job; or with the reported onset of any inflammatory state even if transitory.

This implicated a significant loss of patients but strengthened our findings, yet. Although we tried to reduce as much as possible shortcomings of our study, we recognized that we need a wider initial cohort to improve the power of the study. Also, a control group, better if randomized, could be beneficial to make our speculation stronger. This might be an interesting perspective of the present pilot study.

The interconnection among endovenous hemodynamics and consequent biochemical signaling is widely unknown. The present investigation identifies potential first clues on this topic inside the post-procedural variations of specific cytokines, like IP-10 and TNFα, in the CVD treatment setting. Their correlation with post-operative duplex hemodynamics parameters modifications, paves the way for a further identification of a circulating biomarker to be related to the CVD stage.

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Disclosure

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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