Peripheral venous congestion causes inflammation, neurohormonal, and endothelial cell activation

Paolo C. Colombo[1]*, Duygu Onat[1], Ante Harxhi[1], Ryan T. Demmer[2], Yacki Hayashi[1], Sanja Jelic[3], Thierry H. LeJemtel[4], Loredana Bucciarelli[5], Moritz Kebschull[6], Panos Papapanou[7], Nir Uriel[1], Ann Marie Schmidt[8], Hani N. Sabbah[9], and Ulrich P. Jorde[1]

[1]Division of Cardiology, Department of Medicine, Columbia University Medical Center, New York, NY, USA; [2]Department of Epidemiology, Mailman School of Public Health Columbia University, New York, NY, USA; [3]Division of Pulmonary, Columbia University Medical Center, New York, NY, USA; [4]Section of Cardiology, Tulane University School of Medicine, New Orleans, LA, USA; [5]Cardiovascular and Methabolic Department, IRCCS Multimedica, Milano, Italy; [6]Department of Periodontology, University of Bonn, Bonn, Germany; [7]Department of Periodontology, Columbia University Medical Center, New York, NY, USA; [8]Division of Endocrinology, Department of Medicine, New York University, New York, NY, USA; and [9]Division of Cardiovascular Medicine, Department of Medicine, Henry Ford Hospital, Detroit, MI, USA

Aims	Volume overload and venous congestion are typically viewed as a consequence of advanced and of acute heart failure (HF) and renal failure (RF) although it is possible that hypervolaemia itself might be a critical intermediate in the pathophysiology of these diseases. This study aimed at elucidating whether peripheral venous congestion is sufficient to promote changes in inflammatory, neurohormonal, and endothelial phenotype similar to those observed in HF and RF.
Methods	To experimentally model peripheral venous congestion, we developed a new method (so-called venous stress test) and applied the methodology on 24 healthy subjects (14 men, age 35 $\pm$ 2 years). Venous arm pressure was increased to ~30 mmHg above the baseline level by inflating a tourniquet cuff around the dominant arm (test arm). Blood and endothelial cells (ECs) were sampled from test and control arm (lacking an inflated cuff) before and after 75 min of venous congestion, using angiocatheters and endovascular wires. Magnetic beads coated with EC-specific antibodies were used for EC separation; amplified mRNA was analysed by Affymetrix HG-U133 Plus 2.0 Microarray.
Results	Plasma interleukin-6 (IL-6), endothelin-1 (ET-1), angiotensin II (AII), vascular cell adhesion molecule-1 (VCAM-1), and chemokine (C-X-C motif) ligand 2 (CXCL2) were significantly increased in the congested arm. A total of 3437 mRNA probe sets were differentially expressed ($P < 0.05$) in venous ECs before vs. after testing, including ET-1, VCAM-1, and CXCL2.
Conclusion	Peripheral venous congestion causes release of inflammatory mediators, neurohormones, and activation of ECs. Overall, venous congestion mimicked, notable aspects of the phenotype typical of advanced and of acute HF and RF.
Keywords	Congestive heart failure • Endothelium • Endothelin • Inflammation

Introduction

Heart failure (HF) and renal failure (RF) promote fluid retention and venous congestion thereby shifting human physiology from a healthy biosystem that operates at low pressures to a pathophysiological milieu where organs are forced to function with significantly elevated venous and interstitial pressures several times above normal.

Besides venous congestion, inflammation, neurohormonal activation, and endothelial cell (EC) activation are also notable aspects of the phenotype typical of advanced and of acute HF and RF. Plasma

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levels of cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α),[1–7] vasoactive peptides, such as endothelin-1 (ET-1),[8,9] neurohormones, such as angiotensin II (AII),[10,11] endothelial adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1),[12–16] and endothelial coagulation factors, such as vonWillebrand factor (vWF),[17–19] are elevated in advanced and in acute HF and RF. While prognostic and pathophysiologic relevance of these circulating biomarkers is now widely accepted, the site and source of their production remains the object of intense investigation.

In vitro evidence indicates that the endothelium may become activated and turn into a primary source of pro-inflammatory, vasoconstrictive, and pro-thrombotic mediators in response to biomechanical stress. Interleukin-6,[20] TNF-α,[21] ET-1,[22] AII,[23] VCAM-1,[24] ICAM-1,[25] and vWF[26] can be secreted within hours of exposure of ECs to stretch. Whether mechanical stretch is sufficient to activate the vascular endothelium in humans, for example, in a setting of venous congestion, remains unclear.

We developed a new experimental model of acute, peripheral venous congestion (so-called venous stress test) to test the hypothesis that venous congestion is sufficient (i) to cause local release of cytokines, vasoactive peptides, neurohormones, endothelial adhesion molecules, and coagulation factors and (ii) to cause EC activation in otherwise healthy human subjects. For the second purpose, we coupled venous EC sampling with transcriptomic approaches using microarray technology.

Methods

Study population and protocol

We enrolled 24 subjects who were normotensive, non-smokers with no history of chronic illness, or chronic medication use.

Venous stress test

Blood and ECs[27] were sampled from the antecubital or basilic vein of the non-dominant arm (control arm) at baseline (time 0) and from the dominant arm (test arm) after 75 min of peripheral venous congestion, using angiocatheters and endovascular wires. Local venous pressure was increased to ∼30 mmHg above the baseline level by inflating a tourniquet cuff around the arm proximally, just below the shoulder (*Figure 1*). As a control, blood was collected from the non-dominant (control) arm 75 min after baseline samplings and measurements. Vital signs (control arm) and arterial oxymetry (test arm) were also recorded at 75 min.

Study subjects were offered the option of undergoing EC and blood collection, or blood collection only.

Magnetic beads coated with EC-specific antibodies were used for EC separation. Amplified mRNA was analysed by Affymetrix HG-U133 2.0 Microarray.

A total of 24 normal subjects were enrolled and provided plasma samples. Of the 24, 16 also contributed EC samples 12 of which produced an mRNA product which was adequate for analysis in terms of quality and quantity. Therefore, our plasma protein results are based on a sample of 24 subjects while mRNA results are based on a subsample of 12 subjects.

Commercially available techniques were used to measure plasma IL-6, TNF-α, ET-1, AII, VCAM-1, ICAM-1, and vWF antigen (vWF:Ag).

An expanded Methods section is available in Supplementary material online.
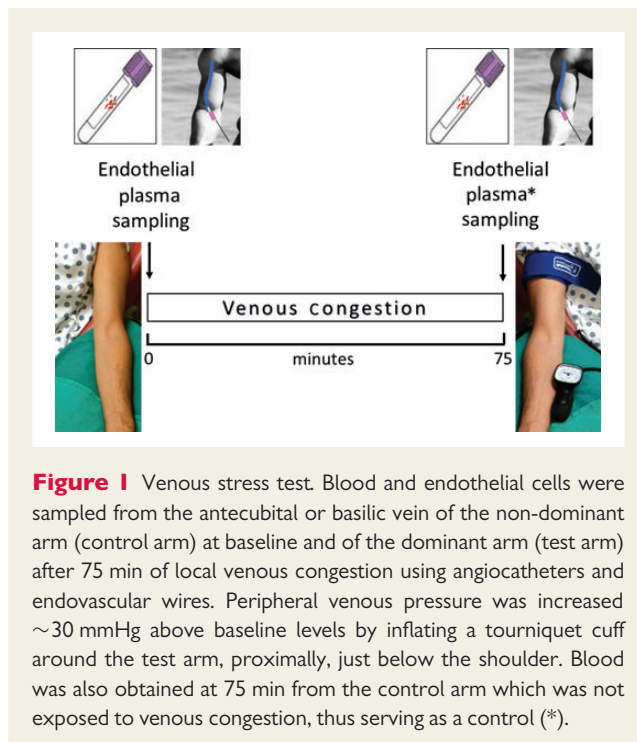

Figure 1 Venous stress test. Blood and endothelial cells were sampled from the antecubital or basilic vein of the non-dominant arm (control arm) at baseline and of the dominant arm (test arm) after 75 min of local venous congestion using angiocatheters and endovascular wires. Peripheral venous pressure was increased ∼30 mmHg above baseline levels by inflating a tourniquet cuff around the test arm, proximally, just below the shoulder. Blood was also obtained at 75 min from the control arm which was not exposed to venous congestion, thus serving as a control (*).

Statistical analysis

All comparisons are based on within person biomarker differences (i.e. either plasma protein level or EC mRNA level) before vs. after induction of experimental venous congestion. mRNA fold-changes are defined by the ratio of post-experiment to pre-experiment absolute transcript levels.

Data are presented as means ± SEM in the text and tables. The Wilcoxon rank-sum test (continuous variables) or χ^2 tests (categorical variables) were used to compare baseline clinical and laboratory characteristics between the 12 subjects with plasma data only, and the 12 subjects with both plasma and endothelial mRNA data. The Wilcoxon signed-rank test was used to test for significant differences in vital signs and plasma measurements before vs. after experimental venous congestion. For all analyses, a two-tailed *P*-value of 0.05 was used to infer statistical significance.

Endothelial cell mRNA (i.e. 'gene expression') data were first normalized and summarized using the log scale robust multi-array analysis[28] with default settings. Differential expression in mRNA after vs. before experimental conditions was assessed using paired *t*-tests. Additional analyses were performed using random effects mixed models in which patients were conditioned as a random effect, gene expression was the dependent variable, and experimental state (pre- vs. post-experiment) was the independent variable. These models accounted for the within person correlation in gene expression and additionally allowed us to include measures of both leucocyte common antigen-1 and α-2-actin expression levels as covariates to minimize the possibility that gene expression from leucocyte or smooth muscle cell (SMC) populations influenced our findings (i.e. confounding by leucocyte or SMC contamination). Similar methods have been recently utilized to isolate effects from specific cell populations in studies of neurodegenerative disease.[29] Statistical significance for gene expression analysis was based on α = 0.05 as well as false discovery rate (FDR)[30] to minimize type 1 error. Gene set enrichment analysis was performed using DAVID to generate a Functional Annotation Chart identifying gene-term enrichment using default settings.[31] Experimental details and

results following the Minimum Information About a Microarray Experiment Standards are available at the Gene Expression Omnibus (accession number GSE38783).

Results

Subject characteristics

Table 1 summarizes the clinical characteristics of the 24 subjects enrolled: 12 subjects with plasma data available and 12 subjects with both plasma and endothelial mRNA data. Overall, study subjects had a normal body mass index, lipid profile, serum glucose, and renal function. Serum creatinine levels were lower in the plasma-only group owing to a higher percentage of women.

Venous stress test
Cuff inflation raised peripheral venous pressure from 5 ± 0 mmHg at baseline to 36 ± 1 mmHg in the congested arm. Endothelial sampling was associated with minor discomfort; pain intensity was scored 0–3 in all cases using a 0–10 numeric scale. One subject developed a painful superficial phlebitis that was otherwise benign and resolved.

Systolic BP (119 ± 2 vs. 120 ± 2 mmHg, $P =$ NS) and diastolic BP (78 ± 1 vs. 77 ± 1 mmHg, $P =$ NS) did not change, while heart rate was higher at baseline than after 75 min of experimental venous congestion (70 ± 2 vs. 66 ± 2 b.p.m., $P = 0.01$).

Experimental venous congestion did not influence arterial O_2 saturation (98 ± 0 vs. $99 \pm 0\%$, $P =$ NS) and venous lactic acid (7.5 ± 0.3 vs. 8.0 ± 0.2 mg/dL, $P =$ NS) in the congested arm. These results suggest that experimental venous congestion did not induce local ischaemia in this model.

Acute venous congestion increases peripheral release of cytokines, vasoactive peptides, neurohormones, and endothelial adhesion molecules

The induction of venous congestion was related to a 60% increase in plasma levels of IL-6 and ET-1 (*Table 2*). Plasma levels of IL-6 also

tended to be higher in the samples collected from the control arm compared with baseline values ($P = 0.07$). Angiotensin II increased by 20% in the congested arm. A marginal but significant increase of plasma VCAM-1 occurred in the congested (test) arm when compared with control arm. Plasma ICAM-1 levels also tended to be higher in the congested arm. Plasma TNF-α and vWF:Ag levels did not change. These results suggest that experimental venous congestion causes local release of cytokines, vasoactive peptides, neurohormones, and endothelial adhesion molecules. Of note, the significant increase in AII suggests volume-dependent activation of tissue angiotensin-converting enzyme and/or chymase in the periphery.

Acute venous congestion promotes endothelial cell activation

Acute venous congestion was related to major changes in the EC transcriptome as assessed via Affymetrix genomic arrays. A total of 3437 probe sets were differentially expressed with $P < 0.05$ before vs. after venous congestion, 96 of which had an absolute fold-change >2.0 (Supplementary material online, *Table S2*). One thousand six hundred and twenty-eight probe sets were differentially expressed with FDR <0.05, among which 72 had an absolute fold-change value >2.0 (*Figure 2*). Results from mixed effects linear regression models that corrected for the presence of residual leucocytes and SMCs within the samples were even stronger than the aforementioned unadjusted results. Specifically, 5332 probe sets were differentially expressed before vs. after venous congestion ($P < 0.05$), among which 143 probe sets had a fold-change >2.0 (Supplementary material online, *Table S3*). Irrespective of the means of analysis, key signatures associated with venous congestion included genes relevant to inflammation, cell migration, and signal transduction, such as ET-1, VCAM-1, CD36, TNF receptor-associated factor 5 (TRAF5), and chemokine (C-X-C motif) ligand 2 (CXCL2). Gene ontology analysis is detailed in Supplementary material online, *Table S4*. On the basis of the above finding, we also assessed plasma CXCL2 ($n = 15$) and found that CXCL2 protein levels were higher in the congested arm compared with the control arm after 75 min of venous congestion (294 ± 110 vs. 254 ± 102 pg/mL, $P = 0.04$). These results suggest that acute venous congestion causes endothelial activation as evidence by significant up-regulation of several pro-inflammatory genes.

Discussion

The current study provides the first direct evidence that peripheral acute venous congestion, without ischaemia, causes inflammation and neurohormonal and EC activation in humans. Overall, this inflammatory, vasoconstrictive phenotype mimics, at least in part and on a local scale, notable aspects of the phenotype which is typical of patients with advanced and of those with acute HF and RF.

Several studies demonstrated elevations in circulating levels of cytokines, vasoactive peptides, neurohomones, endothelial adhesion molecules, and coagulation factors that correlate both with severity and acuity of HF and of RF.[1–18] The source and site of their production is controversial,[2,32–37] and no evidence has so far mechanistically linked peripheral venous congestion to this pro-inflammatory, vasoconstrictive, and activated vascular phenotype in humans. Herein, we introduce a new experimental model of peripheral venous congestion

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Table I Clinical characteristics of the study population by sampling methodology (mean $\pm$ SEM)

Variable	Plasma-only (*n* = 12)	Plasma and mRNA (*n* = 12)	*P*-value
Age (years)	34 ± 3	36 ± 2	0.30
Gender (no. male/female)	4/8	10/2	0.02
Body mass index (kg/m^2)	25 ± 1	24 ± 1	0.11
Total cholesterol (mg/dL)	168 ± 9	185 ± 9	0.31
Low-density cholesterol (mg/dL)	100 ± 8	111 ± 7	0.40
High-density cholesterol (mg/dL)	53 ± 4	60 ± 6	0.40
Triglycerides (mg/dL)	73 ± 7	72 ± 5	0.93
Blood glucose (mg/dL)	87 ± 2	90 ± 2	0.17
Serum creatinine (mg/dL)	0.71 ± 0.05	0.90 ± 0.04	<0.01

Table 2 Plasma measurements (means ± SEM) before and after 75 min of venous congestion

Variable	Baseline (B) (0 min)	Control arm (C) (75 min)	Test arm (T) (75 min)	P-value (T vs. B)	P-value (T vs. C)
IL-6 (pg/mL)	1.37 ± 0.44	1.79 ± 0.53	2.26 ± 0.58	<0.01	<0.01
TNF-α (pg/mL)	1.35 ± 0.08	1.27 ± 0.08	1.35 ± 0.11	0.75	0.22
ET-1 (pg/mL)	1.46 ± 0.19	1.26 ± 0.13	2.43 ± 0.27	<0.0001	<0.0001
AII (pg/mL)	27 ± 3	25 ± 3	32 ± 4	0.01	<0.01
VCAM-1 (ng/mL)	557 ± 26	544 ± 24	589 ± 25	<0.01	<0.01
ICAM-1 (ng/mL)	158 ± 9	158 ± 7	167 ± 9	0.09	0.06
vWF:Ag (%)	105 ± 9	100 ± 6	113 ± 9	0.37	0.15

IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; ET-1, endothelin-1; AII, Angiotensin II; VCAM-1, vascular adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; vWF:Ag, vonWillebrand factor antigen.

(so-called venous stress test) coupled with endothelial sampling[27] and transcriptomic approaches using microarray technology. This approach enables, in a controlled setting, assessment of the acute inflammatory, neurohormonal, and endothelial response to biomechanical stress in humans. An increase in local peripheral venous pressure of ∼30 mmHg caused a >60% increase in plasma IL-6 and ET-1 as well as a 20% increase in plasma AII in the congested arm of healthy subjects. Plasma levels of IL-6 also tended to be higher in the sample collected from the control arm compared with baseline values. While we cannot provide a direct mechanistic justification for this latter result, it is possible that activation of circulating T cells and macrophages in response to a local inflammatory stimulus such as experimental venous congestion caused systemic in addition to local release of IL-6. Peripheral spillover from the congested arm into the systemic circulation may offer an alternatively explanation. However, it appears less likely as the concentration of the other plasma mediators did not increase in the control arm.

Venous congestion promoted deferential expression of >3000 probe sets in venous ECs, including key markers of EC activation such as ET-1 and VCAM-1. These results in ECs parallel and may, at least in part, explain the increase in plasma ET-1 and VCAM-1, which was observed in venous blood collected from the congested arm. Overall, these data expand and provide mechanistic insights to previous observational evidence of EC activation in HF patients who were hospitalized for acute HF.[38]

Whole-genome expression analysis in the EC samples also helped to identify other genes and biological pathways that might be involved in the mechanotransduction of high intravenous pressure. Among the observed probe sets, we recognized other key pro-inflammatory and vasoactive genes such as: (i) CD36, the endothelial receptor for thrombospondin, a potent antagonist of the angiogenic signalling downstream of nitric oxide;[39] (ii) TRAF5 which is induced in atherosclerotic plaques and is one of the key mediators of the pro-inflammatory functions of CD40 ligand in ECs;[40] and (iii) CXCL2, a small pro-inflammatory chemokine belonging to the CXC family. Of note, recent evidence suggests that TRAF5 prolongs the half-life of chemokines, including CXCL2.[41] On the basis of the CXCL2 mRNA finding, we performed a data-driven a priori hypothesis test of CXCL2 plasma protein expression and found that CXCL2 was indeed increased in plasma collected from the congested arm compared with the control arm.

The present study has several limitations. First, the small sample size substantially limited our power to detect findings at the transcript level after adjustment for multiple comparisons. However, the primary aim of the experiment was to investigate the influence of venous congestion on plasma proteins, as they are more clinically relevant. Nevertheless, the mRNA data generated in our current study not only support our findings on plasma proteins, but also provide a rich database (see Supplementary material online, Tables S2−4) that will help us and others to develop focused mechanistic studies that can better explain how venous congestion leads to EC generation of pro-inflammatory mediators and, possibly, to neurohormonal activation. Secondly, this study was conducted in healthy subjects rather than in patients with HF and/or RF. However, the fact that we have studied healthy participants provides a strong proof-of-principle for the concept of hypervolaemia as a fundamental stimulus for the pathophysiology of HF and RF as it is not possible for other aspects of these disease phenotypes, for co-morbidities and/or for background therapy to confound our results. Finally, cuff inflation not only promoted venous congestion, but necessarily caused (i) hydrostatic pooling by reducing venous flow and (ii) reduction in arterial perfusion pressure by impinging on the brachial artery. These latter effects may have also influenced plasma concentration and/or peripheral release of the measured mediators. However, (i) the resulting change in local blood flow did not cause ischaemia in our model and (ii) reduction in perfusion pressure and hydrostatic pooling are typical clinical features of advanced HF where arterial blood pressure progressively declines[42] and chronic venous insufficiency is frequent,[43] thus making our model even more relevant, from a pathophysiological standpoint.

We developed a new human model of acute, local venous congestion (so-called venous stress test) where peripheral venous pressure was increased to ∼30 mmHg above baseline levels for 75 min by inflating a tourniquet cuff around the dominant arm. Transient venous congestion was sufficient to cause local release of inflammatory mediators, neurohormones, and endothelial activation. Additional studies are warranted to further test the hypothesis that venous and tissue congestion might be a causal contributor to (as opposed to a consequence of) the pathophysiology of advanced and of acute HF and RF via their influence on inflammation, neurohormonal, and EC activation. From a clinical perspective, if venous and tissue congestion prove to be fundamental stimuli for the pathophysiology of HF and
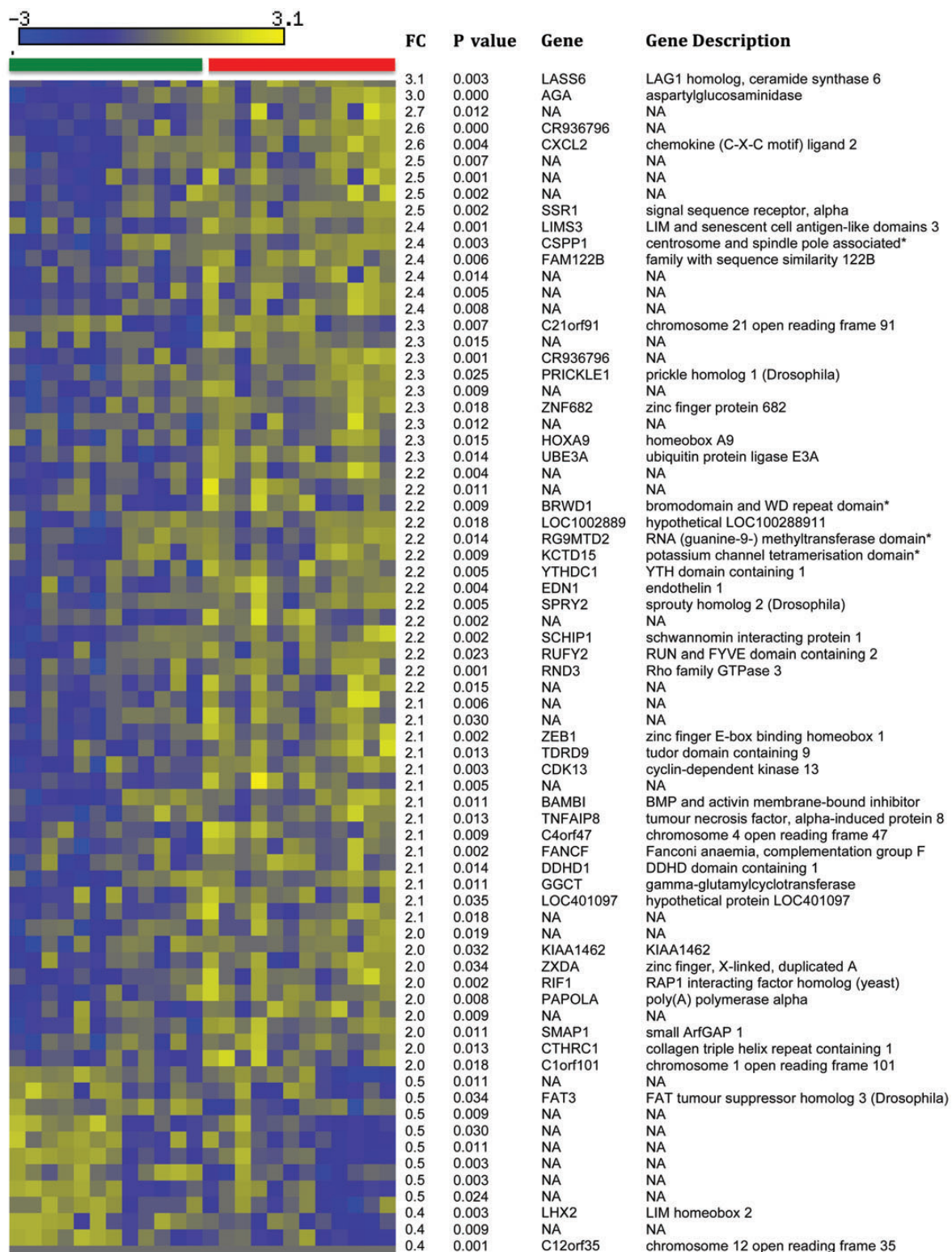
FC	P value	Gene	Gene Description
3.1	0.003	LASS6	LAG1 homolog, ceramide synthase 6
3.0	0.000	AGA	aspartylglucosaminidase
2.7	0.012	NA	NA
2.6	0.000	CR936796	NA
2.6	0.004	CXCL2	chemokine (C-X-C motif) ligand 2
2.5	0.007	NA	NA
2.5	0.001	NA	NA
2.5	0.002	NA	NA
2.5	0.002	SSR1	signal sequence receptor, alpha
2.4	0.001	LIMS3	LIM and senescent cell antigen-like domains 3
2.4	0.003	CSPP1	centrosome and spindle pole associated*
2.4	0.006	FAM122B	family with sequence similarity 122B
2.4	0.014	NA	NA
2.4	0.005	NA	NA
2.4	0.008	NA	NA
2.3	0.007	C21orf91	chromosome 21 open reading frame 91
2.3	0.015	NA	NA
2.3	0.001	CR936796	NA
2.3	0.025	PRICKLE1	prickle homolog 1 (Drosophila)
2.3	0.009	NA	NA
2.3	0.018	ZNF682	zinc finger protein 682
2.3	0.012	NA	NA
2.3	0.015	HOXA9	homeobox A9
2.3	0.014	UBE3A	ubiquitin protein ligase E3A
2.2	0.004	NA	NA
2.2	0.011	NA	NA
2.2	0.009	BRWD1	bromodomain and WD repeat domain*
2.2	0.018	LOC1002889	hypothetical LOC100288911
2.2	0.014	RG9MTD2	RNA (guanine-9-) methyltransferase domain*
2.2	0.009	KCTD15	potassium channel tetramerisation domain*
2.2	0.005	YTHDC1	YTH domain containing 1
2.2	0.004	EDN1	endothelin 1
2.2	0.005	SPRY2	sprouty homolog 2 (Drosophila)
2.2	0.002	NA	NA
2.2	0.002	SCHIP1	schwannomin interacting protein 1
2.2	0.023	RUFY2	RUN and FYVE domain containing 2
2.2	0.001	RND3	Rho family GTPase 3
2.2	0.015	NA	NA
2.1	0.006	NA	NA
2.1	0.030	NA	NA
2.1	0.002	ZEB1	zinc finger E-box binding homeobox 1
2.1	0.013	TDRD9	tudor domain containing 9
2.1	0.003	CDK13	cyclin-dependent kinase 13
2.1	0.005	NA	NA
2.1	0.011	BAMBI	BMP and activin membrane-bound inhibitor
2.1	0.013	TNFAIP8	tumour necrosis factor, alpha-induced protein 8
2.1	0.009	C4orf47	chromosome 4 open reading frame 47
2.1	0.002	FANCF	Fanconi anaemia, complementation group F
2.1	0.014	DDHD1	DDHD domain containing 1
2.1	0.011	GGCT	gamma-glutamylcyclotransferase
2.1	0.035	LOC401097	hypothetical protein LOC401097
2.1	0.018	NA	NA
2.0	0.019	NA	NA
2.0	0.032	KIAA1462	KIAA1462
2.0	0.034	ZXDA	zinc finger, X-linked, duplicated A
2.0	0.002	RIF1	RAP1 interacting factor homolog (yeast)
2.0	0.008	PAPOLA	poly(A) polymerase alpha
2.0	0.009	NA	NA
2.0	0.011	SMAP1	small ArfGAP 1
2.0	0.013	CTHRC1	collagen triple helix repeat containing 1
2.0	0.018	C1orf101	chromosome 1 open reading frame 101
0.5	0.011	NA	NA
0.5	0.034	FAT3	FAT tumour suppressor homolog 3 (Drosophila)
0.5	0.009	NA	NA
0.5	0.030	NA	NA
0.5	0.011	NA	NA
0.5	0.003	NA	NA
0.5	0.003	NA	NA
0.5	0.024	NA	NA
0.4	0.003	LHX2	LIM homeobox 2
0.4	0.009	NA	NA
0.4	0.001	C12orf35	chromosome 12 open reading frame 35

Figure 2 Heat map of the 72 probe sets with an absolute fold-change >2.0 and a false discovery rate <0.05. The green header bar refers to endothelial cell gene expression in samples prior to venous congestion and the red header bar represents endothelial cell gene expression in samples after experimental venous congestion. Blue-coloured cells in the matrix represent relative under expression (i.e. lower expression values) and yellow-coloured cells represent relative over expression (i.e. higher expression values). FC, fold-change.

RF, early and aggressive treatment of hypervolaemia may have a critical role not only in symptom relief, but also in disease progression. This early treatment strategy may include not only diuretics, but also, as one may infer from our results and test in future studies, adjuvant therapies such as short-term anti-inflammatory and vasoactive treatments that may prevent the development of overt decompensation. In the future, our experimental approach may also help identify 'volume-sensitive' patients, who have enhanced endothelial and neurohormonal responses to venous and tissue congestion. This group of patients may be more prone to decompensation episodes and, consequently, more likely to benefit from meticulous monitoring of their volume status, which may then be used to prompt early interventions.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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CARDIOVASCULAR FLASHLIGHT

Vanishing pulmonary oedema, a visual delight!

Sudhir Suryakant Shetkar, Shrenik R. Doshi, and Sandeep Seth*

Department of Cardiology, A.I.I.M.S, Ansari Nagar, New Delhi 110029, India

* Corresponding author. Tel: +91-11-26593322, Fax: +91-11-26588663, Email: drsandeepseth@hotmail.com

A forty-two year male patient with bileaflet mechanical mitral valve prosthesis presented with sudden onset breathlessness and haemodynamic collapse. He was in New York Heart Association (NYHA) class IV status with a pulse rate of 120 b.p.m. and blood pressure of 90/60 mmHg. Bilateral extensive crepitations over lung fields could be auscultated. He had stopped oral anticoagulation for past 15 days and had a subtherapeutic international normalized ratio of 1.5.

Urgent transthoracic echocardiogram revealed an elevated mean diastolic gradient (MDG) of 37 mmHg across mitral prosthesis and cine-fluoroscopy (Supplementary material online, *Video S1* and *Panel C*) showed completely immobile one leaflet with restricted movement of the other leaflet, confirming prosthetic heart valve thrombosis (PHVT). Considering imminent threat to the life and non-availability of surgical team due to logistic issues, thrombolysis with streptokinase was considered. He received an accelerated thrombolytic regime with 1.5 million unit streptokinase intravenously over 1 h followed by 100 000 units per hour continuous infusion.

There was significant clinical improvement within 6 h of therapy, with opening of both valve leaflets (*Panels D* and *E*; Supplementary material online, *Videos S2* and *S3*) and fall in MDG to 6 mmHg. The pre- (*Panel A*) and post- (*Panel B*) therapy chest radiograph taken 24 h apart, showing complete resolution of pulmonary oedema, is testimony for this dramatic clinical response.

Though surgery is recommended treatment for left-sided prosthetic valve thrombosis, thrombolytic therapy is still the first line therapy for PHVT in countries with limited resources. This case demonstrates utility of thrombolytic therapy in managing PHVT.

(*Panel A*) Chest Roentgenogram at presentation shows mitral prosthetic valve (black arrow) and pulmonary oedema. (*B*) Chest Roentgenogram at 24 h shows complete resolution of pulmonary oedema. (*C*) Cine-fluoroscopy shows completely immobile one leaflet with partial movement of other leaflet of the bileaflet mechanical mitral prosthetic valve, confirming prosthetic heart valve thrombosis. (*D*) Completely opened both leaflets of mitral valve prosthesis on cine-fluoroscopy and (*E*) on transthoracic echocardiography.

Supplementary material is available at *European Heart Journal* online.