

Supporting Information

Metabolic phenotyping in venous disease: the need for standardization.

Sarah Onida^{1†*}, Matthew K. H. Tan^{1†}, Marina Kafeza^{1†}, Richmond T. Bergner^{2‡}, Joseph Shalhoub^{1†}, Elaine Holmes^{2,3‡}, Alun H. Davies^{1†}

¹ † Academic Section of Vascular Surgery, Department of Surgery and Cancer, Imperial College London, Floor 4 East, Charing Cross Hospital, Fulham Palace Road, London W6 8RF

² ‡ Section of Computational and Systems Medicine, Department of Surgery and Cancer, Imperial College London Sir Alexander Fleming Building, Prince Consort Road, Kensington, London SW7 2BB

³ ‡ Health Futures Institute, Murdoch University, Discovery Way, Perth, W.A.

* Corresponding Author

Miss Sarah Onida

NIHR Clinical Lecturer in Vascular Surgery, Academic Section of Vascular Surgery

Department of Surgery and Cancer, Imperial College London

s.onida@imperial.ac.uk

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Supplement 1 – Search algorithm

((((((((((dvt[Title/Abstract]) OR (((deep[Title/Abstract]) AND venous[Title/Abstract]) AND thrombosis[Title/Abstract])) OR ((pulmonary[Title/Abstract]) AND embolism[Title/Abstract])) OR ((venous[Title/Abstract]) AND thrombus[Title/Abstract])) OR VTE[Title/Abstract]) OR ((venous[Title/Abstract]) AND thromboemboli*[Title/Abstract])) OR ((venous[Title/Abstract]) AND thrombosis[Title/Abstract])) OR post thrombotic syndrome[Title/Abstract]) OR (((varicose[Title/Abstract]) AND ulcer*[Title/Abstract])) OR ((venous[Title/Abstract]) AND ulcer*[Title/Abstract])) OR (((((((chronic[Title/Abstract]) AND venous[Title/Abstract]) AND insufficiency[Title/Abstract])) OR ((venous[Title/Abstract]) AND insufficiency[Title/Abstract])) OR ((venous[Title/Abstract]) AND disease[Title/Abstract])) OR ((chronic[Title/Abstract]) AND venous[Title/Abstract]) AND disease[Title/Abstract])) OR ((varicose[Title/Abstract]) AND vein[Title/Abstract])) AND (((((((metabolomic*[Title/Abstract]) OR metabonomic*[Title/Abstract]) OR metabolic profiling[Title/Abstract]) OR metabolic phenotyping[Title/Abstract]) OR NMR[Title/Abstract]) OR nuclear magnetic resonance spectro*[Title/Abstract]) OR mass spectro*[Title/Abstract])).

Supplement 2 – Extended tables

Table 1 – CVD and VLU Results – Table presenting the list of articles identified by the systematic review and relevant data extracted.
Tentative / preliminary metabolite allocations in italics.

TABLE 1	H / A	Pathology / Model	Comparator	Assay	T / U	N	Substrate	Statistical analysis	Multiple testing correction	p-value threshold	Control for variables	Upregulated metabolites in cases	Upregulated metabolites in controls	Downregulated metabolites in cases	Comparative testing
Tanaka 2010 (27)	H	CVD C2 - n = 4 C3 - n = 1 C4 - n = 4 C5 - n = 1 Total n = 10	Controls with PAD n = 6	MALDI-IMS	U	16	Tissue – GSV	T test One-way ANOVA	Tukey's test	< 0.05	-	Lysophosphatidylcholine (LPC) (1-acyl 16:0) around valves Phosphatidylcholine (PC) (1-acyl 34:2) ubiquitous PC (1-acyl 36:4) around valves Sphingomyelin (SM) (d18:1/C16:0) around valves	LPC (1-acyl 16:0) uniformly distributed PC (1-acyl 34:2) ubiquitous		-
Tanaka 2012 (28)	H	CVD C2 - n = 16 C3 - n = 11 C4 - n = 21 C5 - n = 2 Total n = 50 (38 patients)	Controls with PAD n = 10	MALDI-IMS	U	48	Tissue – GSV	One-way ANOVA	Tukey's test	< 0.05	-	LPC (1-acyl 16:0) in the intima and media PC (diacyl 16:0/20:4) in the intima and media PC (diacyl 16:0/18:1) ubiquitous Triglyceride (TG) (52:2) and (52:3) in adventitia	PC (diacyl 16:0/18:1) ubiquitous		Histology (HE staining) Lipid staining Biochemical quantitation Immunostaining (Anti vWF and anti D2-40)
Anwar 2012 (26)	H	CVD C2 - n = 8	Non varicose GSV tissue n = 8	MAS NMR	U	16	Tissue – GSV	Multivariate (PCA, OPLS) ROC curve	-	-	-	Creatine; Myo-inositol; Lactate; Glutamate	TG		-
Tanaka 2016 (30)	A	Male Sprague Dawley rats (8 weeks) lymphatic ligation model	Contralateral limb	MALDI-IMS	U	N/A	Tissue – Femoral vein wall	Descriptive	Tukey Kramer test	< 0.05	-	LPC (1-acyl 16:0) in vein wall PC (16:0/20:4) in vein wall TG (52:2) in vein wall PC (diacyl 16:0/18:1) ubiquitous	PC (diacyl 16:0/18:1) ubiquitous		Immunohistochemistry Immunofluorescent staining Adipocyte detection TNF alpha and Caspase 3 expression
Anwar 2016 (29)	A	Male Sprague Dawley rats (12 weeks) Stretch IVC model – 2 g stretch for 4 hours; 2 g stretch for 18 hours n = 5	0.5 g and 2g stretch applied for either 4 or 18 hours (4 groups)	NMR MS	U	5	Tissue - IVC	Multivariate One-way ANOVA Spearman correlation	Benjamini Yekutieli FDR correction	< 0.05	-	Valine; Choline TG (18:2/18:1/18:1); (18:2/18:2/18:1); (16:1/18:1/22:5) Increased in veins stretched for 18h compared to non-stretched 18h			-
Anwar 2017 (31, 37)	H	CVD C2 - n = 4 C3 - n = 66 C4 - n = 9 C5 - n = 1 Total n = 80	Non – CVD controls n = 35	NMR MS	U	115	Tissue – GSV or tributary	Multivariate (PCA, OPLS-DA)	Benjamini Yekutieli FDR correction	< 0.0001	Age, sex past medical history and medications assessed in multivariate analysis	Phosphatidylserines (PS) (16:0/22:6; 18:0/20:1; 18:0/20:3; 18:0/22:6; 16:0/22:6; 18:0/20:1; 18:0/20:3; 18:0/22:4; 18:0/22:6) PC (18:0/20:3; 38:4; 16:0/20:3) SM (d18:2/24:0; d18:2/24:1; d18:2/24:0; d18:2/24:1) Phosphatidylethanolamines (PhE) (O-16:1/22:4; O-16:1/22:6; O-16:1/22:6) Phosphatidylinositol (PI) (18:2/18:0; 36:1) Myo-inositol; Inosine; Taurine; Uridine; Creatine; Alanine; Guanosine; Glutamine; Glutamate	Ceramides (d18:0/22:0, d40:1) Lysophosphocholine (lysoPC) (18:1) TG (12:0/14:0/18:1; 12:0/16:0/16:1; 12:0/16:0/18:1; 14:0/16:1/16:0; 14:0/16:0/18:1; 15:0/16:0/18:1; 14:0/17:0/18:1; 16:0/16:0/18:1)		Messenger RNA (mRNA) expression

Ennis 1994 (41)	H	VLU n = 4	Ischaemic skin samples/ Controls n = 13	NMR	U	17	Ulcer biopsy	One-way ANOVA	Test of Scheffe	< 0.05	-			Phosphocreatine Adenosine triphosphate (ATP)	-
Junka 2017 (43)	H	VLU n = 20	-	NMR	U	20	Wound fluid	Multivariate (PLS - DA)	-	-	-	Lactate; Lipids (L1, L2, L3); Glycerophosphorylcholine (GPC); Lysine; Acetate associated with pseudomonas aeruginosa; Valine; Leucine; Isoleucine; 3- hydroxybutyrate(3-HB); Propylene glycol; Alanine; Ethanol; Aspartate; Urea; Histamine; N-N-dimethylglycine; Phenylalanine; Tyrosine; Glutamate; Pyruvate; Succinate		Microbiology	

Table 2 – VTE Results – Table presenting the list of articles identified by the systematic review and relevant data extracted.

Tentative / preliminary metabolite allocations in italics

TABLE 2	H / A	Pathology / Model	Comparator	Assay	T / U	N	Substrate	Statistical analysis	Multiple testing correction	p-value threshold	Control for variables	Upregulated metabolites in cases	Upregulated metabolites in controls	Downregulated metabolites in cases	Comparative testing
Deguchi 2015 (48)	H	Unprovoked DVT n = 40	Non DVT controls n = 40	MS	T + U	80	Plasma	Mann Whitney Spearman Fisher's exact probability test	-	< 0.05	-			Acylcarnitines (AC) (10:1; 12:0; 12:2; 16:1; 18:1; 18:2); Palmitoleoyl-carnitine (16:1); Decenoyl-carnitine (10:1)	Thrombosis assays
Bujak 2015 (57)	A	Pig (2-3 month old castrated male) PE (polydextran microspheres 100– 300 µm in diameter injected in femoral vein; increase of mPAP >40mmHg for at least 20 minutes) n = 16	Baseline blood	LC – GC – QTOF MS	U	16	Plasma	Univariate Multivariate (PCA, OPLS DA) Welch's paired t test Mann Whitney U test	-	< 0.05	-	Pyruvate; Lactate; Glycerol; Palmitic acid; Oleic acid; 3-hydroxybutyric acid; 2-ketoisocaproic acid; Galacturonic acid PC (O-40:4); Sphingosine; Alpha-tocopherol; Leukotriene C4	Docosatetraenoic acid; Docosapentaenoic acid; Hydroxyoxohexadecanoic acid; Dihydroxyoctadecadienoic acid; Oxoheptadecatrienoic acid; Dodecadienoic acid; Methyltridecanedioic acid; Ceramide (d18:1/22:0)-1P; SM (d18:1/23:0); PS (36:2); PA (18:0/18:3); PI (21:0/10:0; 21:0/13:1); LPE (16:0; 20:4); LPI (20:1); LPA (12:0); Creatine; Arginine; Didesmethyl tocotrienol; Desmosine		-
Obi 2016 (54)	A	Mouse (young and old C57BL/6) DVT (electrolytic IVC model) Young n = 15 Old n = 16	Controls	NMR	U	31	Serum	ANOVA T test	Holm-Šidák test	p < 0.05	Linear regression for association between thrombosis parameters, P and E selectin levels and metabolite concentration	Glutamine; Proline; Phenylalanine			Vein wall P- and E- selectin levels
Cao 2018 (53)	A H	Male Sprague – Dawley rats DVT (IVC ligation model) n = 10 Unprovoked distal DVT n = 61	Controls (rats) n = 10 Sham controls (rats) n = 10 Controls (human) n = 61	NMR	U	30 122	Serum	CV ANOVA Multivariate analysis (PCA, OPLS DA)		< 0.05	-	RAT DVT Lipids ; Leucine; Valine; N-acetylglycoproteins (NAC); O-acetylglycoproteins (OAC); Acetoacetate; Pyruvate HUMAN DVT Lipids; Valine; 3-HB; Lactate; Lysine; Acetate; Glutamine; Acetoacetate; Pyruvate; Creatine; GPC; Glycine; Tyrosine; Phenylalanine; Formate	-	RAT DVT Lactate; Alanine; Glucose; Methanol HUMAN DVT NAG; Acetone; Glutamate; Glucose; Methanol	-

Sung 2018 (55)	A	Mouse (Male Balb/C) DVT (IVC ligation model) n = 10	Sham control n = 10	MS and NMR	U	20	Serum IVC wall	Student's t test Multivariate (PCA, OPLS DA)	FDR correction	< 0.05	-	SERUM Alpha-hydroxyisobutyrate; L carnitine; LPC (16:0, 18:0); PC (18:0/22:6); SM (d18:0/15:1; d18:0/18:1; d18:1/16:0; d18:1/23:0; d18:1/24:0; d18:1/24:1; d18:1/24:1; d18:2/24:0) VEIN WALL Acetylcarnitine; Adenosine; Ceramide; GPC; PC (14:0/16:0; 15:0/20:4 / O-12:4/24:0; 16:0/18:0; 16:0/18:2; 16:0/20:4; 16:0/22:6; 18:1/18:2; O-16:0/16:0; O-16:0/18:2); SM (d18:1/16:0; d18:1/24:0; d18:1/24:1; d18:1/24:2)	-	SERUM Alanine; Allantoin; Citrate Creatine; Fumarate Glycine; Succinate; Taurine Tyrosine; Valine; Acetylcarnitine; Adenine Adenosine; Creatine Indoxylsulfuric acid; L-tyrosine PC (16:0/20:4; 18:0/20:3; 18:0/20:4; 18:1/18:2; 20:3/20:5); Taurine; TG (14:0/20:2/22:6; 16:0/18:1/18:2; 16:1/18:1/22:6; 16:0/18:1/22:6; 16:0/18:2/18:2; 16:0/18:2/20:4; 18:1/18:2/18:3; 18:2/18:2/18:2; 16:0/16:0/22:6) VEIN WALL Choline PA (15:0/18:0) TG (16:0/18:2/20:3)	-
Voils 2018 (49)	H	Critically ill patients with VTE (provoked, blunt trauma); DVT n = 10 and PE n = 10	Critically ill patients without VTE n = 20	UPLC MS	U	40	Plasma	Chi square Fisher's exact test T test Wilcoxon rank sum tests	FDR	< 0.05		N-formylkynurenine; 5-hydroxy N-formylkynurenine		In silico gene expression analysis	
Zeleznik 2018 (52)	H	PE with risk stratification (low n = 46; intermediate n = 28; high n = 18)	-	UPLC MS	U	92	Plasma	Mann Whitney U test Fisher's exact test	Benjamini Hochberg FDR	< 0.02	Multivariable linear regression to differentiate between PE risk	Low vs intermediate risk Xanthosine; Alpha-ketoglutarate; Arachidoylcarnitine; Picolinic acid; Vanillylmandelate (VMA) Intermediate vs high risk Phenylalanine; Lactose; Tyrosine; Caffeine; 3-HIB; Citrate		-	
Jiang 2018 (58)	H	Incident VTE DVT n = 240, PE n = 125	Controls n = 6963	LC-MS	U	7,203	Serum	Multivariate (PCA, OPLS DA) Sensitivity analysis Pearson correlation	Bonferroni correction	< 0.05	Logistic regression for VTE association. Adjustment for BMI	None significant			
Maekawa 2019 (56)	A	Rabbit (male Japanese white rabbits) DVT (jugular venous thrombus; jugular endothelial denudation and vessel ligation) n = 5	-	CE-TOFMS	U	5	Plasma Thrombus	Unpaired t test One way ANOVA Spearman's test	Bonferroni correction	< 0.05		PLASMA Citrate; Glucose-6-phosphate (glucose 6-P); Nicotinamide adenine dinucleotide phosphate (NADP); Tryptophan; Fructose-6-phosphate THROMBUS Lactate; Adenosine monophosphate (AMP); Choline; Hypoxanthine; Guanine monophosphate (GMP); Guanine; Glutathione		Thromboelastometry Cellular contents And Glut 1 expression in human aspirated DVT Histology	

Table 1 and 2 abbreviations: 3-HB – 3-hydroxybutyrate; 3-HIB – 3-hydroxyisobutyrate; A – animal; AC – acylcarnitine; Alpha KG – alpha ketoglutarate; AMP – adenosine monophosphate; ANOVA – analysis of variance; ATP – adenosine triphosphate; CE-TOF MS – capillary electrophoresis time of flight; CV-ANOVA – cross-validated residuals analysis of variance; CVD – chronic venous disease; C2/3/4/5 – CEAP stages; GC – gas chromatography; GMP – guanine monophosphate; Glucose 6-P – glucose 6-phosphate; GPC – glycerophosphocholine; H – human; HE – haematoxylin-eosin; FDR – false discovery rate; IVC – inferior vena cava; LPA – lysophosphatidic acid; LPC – lysophosphatidylcholine; LPE – lysophosphatidylethanolamine; LPI – lysophosphatidylinositol; lysoPC – lysophosphocholine; MAS – magic angle spinning; mPAP - mean pulmonary arterial pressure; NAC – N-acetylglycoproteins; NADP - nicotinamide adenine dinucleotide phosphate; NAG – N-acetylglutamate; OAC - O-acetylglycoproteins; OPLS – DA – orthogonal partial least square regression discriminant analysis; PA – phosphatidic acid; PAD – peripheral arterial disease; PC – phosphatidylcholine; PCA – principal component analysis; PE – pulmonary embolism; PhE – phosphatidylethanolamine; PI – phosphatidylinositol; PS – phosphatidylserine; QTOF – quad time of flight; ROC – receiver operating characteristic curve analysis; SM – sphingomyelin; T – targeted; TG – triglyceride; TMAO – trimethylamine N-oxide; TNF – tumour necrosis factor; U – untargeted; VLU – venous leg ulceration; VMA – vanillylmandelate; VTE – venous thromboembolism; vWF – von Willebrand Factor