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Infusion of foam sclerosants results in a distance-dependent procoagulant activity, haemoconcentration and elevation of D-dimer levels

DE Connor[1,2,3], JE Joseph[2,3], T Exner[4], DDF Ma[2,3] and K Parsi[1,2,3]

Abstract

Objective: To investigate the biological effects of foam sclerotherapy *in vivo*.

Materials and methods: Ultrasound-guided sclerotherapy was performed using a 3% sodium tetradecyl sulphate or polidocanol. A total of 15 mL of foam was injected. Samples were collected from antecubital veins, target saphenous veins and the adjoining deep veins before, immediately after and 1 hour after the procedure. Saphenous vein samples were also taken sequentially at set 15 cm intervals. Clotting times, D-dimer, cell counts and biochemical parameters were measured. D-dimer levels were repeated one week later.

Results: Forty procedures were performed**.** Systemic clotting times were not affected by the procedure. Injection of 0.5 mL of foam 5 cm away from the relevant junctions resulted in procoagulant activity in the adjoining deep veins (sodium tetradecyl sulphate) and the target saphenous veins (sodium tetradecyl sulphate and polidocanol). The procoagulant effect in the target veins reached a peak at 15 cm but normalised at 45 cm. D-dimer levels were significantly increased 1 hour after treatment with either agent and remained elevated one week later. Sodium tetradecyl sulphate and to a lesser degree polidocanol induced biochemical changes consistent with haemoconcentration.

Conclusion: Infusion of foam sclerosants results in a distance-dependent procoagulant activity in the exposed vessels. Foam sclerotherapy results in haemoconcentration and elevation of D-dimer.

Keywords

Foam sclerotherapy, blood cells, coagulation, D-dimer, cardiac enzymes, sclerosants

Introduction

Detergent sclerosants sodium tetradecyl sulphate (STS) and polidocanol (POL) are commonly administered in the foam format during ultrasound-guided sclerotherapy (UGS) to treat varicose veins and vascular malformations. These agents act by inducing endothelial cell lysis, exposure of basement membrane proteins with the ultimate aim of achieving endovascular fibrosis. The procedure may be complicated by rare thromboembolic events such as deep vein thrombosis (DVT),[1,2] pulmonary embolism,[1] transient ischaemic attacks and stroke.[3]

We have previously demonstrated that the *in vitro* administration of high concentration sclerosants to plasma samples significantly prolongs the activated partial thromboplastin time (APTT), prothrombin time (PT) and Factor Xa clotting time (XACT) assays.[4] The *in vitro* incubation of circulating blood cells, as well as cultured endothelial cells with high concentrations of detergent sclerosants, results in cellular lysis, whilst at low concentrations, stimulates the activation of platelets and release of procoagulant platelet-derived microparticles (PMP) that correspond to shortened XACT times.[4,5] We have also shown that blood components and in particular plasma proteins deactivate the sclerosants resulting in a gradual fall in the concentration and

[1]Dermatology, Phlebology and Fluid Mechanics Research Laboratory, St Vincent's Centre for Applied Medical Research, Sydney, Australia
[2]Haematology Research Laboratory, St Vincent's Centre for Applied Medical Research, Sydney, Australia
[3]University of New South Wales, Sydney, Australia
[4]Haematex Research, Hornsby, Australia

Corresponding author:
Kurosh Parsi, Dermatology, Phlebology and Fluid Mechanics Research Laboratory, St. Vincent's Hospital Centre for Applied Medical Research, Level 8, Lowy-Packer Building, 405 Liverpool Street, Darlinghurst, NSW 2010, Australia.
Email: kparsi@stvincents.com.au

a corresponding change from an anticoagulant to a procoagulant profile. Due to the *in vitro* nature of these investigations, only liquid agents were investigated as foam sclerosants could not be incubated with blood samples without affecting the foam structure.

Only a handful of *in vivo* studies have investigated the biological effects of foam sclerotherapy and those published have produced conflicting results.[6–8] One study[6] reported a prolongation of PT at both 30 minutes and 24 hours following liquid sclerotherapy using STS, while another publication[7] reported no change in the systemic clotting times with STS foam. None of these studies investigated the biological effects of foam sclerosants on target vessels or the adjoining deep veins where a deep vein thrombus may actually form.

In the current study, we investigated the *in vivo* effects of foam sclerotherapy on circulating blood cell counts and coagulation assays. Samples were taken from the systemic circulation, target vessels and the adjoining deep veins at set time and distance intervals. In the systemic circulation, we also assessed the effects of sclerotherapy on D-dimer levels and biochemical markers including renal and liver function tests and cardiac enzymes.

Methods

Ethics approval

Approval for this study was obtained from St Vincent's Hospital Human Research Ethics Committee and all research was performed in accordance with the Declaration of Helsinki.

Patient recruitment

Patients with truncal lower limb venous incompetence were recruited. Following informed consent, all patients underwent full history and examination. Duplex ultrasound venous incompetence studies (mapping) of the entire lower limb deep and superficial venous systems were performed in the standing position, using manual compression. Reflux was defined as retrograde flow >0.5 s and was assessed by spectral and colour Doppler flow. Inclusion criteria included consenting patients aged between 18 and 75 years requiring intervention for great (GSV) or small saphenous vein (SSV) incompetence. Exclusion criteria included known allergy to STS or POL, acute deep or superficial venous thrombosis or pulmonary embolism in the previous 12 months, recent (preceding 4 weeks) surgery requiring general anaesthesia or long haul (>5 hours continuous) travel on car, coach or plane, inability to walk daily for 30 minutes at a brisk pace, pregnancy, breast feeding, intake of the oral contraceptive pill, hormone replacement therapy, fish oil, vitamin E, Chinese or other herbal supplements. Two patients on anticoagulant therapy with warfarin for atrial fibrillation were allowed to participate in this study but their clotting time and D-dimer measurements were excluded from the analysis.

Foam preparation

Foam was prepared using a modified Tessari method.[9] In brief, one luerslip syringe (1 mL, Becton Dickson [BD], NJ, USA) containing 0.6 mL of 3% liquid sclerosant was connected to a 3-way stopcock (BD) via a 5 μm Sterifix filter (B-Braun, Melsungen, Germany). One luerlock syringe (3 mL, Terumo, NJ, USA) containing 2.4 mL of room air was connected to the second port of the stopcock. The plungers were moved through a minimum of 10 full strokes to mix the liquid with air. The assembly was inverted at least once during foam preparation to ensure adequate mixing. The foam was collected in the 3 mL luerlock syringe.

Sample collection

Systemic whole blood samples were collected from antecubital veins following the application of a tourniquet 10 cm proximal to the site of venepuncture using a 21 G butterfly needle connected to a Vacutainer™ system (BD). Deep vein and target vein samples were collected under ultrasound guidance using a 21 G needle connected to a Vacutainer system via a 25 cm extension tube (TUTA Healthcare, Sydney, Australia).

Protocol

Sample collection and all UGS procedures were performed by an experienced phlebologist (KP) assisted by an experienced vascular sonographer and a research officer. The protocol was executed in the following order (Table 1):

A. Pre-operative (before) sample collection:
   1. Systemic blood (AB) – Samples were collected from the left antecubital veins (AB, antecubital vein before).
   2. Adjoining deep veins (DVB) and target veins (TVB) – Samples were collected in the proximity (within 2 cm) of saphenofemoral (SFJ) or sapheno-popliteal junctions (SPJ) from relevant veins (DVB, deep vein before; TVB, target vein before).

B. Initial injection

0.5 mL of sclerosant foam (3% STS or POL) was prepared at the bedside. The target vessel was identified on ultrasound. Entry point was selected at 5 cm distal to

Table 1. Protocol.

Steps		Veins	Sample code	Duration[a]	Time point	Sample processed for
1	Pre-op sample collection	Systemic blood (left antecubital veins)	AB	5 min	–5 min	FBC, clotting tests, D-dimer, biochemical markers
2	Pre-op Sample Collection	Deep and target veins	DVB, TVB	10 min	Immediately before	FBC, clotting tests
3	Initial injection of 0.5 mL of 3% foam	Target vein, 5 cm away from junction	-	< 1 min	0	-
4	Post-injection samples	Deep veins	DVA	5 min	5 min	FBC, clotting tests
5	Post-injection samples	Target veins at 15, 30 and 45 cm from the initial injection	TV 15, 30, 45	5 min	10 min	XACT
6	Post-injection samples	Target veins foam front (average 30 cm)	TVA	5 min	15 min	FBC, clotting tests
7	UGS	Remaining incompetent veins	-	30 min	45 min	
8	Post-op walk	-	-	30 min	75 min	
9	Post-op samples[b]	Systemic blood (right antecubital veins)	AA	5 min	80 min	FBC, clotting tests, D-dimer, biochemical markers

AA: systemic blood (antecubital vein) after; AB: systemic blood (antecubital vein) before; DVA: deep vein after; DVB: deep vein before; FBC: Full Blood Count; TV: target vein; TVA: target vein after; TVB: target vein before; UGS: ultrasound-guided sclerotherapy; XACT: Factor Xa Clotting Time.

[a]All durations are approximations.

[b]Post-op systemic samples were collected 30 minutes after the completion of UGS but approximately 80 minutes after the initial 0.5 mL injection of foam.

relevant junctions (SFJ or SPJ). Foam was delivered via a 25 G (40 mm length) needle over 5 seconds. Entry and flow of foam in the target vessel was monitored on ultrasound.

C. Post-injection (after) sample collection from adjoining deep and target veins

1. Deep veins (DVA) – Samples were taken from the adjoining deep veins immediately after the injection. The post-operative collection site was chosen to be approximately 2 cm distal to the pre-op (before) collection site to avoid local haemostatic activation induced by venepuncture (DVA, deep vein after).
2. Target veins (TV)
   (i) Distance intervals (TV 15, 30, 45) – Samples were collected at set distances of 15, 30 and 45 cm away from the initial injection site.
   (ii) Foam front (TVA) – Further samples were collected from the target vein at the foam front, an average distance of 30 cm from the initial injection (TVA, target vein after).

D. Foam UGS

The rest of the incompetent system was treated with foam UGS following a proximal to distal approach using multiple injections to a total foam volume of 14.5 mL per session. Class II full length graduated compression stockings were applied. The procedure and the application of stockings took approximately 30 minutes. The patient was instructed to walk for 30 minutes and return for post-operative sample collection.

E. Post-operative (after) systemic sample collection (AA)

Systemic WB samples were collected from the right antecubital vein (AA, antecubital vein after).

F. Follow-up DVT screening and sample collection (1 week)

One week after the procedure, a duplex ultrasound study was performed to screen for DVT and further systemic blood samples were taken for D-dimer levels.

Sample processing

Blood was collected into 7.2 mg K_2EDTA for full blood counting, 0.105 M sodium citrate for coagulation assays and a serum separator tube for biochemical assays. Blood samples were processed in-house (for XACT) or immediately transported to an accredited pathology laboratory (SydPath, Sydney, Australia) for all other testing.

Coagulation assays and blood cell counting

The APTT assays were performed on an STA automated coagulation analyser (Diagnostica Stago, Asnières sur Seine, France). Two comparable APTT reagents were used in this study by the reference laboratory including Automated APTT (Biomerieux SA, Lyon, France) for all STS samples (normal range, NR 27-42s) and Actin FS (Siemens, Munich, Germany) for all POL samples (NR 25-35s). PT assay was performed using Neoplastin C1 Plus (Diagnostica Stago) on an STA automated coagulation analyser. The normal range was 11–15 seconds. D-dimer levels were measured using Vidas D-Dimer Exclusion assay system (Biomerieux, Marcy-l'Étoile, France), with a reference range of <0.5 mg/L.

The XACT assay (Inter-laboratory Coefficient of Variance 12.08%)[10] was performed as previously described,[11] using an ST4 coagulation analyser (Diagnostica Stago); 25 µL of test samples was added to 25 µL of phospholipid depleted porcine plasma and incubated for 120 to 180 seconds at 37°C before the addition of 100 µL of a Factor Xa solution containing calcium. The time required for clot formation was then measured. Normal range for WB samples was established to be 42.5–65.3 seconds.

Blood cell counting was performed using an automated differential haematology analyser (Beckman Coulter, California, USA).

Systemic biochemical markers

Biochemical markers were measured using standard laboratory methods. These included serum electrolytes, urea, creatinine, liver function tests (LFT; total protein, albumin, total bilirubin, serum alkaline phosphatase [SAP], gamma-glutamyl transferase [GGT], alanine aminotransferase [ALT], aspartate aminotransferase [AST], lactate dehydrogenasae [LDH]), serum lipids (cholesterol, triglycerides, high density lipoprotein [HDL], low density lipoprotein [LDL]), cardiac enzymes (Troponin I, creatine kinase [CK], creatine kinase-muscle and brain isoenzyme [CK-MB]) and c-Reactive Protein (CRP).

Statistical analysis

Statistical analysis was performed using Prism v5.03. Variables with normal distribution are reported as mean ± the standard error of the mean (SEM), unless otherwise stated. Paired data was compared using a paired student's t-test.

Results

Patient demographics

The recruited patients were randomised to treatment with STS (12) or POL (13). All patients completed the treatment protocols. Patient demographics and procedural details are shown in Table 2. The Clinical Etiological Anatomical Pathophysiological classification (CEAP) distribution was C2 (39%), C3 (11%), C4a (22%), C4b (6%), C5 (22%). All measurements and analyses relate to a single procedure. A second procedure was performed on a sub-group of patients one week to one year after the first procedure to treat a different truncal vein (ipsilateral or contralateral limb). These procedures were treated and analysed as separate procedures. Excluding the second procedure from the analyses had no effect on the statistical significance of the elevation in D-dimer, biochemical markers or Factor Xa clotting times.

Clinical follow-up

In one asymptomatic female patient treated with STS, a tongue of thrombus was detected on ultrasound to extend from the treated left GSV into the common femoral vein for 3.2 cm. There were no significant changes in any of the measurements to suggest an increased thrombotic risk. Following the procedure, her APTT in the femoral vein shortened from 35 to 34 seconds while PT remained at 13 seconds. The platelet count in the deep veins remained unchanged from 356 to 354×10^9 platelets/L. The D-dimer concentration increased from 0.17 to 0.66 mg/L 1 hour post procedure. At the time the DVT was diagnosed, the D-dimer concentration was 0.46 mg/L. She was treated with enoxaparin and the clot resolved within 2 weeks. None of the remaining patients developed

Table 2. Subject and procedural details.

Subjects	STS	POL
Number	12	13
Mean Age (y) [range]	64 [44–76]	63 [38–75]
Male/Female	5/7	7/6
Mean Sclerosant volume (mL) [range]	6.4 [1.5–15]	8.2 [0.5–15]
UGS Procedures	STS	POL
Number	25	15
Great Saphenous system	20	9
Small saphenous system	5	6

STS, Sodium Tetradecyl Sulphate; POL, Polidocanol; UGS, ultrasound guided sclerotherapy.

complications and in particular there was no other thrombo-embolic or neurological adverse event.

Coagulation assays

Systemic clotting times were not affected by foam sclerotherapy (Figure 1). However, injection of 0.5 mL of STS foam induced a significant shortening of APTT and XACT in the target veins and the adjoining deep veins. POL induced a significant shortening of XACT in the target veins but not in the adjoining deep veins.

Samples obtained from the target veins demonstrated a distance-dependent procoagulant effect induced by both agents (Figure 2). This procoagulant effect reached a peak at 15 cm but normalised at 45 cm.

D-dimer

The initial mean D-dimer levels prior to sclerotherapy were higher for STS vs POL (0.88 vs 0.64 mg/L), but this difference was not statistically significant ($p = 0.39$). Foam UGS with either agent resulted in a significant

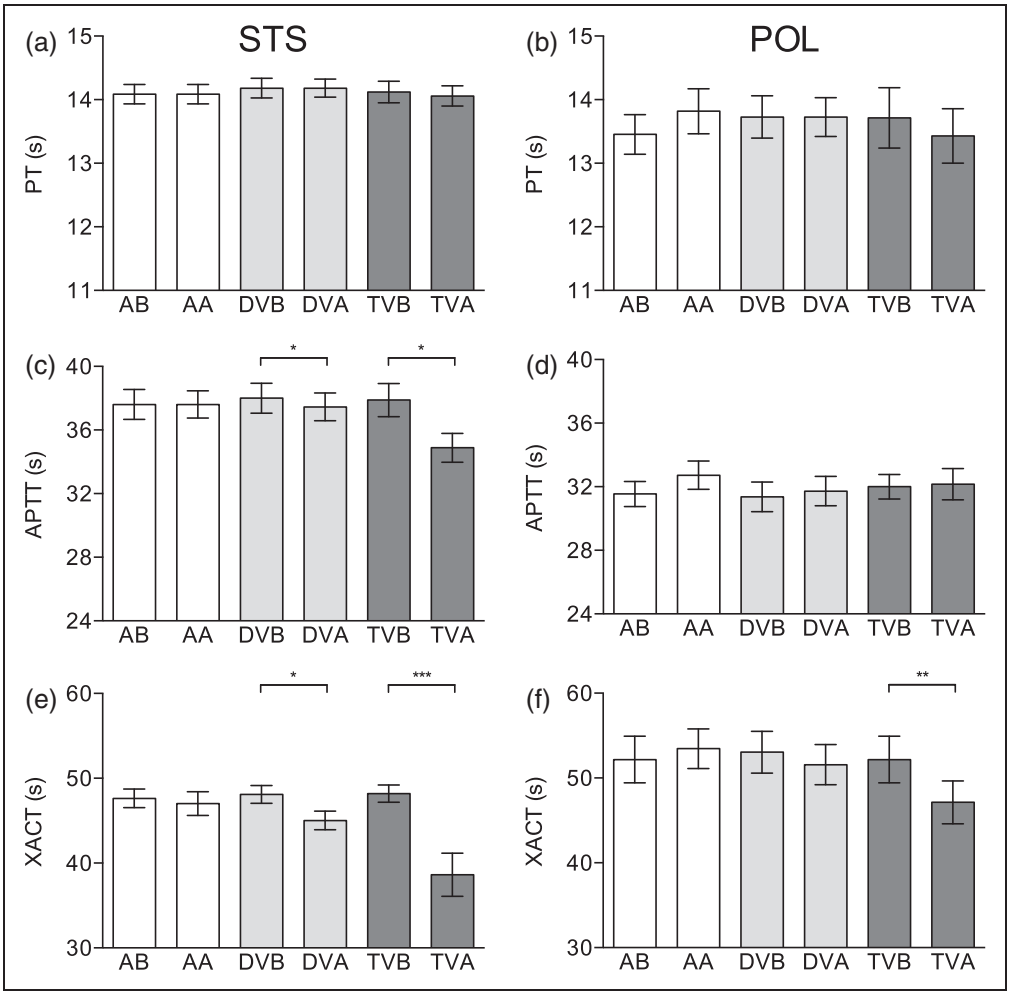

Figure 1. Clotting times: the effect of foam sclerotherapy with sodium tetradecyl sulphate (STS) or polidocanol (POL) on clotting times. Mean prothrombin time (PT) (a and b), activated partial thromboplastin time (APTT) (c and d) and factor Xa clotting time (XACT) (e and f) are shown for STS (n = 24) (a, c, e) and POL (n = 14) (b, d, f). Samples from deep veins and target veins were collected after an injection of 0.5 mL of foam 5 cm away from the relevant saphenous junctions. Samples from systemic blood were collected after a 30-minute walk following the completion of the procedure using a total of 15 mL of foam. Two patients taking warfarin were excluded from prothrombin time testing due to prolonged results. The baseline values for APTT were shorter for POL compared with STS ($p = 0.02$) due to the different APTT reagent used to analyse these samples. The difference in the baseline value did not affect the results which compare the APTT values to the baseline values for the same sclerosant. AA: systemic blood (antecubital vein) after; AB: systemic blood (antecubital vein) before; DVA: adjoining deep vein after; DVB: adjoining deep vein before; TVA: target vein after; TVB: target vein before. Error bars represent the standard error of the mean. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

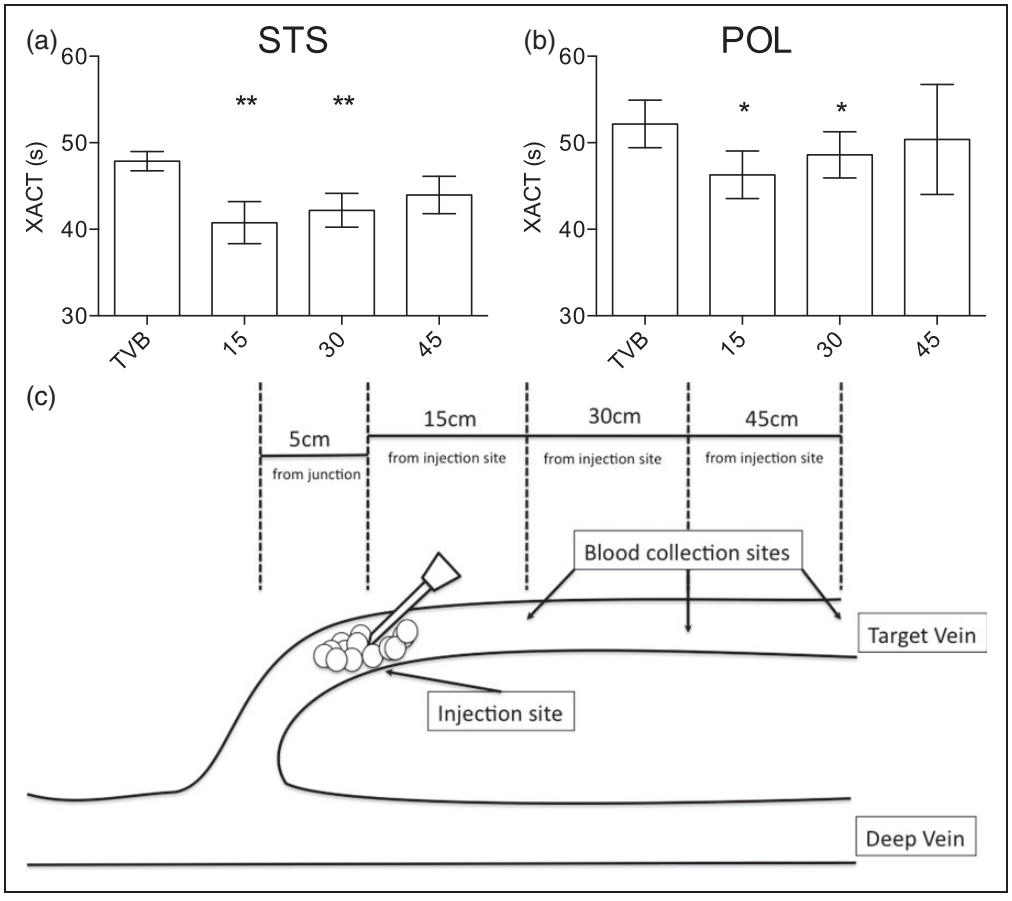

Figure 2. Distance: the effect of distance on factor Xa clotting times (XACT) following foam sclerotherapy with sodium tetradecyl sulphate (STS) or polidocanol (POL). XACT times were measured in samples collected at 15 cm, 30 cm and 45 cm distal to the injection of 0.5 mL of foam into target veins using either (a) STS (n = 12) or (b) POL (n = 9) according to the cartoon representation (c) of the target vein, deep vein and injection site. For reference, the pre-operative XACT times for target veins (TVB) are shown. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs baseline measurements.

rise (159% for STS, 198% for POL) in the systemic D-dimer levels within 1 hour of the procedure (Figure 3). After 1 week, D-dimer levels were still elevated compared to baseline levels.

Blood cell counts

UGS with 15 mL of STS foam resulted in a significant rise in the systemic erythrocyte, leukocyte and platelet counts within 1 hour of the procedure (Table 3). POL induced a significant rise in the systemic leukocyte count only.

Injection of 0.5 mL of STS foam induced a significant decrease in the leukocyte and platelet count in the adjoining deep veins and a significant fall in the platelet count in the target vessels (Figure 4). By contrast, same volume of POL foam did not affect the cell counts in the target vessels or the adjoining deep veins.

Systemic biochemical and cardiac markers

Both agents induced a significant rise in the total protein, total bilirubin, AST, SAP and LDH levels within 1 hour of the procedure. In addition, STS induced a significant increase in the albumin, ALT and GGT levels (Table 3). Serum cholesterol was significantly increased by both agents. POL induced a rise in HDL while STS induced a rise in LDL levels. CK and CK-MB levels were significantly increased by both agents but there was no change in Troponin I. There was a significant increase in CRP following sclerotherapy with STS, but not with POL.

There was a statistically significant increase in the serum potassium levels and a fall in the serum urea after sclerotherapy using STS, however these changes remained within the normal range. There was no significant change in other electrolytes or creatinine levels following sclerotherapy with either agent.

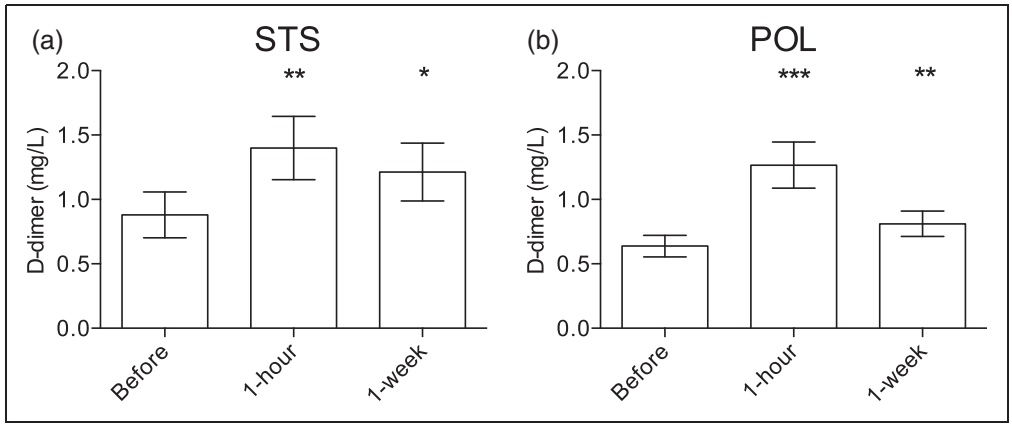

Figure 3. D-dimer: the effect of foam sclerotherapy with sodium tetradecyl sulphate (STS) or polidocanol (POL) on D-dimer concentration. D-dimer levels were measured in the systemic circulation immediately prior to (AB, antecubital vein before) and within 1 hour following foam sclerotherapy using either agent. (a) STS (n = 24) or (b) POL (n = 14). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

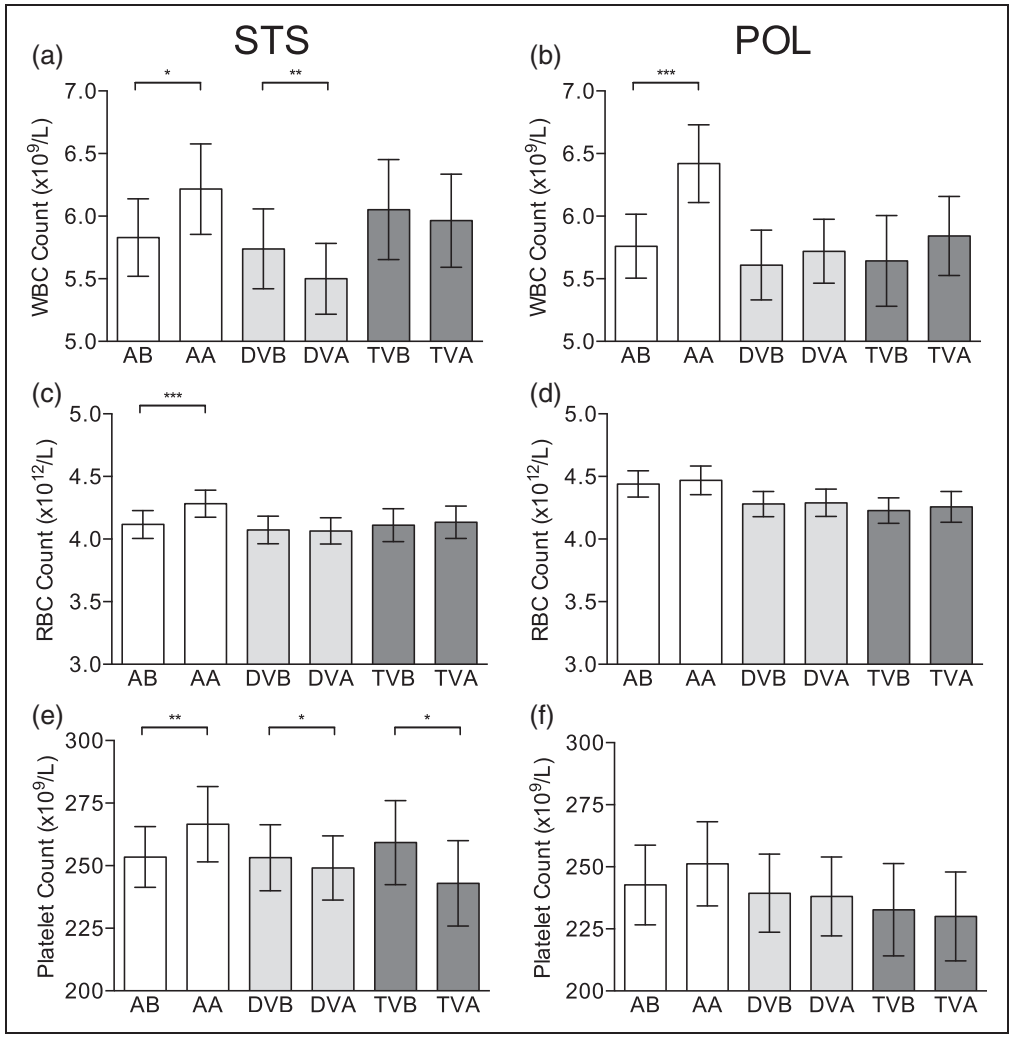

Figure 4. Blood cell counts: the effect of foam sclerotherapy with sodium tetradecyl sulphate (STS) or polidocanol (POL) on blood cell counts. Mean white blood cell (WBC) (a and b), red blood cell (RBC) (c and d) and platelet counts (e and f) are shown for STS (n = 24) (a, c, e) and POL (n = 10) (b, d, f). AA: systemic blood (antecubital vein) after; AB: systemic blood (antecubital vein) before; DVA: adjoining deep vein after; DVB: adjoining deep vein before; TVA: target vein after; TVB: target vein before. Error bars represent the standard error of the mean. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3. The systemic effects of foam sclerotherapy on blood cell counts, serum electrolytes, liver function tests, lipid levels, cardiac and muscle enzymes measured within 1 hour from completion of sclerotherapy using a total of 15 mL of sodium tetradecyl sulphate (STS) or polidocanol (POL) foam. Results are displayed as mean ± standard deviation, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs baseline measurements.

	STS			POL			
	AB	AA	% Change	AB	AA	% Change	Reference range
Full blood count							
Haemoglobin	128 ± 14.2	133 ± 12.7***	3.6	137 ± 12.3	139 ± 13.4	1.3	115–165 g/L (F) 130–180 g/L (M)
Haematocrit	0.38 ± 0.04	0.39 ± 0.04***	3.4	0.41 ± 0.04	0.41 ± 0.04	N/A	0.37–047 (F) 0.40–0.50 (M)
MCV	92 ± 6.3	92 ± 6.3	N/A	92 ± 3.8	92 ± 3.8	N/A	76–96 fL
MCH	31.3 ± 2.1	31.2 ± 2.0	−0.2	30.9 ± 1.3	31.0 ± 1.4	0.3	27.0–32.0 pg
MCHC	340 ± 6.1	340 ± 5.5	N/A	336 ± 4.8	338 ± 4.8	0.5	320–360 g/L
RDW	12.1 ± 0.6	12.0 ± 0.5	−0.6	12.0 ± 0.4	12.1 ± 0.8	1.1	11.5–14.5%
Erythrocytes	4.1 ± 0.5	4.3 ± 0.5***	4.0	4.4 ± 0.3	4.5 ± 0.4	0.7	3.8–5.8 ($\times 10^9$/L) (F) 4.5–6.5 ($\times 10^9$/L) (M)
Platelets	253 ± 59	267 ± 74**	5.2	243 ± 51	251 ± 54	3.5	150–400 ($\times 10^9$/L)
Leukocytes	5.8 ± 1.5	6.2 ± 1.8*	6.6	5.8 ± 0.8	6.4 ± 1.0***	11.5	4.0–11.0 ($\times 10^9$/L)
Neutrophils	3.5 ± 1.0	3.9 ± 1.4*	12.0	3.3 ± 0.9	3.8 ± 1.3**	15.9	2.0–7.5 ($\times 10^9$/L)
Lymphocytes	1.7 ± 0.7	1.8 ± 0.7	2.9	1.5 ± 0.3	1.8 ± 0.5**	15.3	1.5–4.0 ($\times 10^9$/L)
Monocytes	0.6 ± 0.2	0.5 ± 0.4***	−18.2	0.5 ± 0.2	0.5 ± 0.1	N/A	0.2–1.0 ($\times 10^9$/L)
Eosinophils	0.1 ± 0.1	0.1 ± 0.1	N/A	0.1 ± 0.1	0.1 ± 0.1	N/A	0.0–0.4 ($\times 10^9$/L)
Basophils	0.0 ± 0.0	0.0 ± 0.0	N/A	0.0 ± 0.1	0.0 ± 0.1	N/A	0.0–0.1 ($\times 10^9$/L)
Serum electrolytes							
Sodium	138 ± 5.1	139 ± 5.3	0.3	142 ± 2.1	142 ± 2.4	N/A	137–146 mmol/L
Potassium	4.2 ± 0.26	4.4 ± 0.34*	4.7	4.3 ± 0.16	4.5 ± 0.37	4.7	3.5–5.0 mmol/L
Chloride	100 ± 4.8	100 ± 4.4	N/A	104 ± 2.7	104 ± 2.0	N/A	95–110 mmol/L:
Bicarbonate	26 ± 1.2	27 ± 2.2	2.7	27 ± 2.1	26 ± 2.5	−2.6	24–31 mmol/L
Urea	5.9 ± 1.1	5.7 ± 1.1**	−2.8	6.6 ± 1.2	6.5 ± 1.2	−1.2	3.0–8.5 mmol/L
Creatinine	72 ± 14	74 ± 14	1.7	75 ± 9.0	77 ± 8.9	2.0	40–90 μmol/L (F) 60–120 μmol/L (M)
Liver function tests							
Total protein	69 ± 3.9	74 ± 4.1***	6.3	70 ± 3.4	72 ± 2.8*	4.0	66–82 g/L
Albumin	41 ± 2.3	44 ± 2.2***	6.4	42 ± 2.9	43 ± 2.8	3.6	36–52 g/L
Total bilirubin	8.7 ± 3.6	11 ± 4.5***	24.5	9.2 ± 4.3	12 ± 4.8***	26.1	<18 μmol/L
ALP	69 ± 17	73 ± 17***	6.7	70 ± 20	74 ± 20**	5.3	30–100 U/L
GGT	33 ± 24	35 ± 23**	5.9	18 ± 6.9	18 ± 7.5	4.0	0–35 U/L
ALT	21 ± 6.6	23 ± 7.0***	8.0	16 ± 6.0	17 ± 6.2	5.1	0–30 U/L
AST	23 ± 4.8	26 ± 5.5***	14.3	19 ± 3.9	20 ± 4.1**	9.7	<30 U/L
LDH	401 ± 89.3	475 ± 123***	18.5	343 ± 64.5	389 ± 54.4**	13.4	<430 U/L
Cardiac markers							
Troponin I	All < 0.03	All < 0.03	N/A	All < 0.03	All < 0.03	N/A	<0.03 μg/L
CK	135 ± 103	151 ± 99*	11.8	92 ± 54	107 ± 62**	16.4	0–110 U/L (F) 0–130 U/L (M)
CK-MB	3.0 ± 2.2	3.2 ± 2.4*	7.1	1.9 ± 1.4	2.0 ± 1.4*	7.4	0.0–5.0 μg/L
Serum lipids							
Cholesterol	4.6 ± 0.9	5.0 ± 0.9***	7.2	4.9 ± 1.4	5.0 ± 1.4*	3.7	0.0–6.0 mmol/L
Triglycerides	1.7 ± 1.7	1.8 ± 1.7	6.0	1.7 ± 0.8	1.7 ± 0.8	N/A	0.0–2.0 mmol/L
HDL	1.8 ± 2.0	1.5 ± 0.4	−16.7	1.3 ± 0.3	1.4 ± 0.3*	6.1	>1.0 mmol/L
LDL	2.5 ± 0.6	2.8 ± 0.7**	8.9	2.8 ± 1.2	2.9 ± 1.2	4.0	0.0–4.0 mmol/L
Inflammatory marker							
CRP	2.4 ± 3.6	2.5 ± 3.7**	4.6	2.1 ± 1.2	2.2 ± 1.9	3.8	<10 mg/L

AA: arm (antecubital vein) after; AB: arm (antecubital vein) before; ALP: serum Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; CK: Creatine Kinase; CK-MB: Creatine Kinase Muscle and Brain Isoenzyme; CRP: C-Reactive Protein; F: Female; GGT: Gamma Glutamyl Transferase; HDL: High Density Lipoprotein Cholesterol; LDH: Lactate Dehydrogenase; LDL Chol: Low Density Lipoprotein Cholesterol; M: Male. MCH: Mean Cell Haemoglobin; MCHC: Mean Cell Haemoglobin Concentration; MCV: Mean Cell Volume; RDW: Red Cell Distribution Width.

Other variables

There was a trend towards increased D-dimer levels with increasing age in both pre- and post-sclerotherapy samples, however this was not statistically significant (Spearman correlation $p = 0.297$ and 0.0598). There was no significant difference between any of the results for GSV and SSV procedures for either agent. There was no significant difference between with volume of foam injected for STS and POL.

Discussion

In this study, we investigated the biological effects of foam sclerosants in target veins, where the sclerosant concentration is expected to be highest; in the adjoining deep veins where DVT may form and in the systemic circulation where dilution and deactivation should take maximum effect. Here we report a number of novel findings and in particular a distance-dependent increase in procoagulant activity following infusion of foam, shortening of clotting times (APTT and XACT) in the adjoining deep veins within minutes of the procedure and a systemic rise in the D-dimer levels in the absence of a concurrent DVT. We also report biochemical changes consistent with haemoconcentration within 1 hour of the procedure.

Target veins are the first point of entry for sclerosants where the concentration of the sclerosant is at its highest. In our previous *in vitro* studies, the addition of high concentration sclerosants and in particular STS significantly prolonged the clotting times (APTT, PT and XACT) and induced platelet lysis. Here, we aimed to determine whether a similar anticoagulant effect can be found *in vivo*. Furthermore, in order to determine the changes that occur in the adjoining deep veins and the systemic circulation, these changes had to be first defined in the target veins. In this study, no prolongation of any of the clotting tests was detected and despite the high initial concentration (3%) of foam preparations, a procoagulant state was detected in the adjoining deep veins and the target veins. Samples from deep veins and target veins were collected from sites upstream and downstream following the injection of 0.5 mL of foam. Hence, the detected procoagulant activity was due to dilution and deactivation of the high concentration sclerosants in blood and a fall in the final active concentration.

Although foam is presumed to be better than liquid in displacing blood, this study demonstrates that there is significant mixing at the foam front. The procoagulant activity in the target veins reached a peak at 15–30 cm away from the point of entry and in particular at the foam front but normalised at 45 cm. The normalisation is due to excessive mixing and deactivation of sclerosants to undetectable levels downstream from the point of entry. Therefore, our current results are indicative of a distance-dependent progressive fall in the active concentration of the sclerosants in target vessels. Furthermore, it can be concluded that simple infusion and spreading of the sclerosant foam from a single point of entry can result in significant procoagulant activity at the foam front where there is maximum mixing.

An interesting and novel finding of this study was the rise in the D-dimer levels within 1 hour of sclerotherapy and 7 days later. In two previous studies, elevated D-dimer or thrombin-antithrombin levels were found between 1 and 7 days following sclerotherapy using POL[8] and following sclerotherapy using hypertonic saline combined with surgery.[12] In another study, the number of D-dimer-positive subjects increased 30 minutes following sclerotherapy.[6] These results are suggestive of fibrin formation and degradation and release of fibrin degradation products soon after sclerotherapy. Our critical finding is that post-sclerotherapy D-dimer elevation happened soon after the procedure and can occur in the absence of a concurrent DVT or a sclerotic deep vein occlusion.

Another novel finding of this study was the rise in blood cell counts and large plasma proteins within 1 hour of the procedure. The observed elevations in the cell counts were detected in systemic samples collected within 1 hour of sclerotherapy with 15 mL of foam. STS induced a minor but statistically significant increase in red cell, white cell and platelet counts. Large molecules such as albumin (+7.3%), protein (+7.2%), cholesterol (+8.6%) and liver enzymes ALP, ALT, GGT all rose similarly. Meanwhile, small molecules such as sodium, chloride, bicarbonate, urea and creatinine did not rise after STS foam sclerotherapy. These findings are consistent with haemoconcentration due to fluid loss from the vascular compartment. STS induced a rise in the erythrocyte count from 4.1 to 4.3×10^9/L, without a change in the mean corpuscular volume. According to the method of Austin et al.,[13] this indicates a 5.5% and 1.1% decline in plasma volume following STS and POL administration, respectively. Assuming a total blood volume of 5 L, this equates to an approximate loss of 171 mL and 33 mL of plasma volume for STS and POL, respectively. In general, these biochemical changes were quiet minor and do not suggest pathology in the short term. The elevations in the D-dimer levels were of a much higher order of magnitude (158.9% for STS and 211.4% for POL) and hence cannot be attributed to haemoconcentration.

The changes in cell counts and large molecules after treatment with POL were less often statistically significant and less in magnitude e.g. albumin (+2.4%), protein (+2.9%) and cholesterol (+2.0%). The liver

enzymes ALP, ALT, GGT were mixed in their response. These results are consistent with less haemoconcentration with POL compared with STS. The samples were collected similarly and the post-treatment samples were not affected by pre-analytical variables causing more haemoconcentration such as the longer use of a tourniquet. The limited impact of POL is not unexpected as this agent is more significantly deactivated by blood components compared with STS.[5] By removing the endothelial lining of target vessels, both sclerosants compromise the vascular integrity resulting in a subsequent fluid loss from the intravascular space. These findings may further justify the use of compression stockings after sclerotherapy to reduce the post-operative oedema.

We have previously reported venous gas embolism and detection of bubbles in the cardiac chambers within minutes of foam sclerotherapy.[14] In this study, we detected no change in troponin-I but found a significant rise in the CK iso-enzyme levels within the reference ranges. The rise in the CK is expected to occur 8–16 hours after exercise and 3–12 hours after myocardial infarction.[15] Troponin-I is reported to rise 2–12 hours after infarction.[15] Our patients were instructed to go for a 30-minute walk after the procedure and samples were taken after the walk. Given the rapid rise in the CK, the observed elevations were unlikely to be due to the 30-minute walk or a cardiac source although these cannot be fully excluded. Meanwhile, there was a concurrent rise in serum potassium while CK, AST and LDH all rose more than 10%. These changes are consistent with red cell damage and sub-clinical haemolysis, a known complication of sclerotherapy.[16] Furthermore, STS induced a significant fall in the leukocyte and platelet counts in the adjoining deep veins (femoral or popliteal veins) and a significant fall in the platelet count in the target vessels. The observed lytic activities are consistent with our previous *in vitro* reports of erythrocyte, leukocyte and platelet lysis induced by STS.[5] None of the patients developed clinical signs of haemolysis and hence, our findings reflect sub-clinical events in otherwise well patients.

This study may have a number of clinical ramifications. Systemic samples collected within 1 hour of the procedure showed haemoconcentration with biochemical changes within the reference range. Biochemical changes consistent with haemolysis were detected following the use of 15 mL of STS foam, but none of the patients developed clinical haemolysis. We detected no changes in the systemic clotting times although D-dimer levels were elevated. The only clinical complication detected in this study was a tongue of thrombus extending into the common femoral vein from a treated GSV. Despite the biochemical changes detected, there were no other thromboembolic or ischaemic neurological complications in this study. Antithrombotic and fibrinolytic systems provide protective mechanisms that possibly counteract some of the pro-coagulant changes detected in this study resulting in a balanced haemostatic state. Further investigations such as thromboelastometry are required to assess the global effects of sclerotherapy on the coagulation system. The rise in the D-dimer in the absence of deep vein occlusion is significant as it may influence the utility of D-dimer in the assessment of post-sclerotherapy DVT. Further clinical studies are required to assess the role of D-dimer evaluation in this setting.

Considering the rapid onset of ischemic and neurological complications described in a number of case reports,[17] we collected samples from the adjoining deep veins within minutes of the initial injection to capture any immediate biochemical alterations. Procoagulant activity was detected in the exposed deep veins and target veins indicative of platelet activation within minutes of the first injection. Therefore, standard foam sclerotherapy and in particular the infusion technique results in some degree of procoagulant activity in the adjoining deep veins although this may not result in a clinically detectable DVT. Tongues of thrombus originating in the treated saphenous veins have been observed to protrude into the adjoining deep veins and one such incident is reported here in a female patient treated with STS foam. A previous study recommended multiple injections as against a single infusion to reduce the incidence of DVT.[18] Other techniques such as the use of peri-venous tumescent anaesthetics can be employed to reduce the size of the target vessels and hence to maintain the optimal concentration of foam sclerosants.

This study had a number of limitations. As vasospasm occurs quickly following injection, blood samples were often difficult to withdraw from the target veins. Vasospasm and the process of blood collection may induce platelet activation and even lysis. Furthermore, the quality of samples obtained from target vessels may be variable due to the variability in the vessel size and the extent of mixing with the intravascular blood at the foam front. Such variables may influence the absolute values of the measured clotting times. Future *in vivo* studies should incorporate a control group with injection of a NS-stabilised foam or another inert foam to detect possible changes. In this study, we only tested a modified Tessari foam (1 + 4) using a 5-micron filter and room air as the foaming gas. Liquid-air fraction and the composition of foam may influence the biological activity of the sclerosants and other foam compositions may demonstrate different rheological and biological properties. Although technically quite difficult, future studies should aim to measure the active concentration of sclerosing agents

in target vessels, in the adjoining deep veins and in the systemic circulation. Finally, osmolality and volumetric measurements should be performed to further quantify the haemoconcentration phenomenon reported here.

In summary, foam sclerotherapy had no effect on systemic coagulation pathways. Infusion techniques result in a distant-dependent procoagulant state. STS and to a lesser degree POL demonstrated biochemical markers of haemoconcentration.

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Ethical approval

This study was approved by the St Vincent's Hospital Human Research Ethics Committee.

Guarantor

DC.

Contributorship

DC, JJ, TE, DM and KP researched literature and designed the study. KP, JJ and DM gained ethical approval. All procedures were performed by KP. Sample processing was performed by DC, TE and KP and the research assistants mentioned in the acknowledgements. DC and KP wrote the drafts of the manuscript. All authors reviewed and edited the manuscript and approved the final version.

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