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Kjersti Ausen

Topical tranexamic acid to reduce bleeding from surgical wounds

NTNU
Kunnskap for en bedre verden

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Kjersti Ausen

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Avhandling for graden
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Trykket av NTNU Grafisk senter

Topikal traneksamsyre for å redusere blødning fra kirurgiske sår

Fukting av sårflater med traneksamsyre reduserer blødning

- Traneksamsyre er et velkjent og rimelig legemiddel som reduserer blødning. Legemiddelet gis intravenøst eller i tablettform ved kirurgi med stor blødning. Usikkerhet om hvorvidt traneksamsyre kan øke risiko for blodpropp forhindrer rutinebruk ved all kirurgi. Avhandlingen utforsker en alternativ bruksmåte for legemiddelet: Ved å fukte sårflatene med traneksamsyre, påføres middelet der blødningen foregår, mens det blir mindre i kroppen forøvrig.

- Avhandlingen viser at fukting av en kirurgisk sårflate med fortynnet traneksamsyre gir en signifikant reduksjon av blødning og dermed er en alternativ bruksmåte for legemiddelet. Avhandlingen viser også at slik lokal påføring av legemiddel gir svært lave konsentrasjoner i resten av kroppen, og dermed en antatt mindre risiko for bivirkninger.

- Effekt av fukting av sårflater med fortynnet traneksamsyre har blitt undersøkt i to studier. Den ene studien ble gjennomført på 28 kvinner som gjennomgikk reduksjon av begge bryst; den andre på 202 kvinner som fikk fjernet et bryst pga brystkreft. Halvparten av sårene ble fuktet med traneksamsyre og den andre halvparten med placebo (saltvann). I begge studier fant man en reduksjon i blødning med ca. 35% ved bruk av traneksamsyre, og dette tilsvarer effekten man får ved intravenøs bruk av legemiddelet. Fukting av sårflaten med traneksamsyre syntes også å redusere risikoen for å måtte opereres på nytt pga blødning.

- I en tredje studie vurderte vi hvorvidt fukting av sårflater med traneksamsyre fører til opptak av legemiddelet i blodbanen. Pasienter som får fjernet overskuddshud etter stor vektnedgang har et stort sårareal og vil dermed ha mulighet for stor absorpsjon av legemiddel via sårflaten. Vi sammenliknet pasienter som fikk fjernet større mengder hud fra buken (bukplastikk) med hofteprotesepasienter, som får intravenøs traneksamsyre ved operasjon. Vi tok hyppige blodprøver i begge grupper og sammenliknet legemiddelkonsentrasjonen i blodet. Fukting av sårflaten med fortynnet traneksamsyre gav meget lav konsentrasjon i blodet og bør ikke kunne gi noen økt risiko for blodpropp eller andre bivirkninger i kroppen forøvrig grunnet opptak av legemiddel fra såret.

Navn kandidat: Kjersti Ausen
Institutt: Institutt for sirkulasjon og bildediagnostikk
Veiledere: Hilde Pleym, Olav Spigset og Birger H. Endreseth
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Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden PhD.
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Topical tranexamic acid to reduce bleeding from surgical wounds

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ABBREVIATIONS

AC	axillary lymph node clearance
AKF	The Unit for Applied Clinical Research
AMCHA	4-aminomethyl-cyclohexane-1-carboxylic acid
ATACAS	Aspirin and Tranexamic Acid for Coronary Artery Surgery
BART	Blood Conservation Using Antifibrinolytics in a Randomized Trial
CI	confidence interval
Cl	clearance
CNS	central nervous system
CSF	cerebrospinal fluid
EACA	epsilon-aminocaproic acid
EudraCT	European Clinical Trial Database
FDA	Food and Drug Administration
HK	High molecular weight kininogen
iv	intravenous
ln	logarithmic (e^x)
λz	log-concentration
NorCRIN	Norwegian Clinical Research Infrastructure Network
OR	odds ratio
PAI	plasminogen activator inhibitor
SD	standard deviation
SNB	mastectomy with sentinel node biopsy
SM	simple mastectomy
t-PA	tissue-type plasminogen activator
TXA	tranexamic acid
u-PA	urokinase-type plasminogen activator
VAS	Visual analogue scale

LIST OF PAPERS

Paper I

Ausen K, Fossmark R, Spigset O, Pleym H. Randomized clinical trial of topical tranexamic acid after reduction mammoplasty. British Journal of Surgery 2015; 102: 1348–1353.

Paper II

Ausen K, Hagen AI, Østbyhaug HS, Olafsson S, Kvalsund BJ, Spigset O, Pleym H. Randomized clinical trial of topical moistening of mastectomy wounds with diluted tranexamic acid to reduce bleeding. British Journal of Surgery Open; in press.

Paper III

Ausen K, Pleym H, Liu J, Hegstad S, Nordgaard HB, Pavlovic I, Spigset O. Serum concentrations and pharmacokinetics of tranexamic acid after two means of topical administration in massive weight loss skin-reducing surgery. Plastic and Reconstructive Surgery 2019;143(6): 1169e-1178e.

INTRODUCTION

Bleeding is inevitable in surgery but should be kept to a minimum. All surgical bleeding implies the use and change of bandages, swelling and bruising. Larger bleeding may provoke wound ruptures or require cumbersome surgical drains with the associated possible risk of infection[1,2]. Excessive bleeding leads to anemia and increased postoperative morbidity, and can ultimately be fatal. The safety of blood transfusions has greatly increased in the last decades but allogeneic blood is not harmless[3]. Moreover, banked blood is costly and in limited supply[4]. Strategies to minimize postoperative anemia and the use of allogeneic blood include preoperative detection and treatment of anemia or coagulopathies, optimizing per- and postoperative homeostasis, and applying a meticulous surgical technique aided by mechanical and pharmacological means of hemostasis[5,6].

Local injection of adrenaline along wound edges induces vasoconstriction and hence hemostasis. Topical hemostatic agents such as cellulose, collagen, fibrin, thrombin, chitin and synthetic polymers all promote clotting[7]. These agents are costly and mostly applicable for small surface areas only, as excessive triggering of coagulation may lead to unwanted thrombosis.

Despite adequately performed hemostasis during surgery, secondary bleeding may occur if fibrinolysis exceeds coagulation and when the vasoconstrictive effect of adrenaline wears off. Antifibrinolytic agents prevent clotted blood from dissolving and may reduce the risk of secondary bleeding. Antifibrinolytics presently represent the only pharmacological means of a general reduction of bleeding with an acceptable safety profile[8]. The synthetic low-cost drug tranexamic acid (TXA) is the most potent of the antifibrinolytic drugs currently available. Intravenous (iv) use reduces both measurable blood loss and transfusion needs with approximately one third[9]. It is routinely utilized prophylactically in surgery with high volume bleeding, particularly in cardiac and orthopedic joint replacement surgery[10].

A drug which prevents bleeding could theoretically increase the risk of thrombosis. Several large multicenter studies have not seen an increase in thromboembolic events after TXA administration[11-14], while other publications suggest that a risk may be present[15,16]. TXA is also associated with a dose-dependent increase in postoperative seizures in cardiac surgery[17]. Concern regarding potential systemic adverse events may dissuade widespread and general use.

A minimization of bleeding is desirable also in moderate- to low-bleed surgery to reduce patient discomfort and costs associated with follow-up, drains and bandages. A prophylactic measure to reduce bleeding should ideally be low-cost, safe, quick and simple. In contrast to the previously

mentioned topical hemostatic agents, TXA can easily be diluted to a volume large enough to topically treat even very large wound surfaces. Topical application of TXA can provide a sufficiently high drug concentration at the site of the wound and an assumed low systemic concentration, hence with a low risk of systemic adverse effects. Topical use is off-label but has been published from mainly orthopedic and cardiac surgery with reduction in postoperative bleeding and transfusion needs comparable to iv administration[18,19]. Most published studies on topical use of TXA describes the application of a bolus into a confined space such as a joint or the mediastinum, prolonged irrigation, or the superficial application of a soaked medium[19].

The surgical surfaces of plastic surgery are readily available for manual manipulation. In 2012, the Section for Plastic and Reconstructive Surgery at St Olav's University Hospital introduced a prophylactic procedure where surgical wounds were moistened with 20 ml of TXA 25 mg/ml right before wound closure. A video demonstrating our method can be found here: https://youtu.be/-8MAE3NAHfQ. We postulated that this simple manual smearing, leaving a thin film of fluid only, could reduce postoperative bleeding. Proper investigations were however needed to assess effect and potential adverse events. As plastic surgery may involve large wound surfaces, e.g. skin reducing surgery after major weight loss, we also wanted to explore to what extent topical use of TXA could result in significant systemic absorption. These questions constitute the rationale for this thesis.

BACKGROUND

An overview of the physiology of blood coagulation and fibrinolysis

After tissue injury, a balance between coagulation – the formation of blood clots – and fibrinolysis – the dissolving of blood clots – is needed to prevent blood loss yet maintain circulation. Injury will damage small blood vessels with subsequent exposure of the subendothelial collagen. This collagen exposure triggers the intrinsic (contact activation pathway) coagulation cascade, as contact between exposed collagen and circulating platelets and coagulation factors initiates autoactivation of factor XII and platelet activation. The damaged endothelium allows for release of tissue factor from subendothelial tissue, which triggers the extrinsic (tissue factor pathway) coagulation cascade. Both the intrinsic and extrinsic coagulation cascades terminate at the conversion of the inert prothrombin to active thrombin, which proteolytically cuts fibrinogen into small fibrin monomers. Thrombin also activates factor XIII, which catalyzes the crosslinking of the fibrin monomers into an insoluble clot (Figure 1).

The formation of fibrin to plug an injured vessel must be balanced against the need for maintaining an open vessel for continuous tissue perfusion. The fibrinolytic system is triggered by release of tissue plasminogen activator (t-PA) from vessel endothelium, which binds to plasminogen and initiates the conversion of plasminogen to its active fibrinolytic form, plasmin (Figure 1). Plasminogen/plasmin and t-PA all have lysine binding sites which bind to lysine residues exposed on the surface of fibrin[20,21]. This attachment to fibrin allows co-location of plasminogen and t-PA and thus more efficient activation of plasminogen to plasmin. Plasmin bound to fibrin then effectively exerts its fibrinolysis. Should activated plasmin escape from the fibrin clot, it is rapidly inactivated by antiplasmin which is present in plasma[20].

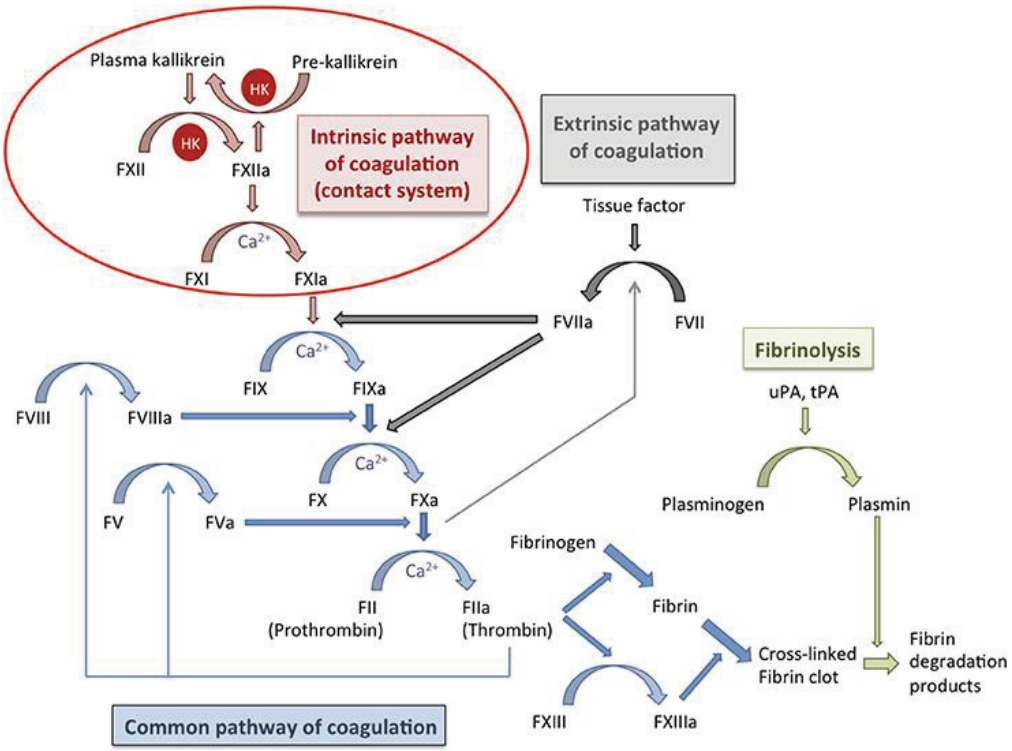

Fig.1. Schematic representation of the coagulation cascade and the fibrinolytic system. The coagulation cascade (blue arrows) can be activated during hemostasis via the intrinsic pathway (contact system; red arrows) or the extrinsic pathway (tissue factor system; gray arrows) that ultimately converge on the common pathway of coagulation. Both pathways lead to the activation of factor X and subsequently of thrombin, which is required for the conversion of fibrinogen into fibrin and for activation of factor XIII. The fibrin clot is cross-linked and stabilized by factor XIII. Fibrinolysis (green arrows) is activated at the same time as the coagulation system but operates more slowly and is important for the regulation of hemostasis. During fibrinolysis, plasminogen is converted into plasmin that degrades the fibrin network. Coagulation factors are indicated with "F" followed by a roman numeral, an additional "a" denotes the activated form; HK, high molecular weight kininogen; uPA, urokinase plasminogen activator; tPA, tissue plasminogen activator. Reprinted with permission from Loof TG et al. *Front. Cell. Infect. Microbiol.* 2014.

Plasminogen and its many functions

The zymogen (enzyme precursor) plasminogen is a ubiquitous substance in the human body. Its activated version, plasmin, has a broad proteolytic capability beyond mere fibrinolysis and is involved in multiple physiological and pathophysiological processes, as reviewed by Miles et al.[22]. In addition to fibrin, extracellular matrix and a variety of cells express surface receptors for both plasminogen and plasminogen activators, and free plasminogen in plasma can also be activated. Plasminogen has

multiple lysine binding sites which facilitate its interactions with other substances; one strong site and several weaker sites[23]. The strong site provides high affinity binding to lysine residues and facilitates the docking to fibrin, extracellular matrix and cell surfaces. The plasminogen activator urokinase (u-PA) is present both in blood and extracellular matrix. It may bind to cell receptors adjacent to docked plasminogen and promote its activation to plasmin. Thus cell surfaces are provided with activated plasmin and proteolytic properties which facilitate multiple functions such as inflammation, cell migration and wound healing[24]. Should activated plasmin escape from its docking site, rapid inactivation by plasma antiplasmin is also mediated through the strong lysine binding site. Mangel et al. (1990) observed that free plasminogen in plasma could undergo an exceptional conformational change from closed to open structure if one of its weaker lysine binding sites were occupied by a ligand[23]. This free-floating open form of plasminogen can bind to and be activated by circulating u-PA. The resulting circulating plasmin may allow for intravascular fibrinolysis[21]. Physiological inhibition of fibrinolysis is either by means of plasminogen activator inhibitors (mainly PAI-1) or by the inhibition of plasmin itself by antiplasmin[25].

Pharmacological inhibitors of fibrinolysis: Serine protease inhibitors and lysine analogues

Plasmin belongs to a large group of proteolytic enzymes classified as serine proteases due to the common presence of the amino acid serine at their protease active sites. Serine protease inhibitors block these active sites and thus inhibit the function of all serine proteases, but the different inhibitors may have a varying and dose-dependent affinity for a specific protease. Serine protease inhibitors are endogenous to eukaryote organisms. Exogenous serine protease inhibitors may have various effects across species. The bovine serine protease inhibitor aprotinin has been widely used to inhibit plasmin and thus fibrinolysis in humans, see later.

Plasminogen bound to fibrin or cells is easily activated by fibrin-bound t-PA or cell-bound u-PA , while plasminogen in solution is not so easily activated as it needs to undergo a conformational change to be accessible for activation in solution[24]. Lysine analogues competitively inhibit the lysine binding sites on both plasminogen, plasmin and the plasminogen activators, thus preventing their binding to fibrin/cells and their respective activations[20,26-28]. This blocking of lysine binding sites both reduces the activation of plasminogen to plasmin, and it prevents activated plasmin from docking to fibrin and exerting its proteolytic effect[20] (Figure 2). However, when hyperfibrinolysis is triggered and ongoing, and antiplasmin is depleted, systemically administered lysine analogues may bind to the lysine binding sites of circulating plasminogen which induces its conformational change and allows for activation of plasminogen in plasma by u-PA. Antifibrinolytics may thus enable activation of

circulating plasminogen to plasmin in a setting where antiplasmin both is scarce and may fail to neutralize circulating plasmin as the target lysine binding site is blocked[29]. This has been proposed as a possible mechanism behind the lack of effect and even possible negative impact of late administration of lysine analogue in trauma[29]. Two synthetic lysine analogues have so far been commercially available: ε-aminocaproic acid (EACA) and the 6-10 times more potent tranexamic acid (TXA). TXA, but not EACA, has also been found to inhibit the active site of u-PA[30]. As u-PA and its receptor may be involved in complex systems such as wound healing, cell migration and adhesion, this inhibition may have effects beyond those of the fibrinolytic system[31,32].

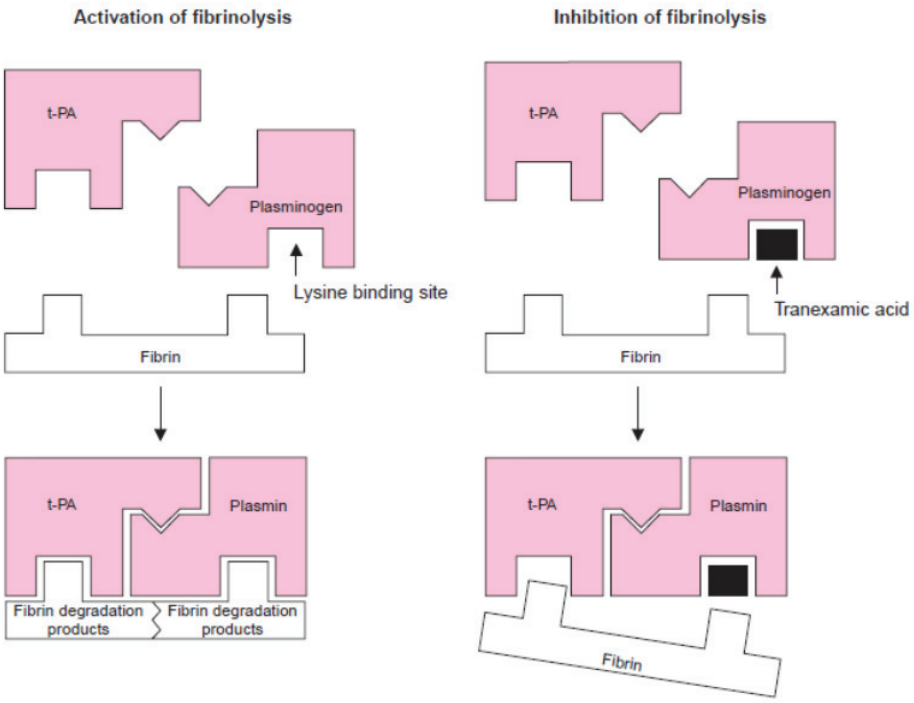

Fig. 2. Simplified antifibrinolytic acion of tranexamic acid. Normally, plasminogen binds to fibrin at a lysine binding site and is converted in the presence of tissue plasminogen activator (t-PA) to plasmin. Tranexamic acid blocks the lysine binding site and prevents access of plasminogen to the fibrin molecule. Tranexamic acid may however also block the binding between t-PA and plasmin as this is also a lysine binding site. Reprinted with permission from Dunn CJ & Goa KL; *Drugs.* 1999.

The emergence of modern surgery and blood preservation

Surgery means inflicting tissue injury with its associated bleeding. Advances in surgery have been closely linked to the ability to control or replace blood loss. From the earliest mechanical means of hemostasis such as compression, tourniquets, simple cautery and ligation[33], an expanding armamentarium of refined mechanical, thermal and pharmacological means of blood preservation has evolved.

Elective surgery was spurred in the latter half of the 1800s both by the introduction of chloroform and ether for anesthesia[34] and the establishment of the antiseptic principles[35]. "The clotting of blood" was also extensively investigated[36]. Various materials such as gelatin and cellulose had been found to enhance clotting[33], while other substances such as snake venoms and leech extract could dissolve clots[37]. Both fibrin and thrombin had been identified by the late 1800s and purified by the early 1900s[38], enabling the use of fibrin and thrombin as topical hemostatic agents. Although many factors involved in the formation of fibrin had been identified, a thorough understanding of the interaction between them had not been established until the coagulation cascade was postulated by MacFarlane in 1964[39,40]. The initiation of the cascade resulting from blood coming into contact with "foreign" surfaces provided a scientific basis for the further development of substances with surface structures designed to initiate coagulation, as well as thrombin and fibrin sealants[41].

Blood transfusions

Methods for safe blood transfusion emerged in the first half of the 1900s as a response to the desperate needs of ongoing wars linked with advances in science and technology[42]. Although successful blood transfusions had been performed before the turn of the century, Landsteiner published in 1901 what would be known as the "Landsteiner reaction"; the identification of blood groups and the testing of their compatibility through cross-matching[43]. Blood grouping prior to transfusion did however not become widespread until the 1920s[42]. The identification of coagulation factors and their association with hemostatic disorders led to targeted substitutional therapies using plasma-derived concentrates[44]. The established transfusion regimes allowed for immense surgical developments through the 60s and 70s. As frontier procedures such as open heart surgery and joint replacement surgery became mainstream, the limited access to banked donor blood struggled to meet the increasing demand[20]. Transfusion reactions still occurred as meticulous subgroup matching beyond the main blood groups was not feasible in emergency settings. Allogeneic blood transfusions were also found to be associated with an increase in patient morbidity[45-47], possibly due to immunomodulatory effects[48]. Unrecognized blood-borne viral infections such as hepatitis C in the

1970s and the emerging AIDS epidemic of the 1980s also grimly illustrated that blood transfusions and plasma-derived substances could have deleterious consequences[49]. This spurred renewed interest in pharmacological blood-preserving measures.

Pharmacological means of blood preservation

Adrenaline had been isolated and purified by 1900[50] and was shortly thereafter shown to have vasoconstrictive and hemostatic properties in surgery, thus representing the first pharmacological means of hemostasis[51]. Its use was however debated among surgeons well into the 1970s[52,53]. Clinical use of the anticoagulants heparin[54] and warfarin[55] was established by the 1960s[56]. Transfusion of plasma could quickly reverse the effect of anticoagulants, and protamine sulfate and vitamin K had also been recognized as pharmacological antidotes[57-59]. The synthetic vasopressin analogue desmopressin was in the late 1970s found to both increase the level of circulating coagulation factors and improve platelet dysfunctions and was thus efficient in mild hemostatic disorders of various origins[60,61]. Recombinant coagulation factors for hemophilia were available by the late 1980s[62].

Blood preservation in patients without bleeding disorders

The pharmacological means of reducing bleeding in patients with various coagulation dysfunctions do not necessarily benefit patients without such dysfunctions. Off-label administration of recombinant coagulation factors to non-hemophilic patients has had little documented effect and may even promote thromboembolic events[63-65]. Whether desmopressin has any hemostatic effect in patients with no coagulation dysfunctions also remains uncertain[66]. Use of procoagulants in surgery has mainly been limited to topical application onto bleeding sites, as systemic administration is considered likely to involve an unacceptable risk of thromboembolic events. Fibrinogen concentrate has received renewed attention as a possible universal hemostatic agent, but adequate documentation on both effect and possible adverse events are still lacking[67]. Thus, the only systemically administered pharmacological intervention to universally reduce bleeding with an acceptable safety profile in patients without bleeding disorders is currently the antifibrinolytic drugs.

Enzymes, their activators and their inhibitors: The emergence of fibrinolytic and antifibrinolytic drugs

W.S. Tillett demonstrated in 1933 that broth cultures of hemolytic streptococci could both dissolve established fibrin clots in human plasma and prevent clotting when added to unclotted plasma[68]. The active agent was initially named *fibrinolysin*. Tillett also observed that the fibrinolysis initiated by the added fibrinolysin occurred faster in experimental fibrinogen preparations than in human plasma, thus postulating that plasma might also contain antifibrinolytic substances[69]. J.H. Milstone (1941) observed that fibrinolysin would only exert its fibrinolysis on purified fibrin clots in the presence of human plasma, but not in the presence of human serum[70]. He postulated a component in human plasma necessary for the initiation of streptococcal fibrinolysis, which was initially termed the *lytic factor*. In 1944, both M.H Kaplan and L.R Christensen demonstrated that this lytic factor was a serum enzyme, similar to trypsinogen, present in plasma in an inactive precursor state[71,72]. The lytic factor could be transformed to its active fibrinolytic form catalyzed by fibrinolysin, similar to how trypsinogen was transformed to its proteolytic form trypsin catalyzed by enterokinase. Fibrinolysin was therefore not a fibrinolytic compound in itself but an activator, a kinase. Christensen and MacLeod proposed to name the precursor variant of the lytic factor *plasminogen* and its proteolytic form *plasmin,* while the catalytic streptococci-derived plasminogen activator was named *streptokinase*[73]. MacFarlane (1946) subsequently proposed the term *antiplasmin* for the antifibrinolytic substance in plasma[74].

The discovery of streptokinase led to widespread utilization to lyse clots and inflammatory fibrinous exudates of multiple conditions[75]. Associated publications of possible allergic reactions increased the awareness of the potential antigenic potential of foreign proteins used as biological drugs[76]. The search continued for a human-derived equivalent product for fibrinolysis.

MacFarlane and Pillig (1947) published the discovery of the presence of plasminogen activator in urine[77], which was named *urokinase* by Sobel et al. in 1952[78], later renamed urokinase-type plasminogen activator (u-PA). Fisher (1946) proposed that an activator of plasminogen might be present in tissue[79] and Astrup and Permin (1947) confirmed that various animal tissue could indeed activate plasminogen. Astrup and Albrechtsen (1957) managed to quantify the contents of tissue-type plasminogen activator (t-PA) in different human tissue[80] and could compare concentration to that of previously measured still-standing postmortem-blood[81]. As this stagnant blood contained a much higher concentration of t-PA than the tissues themselves, a plasminogen activator derived from the endothelium of the vascular bed was proposed by Todd (1959)[82]. Only in 1980 did Rijken et al.[83] prove that this vascular plasminogen activator[84] and plasminogen activators in blood and tissue

were identical, but different from u-PA. Urine was easily obtained and purification of u-PA from human urine had been achieved by the late 1950s[85]. Obtaining t-PA was more challenging; purified t-PA had been obtained by 1979 from perfusion of the lower leg vessels of human cadavers[84], and extracted from large volumes of human uterine tissue[86].

All versions of plasminogen activators can dissolve clots and fibrous exudates. Streptokinase, u-PA, t-PA and recombinant versions thereof have all been used as intravenously administered thrombolytic drugs[87]. All were effective in restoring circulation and reducing ischemic tissue damage, but systemic treatment with large doses involved a significant risk of bleeding – including potentially lethal intracranial hemorrhage. Modern endovascular techniques have however allowed for intravascular targeted treatment of the thrombus (catheter-directed thrombolysis) which has significantly reduced the risk of bleeding[88].

The discovery and isolation of enzymes led to parallel research on enzyme inhibitors. Isolation of streptokinase and later urokinase provided a possibility for scientific evaluation of agents capable of preventing fibrinolysis. A standardized experimental kinase-induced fibrinolysis could be strongly or weakly inhibited according to agent and/or concentration. The search for antifibrinolytic agents was thus initiated[89].

Serine protease inhibitors – the naturally occurring biological drugs

The presence of naturally occurring inhibitors of proteases in plasma had been demonstrated in the early 1900s[90]. A kallikrein inactivator was isolated from both bovine parotid and lung tissue by Kraut et al. in 1928[91], and a potent trypsin inhibitor isolated from bovine pancreas tissue by Kunitz in 1936[92] was found to be almost identical in structure to the kallikrein inactivator[91]. Northrop, Kunitz and Herriot had succeeded in isolating animal-derived proteolytic enzymes as pure, crystalline proteins by the 1930s[93]. The presence of plant-derived trypsin inhibitors was demonstrated in beans by Ham and Bowman independently in 1944/45[94,95]. Mirsky demonstrated in 1944 that trypsin inhibitors derived from both bovine pancreas and soy beans could also prevent fibrinolysis[96].

Bergmann (1937) proposed that enzymes should be classified according to their structural characteristics. The proteolytic enzymes kallikrein, trypsin, plasmin and several others were classified as *serine proteases* due to the presence of the amino acid serine at the enzymes' active sites[97]. The serine proteases were all inhibited by a variety of naturally occurring polypeptides – the *serine protease inhibitors.* A crystalized soybean trypsin inhibitor published by Kunitz in 1945[98] was used to

reverse fibrinolysis *in vivo* by Heuson in 1958[99]. Plant-derived protease inhibitors were however weak antifibrinolytics[100] and were abandoned in favor of the bovine derived protease inhibitors.

Aprotinin

The bovine-derived serine protease inhibitor aprotinin, originally marketed as Trasylol, was initially isolated from bovine pancreatic tissue[98], but commercial preparation has since been done from bovine lung tissue. Aprotinin was originally marketed to alleviate the auto-digestion resulting from the leaking proteolytic enzymes of pancreatitis[101]. As trypsin inhibitors had been described to inhibit fibrinolysis, reports on the use of aprotinin to treat acute fibrinolysis rapidly emerged particularly from cardiac surgery[102-104] but received little attention. The serine protease kallikrein is a potent mediator of inflammation, and in a protocol designed to demonstrate anti-inflammatory and lung-preserving qualities of aprotinin in open-heart surgery, van Oeveren and Royston in 1987 demonstrated an unexpected profound effect of high dose aprotinin on blood loss and transfusion needs[105]. The use of aprotinin as a routine prophylactic measure to reduce bleeding in cardiac surgery spread rapidly thereafter.

Aprotinin – like the naturally occurring plasmin inhibitors in serum – quickly but reversibly binds to the active site of free plasmin in serum. Aprotinin has little interaction with plasmin when plasmin is bound to fibrin, but aprotinin can bind to tissue plasminogen activator (t-PA), thus preventing the t-PA from binding to plasminogen and exerting its catalytic conversion of plasminogen to plasmin. Aprotinin inhibits all serine proteases, but with varying affinity. It has high affinity to trypsin and plasmin, intermediate to kallikrein, and only binds to plasma thrombin at high concentrations. Aprotinin can therefore potentially influence platelet and complement activation, inflammation, coagulation and fibrinolysis, and while low dose may prevent fibrinolysis, a higher dose is needed for anti-inflammatory effect[106]. The exact mechanisms of the net blood-preserving qualities of aprotinin are not understood in full detail[107]. As aprotinin is a bovine peptide, hypersensitivity can develop in about 5% of patients within a few days to weeks of initial exposure and may still persist after 12 months in approximately 0.5-1% of patients[108].

The withdrawal of aprotinin

In 2006, the U.S. Food and Drug Administration (FDA) was alerted to a commissioned retrospective study of 67 000 patients by one of the participating researchers; 30 000 patients had received aprotinin while the others had received lysine analogues. The study had been withheld by Bayer, the manufacturer of aprotinin who had commissioned the trial, as it suggested that aprotinin led to increased risk of multiple adverse events[109,110]. 5-year follow-up also revealed an increased risk of death[111]. A prospective randomized trial - Blood Conservation Using Antifibrinolytics in a Randomized Trial (BART) [112] was commissioned by the FDA but terminated late 2007 due to a trend towards increased mortality in the aprotinin group compared to lysine analogues, and Bayer subsequently withdrew aprotinin from the market. Retrospective analyses were conducted in the aftermath and seemed to confirm the increased mortality associated with aprotinin[113,114], but the withdrawal has been met with much controversy and questioning regarding selection bias in the mentioned studies[115-117]. The ban has been partially lifted, as aprotinin may be the most powerful inhibitor of fibrinolysis in procedures with particularly high volume bleeding and hyperfibrinolysis[118,119]. However, in the wake of the withdrawal of aprotinin, the lysine analogues have dominated as prophylactic antifibrinolytics in surgery.

Lysine analogues – the synthetic drugs

The purification and crystallization of naturally occurring serine protease inhibitors allowed for further analysis of their physiochemical structure, with the associated possibility of creating synthetic selective inhibitors. Herriot (1940) demonstrated that a naturally occurring pepsin inhibitor contained significant amounts of the amino acid arginine[120], and Neurath and Schwert (1950) demonstrated that amino acid esters were sensitive substrates and potential inhibitors for proteolytic enzymes[121]. In Europe, Troll et al (1953) published that for plasmin in particular, lysine and arginine esters seemed to be specific substrates[122].

Meanwhile, in post-war Japan, the husband-and-wife-team Shosuke and Utako Okamoto had targeted the hyperfibrinolysis of pathological bleeding. Assuming hyperfibrinolysis was caused by excessive action of plasmin, they aimed at creating a synthetic antiplasmin and performed a systematic screening of more than 300 chemical compounds for possible antifibrinolytic activity through plasmin inhibition[123]. While lysine exerted a weak inhibition, the Okamotos found that a chemically modified derivative of lysine, ε-aminocaproic acid (EACA), had a tenfold inhibitory effect, mainly through the inhibition of plasminogen activation[124]. A patent claim for EACA was filed in 1953 and granted in 1957[125]. The Okamotos continued their search for more potent inhibitors of

fibrinolysis, and in 1961 patented 4-aminomethyl-cyclohexane-1-carboxylic acid, abbreviated AMCHA[125], of which the trans-stereoisomer was later discovered to carry the antifibrinolytic properties[126-128]. This *trans*-AMCHA was named *tran*examic acid (TXA) and had an approximately 6-10-fold higher antifibrinolytic potency than EACA[129,130]. EACA and TXA were purely synthetic substances and hence much cheaper to produce than aprotinin, and with less antigenic potential. While aprotinin could only be administered intravenously, EACA and TXA could be administered both intravenously, intramuscularly and orally.

Clinical use of lysine analogues

It had been long recognized that elective surgery carried a significant risk of postoperative thromboembolic events, and that prophylaxis with heparin could reduce the risk[131]. Thus, simultaneous use of lysine analogues and postoperative heparin could reduce the risk of postoperative embolism with less increase in postoperative bleeding[26]. Initially, EACA and later TXA was particularly used in patients with bleeding related to prostate and urological surgery, as urine was known to be the source of the fibrinolytic plasminogen activator u-PA which could sustain the local bleeding[26]. However, insoluble clots resulting from use of lysine analogues were reported to lead to urinary retention, kidney infarction and even death from bilateral ureter obstruction[132,133]. Enthusiasm was dampened, and use of TXA in larger surgery was henceforth little explored due to fear of the thrombotic potential of lysine analogues[134]. Through the 1970s, use continued in prostate surgery but was debated[135]. Lysine analogues were explored in the treatment of intracerebral hemorrhage and prophylaxis of aneurysmal rebleedings[136], but effect was questionable and potential negative side effects in the central nervous system(CNS) were suggested[137]. Effect was however well documented to reduce menorrhagia, and use in epistaxis, minor oral surgery, dental extractions, tonsillectomy and cervical conisation was also recommended[138].

The HIV epidemic of the mid 1980s spurred renewed interest in antifibrinolytics to avoid blood transfusions. Aprotinin was documented by Oeveren and Royston (1987) to significantly reduce blood loss in cardiac surgery[105], and Vander et al. and Horrow et al. shortly thereafter demonstrated similar effects from lysine analogues[139,140]. Although benefits were still debated[141], prophylactic use increased in cardiac surgery. In the mid 1990s, the benefits of antifibrinolytics were also recognized in joint replacement surgery[142-144]. Henceforth, prophylactic use of antifibrinolytics became widespread in surgery with high-volume bleeding, and after the withdrawal of aprotinin in 2007, TXA has dominated as prophylactic antifibrinolytic drug.

Lysine analogues in hemophilia

Early research on lysine analogues suggested potential significant benefits in patients with congenital or acquired coagulation deficits[129,145]. As lysine analogues were low-cost and could be administered orally, there was much optimism regarding the potential prophylactic use in the hemophilic population. Studies on continuous prophylactic oral use of TXA to prevent hemorrhagic episodes in patients with hemophilia were few, inadequately powered and could not conclude regarding effect[146]. Hematuria is one of the major challenges in severe hemophilia, but the use of lysine analogues was deemed contraindicated in hematuria after the previously mentioned reports of ureter obstruction[146]. Prophylactic use in larger surgery in hemophiliacs has henceforth been inadequately explored.

Patients with coagulation deficits seem to benefit from on-demand use of TXA in conjunction with smaller oral or nasal bleeds and prophylaxis in connection with dental surgery, but randomized controlled trials are lacking[147]. Lysine analogues used as adjunct therapy along with factors substitution can increase clot stability[148] and thus potentially reduce the amount of coagulation factors needed, and although this is clinically practiced, few controlled studies have been conducted on the possible benefits of combining lysine analogues and coagulation factors[146,148].

Systemic use of TXA

Pharmacokinetics

TXA is excreted largely unchanged via glomerular filtration; its plasma elimination half-life is approximately 80-120 minutes and prolonged in renal failure. After iv administration, 95% can be recovered from urine within the first 24 hours. TXA is retained in tissues with an estimated tissue half-life of 17 h[129], and the tissue retention may account for the non-recovered drug in urine at 24 hours. Oral bioavailability is approximately 35-40%[130,149]. Time from oral ingestion to maximal plasma concentration is approximately 3 hours[129]. TXA given iv diffuses quickly to joint synovium; elimination half-life in synovial fluid is approximately 3 hours, and synovial fluids therefore maintain therapeutic levels longer than plasma[150]. TXA crosses the placenta but no teratogenic properties have been identified[130]. Concentrations in breast milk is approximately 1/100 of that in plasma[151]. TXA crosses the blood-brain barrier and concentration in the cerebrospinal fluid (CSF) is approximately 10% of that in plasma, but this percentage may vary and peak CSF concentration may occur several hours after peak plasma concentration[152,153].

Dose-effect and concentration-effect relationships

In vitro studies suggest the minimum plasma concentration to significantly inhibit fibrinolysis to be about 5 µg/ml in children and 10 µg/ml in adults[26,154,155]. Plasminogen inhibition is achieved at plasma concentrations of 10-15 µg/ml[20,130], while a higher plasma concentrations can also inhibit the plasminogen activators and plasmin itself[26,30]. In theory, a maximal suppression of both plasminogen and its activators would increase hemostatic effect. Fibrinolysis *in vitro* is inhibited by more than 90% at TXA plasma concentrations around 20 µg/ml, and a concentration of 100 µg/ml provides a 98% inhibition as also the plasminogen activators are inhibited[26].

The optimal dosing of TXA *in vivo* to reduce bleeding has been debated. Intravenous regimes have been particularly varied in cardiac surgery, ranging from single doses, continuous infusions, or both, from 1g to 20 g[156] given over time intervals from 20 minutes to 12 hours[119]. While some have advocated high-dose regimes in cardiac surgery[157,158], maintaining serum concentrations above 100-150 µg/ml[159], other studies do not find clinically significant differences in blood saving effect between high- and low-dose regimes[160-163]. There is however no international consensus on dosing protocols and specific definitions of high- and low-dose regimes. A classical high-dose regime yielding serum concentrations between 100-150 µg/ml is the dose recommended by Dowd et al.[158] which was used in the commissioned BART trial comparing adverse effects of aprotinin to that of TXA[112]. The BART regime consists of a loading dose of TXA 30 mg/kg and then an ongoing infusion of 16 mg/kg/h. Conversely, the lowest dose among the low-dose regimes in cardiac surgery is a loading dose of TXA 10 mg/kg with or without a continuous infusion of 1 mg/kg/h. TXA does not yield a linear dose-response relationship, and there seems to be little added clinical benefit from iv doses exceeding 10-20 mg/kg, which is recommended by the manufacturers[151]. While TXA administration in cardiac surgery often consists of an initial bolus plus continued infusion throughout the procedure both due to the use of cardiopulmonary bypass and the relatively long operating time, orthopedic administration is typically a single bolus of TXA 10-20 mg/kg. Repeated administration may however yield added benefits compared to single dose administration only, as the hyperfibrinolysis induced by surgery may persist up to 24 hours[164,165].

Different types of surgery are associated with varying magnitudes of bleeding. Although the reduction in bleeding resulting from iv TXA as measured in ml varies between procedures, the proportional reduction is rather constant with an approximate reduction of one third in both measured bleeding volume and transfusion needs[9,166]. The incidence of explorative re-operations due

to hemorrhage in cardiac surgery was halved after iv TXA in two different meta-analyses[119,167]. Orthopedic studies rarely report need for re-operation due to hemorrhage as a separate outcome.

Thromboembolic adverse events

A drug which prevents bleeding could in theory promote thromboembolic events, and this was a major fear when use of TXA was initiated. After the introduction of antifibrinolytic drugs, anecdotal reports of occluded grafts after coronary bypass[168], increased incidence of myocardial infarction[141] and pulmonary embolism/ deep venous thrombosis[15] have maintained an uncertainty regarding this potential adverse effect. Large multicenter studies investigating the effect of iv TXA in thousands of arthroplasties[13], trauma[14], postpartum hemorrhage[11], or cardiac surgery[12] have not found an association between TXA and thromboembolic events. However, case reports of thromboembolic events maintain uncertainty[15], and a recent large retrospective study by Myers et al. diverges from the above mentioned studies and suggests an increase in venous thromboembolic events among trauma patients receiving TXA[16]. Adequately powered studies to specifically address potential adverse effects have been lacking. The Aspirin and Tranexamic Acid for Coronary Artery Surgery (ATACAS) study was a multicenter prospective randomized study designed to investigate potential adverse effects of TXA in high-risk patients undergoing coronary artery surgery, "high risk" being defined as advanced age, pulmonary/cardiac/kidney failure or obesity. The primary outcome was a composite endpoint of death and thrombotic complications (nonfatal myocardial infarction, stroke, pulmonary embolism, renal failure, or bowel infarction) within 30 days after surgery[169]. There was a slightly lower incidence of an unfavorable outcome in the TXA group, and in particular no increase in thromboembolic events. This trend persisted at a one-year follow-up of the population[170]. However, former thromboembolic events or ongoing anticoagulation for whatever reason at the time of surgery were exclusion criteria in this study population, and large randomized controlled trials on the use of iv TXA in populations with known increased risk of thromboembolic events are still lacking. Unease regarding this possibility persists, limiting routine prophylactic use of TXA to surgical procedures associated with high-volume bleeding and transfusion needs.

CNS adverse events

As TXA so clearly reduced bleeding, administration to reduce re-bleeding after ruptured intracranial aneurysms became widespread in the 1970s. Although re-bleeding was reduced, there was concern regarding observed possible ischemic and thromboembolic events[137]. In 1981, a retrospective

analysis of ruptured intracerebral aneurysms which had received either a combination of low-dose TXA in combination with aprotinin versus high-dose TXA alone found a significant increased rate of cerebral ischemic complications in the high-dose TXA group[137]. The net benefit of prolonged use of TXA after cerebral bleeding has been questioned[171], while short-term treatment may be advantageous[172,173].

Potentially dramatic effects of lysine analogues coming into direct contact with the CNS were reported in the early 1980s; animal models investigating the effect of TXA in cerebral hemorrhage found that TXA induced ischemia, and subdural application of fibrin sealants containing TXA in animals evoked epileptic convulsions[174-176]. Accidental intrathecal administration of TXA has occurred on many occasions in settings where vials of TXA were mistaken for bupivacaine for spinal anesthesia because they were visually similar. This has resulted in lower limb myoclonus, generalized seizures, ventricular refractory arrhythmias and several deaths[177-181].

Fig 3. Ampules of bupivacaine and tranexamic acid. The similar size of ampules led to a medication administration error during spinal anesthesia.
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When the blood-brain barrier is intact, iv TXA administration renders the concentration in the CSF approximately 1/10 of that in serum[152]. Ongoing intracranial bleeding would expose the CNS to the serum concentration of TXA, and intrathecal administration would expose the CNS to exceedingly high levels. Yet reports from cardiac surgery indicate that even with an intact blood-brain barrier, TXA can increase the risk of seizures[182].

In the wake of the withdrawal of aprotinin from the marked in 2007, several studies were published comparing the safety profiles of TXA vs. aprotinin. Martin et al. (2008) were the first to report the significant association between administration of TXA and seizures in cardiac surgery[182]. Several studies confirmed this observation[183-186], and although overall incidence of seizures is normally in the range of 0.5 – 3%, meta-analyses suggested that TXA was associated with at least a four-fold increase in risk[17,187]. The increase in seizures seemed to be dose-dependent and was particularly seen in settings with an increased risk of accumulation of TXA - such as open cardiac surgery where a prolonged procedure also implies an extended continuous infusion, and in patients with renal failure with subsequent delayed elimination. Benchmark studies such as the BART trial[112] and the ATACAS[169] study also found increased incidence of seizures in the TXA population. Both studies administered

high-dose TXA regimes; the ATACAS study reduced their TXA administration from single dose 100 mg/kg to 50 mg/kg after awareness regarding the possibility of seizures was raised, yet this did not significantly reduce the incidence of seizures in the TXA group. Seizures are generally associated with an increased risk of death in the postoperative period[170,186], albeit it has not been determined whether this holds true for TXA-induced seizures.

Possible underlying mechanisms for the TXA-induced hyperexcitability was first proposed by Furtmüller (2002)[188], who demonstrated that TXA could bind antagonistically to $GABA_A$ receptors in the CNS and thus prevent the inhibitory action of the neurotransmitter GABA. The studies by Furtmüller were based on the observations of topical/intrathecal/hemorrhagic TXA exposure of the CNS, thus representing high concentration exposures. The concentrations needed for $GABA_A$ receptor inhibition were observed at drug concentrations in the mg/ml range, while CSF concentrations obtained from therapeutic iv administrations in a setting with an intact blood-brain barrier should normally not exceed low µg/ml levels.

Lysine analogues are chemically related to the amino acid glycine, and Lecker et al. (2012)[153] showed that TXA also acts as a competitive antagonist to the inhibitory effect of glycine via glycine receptors in the CNS. Based on *in vitro* studies, CSF concentrations of approximately 15 µg/ml may be a threshold value for a potentially excitatory effect, which is much lower than for the $GABA_A$ mechanism. There may also be a synergy between the two means of blocking cerebral inhibition[153,189].

The CSF achieves approximately 10% of the TXA concentration of plasma, but this proportion may vary[152]. TXA dosing regimens advocating the maintenance of plasma concentrations above 100 µg/ml for an extended period may therefore be at risk of reaching threshold concentrations in the CSF. Sharma et al. have documented that high-dose administration as defined by a 30 mg/kg bolus followed by 16 mg/kg/h infusion (The BART study dose for high-risk cardiac surgery) maintains a plasma level above 100 µg/ml and therefore above the theoretical threshold for evoking seizures[186].

A single bolus injection of 10-20 mg/kg would in all likelihood not be sufficient to reach the threshold concentration in the CSF, but factors which may increase patient susceptibility are unexplored[189].

Intravenous TXA in all surgery?

Based on the evidence reviewed in the previous sections, a single bolus of 10-20 mg/kg TXA as a prophylactic measure at the initiation of surgery would seem like a safe prophylactic measure to reduce surgical bleeding. However, an unease regarding potential unrecognized adverse events may

dissuade clinicians from using iv TXA in low- to medium-bleed surgical procedures. Intravenous TXA as a routine pharmacological means of reducing bleeding has therefore mainly been adopted in surgery with high-volume bleeding and potential high transfusion rates.

Topical use of TXA

Effect

Theoretically, direct application of TXA onto surgical wound surfaces would be expected to give high local concentrations with limited systemic exposure, thereby reducing bleeding with a lowered risk of systemic adverse events.

Topical application of TXA to reduce bleeding has been sporadically published since the 1970s[190,191]. Sindet-Pedersen et al. demonstrated in the 1980s the hemostatic properties of a 50 mg/ml TXA mouthwash in patients on anticoagulation and hemophiliacs undergoing dental extractions[192,193]. During the 1990s, sporadic reports of alternative topical use emerged, such as moistened gauze on a surgical wound[194], and enema administration for intestinal bleeding[195,196].

In 2000, De Bonis et al. published the first study in cardiac surgery where 100 ml of either 10 mg/ml TXA or 0.9% saline was poured into the pericardium and mediastinal tissue prior to sternal closure, with a significant 22% reduction in postoperative drain production[197]. Further studies from cardiac surgery have been few and not unambigous[198-202], although meta-analyses suggests a beneficial effect[203,204]. The use of cardiopulmonary bypass in cardiac surgery leads to an increased peroperative triggering of the fibrinolytic system, and pre/peroperative iv administration has been the route of choice to ensure perioperative antifibrinolytic effect. The increasing awareness of associated seizures has however spurred renewed interest in topical alternative use in cardiac surgery[205].

Orthopedic surgery has to a much larger extent explored the field of topical TXA. Krohn et al. (2003)[206] irrigated the wound of low-back pain patients undergoing lumbar fusion with 50 ml of 10 mg/ml TXA for 2-5 minutes prior to closure and found a significant 40% reduction of postoperative blood loss compared to placebo. Wong et al. (2010)[207] published the first arthroplasty study in knee joint replacement surgery, exposing the open joint to 100 ml of either 15 or 30 mg/ml TXA for five minutes, suctioning away the excess fluids prior to closure. Wong et al. found a significant decrease of postoperative bleeding and a maintenance of higher Hb levels in the TXA groups, with no significant difference between the different concentrations. Instilling various concentrations in conjunction with hip and knee arthroplasties has since been extensively published along with an

increasing number of meta-analyses demonstrating that the reduction in bleeding after topical administration of TXA is non-inferior to iv use[19,208-211].

Publications regarding topical use in spine surgery have been scarce since the original publication by Krohn[206], but there is an increasing awareness of the possibility of topical use reflected in a notable increase in publications, systematic reviews and meta-analyses based on the rather few studies available[212-217]. It is however worth noting that none of the authors mention the dangers of intrathecal or topical contact of TXA with the CNS, as dural lesions and possibilities of leakage could be an issue in spine surgery.

Topical use of TXA outside orthopedic, cardiac and spine surgery has until now only been anecdotally published, as demonstrated by a recent review by Montroy et al.[218]. Topical use is off-label, and there is no consensus as to the optimum dose and mode of application. Most published methods of application involve instilling a bolus into a confined space, prolonged irrigation, or application of a moistened carrier[19], and concentration in the applied fluids varies from 1 to 100 mg/ml, without any apparent dose-response relationship[218]. Arthroplasty studies have generally used topical solutions with concentrations in the range of 15-100 mg/ml[219], while the studies in cardiac surgery have generally used somewhat weaker solutions with concentrations between 10 and 20 mg/ml[203].

Systemic concentrations and adverse effects

Although it is assumed that topical application of TXA will result in low systemic concentrations, few studies have actually investigated the systemic absorption and then only at a single postoperative time point[193,197,207,220], rendering peak levels uncertain. Pharmacokinetic studies after administration of a 2 g TXA enema[221] or a 2-minute mouth rinse with TXA 50 mg/ml[192] resulted in systemic concentrations below 2 µg/ml. Wong et al. irrigated the knee joint with TXA after arthroplasty for 2-5 minutes[207]. Serum samples taken one hour after tourniquet release showed that irrigation with TXA 15 mg/ml (at a volume of 100 ml) yielded a concentration of 4.5 µg/ml , while a double concentration of 30 mg/ml (at a volume of 100 ml) led to a concentration of 8.5 µg/ml. It seems reasonable that systemic absorption after topical application of a given volume should be proportional to the drug concentration. Well-vascularized tissue may also absorb more of a topically applied drug than less vascularized tissue. Whether topical application can lead to systemic concentrations above the therapeutic threshold value of 5 µg/ml in children or 10 µg/ml in adults needs clarifying.

Local adverse effects from topical use

The potential local toxicity of topical TXA has been insufficiently explored. The dramatic effect of lysine agonists on inhibitory neuroreceptors in the CNS illustrates the possibility of unexpected adverse effects of an apparently safe drug[17,177]. As topical TXA has become most widespread in orthopedic arthroplasty, studies regarding potential local toxicity have mainly been conducted on cartilage, tendon and synovial tissue. Published studies on the effect of topical TXA on chondrocytes and cartilage tissue suggest a threshold for toxicity of around 25 mg/ml given at least 3 hours of exposure, and cells embedded in a natural (cartilage) or artificial (hydrogel) matrix are more resilient[172,222-224]. Marmotti et al. found that chondrocytes, tenocytes and synoviocytes were not affected by a two-week exposure to 7 mg/ml TXA[225]. In other tissues, Furst et al.[226] reported 50% viability in human fibroblasts exposed to 100 mg/ml TXA and 65% viability after exposure to 50 mg/ml for 100 minutes, while Bergenholtz et al.[227] found that TXA concentrations of 12 mg/ml or lower did not prevent wound healing in vitro of incisional wounds through palatal mucosa from cats although re-epithelialization took place with abnormal keratinocyte migration.

AIMS OF THE THESIS

The purpose of the project “Topical tranexamic acid to reduce bleeding from surgical wounds” was to assess the effect of a simple topical moistening of surgical wounds with TXA 25 mg/ml, to register possible adverse events and assess the systemic concentration resulting from topical application. We wanted to achieve this through clinical studies measuring the effect of the intervention on postoperative bleeding and registering possible adverse events. We also wanted to compare the systemic pharmacokinetics of TXA after topical administration to iv administration.

The specific aims of the studies described in the thesis were:

- To investigate whether a single moistening of a surgical wound surface prior to wound closure with TXA 25 mg/ml would reduce postoperative bleeding as measured by wound drain production at 24 h using patients undergoing reduction mammoplasty and mastectomy as study models (Studies I and II)

- To investigate whether a single moistening of a surgical wound surface prior to wound closure with TXA 25 mg/ml would influence late seroma formation in patients undergoing mastectomy (Study II)

- To investigate whether a single moistening of a surgical wound surface prior to wound closure with TXA 25 mg/ml would be associated with an increase in postoperative complications or adverse effects (Studies I and II).

- To investigate the degree of systemic absorption of TXA after two different means of topical application in patients having large wound surface areas after abdominal major weight loss skin reducing surgery, and compare the systemic TXA concentrations achieved by these two methods with standard intravenous prophylactic administration of 1 g of tranexamic acid in hip replacement surgery (Study III).

METHODS

All studies were prospective clinical studies on patients receiving topical TXA. Studies I and II were randomized double-blinded clinical intervention trials to assess the effect of moistening a surgical wound surface with TXA, while Study III was a pharmacokinetic study investigating systemic concentrations of TXA after topical versus systemic administration. All studies were approved by the Norwegian Medicines Agency and The Regional Committee for Medical Research Ethics, Mid Norway. All participants gave written informed consent prior to inclusion. In all studies, patients who were to receive topical TXA were not considered eligible if they 1) had a known allergy to TXA, 2) were pregnant or nursing, 3) had a known history of a thromboembolic event, or 4) were less than 18 years old. Study I did not have a monitor protocol, while studies II and III underwent formal monitoring in cooperation with the Norwegian Clinical Research Infrastructure Network (NorCRIN). All studies were registered in ClinicalTrials.gov and in the European Clinical Trial Database (EudraCT). Assistance with study registrations, conduction of computer-generated randomization, development of electronic study forms and assistance on statistical analyses were provided by the Unit for Applied Clinical Research (AKF), Norwegian University of Science and Technology – NTNU. Statistical analyses were performed using SPSS version 25 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA). Values of $p < 0.05$ were considered statistically significant.

Specific methodological aspects in the individual studies

Studies I and II

Studies I and II were randomized clinical trials to evaluate the effect of moistening a surgical wound surface with 20 ml of TXA 25 mg/ml (intervention) versus 20 ml of NaCl 0.9% (placebo) prior to wound closure. Study I was conducted on patients undergoing bilateral reduction mammoplasty at the Section for Plastic and Reconstructive Surgery at St Olav's University Hospital during the years 2013 – 2014. Study II was conducted on patients undergoing mastectomy with or without lymph node clearance at the Departments of Breast and Endocrine Surgery at St Olav's University Hospital and Ålesund Hospital during the years 2016 – 2018. Computer generated randomization and production of corresponding sealed randomization envelopes were performed by AKF, Norwegian University of Science and Technology – NTNU, who also held the randomization code. Preparation of study solutions was performed by a nurse not otherwise connected to the operation. Patients and all personnel involved in surgery and postoperative follow-up, data collection and statistical analysis were blinded to the randomization. Primary outcome in both studies was drain production at 24 h after surgery. Possible adverse events such as hemorrhage warranting re-operation, wound infection

or wound rupture were defined as secondary endpoints in both studies. All patients were contacted approximately three months postoperatively to ensure correct registration of data and possible adverse events.

Study I

Bilateral reduction mammoplasty was chosen for this study as it is one of the few surgical procedures where the patient has two fairly identical wounds, allowing for a paired sample randomized controlled study using a within-patient design. A total of 28 patients were included, 56 breasts in total. In every patient, one breast was moistened with 20 ml of TXA 25 mg/ml and the other breast with 20 ml of NaCl 0.9%. Breast specimen weight was recorded. As the patient served as her own control, patient characteristics were not registered.

To ensure as homogenous wounds as possible when two surgeons operate one side each, the surgeons switched sides and controlled the hemostasis of the opposite side prior to the application of study fluid. Care was taken to ensure that no study fluid from one side came into contact with the opposite side. The surgeon who applied the fluid onto one breast also performed the surgical closure of that breast. Finally, based on the assumption that bleeding is correlated to the size of a wound and using breast specimen weight as a surrogate measure of wound size, we could calculate both crude and adjusted differences in drain production between breasts. The adjustment was done by dividing right breast weight with left breast weight, and multiplying left breast drain production with this factor.

To identify possible irritative properties from TXA in this pilot study, postoperative pain was registered for each breast both 3 and 24 h after surgery, using a 10 cm visual analogue scale (VAS) from 0 (no pain) to 10 (unbearable).

Statistical analyses in Study I

Making a "best guess" estimate of the difference in drain production from two paired breasts in bilateral reduction mammoplasties based on our in-house experience, we set the standard deviation to 0.4 when calculating power. We also determined that for an effect to be clinically relevant, there should be at least a 25% difference between treatment and placebo groups. Using a paired-samples t-test to detect a difference of 0.25, and a standard deviation of 0.4, α of 0.05 and power 0.80, a sample size of 23 was needed[228]. As we expected non-normally distributed data in this small pilot

study, we included 30 patients as it is recommended to add approximately 15% to the study population when using non-parametric tests[229]. Two patients were excluded and 28 remained for final analyses. We analyzed postoperative drain production and postoperative pain VAS scores using a paired samples Wilcoxon signed rank test. For categorical variables such as presence or absence of defined possible adverse events, a Fischer's exact test was used.

Study II

Study II was designed to further explore the suggested positive effect in Study I on surgical bleeding by moistening the wound surface prior to closure with 25 mg/ml TXA. Mastectomy was chosen as a suitable study model for bleeding as it is a common and standardized surgical procedure with homogenous wounds and little occult blood loss, but with higher postoperative drain production than reduction mammoplasties. Postoperative seroma is a major adverse event after mastectomy, and particularly after lymph node clearance in connection with mastectomy. Our choice of study model therefore led us to additionally investigate whether topical TXA might also influence seroma formation.

The two largest breast cancer centers in Mid Norway, St Olav's University Hospital and Ålesund Hospital, participated in the study. Patients who were to undergo simple mastectomy (SM), mastectomy with sentinel node biopsy (SNB) or axillary lymph node clearance (AC) were consecutively identified from the operation planning registries. Of 239 patients assessed for eligibility at St Olav's, 154 (67%) were included, versus 48 out of 81 (60%) in Ålesund. A total of 202 patients were thus included. Randomization was stratified according to study center. It was discussed whether to sub-stratify the randomization according to type of surgical procedure, as the presence or absence of lymph node clearance is known to have a significant impact on drain output. As randomization for practical purposes was done preoperatively and a planned procedure might be converted perioperatively, we chose to rather adjust for type of surgery in the statistical analyses. As patients were no longer their own control, we wanted to be able to correct for individual effect modifying variables. A range of potentially relevant patient characteristics were obtained from the medical journal, and both breast specimen weight and wound surface area were registered (Table 1).

Of the 202 included patients, 101 had their wound moistened with 20 ml of TXA 25 mg/ml and 101 with 20 ml of NaCl 0.9%. Stratifying for type of surgery, 137 patients underwent SM/SNB (TXA = 67, Placebo = 70), and 65 patients underwent AC (TXA = 34, placebo = 31).

Operation technique is similar at the two centers. It was however noted, well into the study, that drain production at 24 h seemed to be systematically lower in Ålesund. Re-evaluation of routines revealed that Ålesund routinely applied a circular compression bandage for the first postoperative 24 h. Postoperative compression is an active intervention to reduce bleeding but was not accurately registered in the medical records nor accurately remembered by patients in retrospect. It could therefore not be added as a post-hoc variable at the individual patient level but had to be regarded an inherent possible mediator when adjusting data for study center.

Table 1. Patient characteristics at baseline

Characteristic	TXA (n=101)	Placebo (n=101)
Female sex	98 (97.0)	100 (99.0)
Age*† (yr)	66.2 (13.3)	62.3 (12.8)
Body mass index (kg/m^2)*	26.9 (4.9)	27.1 (4.7)
Active smoker	19 (18.8)	13 (12.9)
Recruited at Center A	77 (76.2)	77 (76.2)
Operated by senior surgeon	50 (49.5)	50 (49.5)
Neoadjuvant treatment	32 (31.7)	41 (40.6)
Irradiated tissue	14 (13.9)	11 (10.9)
Perioperative anticoagulation	32 (31.7)	37 (36.6)
Axillary clearance	34 (33.7)	31 (30.7)
Weight of breast specimen*‡ (g)	780 (450)	746 (359)
Wound surface*‡ (cm^2)	292 (117)	279 (84)

TXA = tranexamic acid

Data are number (%) unless stated otherwise

*Data are mean (standard deviation)

†Significant difference

‡Axillary component included

Primary end point was drain production at 24 h. In this study, further drain production and late seroma needing aspiration were secondary endpoints. Drain production was registered daily until drain removal, and all later aspiration of seromas were also registered. Ongoing seroma aspirations beyond 3 months was registered as chronic seroma. Postoperative pain was not registered as Study I had not suggested any effect on this parameter. Postoperative hemorrhage warranting re-operation

before drain removal was registered, and potential adverse effects were otherwise registered as for Study I.

Statistical analyses in Study II

A between-group difference in mean drain production of 25% was considered clinically significant. We proposed a standard deviation of 0.6 based on published drain volumes[228] and an in-house clinical estimate. Using an unpaired samples t-test analysis to detect a difference of 0.25, and a standard deviation of 0.6, alpha of 0.05 and power 0.80, a sample size of 92 patients in each group was needed. We originally planned to include a total of 210 patients to ensure additional power; 202 patients were included for final analyses.

Continuous patient characteristics data were calculated as mean ± standard deviation (SD) and compared using independent samples t-tests. Categorical patient characteristics data were calculated as frequency counts and percentages and compared using chi-square tests, but variables with ≤5 cases were analysed using Fisher exact tests.

Continuous outcome data were presented as mean (95% Confidence Interval (CI)). Categorical outcome data were presented as frequency counts and percentages. For presentation of unadjusted effect size of continuous data, mean difference (95% CI) between groups was used. Effect size of categorical data was presented as odds ratio (OR) (95% CI). For statistical analyses of unadjusted effect size, continuous outcome data were analysed using independent samples t-tests while categorical outcome data were compared using chi-square tests or Fisher exact tests where appropriate.

For adjusted statistical analyses, non-normally distributed continuous data underwent logarithmic (ln) transformation. Data were then analysed using a general linear model . We included all variables registered in Table 1 and performed a stepwise removal of non-significant variables ($P > 0.05$) to obtain a final model that included only individually significant variables. Results were calculated as differences between means as percentage (95% CI). Non-normally distributed continuous data that could not undergo logarithmic transformation due to the appearance of zero values were dichotomized and analysed using a logistic regression model adjusting for significant variables. Results were given as odds ratio (OR) with 95% CI. Analyses were performed in accordance to the "intention to treat" principle.

Study III

Patients undergoing hip replacement surgery at the Department of Orthopedic Surgery at St Olav's University hospital routinely receive TXA 1 g iv as bleeding prophylaxis (iv group). They therefore represent a population receiving iv TXA in accordance with established international recommendations. Patients undergoing skin reduction abdominoplasty after massive weight loss have very large wound surface areas and thus represent a study model with a possibility for significant absorption of topically applied drug. The Section for Plastic and Reconstructive Surgery at St Olav's University Hospital moistens the wound surface prior to closure with 20 ml of TXA 25 mg/ml (moistening group), while Aleris Medical Center instill a 200 ml bolus of 5 mg/ml TXA retrograde through drains after wound closure and keep drains clamped for 1 h postoperatively (bolus group). We wanted to investigate systemic pharmacokinetics after both moistening and bolus mode of topical administration and compare this to iv administration. Patients with preoperative renal insufficiency defined as glomerular filtration rate less than 60 ml/min were excluded from the study as elimination of TXA is almost purely renal. Twelve patients were included from each patient group during the years 2017 – 2018.

Age, sex, height, body weight, body mass index, serum creatinine concentration, and estimated glomerular filtration rate were registered for all participants. The weight of the resected tissue was registered for the abdominoplasty groups and the maximum width and length of the wound were measured to allow calculation of an elliptical wound surface area (in square centimeters) as $\pi \times (\text{length}/2) \times (\text{width}/2)$. A total of eight blood samples for the analysis of TXA were obtained: Before drug administration, and at regular intervals thereafter for up to 6 hours for the iv group and until the next morning for the two topical groups. Samples were centrifuged within 30 minutes and serum secured for storage at -80°C until analysis. Assistance with blood sampling was provided by the Clinical Research Facility at St Olav's University Hospital.

TXA concentrations in serum were determined by an ultra-high performance liquid chromatography tandem mass spectrometry method specifically designed for precise analysis of low TXA concentrations. The limit of quantification was 0.10 μg/ml. This analysis was developed by the Department of Clinical Pharmacology at St Olav's University Hospital. Details on the analysis is presented in the Appendix linked to Paper III.

Pharmacokinetic analysis in Study III

Maximum measured peak serum concentration and the times to achieve these concentrations were obtained directly from the measured values. Other pharmacokinetic variables were calculated by using the pharmacokinetic program package Kinetica, version 5.0 (ThermoFisher Scientific, Waltham, MA, USA). Area under the time–serum concentration curve was calculated using a mixed log-linear model with extrapolation to infinity. Clearance (Cl) was calculated as dose per area under the time–serum concentration curve. By applying a non-compartment model, the parameter estimate describing the decrease of the log-concentration (λz) was calculated using the best-fit log-linear regression line of the samples representing the elimination phase. The elimination half-life was calculated as ln2/λz. Volume of distribution was calculated as Cl/λz. Mean residence time was calculated as area under the serum concentration-time product versus time curve from zero to infinity/ area under the time–serum concentration curve.

Statistical analyses in Study III

Descriptive data are presented as mean ± SD or median (interquartile range) as appropriate. Categorical variables were compared using Fisher's exact test, and continuous variables were compared using an independent samples t test. Associations between continuous variables were analyzed using the Pearson correlation coefficient.

SUMMARY OF RESULTS

Study I

Postoperative drain production was not normally distributed and we therefore chose to present median values for effect size. Median drain production in breasts treated with TXA was 12.5 ml (range 0-44 ml) while median drain production in breasts treated with placebo was 20.5 ml (range 0-100 ml) (p=0.038). When adjusting drain production for breast specimen weight, median drain production for TXA versus placebo was 13 vs. 22 ml (p=0.017). The practiced cutoff-value for drain removal in reduction mammoplasties at the time was ≤40 ml/24 h. Only one of the TXA breasts produced ≥40 ml of drain fluid at 24 h (44 ml), while 9 of the placebo breasts did (range 40 – 100 ml) (p=0.016).

There were no significant differences in postoperative pain scores or possible adverse effects between groups. There were two re-bleedings needing re-operation in the placebo breasts. Three patients had signs of infection or suture reactions on the TXA side only, while two patients had such reactions on the placebo side only. Another four patients had signs of infection or suture reactions which were bilateral. All these complications were conservatively managed.

Study II

Crude unadjusted mean drain production at 24 h was 110 ± 67 ml in the TXA group versus 144 ± 113 ml in the placebo group, yielding a difference between means of 34 ± 13 ml. As data were non-normally distributed, ln transformation was performed, yielding an unadjusted difference between means of 27 ± 10 ml (P = 0.011). Hematomas warranting re-operation within the postoperative drain period occurred in seven placebo patients and one TXA patient (P= 0.057). There were no differences between groups regarding other complications. No thromboembolic events were registered.

Of the patient variables listed in Table 1, the following independently and significantly affected outcome: 1) Administration of TXA, 2) type of surgery, 3) study center, 4) wound surface area and 5) surgeon seniority. Perioperative anticoagulation did not affect the outcome variables. Outcome adjusted for the significant variables showed that moistening the wound surface with TXA significantly reduced 24 h drain production with 32.4% (95% CI -51.4 to -15.8, P < 0.001). Total drain volume was reduced with 33.0% (95% CI -60.0 to -10.4, P = 0.003). Patients in the TXA group were significantly more likely to have drains removed on the first day (OR 3.00, 95% CI 1.44 to 6.22, P = 0.003) and had a significantly shorter drain period (-15.6%, 95% CI -30.2 to -2.6, P = 0.017).

Stratified outcomes

In patients who had undergone AC, TXA had significant effect on drain production at 24 h compared to placebo (-26.7%, 95% CI -55.9 to -2.9, P = 0.026), but total drain production was not significantly reduced (-27.3%, 95% CI -91.9 to 18.4, P = 0.244). They later had significantly *increased* odds compared to placebo of both needing seroma aspiration (OR 5.71, 95% CI 1.16 to 28.2, P = 0.032) and of a cumulative aspirated seroma volume ≥ 500 ml (OR 5.72, 95% CI 1.50 to 21.9, P = 0.011), but no increased risk of chronic seroma (OR 1.50, 95% CI 0.23 to 9.73, P = 0.671). In SM/SNB patients, there was a greater effect of TXA compared to placebo on both drain production at 24 h (-33.4%, 95% CI -58.1 to -12.6, P = 0.001) and on total drain production (--32.3%, 95% CI -62.4 to -9.4, P = 0.005). There was no difference regarding neither need for seroma aspirations nor number of aspirations or cumulative aspirated seroma volume, but there was a significantly reduced OR of chronic seromas among SM/SNB patients who had received TXA (OR 0.18, 95% CI 0.04 to 0.89, P = 0.035).

Stratifying for study center, patients operated at St Olav's Hospital had 60.3% higher drain production at 24 h than patients from Ålesund Hospital (95% CI 36.1 to 88.9, P < 0.001). St Olav's Hospital accepts larger ongoing drain production at removal than Ålesund Hospital yet despite earlier drain removal at St Olav's Hospital, they had a 33.5% larger total cumulative drain production than Ålesund Hospital (95% CI 6.5 to 67.4, P = 0.013).

As AC and study center had such major influence on drain production at 24 h, a subgroup analysis was done in the population who neither was operated at Ålesund Hospital nor underwent AC. These were the SM/SNB patients at St Olav's Hospital (N=108, TXA=52, Placebo=56). Adjusted for wound surface area, those receiving TXA in this subpopulation had a 39% reduction in 24-hour drain production (95% CI -69.2 to -14.3, P = 0.001), a 46% reduction in total drain output (95% CI -80.9 to -17.9, P = 0.001), no significant difference on late seroma aspirations, but a suggested lower risk of chronic seroma (OR 0.18, 95% CI 0.04-0.89, P = 0.035).

Study III

Patient characteristics were similar in the abdominoplasty populations. Due to the nature of the procedure, patients undergoing hip replacement surgery were on average 20 years older (P < 0.001) and had a significantly lower estimated glomerular filtration rate (P = 0.002) than the abdominoplasty patients.

After iv administration of 1 g, TXA peak serum concentration (mean 66 ± 13 µg/ml) occurred within the first few minutes and stayed above the assumed threshold concentration of 10 µg/ml for at least 2.5 hours. One patient in the moistening group had a peak serum concentration above the threshold value (10.9 µg/ml); otherwise the patients in both abdominoplasty groups had sub-threshold peak values. In the moistening group, the time-concentration curves were homogenous with peak concentrations occurring within 1-2 hours and with all patients having serum TXA concentrations of 2 µg/ml or below at 6 hours. In the bolus group, the time-concentration curves were more heterogeneous with peak concentrations occurring 4 to 8 hours after administration. In the topical groups, peak serum concentration was not significantly correlated to body weight or wound surface area, while there was a significant inverse correlation between serum concentration after iv administration and body weight.

DISCUSSION

Clinical implications of the findings

Studies I-II suggest that moistening a surgical wound surface with TXA 25 mg/ml significantly reduces surgical bleeding as defined by drain production at 24 h. Effect measured as percentage reduction of blood loss seems comparable to the effect of iv prophylactic use, as iv administration in various types of surgery seems to yield a reduction of both measured bleeding and transfusion needs with approximately one third[10]. We found a 39% difference between medians in Study I and a 32% difference between adjusted means in Study II. In Study I, the fact that only one TXA breast had a 24 h drain production above the threshold value for drain removal of 40 ml – and then only barely above at 44 ml – led to the discontinuation of routine use of drains in reduction mammoplasties receiving topical TXA at St Olav's University Hospital.

In Study II, TXA significantly reduced the cumulative drain production until drain removal by 33%. Subtracting the drain production for the first 24 h, there was no longer a significant difference between TXA and placebo regarding further drain production until drain removal. This may suggest that the single moistening does not induce an ongoing effect beyond 24 h, but at the same time there does not seem to be any rebound effect when the TXA effect subsides.

Moistening the wound with 25 mg/ml TXA may reduce the risk of postoperative hematoma warranting re-operation. Although neither study was powered to investigate this variable, a possible effect is strongly suggested as the two studies combined had ten re-bleedings, of which nine occurred in placebo breasts (Fisher's exact test of all breasts in Studies I and II: P=0.010).

No difference in late hematomas, wound ruptures or wound infections were seen in Studies I-II. No patients had thromboembolic events or seizures. However, patients in Study II who had undergone AC and had received TXA had significantly increased odds compared to the placebo group of both needing seroma aspiration (OR 5.7, P = 0.032) and of a cumulative aspirated seroma volume ≥ 500 ml (OR 5.7, P = 0.011), but no increased risk of chronic seroma (OR 1.5, P = 0.671). This is to our knowledge the first publication of a possible adverse effect of topical TXA.

Study III renders the risk of systemic adverse events, such as thromboembolism or seizures, highly unlikely after topical moistening of a large wound surface with TXA 25 mg/ml. We therefore postulate that in patients with an increased risk of such adverse events, topical administration should constitute a safe alternative to iv administration.

Methodological issues

Drain production as a surrogate measure for bleeding

The primary endpoint in both our intervention studies was postoperative bleeding at 24 h as measured by drain production. There is no gold standard for the estimation of blood loss[230]. Methods applied include comparing pre- versus postoperative hemoglobin (Hb), transfusion needs, and visual and physical calculations from suctioned fluids and surgical sponges. Several formulae have been proposed for the calculation of occult blood loss based on clinical parameters, but their accuracies are debatable[230].

As our prophylactic intervention to reduce bleeding is performed at the end of surgery and after the completion of hemostasis, any perioperative bleeding will naturally not be affected by the intervention. Hence any comparison of pre- and postoperative Hb or registration of transfusion needs would falsely take into account the perioperative bleeding. To be able to assess the effect of our intervention as accurately as possible, we wanted study models with little possibility for occult bleeding and where measured bleeding must have occurred after the intervention.

Breast surgery is performed in tissue adherent to the thoracic wall. There is little deep space to accommodate occult bleeding. Visual assurance of a dry surgical field prior to wound closure can be obtained, and a drain activated after closure would deliver postoperative fluids only. We therefore chose drain production in breast surgery as the most suitable primary endpoint to evaluate the effect of our intervention on postoperative bleeding.

Drain fluids generally become more serous over time; we therefore deemed drain production at 24 h as the best measurement of blood loss in our study settings, although this is a surrogate measure. To accurately analyze the contents of collected drain fluids is a major technical challenge which we did not undertake. However, the composition of drain fluids may have varied significantly in our study populations, and in an ideal situation, the composition of the drain fluids should be analyzed to differ between blood and serous fluids.

In Study I, 37 of the 56 breasts had drain production ≤20 ml. This is such a low production that leftover infiltrated local anesthetics and general wound exudate may constitute a major part of the volume at 24 h. Hence, the true effect of TXA on bleeding may have been more correctly observed in the patients with larger volume bleeding in Study I (Fig. 4). This also demonstrated that if we were to use drain production as primary endpoint, we should use a study population with higher volume bleeding in a confirmatory study. We therefore performed Study II on mastectomy patients, who

generally bleed more. However, in Study II, patients undergoing AC were likely to have lymph leakage which could constitute a significant proportion of the measured drain volume. Hence the most accurate effect of TXA on pure surgical bleeding would probably be observed in patients who did not undergo lymph node clearance. We therefore performed stratified analyses for this subgroup.

Non-normally distributed data in smaller versus larger studies

In both our studies, drain production at 24 h was not normally distributed. Non-normally distributed data can either be transformed to a more normal distribution by means of e.g. logarithmic transformation and then analyzed using tests for normally distributed data, or data can be analyzed with a non-parametric test.

In Study I, two re-bleedings constituted outlier values with significantly higher drain volumes than the rest of the breasts. On the illustration figure in the article (Fig. 4), we chose to define the two re-bleedings as "100 ml", to allow for a reasonable Y-axis although the real volume was much larger. A non-parametric Wilcoxon Signed Ranks test allows for an estimation of the significance of the trend in the treatment effect, but does not take into consideration the absolute volumes of outlier data.

In Study II, the larger data set from 202 patients allowed for a logarithmic transformation of non-normally distributed data and we used the transformed values for further statistical analyses. However, when registering aspirations of late seroma, patients not in need of seroma aspirations would present with the value "0". As non-normally distributed continuous data containing zero values cannot undergo logarithmic transformation, these data had to be dichotomized and analysed using a logistic regression model. The need for such dichotomization had not been foreseen and which cut-off values to use had not been discussed in the protocol phase; these were therefore established in the aftermath based on clinical reasoning and the general distribution observed in the study. Different cut-offs for the dichotomization could have yielded different results. The significantly increased risk of needing at least one seroma aspiration after TXA administration does not depend on any cut-off value. Had we however set the cut-off for "large cumulative seroma volume" at 1000 ml instead of 500 ml, the OR would still have been higher after TXA administration (OR 3.28 at 1000 ml cut-off versus 5.72 at 500 ml cut-off) but the increased OR would no longer be statistically significant (p=0.076).

Presenting effect sizes

In Study I, we debated how to present the effect size. The calculation of mean paired difference in drain volumes (13 ± 27 ml) was not normally distributed. As 13 of the participating women had drain volumes below 20 ml in both breasts, the difference was skewed towards lower values, while the two

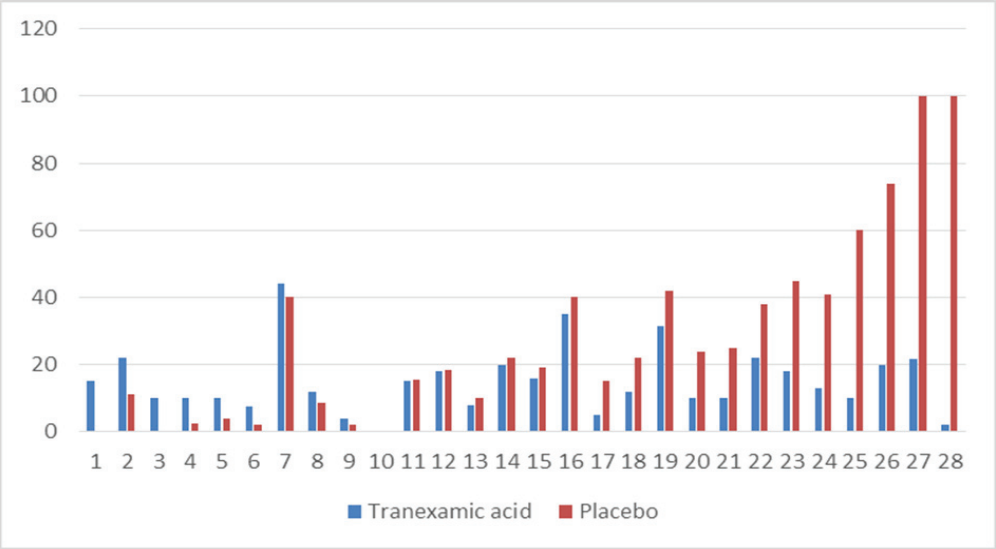

Fig. 4 Study I. Revised figure. Drain production in TXA breast and placebo breast for each patient; patients sorted in ascending order according to calculated difference in volume (placebo breast minus TXA breast).

re-bleedings also represented significant outliers even when substituting the actual high drain volume in re-bleedings with a volume of 100 ml. Four breasts had zero drain production and would be excluded if we attempted to logarithmically transform the data. Excluding the two re-bleedings would not have been in accordance with protocol. We therefore chose to present the difference in drain production as the unpaired median drain production in TXA breasts (12.5 ml) versus the median of placebo breasts (20.5 ml), which would convey a conservative estimate of effect size. A published comment to Study I (Appendix, Paper I) correctly pointed out that in a paired study, we should present paired values, and that the median paired difference was only 2.5 ml. This comment led us to revise the figure originally published in the paper – the original figure had an unfortunate chronological sorting of included patients (Fig. 1, Paper I), while the revised figure (Fig.4) sorted patients according to the difference in drain volume between TXA and placebo breast.

To report the paired median difference in this material (the average between patients 14 and 15) as suggested in the comment would clearly underreport the fact that differences in drain volume to the right of the median far exceeds the differences to the left of the median. Figure 4 illustrates an

increased paired difference in breasts with larger bleeding volumes. In 13 of the 15 patients in whom one or both breasts had more than 20 ml drain production, the difference was in favor of TXA. We felt the revised figure was the best way to convey the results of the study.

Small volumes and large variances

The published comment to Study I (Appendix Paper I) also mentioned that the actual variance in Study I was much higher than the SD estimated in our power analysis, and that the study therefore was significantly underpowered. This comment illustrates methodological challenges in Study I when using a study model with little postoperative bleeding, and simultaneously using a simplified surrogate measurement of that bleeding which may be increasingly inaccurate with lower values. This is a situation where reliable SD values for the specific situation to be studied do not exist and thus represents a challenge in the power analyses.

Reduction mammoplasties have large wound surfaces yet often bleed little, as fatty mammary tissue is less vascularized than e.g. muscle, skin and fascia. Although reduction mammoplasty offers a study model with an alluring possibility of within-patient randomization, low drain volumes allow for large variances and misleading proportions between treated and untreated breasts, as mentioned previously. Cases with a 3 ml production on one side versus 18 ml on the other contribute to large variances but have little clinical significance. We reached non-parametric significance at the $p<0.05$ level but not at the $p<0.01$ level in our pilot study. Had more patients had bilateral low volume bleeding, we might not at all have reached statistical significance.

Effect modifying variables and mediators

In Study I, we did statistical analyses both on crude drain volumes and on drain volumes adjusted for breast specimen size. The rationale behind this simple adjustment was the assumption that larger wounds will bleed more. The wound surface area of reduction mammoplasties is much more complex than the oval wound bed of a standardized mastectomy; hence actual wound surface area could not be calculated with sufficient precision in Study I. The weight of the resected tissue was therefore registered as a surrogate measure of wound area. As mentioned in the Methods chapter, adjustment was done by multiplying left breast drain volume with the factor obtained from dividing right breast weight with left breast weight. P values of the difference in drain production using the Wilcoxon signed rank test then decreased from 0.038 to 0.017.

In Study II, case and control wounds were no longer in the same patient. In an optimal study setting, randomization should sufficiently distribute patient characteristics. We knew in advance that variables such as presence or absence of axillary lymph node dissection were definite effect modifying variables which would significantly affect primary outcome. We also suspected that several other factors such as wound surface area, use of anticoagulants and surgeon seniority might be effect modifying variables. As mentioned in the Methods chapter, we discovered well into the study that being operated at Ålesund Hospital seemed to obviously reduce drain production at 24 h. Although there may be many small unrecognized differences between hospitals that will justify a separate stratified randomization for each study center when conducting a randomized clinical trial, there was at study initiation few well-founded suspicions that differences between study centers should be significantly effect modifying. We ultimately found an adjusted 60% difference between drain production at 24 h between study centers, which suggested a significant "study center" effect.

Compression reduces bleeding. Park et al. have demonstrated in knee arthroplasties that the intraarticular compression induced by clamping the drain for three hours reduces bleeding equal to low-dose intraarticular TXA in combination with drain clamping for 30 minutes[231]. We had not noted during study planning that Ålesund Hospital practiced a 24 h circular compression bandaging. This clinical intervention to reduce bleeding was very likely to compete with the effect of the application of TXA. It may also explain the relative lack of effect of TXA on 24 h bleeding at Ålesund hospital, as mean bleeding at 24 h in placebo patients in Ålesund was lower than mean bleeding among TXA intervention patients in Trondheim (Study II, supplementary tables 4 and 5). Yet we could not in retrospect register "postoperative compression" as a separate effect modifying variable at the individual level but had to include this as an assumed mediator in the broader "study center" variable. However, a study designed to evaluate the isolated effect of 24 h postoperative compression as practiced by Ålesund Hospital is needed to confirm this assumption.

There was no significant difference between the proportion of patients who at some point needed seroma aspiration at St Olav's Hospital (76%) versus Ålesund Hospital (66%) (P = 0.212), but St Olav's Hospital had a significantly increased total number of aspirations (OR = 9 for ≥ 5 aspirations, 95% CI 2 to 33, P < 0.001) and cumulative aspirated volume (OR = 4 for ≥ 500 ml cumulative seroma, 95% CI 2 – 11, P= 0.004). As published studies[232,233] have not found effect of prolonged postoperative compression on seroma formation, it would seem strange if a 24 h compression regime should have a major impact. An alternative explanation lies in the fact that Ålesund Hospital serves a population with long travel distances while St Olav's Hospital has a much larger proportion of its patient population in close proximity. Seroma aspiration is very accessible at St Olav's Hospital, it is performed by the nurses at the outpatient clinic and patients can merely call or drop by. At Ålesund

Hospital, seroma aspirations are mainly performed by the surgeons and requires an outpatient appointment.

Stratified randomization or regression analyses?

When planning Study II, we were well aware that there are significant differences between mastectomy surgeries. A mastectomy with an associated AC will both have a larger and more complex wound surface and added damage to large lymph vessels. All surgeons are aware that these extended mastectomies have much larger drain volumes and increased risk of need for late seroma aspirations. We could have designed Study II with two arms and stratified the randomization not only for study center, but also for type of surgery. A two-armed study would have needed separate power calculations for each arm. Variance in drain production is much larger after AC than after mastectomy only, and there is approximately one mastectomy with AC to every two SM/SNB mastectomies. A two-armed study would require a significantly larger study population of AC patients, and the study would have required a significantly longer inclusion period. As the decision to perform AC is sometimes not made until well into the operation, such a stratification would not have been easily compatible with a pre-surgery randomization.

Regression analyses take into account the effects of possible effect modifying variables on a defined outcome. Stratifying for each of these variables would have produced small study populations possibly without the individual power to significantly demonstrate an effect.

We extensively used regression analyses in Study II. For the primary outcome, drain production at 24 h, we used a General Linear Model (GLM). This model allows for a continuous dependent variable to be adjusted for both continuous (e.g. wound surface area) and categorical (e.g. type of surgery) variables. For dichotomized dependent variables, such as need for late seroma aspiration (Y/N), we used binary logistic regression which also allows for both continuous and categorical adjusting variables.

Our regression analyses adjusted for those variables in Table 1 which independently significantly affected outcome. Our final model explained 50% of the observed variation in the drain production at 24 h (R^2 = 0.496), and the order of importance according to partial Eta squared values were AC (0.233), study center (0.141), wound surface area (0.117), administration of TXA (0.080) and surgeon seniority (0.027). Clinical studies on bleeding in surgery most often present absolute and unadjusted volumes, but surgery is a field with extensive possibilities for effect modifying factors and surgical study populations are often relatively small. Study II illustrates the importance of adjusting for

possible significant variables in surgical research as many variables had greater influence on the variance in postoperative bleeding than the application of TXA – yet the adjusted data confirms the effect of TXA, as the significance of the effect increases from P = 0.011 in an unadjusted Student's t-test to P < 0.001 for adjusted data.

Regression analyses can identify potential variables which may justify stratified analyses or inspire tailored investigation in future studies, particularly in pilot studies such as ours. Two major findings from Study II thus need further exploration: The aforementioned influence of study center (with the postulated mediator "circular compression"), and the effect of TXA on seroma in the presence/absence of lymph node clearance.

New questions arising from the studies

Can topical TXA increase lymphatic seroma formation?

Seroma can be defined as the collection of fluid within the dead space created by extensive dissection. Seroma as a postoperative complication is mainly addressed in plastic and breast surgery, and reported incidence in breast cancer surgery varies greatly from 5 – 80%[234]. The exact composition of post-mastectomy seroma remains somewhat unclear. Analyses by Walt-Boolsen et al. (1989)[235] suggested that post-mastectomy seroma is mainly an exudate secondary to the inflammatory reaction that constitutes the first phase of surgical wound repair, and analyses of seroma fluids in abdominoplasty supports an exudate composition[236]. However, Bonnema et al. (1999)[237] demonstrated that seroma after axillary dissection contains virtually no fibrinogen and that its composition must therefore be mainly lymph-derived, as lymph– but not exudate– is void of fibrinogen. Bonnema therefore questions the proposed possible reduction in seroma formation in a published study on the effect of intravenous TXA in breast cancer patients by Oertli et al.[238], as a mechanism to explain such an effect "would remain obscure given the absence of fibrinogen in seroma". These studies also demonstrate that "seroma" may be of different pathophysiological origin and that "lymphatic seroma" should perhaps be defined as a separate condition. We have not been able to identify studies comparing the constituents of seromas after SM versus AC.

The suggested *increase* in postoperative seroma after lymph node clearance in Study II may represent a not previously described adverse effect of topical TXA, and may be applicable only to lymph-derived seroma, as no such trend was seen in the mastectomies not undergoing AC. On the contrary, SM/SNB patients actually had a significantly decreased OR of developing chronic seroma after TXA administration (OR = 0.18, P = 0.035). As patients with postoperative complications were

excluded from seroma analyses as the complications themselves may cause seroma, only 52 of 65 patients undergoing AC and 128 of 137 patients undergoing SM/SNB remained for seroma analyses. Our study centers remove drains after axillary clearance after a mean 2.7 days, which is early by international standards. Our centers also have a very high incidence of seroma aspirations (77% at St Olav's Hospital and 67% at Ålesund Hospital): As stated by one reviewer in Study II, the participating study centers "seemed to trade drain time for seroma".

Whether moistening the wound surface with TXA 25 mg/ml in patients undergoing lymph node clearance increases the need for late seroma aspirations needs confirmation. Our material does suggests a different effect of TXA on what we assume is exudate-dominated seroma (SM/SNB-patients) versus lymph-dominated seroma (AC-patients). Our findings need confirmation as significance is at the $P<0.05$ level but not at the $P<0.01$ level. We propose a randomized controlled study in patients undergoing lymph node clearance, regardless of location, but with a standardized regime for drain removal. End points could be number of and cumulative volume of late seroma aspirations. Should an increase in seroma be confirmed, explanatory mechanisms must be investigated, and a repeat study comparing lower doses, e.g. 5 mg/ml TXA versus 25 mg/ml TXA versus placebo should be performed to investigate whether the phenomenon is dose-dependent within the concentration range used clinically. It would also be interesting in a later study to evaluate early blood/lymph ratio in the drain fluids after exposure to TXA versus placebo in lymph node clearance patients.

Possibility of systemic effect after topical TXA application?

Study III demonstrated that in large skin reduction abdominoplasties, moistening the wound surfaces with 20 ml of 25 mg/ml TXA or instilling a 200 ml bolus of 5 mg/ml TXA into the wound cavity after wound closure resulted in very low serum levels of TXA compared to iv administration.

To our knowledge, Study III is the first extensive pharmacokinetic study on TXA serum concentrations after topical administration. A sensitive analytical method specifically designed to detect low serum levels of TXA was developed and is a major strength of the study (Appendix 1, Paper III).

Using the specific moistening technique, 20 ml was sufficient to moisten even the largest participating wounds. Depending on wound surface area, varying fractions of the administered 20 ml would spill and the total amount of drug which remained adherent to the wound was uncertain. We did not collect or analyze drug from absorbent materials or drains. In the study setting, we estimated actual absorbed drug by comparing the area under the time–serum concentration curve values in the

moistening group with the area under the time–serum concentration curve values in the intravenous group, assuming that the true clearance in the two groups was the same. Mean net absorbed dose in the moistening group could then be estimated to be 316 ± 98 mg of the 500 mg administered, but the dose was somewhat surprisingly not correlated to wound surface area. This led us to speculate whether tissue micro-topography or individual tissue vascularization may affect absorption.

When administering TXA via the instillation of a voluminous bolus of diluted drug, Study III demonstrated that this administration might give an unpredictable systemic absorption from large wounds such as abdominoplasties. Such large wounds have nooks and crevices where study fluid may reside, and patient mobilization may influence whether study fluid actually reaches all areas of the cavity. Smaller cavities, such as knee and hip joints, may however be good candidates for bolus administrations. Although serum concentrations remained below threshold values when using a 200 ml bolus of a low concentration (5 mg/ml) of TXA, elimination was considerably slower and drug can be expected to linger much longer in the tissues, increasing exposure time compared to the moistening method.

For both topical modes of administration, the TXA serum concentrations can be expected to correlate to the TXA concentration in the solution administered. Wong et al.[207], who introduced the intraarticular topical bolus administration of TXA in arthroplasties, demonstrated that the serum concentration after a 100 ml intraarticular bolus of either 15 or 30 mg/ml tranexamic acid in knee arthroplasty resulted in serum concentrations of 4.5 µg/ml versus 8.5 µg/ml at the same single time point postoperatively. Similarly, we assume that a moistening technique with more concentrated solution will give a correspondingly higher serum concentration. Systemic concentrations after topical application may therefore exceed the threshold for therapeutic effect if sufficiently high TXA concentrations are used in the fluid applied.

Local adverse effects after topical application?

When introducing a moistening of the wound surface as a prophylactic measure to reduce bleeding, the concentration of 25 mg/ml was somewhat arbitrarily chosen based on the available literature. Wong et al. had demonstrated similar effect from both 15 and 30 mg/ml solutions[207], and when leaving a thin film instead of a bolus, it seemed reasonable to go for the higher concentration as dilution from tissue fluids would be rapid. Few studies on potential local toxicity were available; chondrocyte studies suggested that 25 mg/ml was safe[222,223]. Potential local adverse effects of topical TXA has however not been sufficiently investigated. In our clinical experience from plastic surgery, we have not seen any obvious increase in wound ruptures, wound infections or seroma aspirations

after the introduction of topical TXA as routine bleeding prophylaxis. In our two RCTs, there has been no difference in wound infections or wound ruptures between the TXA and placebo groups, but our studies were effect studies and not designed or powered to identify potential adverse effects. However, the aforementioned increase in seroma in patients having undergone AC triggered a search for possible explanations.

Our group has recently exposed *in vitro* keratinocytes and fibroblasts to various clinically relevant concentrations of TXA, and we have also investigated the effect of topical TXA on wound re-epithelialization in an *ex vivo* human skin model[239]. While short exposure (10 minutes of exposure to drug-containing medium, pour off without rinsing, replace with drug-free medium) even to high concentrations had little cytotoxic effect and did not significantly affect wound re-epithelialization, prolonged exposure (continuous exposure to drug-containing medium for up to 72 h in cells/8 days in skin wounds) was increasingly cytotoxic with increasing exposure time and drug concentration. Prolonged exposure to 25 mg/ml TXA completely prevented wound re-epithelialization which otherwise would occur within 3-4 days. Prolonged exposure to 50 and 100 mg/ml induced epidermiolysis in the human skin model, but without signs of cell death and thus the mechanism seemed non-toxic. Prolonged exposure to the low concentration of 6.25 mg/ml did not significantly affect wound re-epithelialization.

The absence of re-epithelialization in the study by our group was seen after prolonged exposure, yet the possible increase in seroma formation in mastectomy patients undergoing AC was after an *in vivo* short exposure. Both phenomena may be explained by an antiadhesive/antimigratory effect of TXA, which may have delayed the healing of lymph vessels and prevented the migration of keratinocytes.

Findings from other studies may also suggest a potential effect of TXA on cell migration and cell adhesion. Bergenholtz et al. have shown in an *ex vivo* model on incisional wounds in palatal mucosa from cats that prolonged exposure to anti-plasminogen activators caused proliferating epithelium to change direction in a non-physiological manner. They even observed the proliferation of epithelial cells onto sheets of other epithelial cells, which does not occur during normal wound healing[227]. The authors state that “The cause of the changed direction of epithelial migration is obscure”, but they postulate that the lack of fibrin degradation/remodeling resulting from the presence of antifibrinolytics may leave strands of fibrin guiding keratinocytes in non-physiological directions. The changed pattern of keratinocyte migration was seen after prolonged exposure to TXA 0.12, 1.2 and 12 mg/ml. Epithelial fusion did occur at all concentrations, also after prolonged exposure to TXA 12 mg/ml, which is in accordance with our findings that threshold levels for inhibition of wound re-epithelialization given chronic exposure to TXA must be somewhere in the range of 5-25 mg/ml.

Studies on fibrin sealants may suggest an effect of TXA on cell adhesion. Commercial fibrin sealants supplemented with TXA to prevent the breakdown of fibrin have demonstrated inferior adhesive strength compared with sealants supplemented with aprotinin[226]. Simultaneously, such TXA-supplemented fibrin sealants have caused fewer unwanted intra-abdominal adhesions compared with sealants without TXA in animal studies[240,241], which may be a clinically beneficial effect of the proposed reduced adhesive strength.

If prolonged exposure to above-threshold concentrations of TXA may prevent re-epithelialization, reduce cell adhesions or cause cell detachment, several questions are raised. Can such exposure reduce tensile strength in various healing tissues, delay re-epithelialization in large wound surfaces such as burns and split-thickness skin grafts, or prevent unwanted postsurgical adhesions or capsular contractures? More research is clearly needed to clarify these issues.

Circular compression for 24 h to reduce bleeding in breast surgery?

It is a well-known surgical principle that compression reduces bleeding. In breast surgery, focus has been primarily on postoperative seroma formation and less on the surgical bleeding. Hence, the few published studies on the effect of postoperative application of a compression garment have mainly focused on – and failed to find – an effect on seroma[232,233]. In these studies compression is generally left for several days, which may both induce tissue ischemia and restrict patient respiration. Circular compression has therefore been somewhat forfeited in breast surgery, but proper randomized studies on the effect on postoperative bleeding and re-operation due to hemorrhage are lacking. It is important to distinguish postoperative bleeding and late seromas as two distinctly separate pathophysiological phenomena, and the lack of effect on seroma should not prevent proper intervention to reduce postoperative bleeding. In Study II, being operated at Ålesund Hospital was more effective than TXA in reducing postoperative bleeding at 24 h (60% reduction, 95% CI 36 – 89%), and we postulate that this was due to circular postoperative compression. A separate study randomizing patients to circular compression bandaging as practiced by Ålesund Hospital versus standard bandaging as practiced by St Olav's Hospital is warranted. The patients should not receive TXA.

Future perspectives

Optimal dose and mode of administration of topical TXA

Minimizing perioperative bleeding is fundamental, and topical use of TXA as a prophylactic measure to reduce surgical bleeding has relevance for many surgical specialties and procedures. Topical use is however still off-label, and minimum effective dose, mode of administration and duration of contact still needs clarification. The possibility of local effects of TXA beyond its antifibrinolytic properties is also inadequately explored.

We have shown that moistening a wound surface with 25 mg/ml TXA reduces bleeding. The lowest effective dose in topical administration is undetermined, but TXA concentrations as low as 4 mg/ml have been found effective[201]. The dose-response relationship regarding the effect of topical TXA remains unclear; most studies have not demonstrated significant differences in bleeding when using equal volumes with different drug concentrations[202,207,242]. A recent study from knee arthroplasty suggests that 3 g is more effective than 500 mg when given as an intraarticular injection[243], but this is administered as a concentration of 50 mg/ml as an either 10 ml or 60 ml bolus. Hence the volume injected is six fold higher in the 3 g group and whether the difference in effect is attributable to the higher total TXA dose or the counter pressure of the larger volume in the enclosed space of a knee joint is unknown. However, other studies have suggested increased effect from higher concentrations when using equal volumes, although those differences have not been statistically significant[207,244]. Determining the lowest effective dose in our moistening technique could be explored in patients undergoing mastectomy without lymph node clearance, and with a strict protocol for postoperative bandaging and drain removal. Randomization could then be into three groups: TXA 5 mg/ml, TXA 25 mg/ml and saline. We still favor mastectomies as study model due to the homogeneity of the surgical wounds and the limited possibility of occult bleeding. To restrict inclusion to patients undergoing mastectomy without lymph node clearance, final inclusion and randomization should preferably be determined perioperatively when the choice of surgery is definite. Instant access to pre-made study drug vials would allow for such a last-minute randomization.

Topical TXA to reduce risk of postoperative hemorrhage warranting re-operation

A reviewer of Study II suggested that postoperative hemorrhage warranting re-operation would be the best primary end point to measure the effect of topical TXA as a prophylactic measure to reduce bleeding. To our knowledge, no study on topical TXA has had this variable as primary endpoint but an

adequately powered study or meta-analysis to investigate the prophylactic effect on re-bleeding is needed. As postoperative hemorrhage is relatively rare (4% in our material, which reviewers thought was above average), much larger study populations are needed using to address postoperative re-bleedings.

Synergistic effect of topical tranexamic acid and topical adrenaline

When suggesting a prophylactic moistening of the wound surface with TXA to reduce bleeding, we reasoned that there was in all likelihood a synergistic effect between the vasoconstriction induced by adrenaline to allow for more efficient clotting and preservation of clotting by TXA. To evaluate the isolated effect of topical TXA in our pilot effect studies, we have diluted TXA with saline only. However, we postulate that diluting one vial of 5 ml TXA 100 mg/ml with 15 ml of standard local anesthesia containing adrenaline may give both the added effects of analgesia and increased hemostasis through a synergistic effect between adrenaline and TXA.

Chinese publications have recently found an increased effect of topical TXA in combination with low-dose intravenous adrenaline[245]. To evaluate whether the synergistic effect of topical TXA and topical adrenaline may be even more efficient than topical TXA alone in reducing surgical bleeding, and to evaluate whether added topical local anesthesia may improve analgesia, repeat randomized studies with topical application of TXA with/without adrenaline and with/without local anesthesia are needed.

Use of topical TXA on superficial wounds

Burn patients, trauma patients and patients with necrotizing soft tissue infections all have large wound surface areas in need of ongoing revisions and extensive split skin grafting. Such patients are particularly prone to massive blood loss and wound oozing with associated loss of proteins and electrolytes[246], both from their revised wounds, and from the split skin graft donor sites. These patients are mostly in a hyperfibrinolytic state and the risk of potential systemic adverse effects of TXA in hyperfibrinolysis has not been clarified[247,248]. Topical administration to these patients might be an alternative to avoid systemic effect yet reduce bleeding.

Donor wounds after split skin graft harvesting enter the superficial dermis and leave adnexal structures of the skin intact. Hair follicles and sweat glands contain epithelium which migrate to cover the wound, and most donor wounds have healed by secondary intent within 10-14 days.

Strategies to reduce bleeding must not delay healing, as only wound healing will permanently terminate ongoing oozing.

We had prepared a protocol for a study on topical TXA on donor site wounds in burn patients (ClinicalTrials.gov NCT02918201). The proposed study awaited the results from our effect studies and also the results from our cytotoxicity studies. We do believe that a reduction in bleeding from topical application will be of great value in this study population, but as our studies on wound re-epithelialization have postulated a potential negative effect of TXA, we must revise the protocol and we have not yet initiated this study. The initial protocol proposed covering the superficial wounds with moistened bandages containing TXA. As the effect of prolonged exposure is undetermined *in vivo*, we will now initially use only a moistening method directly onto the wound as described in Studies I and II, and we will primarily evaluate wound re-epithelialization in smaller donor site wounds. If no delay in wound epithelialization is seen compared to placebo, we can progress to an evaluation of bleeding volume using the moistening method only/alternatively re-epithelialization studies on smaller wounds using moistened bandages.

Mechanisms behind potential adverse effects of topical tranexamic acid

Caution is warranted when promoting off-label use of a registered drug. Whether the effects of topical TXA corresponds to dose or exposure time, or may be dependent of threshold levels, remain undecided. While topical use of TXA seems safe regarding potential systemic adverse events, topical TXA coming into contact with the CNS can induce potentially life-threatening seizures[178]. To our knowledge, clinical adverse effects from prophylactic use of topical TXA to reduce bleeding has until now not been published. However, our *ex vivo* studies in human skin suggest that prolonged exposure of superficial wounds to higher concentrations of topical TXA may inhibit re-epithelialization[239]. Results from Study II raises the questions whether damaged lymph vessels may seal more slowly after exposure to topical TXA. Possible adverse effects of topical TXA on various tissues therefore warrant tissue-specific studies, and potential mechanisms behind a possible antimigratory or antiadhesive effect need to be explored.

Cox and colleagues[249] have demonstrated that the presence of TXA 50 mg/ml both prevents fibroblast adherence and causes fibroblast detachment *in vitro*, proposing a non-cytotoxic mechanism with changes in integrin interaction.

The hemidesmosomes which attaches the keratinocytes and thus the epidermis to the basal membrane utilizes integrins, and integrins are also involved in migration of both keratinocytes and

various other cells[250]. On a molecular level, plasminogen, plasmin and in particularly u-PA serve as ligands and interact with both integrins and other cell surface receptors involved in cellular migration, adhesion and signaling, and these events may be quite separate from the functions related to fibrinolysis [22,30,251-253]. Ploskota et al. have even described a convergence of the adhesive and fibrinolytic systems through a mechanism where a single u-PA ligand crosslinks integrin receptors to form a complex which may simultaneously play an important role in the control of cell migration and vascular homeostasis[253].

Human receptors with affinity for lysine analogues and the consequences of such bindings are inadequately explored. Lysine analogues may cause convulsive seizures through their competitive binding to GABA and glycine receptors in the CNS[189]. Our observations regarding possible effect on cell adhesions and cell migration may be in accordance with described mechanisms at cellular receptor level involving integrins, plasminogen and u-PA. Off-label use of both topical and systemic use of TXA to treat skin hyperpigmentation and melasma has been increasingly published in the last decade[254], and a possible mechanism could be interference with melanosome transfer from melanocyte to keratinocytes. Lysine binding sites may be widespread in the human organism and possible effects of TXA clearly needs further investigation.

CONCLUSIONS

- Topical moistening of a surgical wound surface with TXA 25 mg/ml reduces postoperative bleeding as measured by drain production with approximately one third, which is comparable to the effect of routine intravenous use of TXA.

- Topical moistening of a surgical wound surface with TXA 25 mg/ml may reduce the risk of re-bleeding warranting re-operation.

- Seroma formation after lymph node clearance may be increased after topical moistening of the surgical wound with TXA 25 mg/ml. This phenomenon needs confirmation, and the potential underlying pathophysiological mechanism is unknown.

- We did not observe an increase in thromboembolic events, wound infection, wound rupture, suture reactions or late hematomas in wounds treated with topical TXA 25 mg/ml.

- Systemic concentrations of TXA does not exceed therapeutic threshold value for antifibrinolytic effect in large abdominoplasties after topical moistening of the wound surface with 20 ml of TXA 25 mg/ml or instillation of 200 ml of TXA 5 mg/ml into the wound cavity.

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CORRECTIONS

Paper I, page 1351, right central column:

Corrected version: A mouthwash containing 48 mg/ml tranexamic acid was effective after dental extraction

PAPERS

Paper I including comment and reply to study

Paper II

Paper III including appendix regarding the development of the analysis for tranexamic acid

Paper I

Randomized clinical trial

Randomized clinical trial of topical tranexamic acid after reduction mammoplasty

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Background: **The antifibrinolytic drug tranexamic acid is currently being rediscovered for both trauma and major surgery. Intravenous administration reduces the need for blood transfusion and blood loss by about one-third, but routine administration in surgery is not yet advocated owing to concerns regarding thromboembolic events. The aim of this study was to investigate whether topical application of tranexamic acid to a wound surface reduces postoperative bleeding.**

Methods: **This was a randomized double-blind placebo-controlled trial on 30 consecutive women undergoing bilateral reduction mammoplasty. On one side the wound surfaces were moistened with 25 mg/ml tranexamic acid before closure, and placebo (saline) was used on the other side. Drain fluid production was measured for 24 h after surgery, and pain was measured after 3 and 24 h. Postoperative complications including infection, seroma, rebleeding and suture reactions were recorded.**

Results: **Topical application of tranexamic acid to the wound surface after reduction mammoplasty reduced drain fluid production by 39 per cent (median 12·5 (range 0–44) *versus* 20·5 (0–100) ml; $P = 0{\cdot}038$). Adverse effects were not observed. There were no significant differences in postoperative pain scores or complications.**

Conclusion: **Topical application of dilute tranexamic acid reduced bleeding in this model. The study adds to the evidence that this simple procedure may reduce wound bleeding after surgery. Registration number: NCT01964781 (http://www.clinicaltrials.gov).**

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Introduction

When fibrinolysis exceeds coagulation, unwanted surgical bleeding may occur despite adequate haemostasis. Tranexamic acid is the most commonly used medication to prevent fibrinolysis. It acts by blocking the lysine-binding sites on plasminogen, thereby preventing the activation of plasminogen to plasmin[1]. Tranexamic acid can be administered orally or intravenously, but topical use is being reported increasingly.

Intravenous administration of tranexamic acid during major surgery has been shown to reduce the need for blood transfusion by 32–37 per cent, as well as measurable postoperative bleeding by 34 per cent[2,3]. The dose has varied considerably[4], from 1 to 20 g administered intravenously over 20 min to 12 h. Suggested single intravenous doses are 1–2 g[2,5,6]. The minimal effective plasma concentration is unknown[5,7–9]. Safety concerns have included thrombosis, renal impairment, and increased risk of seizures associated with high doses (above 2 g)[4,10]. Definitive causal relationships have not been established[4,11,12] but, as adverse effects may be dose-related, and doses above 1–2 g seem to provide no added benefit, high-dose intravenous administration is discouraged. Because of uncertainty about the effect of tranexamic acid, particularly on vascular occlusive events, it is still not recommended for routine use during most surgical procedures[13].

Topical application of tranexamic acid provides a high drug concentration at the site of the wound and a low systemic concentration[14,15]. Studies from cardiac and orthopaedic surgery have shown an equal or superior effect of topical compared with intravenous tranexamic acid on both bleeding and transfusion requirement[16–20]. Topical treatment is cost-effective[21], and adverse effects or drug interactions have not been reported.

In previous studies, topical tranexamic acid was instilled mainly as a bolus into confined spaces such as a joint, the mediastinum or the pericardium[20,22], or applied to accessible wounds using soaked gauze[23–25]. A few studies[26–28] have described simple moistening of a wound surface. Whereas most topical haemostatic agents can cover only a small surface area[29], tranexamic acid diluted in saline can moisten large areas, such as after massive weight loss surgery, or in patients with burns[30,31].

The aim of this study was to investigate whether moistening a wound surface with tranexamic acid reduces bleeding. This hypothesis was tested in a randomized double-blind placebo-controlled study of women undergoing bilateral reduction mammoplasty, where effects of intervention on one breast can be evaluated by comparison with the other.

Methods

Consecutive women above 18 years of age undergoing bilateral reduction mammoplasty at the Department of Plastic Surgery, St Olav's University Hospital, Trondheim, Norway, were eligible for inclusion in the study. Exclusion criteria were: a history of any thromboembolic disease, pregnancy or severe co-morbidity (American Society of Anesthesiologists (ASA) fitness grade III or IV). Women using platelet-inhibiting drugs or anticoagulants were not excluded automatically. Written informed consent was obtained from all participants before inclusion.

Randomization and masking

The women were randomized to receive topical tranexamic acid to one breast and placebo to the other after reduction mammoplasty. Randomization was performed electronically by the Unit of Applied Clinical Research at the Norwegian University of Science and Technology (NTNU-Trondheim) (http://www.ntnu.edu/dmf/akf/randomisering). Sealed envelopes were prepared stating whether the right or left side was to receive tranexamic acid according to the randomization. All personnel involved in surgery and postoperative follow-up were blinded to the randomization. The randomization code was kept at the Unit of Applied Clinical Research and was not broken until 4 weeks after the last patient had been enrolled.

Intervention

As the specific mode of topical application of tranexamic acid has not been published previously, the concentration of tranexamic acid needed to achieve an antifibrinolytic effect was unknown. To ensure a sufficiently high concentration, the tranexamic acid was diluted only to a volume sufficient to moisten a fairly large wound surface[32]: 20 ml moistens at least 1500 cm^2. On the morning of surgery, a nurse not involved in the procedure prepared two identical plastic bottles of 20 ml saline (0·9 per cent sodium chloride), marking them right and left. She then opened the sealed envelope with the patient's project identification number. On the side stated in the envelope, 5 ml saline was extracted from the bottle and replaced with 5 ml of 100 mg/ml tranexamic acid. The prepared solution thus contained 20 ml of 25 mg/ml tranexamic acid. The other bottle (placebo) received only an identical needle puncture. The solutions were colourless, so the two bottles looked identical apart from their right and left markings. The bottles were then brought to the operating theatre.

Two surgeons performed the operation, operating on one breast each. Equal amounts of local anaesthetic were injected into the breasts at the end of surgery in accordance with the hospital's prevailing routine for reduction mammoplasty (20 ml of 1 mg/ml lidocaine with 5 μg/ml adrenaline and 20 ml of 2·5 mg/ml bupivacaine (AstraZeneca, London, UK) diluted in 120 ml saline for each breast). After resecting the breast tissue, the surgeons switched sides to evaluate/improve haemostasis, thus securing homogeneous wounds and equivalent operating times. The weight of resected tissue was recorded. The contents of the appropriate bottles containing tranexamic acid or placebo were smeared on to the wound surfaces before closure, taking care not to use moist swabs or gloves on the opposite side. Vacuum drains (Exudrain™ FG 14; AstraZeneca) were placed symmetrically, the wounds were closed with standard subcutaneous and intracutaneous suturing, and standard compression garments were fitted. The patients received oral analgesics after surgery, but no routine thromboprophylaxis; this is standard practice after reduction mammoplasty at this hospital.

Follow-up

All women were interviewed by a nurse 3 and 24 h after surgery. Drain fluid volume was recorded 24 h after surgery, and drains were removed when production was below 40 ml per 24 h, according to hospital routine. Haematoma needing reoperation within 24 h was registered. All women also had an outpatient appointment with one of the surgeons 3 months later. The women were encouraged to contact the hospital if any complications occurred.

Outcomes

The primary outcome was drain fluid production in the first 24 h after surgery. The drain fluid was not analysed

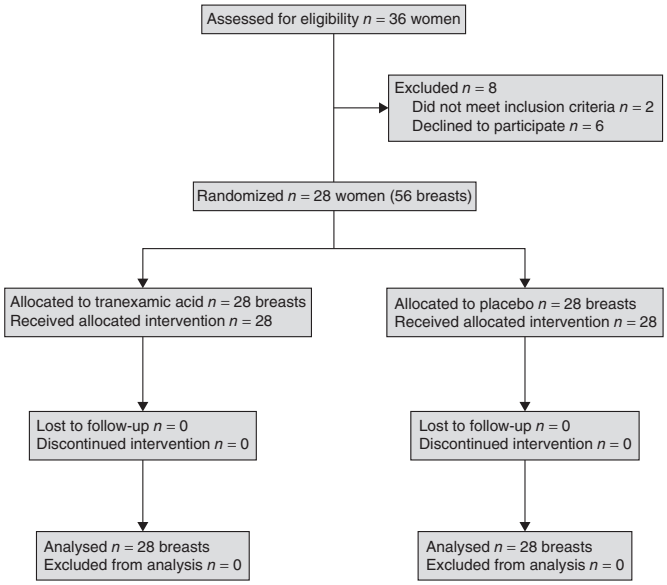

Fig. 1 CONSORT diagram for the study

further. A secondary outcome was postoperative pain, which was registered for each breast both 3 and 24 h after surgery, using a visual analogue scale from 0 (no pain) to 10 (unbearable). Medical and surgical serious adverse events were recorded at the scheduled follow-ups.

Statistical analysis

A 25 per cent difference in drain volume between treated and untreated wounds was considered clinically important. As each woman was her own control, using a paired-samples *t* test to detect a difference of 0·25, and a standard deviation of 0·4, α of 0·05 and power 0·80, a sample size of 23 was needed[33]. To ensure additional power when using non-parametric statistical tests[34] and to counter unforeseen events, 30 women were included.

Descriptive data are presented as mean(s.d.) or median (range), as appropriate. Differences in drain fluid volume and pain between breasts were analysed using a paired Wilcoxon signed-rank test. Differences in categorical variables between groups were evaluated with Fisher's exact test. $P < 0{\cdot}050$ was considered significant. All analyses were done using SPSS® version 22.0 (IBM, Armonk, New York, USA).

Results

Of 36 women eligible for inclusion, six declined participation and 30 women were therefore enrolled. No patient withdrew after enrolment. Two were, however, excluded just before surgery (1 had a complete mastectomy instead of reduction mammoplasty; the other was recognized to have had a previous myocardial infarction and was considered ASA grade III). Tranexamic acid and placebo were not administered to these patients. Twenty-eight women remained eligible for final analyses (*Fig. 1*); their median age was 45 (18–67) years. None of the women used platelet inhibitors or anticoagulants. Mean resected tissue weight per breast was 415(162) g.

Drain production was 39 per cent lower in breasts treated with tranexamic acid compared with breasts treated with placebo: median 12·5 (0–44) *versus* 20·5 (0–100) ml respectively ($P = 0{\cdot}038$) (*Table S1* and *Fig. S1a*, supporting information). To adjust for possible differences in wound surface areas owing to unequal size of the two breasts, a separate analysis was done with drain volume adjusted for resected weight. This analysis showed 42 per cent lower drain fluid production in breasts treated with tranexamic acid: median 12·9 (0–47) *versus* 22·0 (0–100) ml respectively ($P = 0{\cdot}017$) (*Table S1* and *Fig. S1b*, supporting information). Only one of 28 breasts treated with tranexamic acid produced 40 ml or more of fluid (44 ml), compared

with nine of 28 placebo-treated breasts ($P = 0{\cdot}016$). Two of these in the placebo group required reoperation and evacuation of haematoma.

Pain scores were similar in breasts treated with tranexamic acid or placebo on the day of surgery (median 2·5 (0–6) *versus* 2·0 (0–6); $P = 0{\cdot}179$) and after 24 h (2·0 (0–6) *versus* 2·0 (0–5·5) respectively; $P = 0{\cdot}428$).

No adverse effects were recorded after topical tranexamic acid. Five patients had a wound infection treated with antibiotics or outpatient wound drainage within 6 weeks of surgery. Another six women had a superficial inflammatory reaction related to the sutures. These had no relation to treatment with tranexamic acid or placebo. One woman had bilateral wound seromas which were evacuated.

Discussion

The present study showed that topical application of tranexamic acid significantly reduced wound drainage after reduction mammoplasty.

The main strength of the study is the model. Surface wounds can be diverse (burns, massive weight loss surgery), and it is challenging to design a study with equivalent wounds. Women undergoing bilateral reduction mammoplasty provide two almost identical wounds in an ordinary clinical setting. A small study can thus provide adequate statistical power.

The study, however, has several weaknesses. It was conducted using a surgical procedure that is not usually associated with major bleeding. Drain volumes, not blood loss, were measured; drain fluids consist of both blood and transudates, with a transition over time to more serous drain fluid. The breasts had also been injected with local anaesthetic containing adrenaline (epinephrine), but with equal volumes in each breast. The constituents of the drain fluids were not analysed, but registration took place during the first 24 h when the drainage is mostly blood. Whether topical tranexamic acid can influence transudate production or even seroma formation through its inhibition of fibrinolysis remains unknown.

The wounds after reduction mammoplasty are not completely identical, particularly when two surgeons operate, one side each. To correct for this, the surgeons undertook haemostasis on the contralateral side, and a separate analysis of drain volumes was done with adjustment for resected tissue weight.

Serum concentrations of tranexamic acid were not measured. Systemic absorption of topical tranexamic acid could in theory affect bleeding from the contralateral placebo wound. *In vitro*, a minimum concentration of 5–10 μg/ml is needed to inhibit fibrinolysis[1,7]. Studies in orthopaedic and cardiac surgery have found a negligible systemic concentration from application of boluses of tranexamic acid in confined spaces[14,15]. The tranexamic acid dose in the present study was 20 ml of 25 mg/ml, a total of 500 mg; this method should give a very low systemic concentration[14,15].

The overall reduction in drain fluid production of about 40 per cent after topical administration of tranexamic acid here accords with previously published studies[3,16–20], which consistently reported a reduction in transfusion need and measurable bleeding of between 30 and 40 per cent after both intravenous and topical tranexamic acid administration. There is little information on the concentration of tranexamic acid needed for topical effect. Instillation of a bolus of 1–3 g tranexamic acid diluted in 100 ml saline (concentration 10–30 mg/ml) has been studied in patients undergoing cardiac[15,16] and orthopaedic[17] surgery, whereas epistaxis has been treated with sponges moistened with undiluted tranexamic acid for intravenous use (100 mg/ml)[23]. This present study opted for a high concentration of 25 mg/ml, but still dilute enough to provide a volume sufficient to moisten large surface areas. There are few published studies where the mode of application was comparable to the moistening used in this study. A mouthwash containing 4·8 mg/ml tranexamic acid was effective after dental extraction[28], whereas Hinder and Tschopp[26] found no significant effect of gargling 1·7 mg/ml tranexamic acid solution after tonsillectomy. Athanasiadis and colleagues[27] reported a significant effect after spraying the wound surfaces with tranexamic acid in endoscopic sinus surgery. The optimal concentration remains unknown.

The use of drains after reduction mammoplasty has little scientific evidence[35] but is nevertheless common. At the time of study, the departmental routine was to use drains until fluid production was below 40 ml per 24 h. The present study suggests that topical tranexamic acid reduces drain fluid production after reduction mammoplasty to below this cut-off value in almost all patients, and may obviate the need for a drain.

There were two postoperative bleeds in the study, both in untreated breasts. The study was not powered to evaluate whether topical tranexamic acid could prevent postoperative haematoma. Ngaage and Bland[4] reported that intravenous tranexamic acid reduced the risk of reoperation by 48 per cent after coronary surgery, and a significant reduction in rebleeding after tonsillectomy was described for the lysine analogue aminocaproic acid[36]. Few studies on tranexamic acid have reported rebleeding as an outcome measure[3,37].

Tranexamic acid is inexpensive. Its cost-effectiveness has been thoroughly documented in orthopaedic surgery[21]. Even after operations where bleeding is less common,

topical application of tranexamic acid may reduce the need for drains and outpatient visits. This simple method has potential for widespread application after surgery.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Drain fluid production measured 24 h after surgery in patients undergoing bilateral reduction mammoplasty (Word document)

Fig. S1 a Drain fluid production measured 24 h after surgery and **b** drain fluid volume adjusted for resected tissue weight (Word document)

Editor's comments

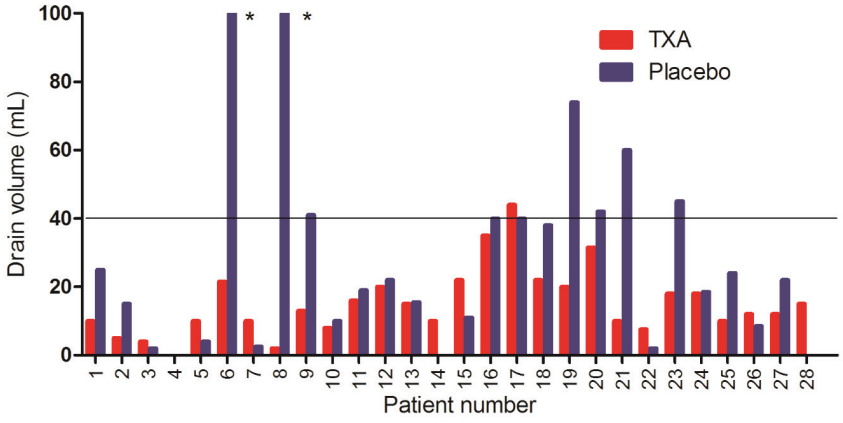
This paper, and the FIBER Study preceding it (*Br J Surg* 2015; **102**: 1338–1347), both explore pharmacological means to reduce perioperative bleeding. *BJS* does not normally publish cardiac papers, but makes an exception because of the high quality of the study. Although essentially a negative trial, the FIBER Study has valuable clinical implications, and is a reminder of the importance of publishing such trials. This study by Ausen *et al*. also has a favoured within-patient randomization design using an established agent (tranexamic acid) topically. This study has a positive result (median reduction of 8 ml in drain fluid) but the clinical relevance remains unclear, as does whether it would stand up to a formal cost-benefit analysis. Minimizing perioperative bleeding is fundamental, and the methods used in both these RCTs have relevance in other specialties and procedures. Only by conducting high-quality research can the role of pharmacological agents be clarified.

J. J. Earnshaw
Joint Chief Editor, BJS

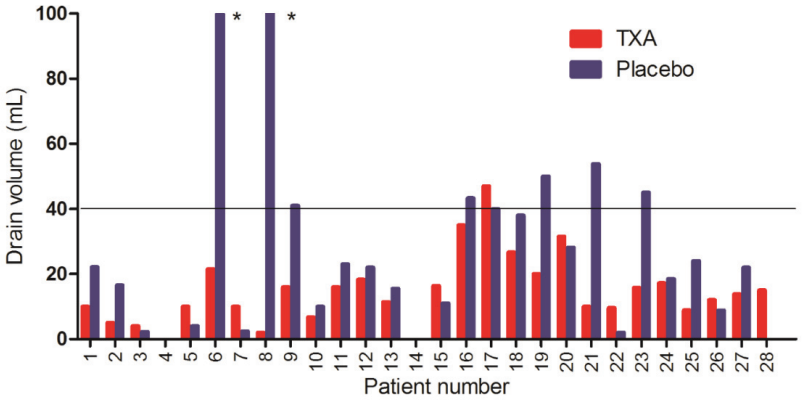
Supplemental digital content BJS9878

Randomized clinical trial of topical tranexamic acid after reduction mammoplasty

K. Ausen, R. Fossmark, O. Spigset and H. Pleym



a Drain fluid volume



b Drain fluid volume adjusted for resected tissue weight

Fig. S1 a Drain fluid production measured 24 h after surgery and **b** drain fluid volume adjusted for resected tissue weight, assuming direct proportion between resected weight and size of surgical wound, in patients undergoing bilateral reduction mammoplasty. *Rebleeding/haematoma requiring surgical evacuation; real volume much higher than 100 ml. TXA, tranexamic acid

Table S1 Drain fluid production measured 24 h after surgery in patients undergoing bilateral reduction mammoplasty

Patient no.	Drain fluid volume (ml)		Weight-adjusted drain fluid volume (ml)		Resected tissue (g)	
	Tranexamic acid	Placebo	Tranexamic acid	Placebo	Tranexamic acid	Placebo
1	10.0	25.0	10.0	22.1	612	692
2	5.0	15.0	5.0	16.5	207	188
3	4.0	2.0	4.0	2.3	315	275
4	Excluded					
5	0.0	0.0	0.0	0.0	635	600
6	Excluded					
7	10.0	4.0	10.0	4.0	78	80
8	21.5	Rebleeding*	21.5	Rebleeding*	607	445
9	10.0	2.5	10.0	2.4	238	244
10	2.0	Rebleeding*	2.0	Rebleeding*	670	636
11	13.0	41.0	16.0	41.0	367	450
12	8.0	10.0	6.7	10.0	457	380
13	16.0	19.0	16.0	23.0	376	326
14	20.0	22.0	18.3	22.0	347	318
15	15.0	15.5	11.4	15.5	624	476
16	10.0	0.0	10.0	0.0	471	306
17	22.0	11.0	16.3	11.0	222	164
18	35.0	40.0	35.0	43.3	419	387
19	44.0	40.0	47.0	40.0	433	462
20	22.0	38.0	26.7	38.0	376	457
21	20.0	74.0	20.0	50.0	506	750
22	31.5	42.0	31.5	28.1	432	646
23	10.0	60.0	10.0	53.8	475	530
24	7.5	2.0	9.6	2.0	339	433
25	18.0	45.0	15.8	45.0	727	640
26	18.0	18.5	17.2	18.5	535	512
27	10.0	24.0	8.9	24.0	496	443
28	12.0	8.5	12.0	8.8	242	234
29	12.0	22.0	13.8	22.0	340	390
30	15.0	0.0	15.0	0.0	250	293

*Drain fluid volume considerably higher than 100 ml.

Comment and reply to Study !

Comments to Study 1:

We read with interest the randomised controlled trial regarding the use of topical tranexamic acid in breast reduction surgery by Ausen et al. (1), but found some inconsistencies in the statistical analyses that may mislead readers. Firstly, the authors appropriately analysed the efficacy of the intervention over control using a paired Wilcoxon signed-rank test, but incorrectly used an unpaired method to estimate its magnitude. They reported the unpaired 8ml difference of medians of drain output between the two groups (39% reduction). However, the paired median difference was only 2.5ml (12% reduction). Analysis of a paired study must not ignore the level of dependency within each pair. Secondly, the variance in drain output ($\delta 2 = 0.16$) used to determine the trial sample size was much underestimated. Using the trial data we conservatively calculated a bootstrap estimate of the actual variance as 1.09 (95% confidence interval (CI): 0.26-1.93), excluding the three placebo group cases with 0ml. The assumed variance was far below the 95% CI of the actual variance and consequently, this study's power to test its hypothesis was only around 30%. If we conservatively assume a variance of 0.9, then the required study size is 113 patients, 4 times larger. The 2.5ml median reduction in drain output in this study may not accurately represent the true effect size, since the variance was very large and the study very underpowered. Based on the promising preliminary data from this study, an adequately powered study to definitively answer the hypothesis should now be conducted.

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1. Ausen K, Fossmark R, Spigset O, Pleym H. Randomized clinical trial of topical tranexamic acid after reduction mammoplasty. Br J Surg 2015; 102: 1348-1353.

Reply:

Thank you for giving us the opportunity to further clarify some aspects of our study (1). This small study attempted to provide a proof of concept, and there was a significant difference in drain production between wounds treated with tranexamic acid and placebo (p=0.038). Reduction mammoplasties generally bleed little and are not in need of haemostatic interventions, but it is one of few rare models where two almost identical wounds coexist in the same patient. We agree that median difference should be calculated from the paired numbers. However, to report the paired median difference in this material clearly underreports the magnitude of the difference that exists in favour of tranexamic acid. If one sorts the patients in the study according to difference in drain volume between their two breasts, the differences in drain volume to the right of the median far exceeds the differences to the left of the median.

Looking at the paired difference between a treated and untreated breast here, the absolute paired difference is generally considerably larger in breasts with larger bleeding volumes – and then in favour of tranexamic acid. In 13 of the 15 patients in whom one or both breasts had more than 20 ml drain production, the difference was in favour of tranexamic acid. Our power calculations were performed in advance, and it should again be emphasized that we found a statistically significant difference. In future studies, new power calculations based upon the findings in the present study should obviously be carried out. Larger studies are needed; preferably in patient groups with expected larger bleeding volumes.

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Reference:

1 Ausen K, Fossmark R, Spigset O, Pleym H. Randomized clinical trial of topical tranexamic acid after reduction mammoplasty. BJS 2015; 102: 1348-1353.

Paper II

Randomized clinical trial of topical moistening of mastectomy wounds with diluted tranexamic acid to reduce bleeding

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Manuscript category: Randomized clinical trial. ClinicalTrials.gov (NCT02627560)

Previous presentation of the trial: An abstract entitled *«Topical moistening of the wound surface with tranexamic acid 25 mg/ml reduces bleeding equal to intravenous administration and may reduce need for re-operation of postoperative haemorrhage»* was presented as an abstract and a single slide/four minute oral presentation at the European Association of Plastic Surgeons Research Council in Helsinki, Finland on May 21st 2019. This abstract included results both from this study and our former pilot study.

Abstract

Background Topical administration of tranexamic acid (TXA) may be an alternative to intravenous administration to reduce bleeding with less risk of systemic adverse events. The objective of this study was to investigate whether moistening a surgical wound with TXA 25 mg/ml prior to closure, leaving a thin film of drug only, reduces postoperative bleeding.

Methods This was a two-centre stratified, parallel-group, placebo-controlled, double-blind randomized clinical trial. Patients undergoing mastectomy with/without axillary lymph node clearance were randomized 1:1 to moistening of wound surface prior to closure with either TXA 25 mg/ml or NaCl 0.9% (placebo). Primary endpoint was postoperative bleeding as measured by drain production first 24 hours. Secondary endpoints were early hematoma, total drain production, postoperative complications, and late seroma aspirations within 3 months.

Results Between January 1st 2016 and August 31st 2018, 208 patients were randomized. Two patients were converted to a different surgical procedure perioperatively, and four patients did not receive intervention due to technical errors. Thus, 202 patients were included in the study (101 received TXA and 101 received placebo). TXA reduced drain production at 24 hours (110 vs. 144 ml, effect size 34 ml, 95% CI 8 to 60 ml, *P*=0.010). Early hematoma occurred in one TXA patient versus seven placebo patients (OR 0.13, 95% CI 0.02 to 1.07, *P*=0.057). There was no significant difference in postoperative complications between TXA and placebo (13 vs 10, OR 1.11, 95% CI 0.45 to 2.73, *P*=0.824) or need for late seroma aspirations (79.3 vs 66.6%, OR 1.83, 95% CI 0.91 to 3.68, *P*=0.089).

Conclusion Moistening the wound prior to closure with TXA 25 mg/ml reduces postoperative bleeding within first 24 hours in patients undergoing mastectomy.

ClinicalTrials.gov (NCT02627560)

Introduction

In healthy patients without coagulation deficiencies, the only systemically administered pharmacological intervention to universally reduce surgical bleeding with an acceptable safety profile is antifibrinolytic drugs[1]. Tranexamic acid (TXA) has been the drug of choice since the withdrawal of aprotinin from the market in 2007[2]. Intravenous (iv) use of TXA reduces surgical bleeding and need for blood transfusion with about one third[3] and is routinely used in surgery associated with significant blood loss. Even high doses of iv TXA has not been associated with increased incidence of thromboembolic events, but large prospective studies designed to particularly evaluate this risk are lacking[4]. A dose-dependent increase in non-ischemic seizures has been described after iv TXA in cardiac surgery[5-7]. Although minimization of blood loss is desirable in all surgery, general use of iv TXA has not been advocated due to unease regarding possible systemic adverse effects[8, 9].

A prophylactic intervention to minimize postoperative blood loss should be low-cost, easy, efficient and safe. Topical use of TXA may provide a high drug concentration on the wound surface with negligible systemic concentrations[10]. Topical use is still off-label with no consensus as to optimal TXA concentration in the solution applied, mode of application, or duration of contact[11, 12]. Most publications come from joint replacement surgery, where instilling TXA as a bolus into the joint reduces bleeding equal to iv administration[13-15]. We have previously introduced a novel procedure where the wound surface is simply moistened with 25 mg/ml tranexamic acid prior to closure, finding a mean 39% reduction in drain volume in a small “proof-of-concept” randomized placebo-controlled study in bilateral reduction mammoplasties[16]. Such moistening exposes the wound surface to TXA for a considerably shorter time than topical boluses[10].

The aim of this study was to investigate the effect of our novel moistening method in a larger population and in a different study model. Mastectomy was chosen as a suitable study model for bleeding as it is a common and standardized surgical procedure with homogenous wounds and little

occult blood loss. As postoperative seroma is a major adverse event after mastectomy[17, 18], we also wanted to investigate whether topical TXA would influence seroma formation.

Methods

The study was a two-centre stratified, parallel-group, placebo-controlled, double-blind randomized clinical trial. The study was registered in ClinicalTrials.gov (NCT02627560) and approved by the involved departments, the Regional Committee for Medical and Health Research Ethics in Mid Norway (2015/1722) and the Norwegian Medicines Agency (15/11405-7). The trial was performed in accordance with the principles of the Declaration of Helsinki and was monitored in accordance with the Good Clinical Practice directive of the European Medicines Agency.

Study population

Patients above 18 years of age who were to undergo simple mastectomy (SM), mastectomy with sentinel node biopsy (SNB) or axillary lymph node clearance (AC) were consecutively identified from the operation planning registries at St Olav's University Hospital (Centre A) and Ålesund Hospital (Centre B) between January 1st 2016 and August 31st 2018. Exclusion criteria were 1) known thromboembolic disease or high risk of thromboembolism warranting extra anticoagulation in connection with the procedure, 2) pregnancy/nursing and 3) known allergy to TXA. Eligible patients were informed about the study after diagnosis but prior to surgery by nurses and doctors connected to the respective breast cancer centres. Patients were enrolled if written informed consent was obtained.

Treatment allocation

Computer generated randomization was done in permuted blocks of 10, 20 or 50 patients stratified according to study centre. Sealed and numbered opaque randomization envelopes were produced accordingly. Details on screening and randomization are shown in *Fig.1*. All randomization and organization of electronical Case Report Forms was performed by the Unit of Applied Clinical Research[19] at the Norwegian University of Science and Technology-NTNU, Trondheim, Norway. Participants and all personnel involved in surgery and postoperative follow-up, data collection and statistical analysis were blinded to the randomization.

Study interventions

Participants were randomly assigned in a 1:1 ratio to moistening of their wound surface prior to closure with either 20 ml of TXA 25 mg/ml or NaCl 0.9% (placebo). Opening of the randomization envelope and drug preparation was done by personnel not connected to the surgery, postoperative follow-up, data collection or statistical analyses. Envelopes were opened in numerical order. TXA 25 mg/ml was prepared by extracting 5 ml from a 20 ml bottle of saline (NaCl 0.9%) and adding 5 ml of tranexamic acid 100 mg/ml to the same bottle, thus obtaining 20 ml of TXA 25 mg/ml. Placebo was an identical 20 ml bottle of saline, which was perforated by a needle for identical appearance.

The moistening method is illustrated in this video: https://www.youtube.com/watch?v=-8MAE3NAHfQ. All involved surgeons were visually instructed on the details of the moistening technique by watching this video, and also instructed to cover all surfaces and use the entire 20 ml although much would spill. Breast specimen weight, height and width were measured during surgery. Wound surface area was calculated as an ellipse (height/2 x width/2 x π). Patients received active vacuum drains (Exudrain FG 14, Wellspect, Oslo, Norway) marked with the exact timepoint to register drain production 24 h after completion of the surgery. Preoperative evaluation, performance of the surgery, postoperative treatment and drain removal were otherwise in accordance with

prevailing routine at the participating study centres and randomization was therefore stratified according to study centre.

It was discussed whether to sub-stratify the randomization according to type of surgical procedure as well, as the presence or absence of lymph node clearance is known to have a significant impact on drain output. As randomization for practical purposes was done preoperatively and a planned procedure might be converted perioperatively, we chose to rather adjust for type of surgery in the statistical analyses.

Study outcomes

The primary outcome was postoperative bleeding as defined by the volume of drain production the first 24 hours after surgery. Secondary outcomes were total drain production and drain time, early hematoma, postoperative complications and seroma formation. Seroma was defined as fluid accumulation warranting aspiration after drain removal. Chronic seroma was defined as persistent seroma at three months. Patients experiencing postoperative complications defined as hematomas, infections and wound ruptures were excluded from the seroma analyses as these may influence seroma formation.

Drain production was recorded at 24 hours after termination of surgery and on a daily basis thereafter until drain removal. Variables which according to protocol could influence the defined outcomes were obtained from the medical record (*Table 1*). The staff was instructed to accurately document seroma aspirations throughout the study period, and patients were instructed to request accurate measuring should they have aspirations performed elsewhere. Patient follow-up lasted for three months and all patients received a final phone call to ensure or correct registered data and identify unregistered adverse events.

An evaluation of the surgical techniques at the two centres had been performed before study initiation. Use of tumescence, dissection with diathermia and choice of surgical levels had been found to be comparable. However, during data collection it was noted that Centre B seemed to have notably lower drain productions. A post-hoc re-evaluation revealed that Centre B routinely applied a circular compression bandage for the first 24 h postoperatively, which had not been recognized during the study design phase. Postoperative compression is an active intervention to reduce bleeding but was not accurately registered in the medical records nor accurately remembered by patients in retrospect. It could therefore not be added as a post-hoc variable at the individual patient level but had to be regarded an inherent factor when adjusting data for study centre.

Statistical analyses

A between-group difference in mean drain production of 25% was considered clinically significant. Estimating a standard deviation of 0.6[20], alpha of 0.05 and power 0.80, a sample size of 92 patients in each group was needed[21]. We planned to include a total of 210 patients to ensure additional power.

Continuous patient characteristics data were calculated as mean ± standard deviation (SD) and compared using independent samples t-tests. Categorical patient characteristics data were calculated as frequency counts and percentages and compared using chi-square tests, but variables with ≤5 cases were analysed using Fisher exact tests.

Continuous outcome data are presented as mean (95% Confidence Interval (CI)). Categorical outcome data are presented as frequency counts and percentages. For presentation of unadjusted effect size of continuous data, mean difference (95% CI) between groups is used. Effect size of categorical data is presented as odds ratio (OR) (95% CI). For statistical analyses of unadjusted effect size, continuous outcome data were analysed using independent samples t-tests while categorical outcome data were compared using chi-square tests or Fisher exact tests where appropriate.

For adjusted statistical analyses, non-normally distributed continuous data underwent logarithmic (ln) transformation. Data were then analysed using a general linear model. We included all variables registered in *Table 1* and performed a stepwise removal of non-significant variables ($P > 0.05$) to obtain a final model that included only individually significant variables. Results are calculated as differences between means as percentage (95% CI). Non-normally distributed continuous data that could not undergo logarithmic transformation due to the appearance of zero values were dichotomized and analysed using a logistic regression model adjusting for significant variables. Results are given as mean difference or odds ratio (OR) with 95% CI. Two-tailed P-values < 0.05 were considered statistically significant. Analyses were performed in accordance to the "intention to treat" principle. Analyses were performed using SPSS version 25 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA).

Results

Between January 1st 2016 and August 31st 2018, 208 patients were randomized, and 202 were included in the study (*Fig. 1*). Of these, 101 received TXA and 101 received placebo. Patient characteristics are presented in *Table 1*. Patients receiving TXA were on average 3.9 years older (P = 0.033), otherwise there were no differences between the groups. Patient data stratified for study centre and type of surgery defined as AC or SM/SNB are presented in *Tables S1-S2*.

Outcome Data

Outcome data are presented in *Table* 2. Data stratified for type of surgery and study centre are presented in *Tables S3 – S5*. Mean drain production at 24 h was 110 ± 67 ml in the TXA group versus 144 ± 113 ml in the placebo group (mean difference 34 ml (95% 8 to 60 ml, P = 0.011).

The following patient variables independently and significantly affected outcome: 1) Administration of TXA, 2) type of surgery, 3) study centre, 4) wound surface area and 5) surgeon seniority. Perioperative anticoagulation did not affect the outcome variables. The independent significances of effect of all patient variables on the primary outcome is presented in *Table S6*.

Outcome variables adjusted for the significant patient variables showed that moistening the wound surface with TXA significantly reduced 24 h drain production with 32.4% ($P < 0.001$) (*Table 2*) . While total drain volume was not significantly reduced in the unadjusted analyses, adjusted outcome showed that total drain volume was reduced with 33.0% ($P = 0.003$). Patients in the TXA group were significantly more likely to have drains removed on the first day (OR 3.0, $P = 0.003$) and had a significantly shorter drain period (-15.6%, $P = 0.017$).

Early hematomas warranting re-operation occurred in seven placebo patients and one TXA patient ($P = 0.057$). There were no differences between groups regarding other complications. No thromboembolic events were registered.

Subgroup analyses

Prior to study initiation, AC was assumed to significantly influence drain production and subgroup analyses were planned according to type of surgery. The recognition of the significant influence of study centre led to an additional post-hoc subgroup analysis according to study centre. The adjusted effect of type of surgery and study centre on outcome variables are presented in *Table S7.*

Subgroup analysis for type of surgery showed that patients undergoing AC and receiving TXA had significant effect on drain production at 24 h (-26.7%, $P = 0.026$) albeit less than in the SM/SNB group (-33.4%, $P=0.001$) (*Table 3*). Total drain production was not significantly reduced in patients undergoing AC (-27.3%, $P = 0.244$) while it was significantly reduced in the SM/SNB patients (-32.3%, $P=0.005$). The subpopulation who had undergone lymph node clearance had significantly *increased*

odds compared to placebo of both needing seroma aspiration (OR 5.7, *P* = 0.032) and of a cumulative aspirated seroma volume ≥ 500 ml (OR 5.7, *P* = 0.011) but no increased risk of chronic seroma (OR 1.5, *P* = 0.671).

Patients operated at Centre A had 60.3% higher drain production at 24 h than patients from Centre B (*P* <0.001) (*Table S7*). Centre A practiced a significantly higher threshold value for ongoing drain production at removal (97.4% more; *P* < 0.001) and kept drains significantly shorter (-29.8%, *P* <0 .001) than Centre B, yet Centre A had a 33.5% *larger* cumulative drain volume at the time of removal (*P* = 0.013). This difference was postulated to be influenced by the postoperative compression used at Centre B.

Patients who neither received compression nor underwent lymph node clearance were the SM/SNB patients at Centre A (n=108, TXA=52, Placebo=56). To illustrate the isolated effect of TXA we separately analysed this subpopulation (*Table 4*), and found a 39% reduction in 24-hour drain production (*P* = 0.001), and a 46% reduction in total drain output (*P* = 0.001).

Discussion

To our knowledge, we are the first to examine the effect of merely leaving a thin film of TXA fluid on the wound surface before closure[16]. This confirmatory study demonstrates that a simple single moistening of a wound surface with TXA 25 mg/ml significantly reduces both postoperative bleeding and total drain production with about one third. This is comparable to the effect of established intravenous prophylactic use of TXA[22] and is thus an alternative mode of administration. We also postulate that topical TXA may reduce the risk of postoperative hematomas warranting reoperations, as 7 out of 8 re-bleedings occurred in placebo patients, and in our previous pilot study[16] the two re-bleedings observed were both in the placebo group. Hence, in our two intervention studies in 258 breasts in total, 9 out of 10 haematomas were in placebo breasts (P=0.019, Fisher's exact test). In

the few existing studies on use of iv TXA in breast surgery, a reduction in bleeding and also haematomas has been suggested[20, 23, 24].

An unexpected finding was a possible negative effect of TXA on lymph leakage. The subgroup of TXA patients who underwent lymph node clearance had less beneficial effect of TXA on postoperative drain production, and they were later significantly more likely to need seroma aspiration and had an increased cumulative seroma volume, although we saw no increase in chronic seromas.

The purpose of this study was not to demonstrate reduced bleeding in mastectomies per se. Minimizing perioperative bleeding is fundamental, and the method has relevance in many procedures other than breast surgery. Mastectomy is however a suitable study model where homogenous wounds and little occult bleeding will provide relatively unbiased results. A major strength of our study was the identification of significant effect modifying variables such as wound surface area, performance of lymph node clearance and inherent differences between study centres.

Drain production is a surrogate variable for bleeding and could be considered a weakness of this study. Topical application of TXA at the end of surgery will naturally not affect perioperative bleeding and hence comparing pre- and postoperative haemoglobin concentrations would not be an appropriate measure on the effect of this mode of administration. Drain fluids consist of both blood and transudate with a transition to more serous fluids over time. We therefore regarded drain production at 24 h as a more appropriate measure of postoperative bleeding than total drain production.

A major weakness of this study was the failure to register “application of postoperative compression” as a separate variable, as this was recognized in retrospect to be a routine procedure at Centre B. Closing of dead space reduces bleeding[25]. Compression is thus an active intervention which may interact with the effect of TXA, possibly contributing to the reduced drain production and the lower beneficial effect of TXA observed at Centre B. Use of compression to reduce bleeding and seroma has been somewhat forfeited in breast surgery – the few existing studies showing little effect particularly

on late seroma[26, 27]. However, these studies describe ongoing compression for several days, which may induce both discomfort and secondary reactions to ischemia. Moreover, seroma is a different endpoint than postoperative bleeding. A proper randomized evaluation of the effect of a 24 h postoperative compression bandage as practiced at Centre B on postoperative bleeding and haematoma is warranted.

When analysing only the subpopulation which had neither lymph node clearance nor postoperative compression (SM/SNB patients at Centre A), the isolated effect of TXA increased to a 39% reduction in 24-hour drain production, and a 46% reduction in total drain output. This may be the most appropriate model for the true isolated effect of topical TXA.

Studies on topical use of TXA have been published since the 1970's[13]. Topical administration has however mostly been done by instilling boluses into closed spaces such as joints or the mediastinum, application of soaked gauze or repeated irrigation[13]. As topical use is off-label and the possible local effects of prolonged tissue exposure are largely unknown, keeping drug concentration and contact time as low as possible would be a sensible precaution when advocating routine prophylactic use. While bolus and gauze administrations may lead to prolonged exposure to TXA, we have in a previous study demonstrated that our topical moistening both has predictable and swift elimination and renders systemic concentrations negligible[10].

Although there was no increase in wound ruptures, wound infections or chronic seromas in the TXA group, the increase in seroma volume after TXA exposure in lymph node clearance patients raise the question of whether tissue adhesion or healing of lymph vessels may be delayed from topical application of TXA. As our population may have presented with exceedingly high rates of seroma due to the practice of early drain removal (>70% needed at least one seroma aspiration thereafter), our findings need confirmation from other populations with different drain protocols.

Whether TXA may have unrecognised antiadhesive properties need further exploration. TXA inhibits plasminogen, which is ubiquitous in tissue matrix and has numerous functions beyond cleavage of

fibrin. Studies have suggested that TXA may affect cell adhesion[28, 29], and inhibition of TXA on tumour growth and spread was investigated in the 1970s and 80s[30]. We made the unexpected observation in a previous study that prolonged exposure to high concentrations of topical TXA caused lack of re-epithelialization and even epithelial detachment in an *ex vivo* human skin wound model[31].

TXA does not only bind to plasminogen but may act as a competitive antagonist to the inhibitory neurotransmitters $GABA_A$ and glycine[5, 32] in the central nervous system (CNS). Both topical application of TXA to the brain in animal experimental settings[33, 34] and intrathecal accidental administration of TXA in humans[35] have been shown to cause seizures, and topical TXA should not be used in surgery with exposed CNS.

These findings demonstrate that the local effect of TXA is insufficiently explored and that caution may be warranted with regard to dose and exposure time.

In conclusion, we propose simple moistening of most surgical wound surface prior to closure with TXA 25 mg/ml as a low-cost, simple and quick routine prophylactic measure to reduce bleeding and possibly prevent re-operation due to haemorrhage. Further research must determine the lowest effective topical dose when using a moistening technique. Adequately powered studies or meta-analyses on the ability of topical TXA to prevent reoperations due to bleeding are also lacking, and possible adverse effects of topical application need further exploration.

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Legend to figure

Figure 1 Flow diagram of enrolment and randomization

List of supporting information

Project Protocol

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Figure 1 Flow diagram of enrolment and randomization

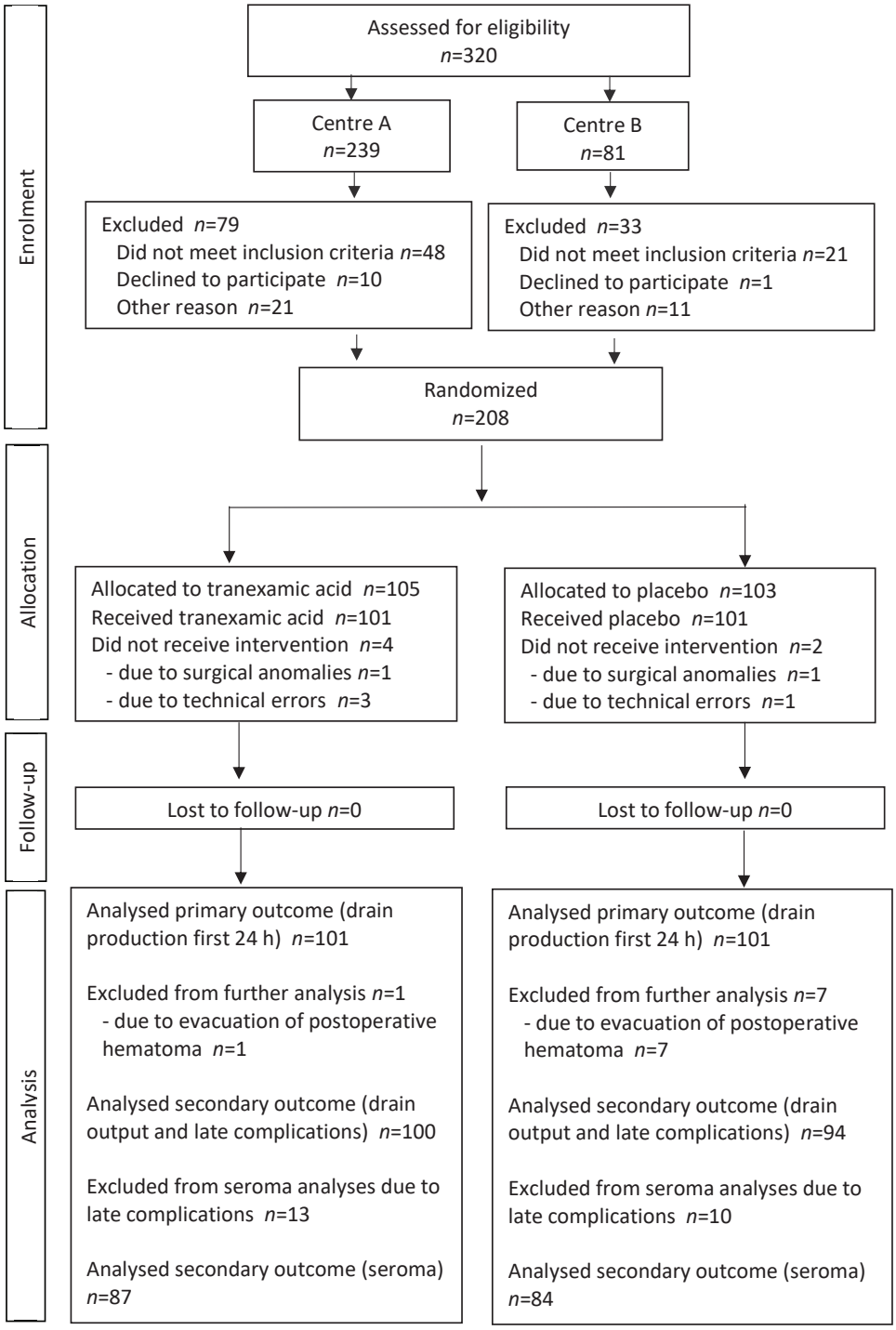

Table 1. Patient characteristics at baseline

Characteristic	TXA (*n*=101)	Placebo (*n*=101)
Female sex	98 (97.0)	100 (99.0)
Age*† (yr)	66.2 (13.3)	62.3 (12.8)
Body mass index (kg/m^2)*	26.9 (4.9)	27.1 (4.7)
Active smoker	19 (18.8)	13 (12.9)
Recruited at Centre A	77 (76.2)	77 (76.2)
Operated by senior surgeon	50 (49.5)	50 (49.5)
Neoadjuvant treatment	32 (31.7)	41 (40.6)
Irradiated tissue	14 (13.9)	11 (10.9)
Perioperative anticoagulation	32 (31.7)	37 (36.6)
Axillary clearance	34 (33.7)	31 (30.7)
Weight of breast specimen*‡ (g)	780 (450)	746 (359)
Wound surface*‡ (cm^2)	292 (117)	279 (84)

TXA = tranexamic acid
Data are number (%) unless stated otherwise
* Data are mean (standard deviation)
† Significant difference
‡ Axillary component included

Table 2. Outcome data

Primary outcome	**TXA (*n*=101)**	**Placebo (*n*=101)**	**Effect size (95% CI) TXA vs. Placebo**	**Adjusted difference‖ (95% CI) TXA vs. Placebo**
Drain production first 24h (ml) Mean (95% CI)	110 (97 to 123)	144 (122 to 167)	-34 ml (-60 to -8) *P*=0.011‡	-32.4% (-51.4 to -15.8) *P*<0.001Γ
Secondary outcomes*	**TXA (*n*=100)**	**Placebo (*n*=94)**		
Early hematoma*	1 (1.0)	7 (6.9)	OR 0.13 (0.02 to 1.11) *P*=0.065§	OR 0.13 (0.02 to 1.07) *P*=0.057#
Total drain production (ml) Mean (95% CI)	189 (145 to 234)	214 (165 to 264)	-25 ml (-91 to 41) *P*=0.461‡	-33.0% (-60.0 to -10.4) *P*=0.003Γ
Days with drain (d) Mean (95% CI)	1.7 (1.4 to 1.9)	1.8 (1.6 to 2.1)	-0.1 d (-0.7 to 0.2) *P*=0.341‡	-15.6% (-30.2 to -2.6) *P*=0.017Γ
Drain removed at 24h	65 (65.0)	47 (50.0)	OR 1.86 (1.04 to 3.31) *P*=0.035¶	OR 3.00 (1.44 to 6.22) *P*=0.003#
Drain production last 24h before removal (ml) Mean (95% CI)	93 (82 to 105)	91 (80 to 102)	2 ml (-13 to 18) *P*=0.783‡	-6.4% (-22.3 to 8.1) *P*=0.083Γ
Late hematoma/postoperative infection/wound rupture	13 (13.0)	10 (10.6)	OR 1.26 (0.52 to 3.02) *P*=0.721¶	OR 1.11 (0.45 to 2.73) *P*=0.824#
Thromboembolic event	0 (0.0)	0 (0.0)		
Seroma†	**TXA (*n*=87)**	**Placebo (*n*=84)**		
Seroma aspiration was needed	69 (79.3)	56 (66.6)	OR 1.92 (0.96 to 3.82) *P*=0.062¶	OR 1.83 (0.91 to 3.68) *P*=0.089#
≥5 seroma aspirations	19 (21.8)	17 (20.2)	OR 1.10 (0.53 to 2.30) *P*=0.797¶	OR 0.87 (0.38 to 2.00) *P*=0.740#
Cumulative seroma ≥500 ml	34 (39.1)	20 (23.8)	OR 2.05 (1.06 to 3.98) *P*=0.033¶	OR 1.99 (0.94 to 4.23) *P*=0.073#
Chronic seroma	6 (6.9)	11 (13.1)	OR 0.49 (0.17 to 1.40) P=0.182¶	OR 0.41 (0.14 to 1.21) P=0.107#

Data are number (%) unless stated otherwise. TXA = tranexamic acid

* Patients with early hematoma calculated from all patients; patients with early hematoma excluded from other secondary postoperative outcome registrations.

† Patients with early or late hematoma, infection or wound rupture excluded from seroma registrations.

‡ Independent samples student's t-test. Difference between means (95% CI).

§ Fisher's exact test. Odds ratio comparing TXA to placebo (95% CI)

¶ Chi square test. Odds ratio comparing TXA to placebo (95% CI)

‖ Regression analyses- adjusted according to axillary clearance, study centre, wound surface area and surgeon seniority.

Γ General linear model. Ratio between ln mean as percentage difference (95% CI).

Logistic regression model. Odds ratio comparing TXA to placebo (95% CI).

Table 3. Exploratory subgroup analysis. Adjusted primary and secondary outcomes according to administration of TXA vs. placebo, stratified for type of surgery.

	SM/SNB (n=137)	AC (n=65)
Outcome	**TXA (*n*=67) vs placebo (*n*=70)**	**TXA (*n*=34) vs placebo (*n*=31)**
Primary outcome		
Drain production first 24h*†	-33.4% (-58.1 to -12.6) P=0.001	-26.7% (- 55.9 to -2.9) P=0.026
Secondary outcomes‡		
Early hematoma‡§	n=1 vs n=3‖ P=0.620	n=0 vs n=4‖ P=0.046
Total drain production*†	-32.3% (-62.4 to -9.4) P=0.005	-27.3% (-91.9 to 18.4) P=0.244
Days with drain*	-11.6% (-24.9 to -0.2) P=0.054	-23.0% (-65.4 to 9.2) P= 0.166
Drain removed at 24 h‡	2.41 (1.02 to 5.68) P=0.044	5.70 (1.27 to 25.6) P=0.023
Drain volume last 24 h before removal*	-11.3% (-30.3 to 5.3) P=0.185	5.2% (-39.9 to 26.4) P=0.721
Late hematoma/ postoperative infection/ wound rupture‡	1.01 (0.33 to 3.06) P=0.985	1.27 (0.24 to 6.62) P=0.778
Seroma¶		
Seroma aspiration needed§	1.32 (0.59 to 2.97) P=0.500	5.71 (1.16 to 28.2) P=0.032
≥5 seroma aspirations§	0.47 (0.15 to 1.50) P=0.203	1.88 (0.54 to 6.49) P=0.320
Cumulative seroma ≥500 ml§	1.08 (0.41 to 2.81) P=0.880	5.72 (1.50 to 21.9) P=0.011
Chronic seroma§	0.18 (0.04 to 0.89) P=0.035	1.50 (0.23 to 9.73) P=0.671

TXA = tranexamic acid, AC = axillary clearance, SM/SNB = simple mastectomy/sentinel node biopsy
* Ratio between ln mean as percentage difference comparing group I to group II (95% CI). Univariate general linear model adjusted for study centre and wound surface area.
† Additionally adjusted for surgeon seniority.
‡ Cases with early hematoma calculated from all patients; patients with early hematoma excluded from other secondary postoperative outcome registrations.
§ Odds ratio comparing group I to group II (95% CI). Logistic regression model adjusted for study centre and wound surface area.
¶ Patients with early or late hematoma, infection or wound rupture excluded from seroma registrations.
‖ Fisher's exact test due to too few cases for logistic regression analysis.

Table 4. Exploratory subgroup analysis. Adjusted primary and secondary outcomes according to administration of TXA vs. placebo in SM/SNB patients at Centre A.

Outcome	TXA (*n*=52) vs placebo (*n*=56)
Primary outcome	
Drain production first 24h*†	-39.1% (-69.2 to -14.3) P=0.001
Secondary outcomes‡	
Early hematoma‡§	n=0 vs n=3‖ P=0.244
Total drain production*†	-46.1% (-80.9 to -17.9) P=0.001
Days with drain*	-23.0% (-83.0 to -9.5) P=0.001
Drain removed at 24 h‡	6.6 (2.07 to 21.22) P=0.001
Drain volume last 24 h before removal*	-9.4% (-30.2 to 8.8) P=0.309
Late hematoma/ postoperative infection/ wound rupture‡	n=5 vs n=6‖ P=1.000
Seroma¶	
Seroma aspiration needed§	1.11 (0.44 to 2.79) P=0.821
≥5 seroma aspirations§	0.47 (0.15 to 1.50) P=0.203
Cumulative seroma ≥500 ml§	1.05 (0.38 to 2.90) P=0.930
Chronic seroma§	0.18 (0.04 to 0.89) P=0.035

TXA = tranexamic acid, SM/SNB = simple mastectomy/sentinel node biopsy
* Ratio between ln mean as percentage difference comparing group I to group II (95% CI). Univariate general linear model adjusted for drug wound surface area.
† Additionally adjusted for surgeon seniority.
‡ Cases with early hematoma calculated from all patients; patients with early hematoma excluded from other secondary postoperative outcome registrations.
§ Odds ratio comparing group I to group II (95% CI). Logistic regression model adjusted for wound surface area.
¶ Patients with early or late hematoma, infection or wound rupture excluded from seroma registrations.
‖ Fisher's Exact test due to too few cases for logistic regression analysis.

Table S1 Patient characteristics at baseline stratified according to type of surgery and study centre

Stratified according to type of surgery	All cases (n=202)		SM/SNB (n=137)		AC (n=65)		Comparison surgery (n=202)	
Characteristic	**TXA (n=101)**	**Placebo (n=101)**	**TXA (n=67)**	**Placebo (n=70)**	**TXA (n=34)**	**Placebo (n=31)**	**SM/SNB (n=137)**	**AC (n=65)**
Female sex	98 (97.0)	100 (99.0)	65 (97.0)	69 (98.6)	33 (97.1)	31 (100)	134 (97.8)	64 (98.5)
Age* (yr)	66.2 (13.3)†	62.3 (12.8)	66.2 (11.6)	62.3 (1.9)	66.2 (15.9)	62.3 (14.8)	64.2 (11.9)	64.3 (15.4)
Body mass index (kg/m^2)*	26.9 (4.9)	27.1 (4.7)	26.6 (4.7)	26.7 (4.4)	27.5 (5.2)	28.0 (5.5)	26.7 (4.5)	27.7 (5.3)
Active smoker	19 (18.8)	13 (12.9)	13 (19.4)	9 (12.9)	6 (17.6)	4 (12.9)	22 (16.1)	10 (15.4)
Recruited at Centre A	77 (76.2)	77 (76.2)	15 (22.4)	14 (20.0)	9 (26.5)	10 (32.3)	29 (21.2)	19 (29.2)
Operated by senior surgeon	50 (49.5)	50 (49.5)	36 (53.7)	37 (52.8)	14 (41.2)	13 (41.9)	73 (53.3)	27 (41.5)
Neoadjuvant treatment	32 (31.7)	41 (40.6)	14 (20.9)	20 (28.6)	18 (52.9)	21 (67.7)	34 (24.8)†	39 (60.0)
Irradiated tissue	14 (13.9)	11 (10.9)	12 (17.9)	10 (14.3)	2 (5.9)	1 (3.2)	22 (16.1)†	3 (4.6)
Perioperative anticoagulation	32 (31.7)	37 (36.6)	15 (22.3)	21 (30.0)	17 (50.0)	16 (51.6)	36 (26.3)†	33 (50.8)
Weight of breast specimen*‡ (g)	780 (450)	746 (359)	732 (454)	698 (339)	875 (432)	857 (383)	715 (398)†	866 (407)
Wound surface*‡ (cm^2)	292 (117)	279 (84)	262 (100)	258 (73)	350 (127)	328 (87)	260 ± 87†	340 (109)
Stratified according to study centre	**All cases (n=202)**		**Centre A (n=154)**		**Centre B (n=48)**		**Comparison study centre (n=202)**	
Characteristic	**TXA (n=101)**	**Placebo (n=101)**	**TXA (n=67)**	**Placebo (n=70)**	**TXA (n=34)**	**Placebo (n=31)**	**Centre A (n=154)**	**Centre B (n=48)**
Female sex	98 (97.0)	100 (99.0)	76 (98.7)	76 (98.7)	22 (91.7)	24 (100.0)	152 (98.7)	46 (95.8)
Age* (yr)	66.2 (13.3)	62.3 (12.8)	65.6 (13.6)	62.9 (13.0)	68.2 (11.3)	60.3 (12.2)	64.2 (13.3)	64.3 (12.3)
Body mass index (kg/m^2)*	26.9 (4.9)	27.1 (4.7)	26.8 (4.9)	27.1 (4.7)	27.4 (5.0)	27.1 (4.9)	26.9 (4.8)	27.2 (4.9)
Active smoker	19 (18.8)	13 (12.9)	13 (16.7)	9 (11.7)	6 (25.0)	4 (16.7)	22 (14.3)	10 (20.8)
Operated by senior surgeon	50 (49.5)	50 (49.5)	33 (42.9)	32 (41.6)	17 (70.8)	18 (75.0)	65 (42.2)†	35 (72.9)
Neoadjuvant treatment	32 (31.7)	41 (40.6)	26 (33.8)	32 (41.6)	6 (25.0)	9 (37.5)	58 (37.7)	15 (31.3)
Irradiated tissue	14 (13.9)	11 (10.9)	12 (15.6)	6 (7.8)	2 (8.3)	5 (20.8)	18 (11.7)	7 (14.6)
Perioperative anticoagulation	32 (31.7)	37 (36.6)	26 (33.8)	33 (42.9)	6 (25.0)	4 (16.7)	59 (38.3)†	10 (20.8)
Axillary clearance	34 (33.7)	31 (30.7)	21 (27.3)	25 (32.5)	9 (37.5)	10 (41.7)	46 (29.9)	19 (39.6)
Weight of breast specimen*‡ (g)	780 (450)	746 (359)	756 (389)	746 (364)	860 (608)	748 (350)	751 (376)	804 (494)
Wound surface*‡ (cm^2)	292 (117)	279 (84)	289 (114)	277 (84)	301 (126)	287 (83)	283 (100)	294 (106)

TXA = tranexamic acid, AC = axillary clearance, SM/SNB = simple mastectomy/sentinel node biopsy
Data are number (%) unless stated otherwise
*Data are mean (standard deviation)
†Significant difference
‡Axillary component included

Table S2 Patient characteristics at baseline stratified according to type of surgery at Centre A and Centre B

Centre A: Stratified according to type of surgery	**All cases (n=154)**		**SM/SNB (n=108)**		**AC (n=46)**		**Comparison surgery (n=154)**	
Characteristic	**TXA (n=77)**	**Placebo (n=77)**	**TXA (n=52)**	**Placebo (n=56)**	**TXA (n=25)**	**Placebo (n=21)**	**SM/SNB (n=108)**	**AC (n=46)**
Female sex	76 (98.7)	76 (98.7)	51 (98.1)	55 (98.2)	25 (100.0)	21 (100.0)	106 (98.1)	46 (100.0)
Age* (yr)	65.6 (13.6)	62.9 (13.0)	65.8 (11.9)	62.7 (11.8)	65.2 (16.9)	63.4 (16.1)	64.2 (11.9)	64.4 (16.4)
Body mass index (kg/m^2)*	26.8 (4.9)	27.1 (4.7)	26.3 (4.5)	26.8 (4.5)	27.9 (5.5)	28.0 (5.4)	26.5 (4.4)	28.0 (5.4)
Active smoker	13 (16.7)	9 (11.7)	8 (15.4)	6 (10.7)	5 (20.0)	3 (14.3)	14 (13.0)	8 (17.4)
Operated by senior surgeon	33 (42.9)	32 (41.6)	24 (46.2)	25 (44.6)	9 (36.0)	7 (33.3)	49 (45.4)	16 (34.8)
Neoadjuvant treatment	26 (33.8)	32 (41.6)	12 (23.1)	16 (28.6)	14 (56.0)	16 (76.2)	28 (25.9)†	30 (65.2)
Irradiated tissue	12 (15.6)	6 (7.8)	10 (19.2)	6 (10.7)	2 (8.7)	0 (0.0)	16 (14.8)	2 (4.3)
Perioperative anticoagulation	26 (33.8)	33 (42.9)	14 (26.9)	20 (35.7)	12 (48.0)	13 (61.9)	34 (31.5)†	25 (54.3)
Weight of breast specimen*‡ (g)	756 (389)	746 (364)	686 (347)	698 (339)	900 (439)	874 (404)	692 (341)†	888 (418)
Wound surface*‡ (cm^2)	289 (114)	277 (84)	254 (89)	253 (72)	363 (127)	338 (86)	254 (80)†	352 (110)

Centre B: Stratified according to type of surgery	**All cases (n=48)**		**SM/SNB (n=29)**		**AC (n=19)**		**Comparison surgery (n=48)**	
Characteristic	**TXA (n=24)**	**Placebo (n=24)**	**TXA (n=15)**	**Placebo (n=14)**	**TXA (n=9)**	**Placebo (n=10)**	**SM/SNB (n=29)**	**AC (n=19)**
Female sex	22 (91.7)	24 (100.0)	14 (93.3)	14 (100.0)	8 (88.9)	10 (100.0)	28 (96.6)	18 (94.7)
Age* (yr)	68.2 (11.3)†	60.3 (12.2)	67.6 (10.8)	60.6 (12.8)	69.1 (12.9)	59.9 (12.0)	64.2 (12.1)	64.3 (13.0)
Body mass index (kg/m^2)*	27.4 (5.0)	27.1 (4.9)	27.9 (5.6)	26.5 (4.2)	26.6 (4.2)	28.0 (5.8)	27.2 (4.9)	27.3 (5.0)
Active smoker	6 (25.0)	4 (16.7)	5 (33.3)	3 (21.4)	1 (11.1)	1 (10.0)	8 (27.6)	2 (10.5)
Operated by senior surgeon	17 (70.8)	18 (75.0)	12 (80.0)	12 (85.7)	5 (55.6)	6 (60.0)	24 (82.6)	11 (57.9)
Neoadjuvant treatment	6 (25.0)	9 (37.5)	2 (13.3)	4 (28.6)	4 (44.4)	5 (50.0)	6 (20.7)	9 (47.4)
Irradiated tissue	2 (8.3)	5 (20.8)	2 (13.3)	4 (28.6)	0 (0.0)	1 (10.0)	6 (20.7)	1 (5.3)
Perioperative anticoagulation	6 (25.0)	4 (16.7)	1 (6.7)	1 (7.1)	5 (55.6)	3 (30.0)	2 (6.7)†	8 (42.1)
Weight of breast specimen*‡ (g)	860 (608)	748 (350)	893 (705)	695 (351)	805 (431)	822 (354)	798 (562)	813 (381)
Wound surface*‡ (cm^2)	301 (126)	287 (83)	292 (129)	274 (79)	314 (127)	305 (88)	284 (106)	310 (105)

TXA = tranexamic acid, AC = axillary clearance, SM/SNB = simple mastectomy/sentinel node biopsy
Data are number (%) unless stated otherwise
*Data are mean (standard deviation)
†Significant difference
‡Axillary component included

Table S3 Primary and secondary outcome measures stratified for type of surgery – crude data

	All cases (n=202)		SM/SNB (n=137)		AC (n=65)	
Primary outcome	**TXA (N=101)**	**Placebo (N=101)**	**TXA (N=67)**	**Placebo (N=70)**	**TXA (N=34)**	**Placebo (N=31)**
Drain production first 24h (ml)						
Mean (SD)	110 (67)	144 (113)	81 (47)	118 (110)	167 (64)	205 (96)
95% CI	97 to 123	122 to 167	70 to 93	91 to 144	145 to 190	170 to 240
Secondary outcomes*	**TXA (n=100)**	**Placebo (n=94)**	**TXA (n=66)**	**Placebo (n=67)**	**TXA (n=34)**	**Placebo (n=27)**
Early hematoma*	1 (1.0)	7 (6.9)	1 (1.5)	3 (4.3)	0 (0.0)	4 (12.9)
Total drain production (ml)						
Mean (SD)	190 (223)	214 (242)	98 (79)	130 (97)	368 (295)	423 (349)
95% CI	145 to 234	165 to 264	78 to 117	106 to 154	265 to 471	285 to 561
Days with drain (d)						
Mean (SD)	1.7 ± 1.2	1.8 ±1.1	1.3 (0.6)	1.4 (0.6)	2.5 (1.6)	2.9 (1.5)
95% CI	1.4 to 1.9	1.6 to 2.1	1.1 to 1.4	1.3 to 1.6	1.9 to 3.0	2.3 to 3.5
Drain removal after first 24h	65 (65.0)	47 (50.0)	52 (78.8)	44 (65.7)	13 (38.2)	3 (11.1)
Drain volume last 24h (ml)						
Mean (SD)	93 (57)	91 (54)	73 (43)	80 (42)	132 (61)	119 (69)
95% CI	82 to 105	80 to 102	63 to 84	70 to 90	111 to 153	92 to 147
Late hematoma	3 (3.0)	2 (2.1)	2 (3.0)	2 (3.0)	1 (2.9)	0 (0.0)
Postoperative infection/wound rupture	10 (10.0)	8 (8.5)	5 (7.6)	5 (7.5)	5 (14.7)	3 (11.1)
Thromboembolic event	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Seroma†	**TXA (n=87)**	**Placebo (n=84)**	**TXA (n=59)**	**Placebo (n=60)**	**TXA (n=28)**	**Placebo (n=24)**
Number of seroma aspirations						
Mean (SD)	3.2 (4.0)	2.6 (3.1)	1.9 (1.7)	2.3 (2.7)	5.9 (5.9)	3.3 (4.0)
95% CI	2.4 to 4.1	1.9 to 3.2	1.5 to 2.4	1.6 to 3.0	3.6 to 8.2	1.6 to 5.0
Cumulative seroma volume (ml)						
Mean (SD)	688 (971)	429 (690)	326 (370)	295 (406)	1450 (1350)	762 (1064)
95% CI	481 to 895	279 to 5	230 to 422	191 to 400	926 to 1973	313 to 1211
Seroma aspiration was needed	69 (79.3)	56 (66.6)	44 (74.6)	41 (68.3)	25 (89.3)	15 (62.5)
≥5 seroma aspirations	19 (21.8)	17 (20.2)	6 (10.2)	10 (16.7)	13 (46.4)	7 (29.2)
Cumulative seroma ≥500 ml	34 (39.1)	20 (23.8)	13 (22.0)	11 (18.3)	21 (75.0)	9 (37.5)
Chronic seroma	6 (6.9)	11 (13.1)	2 (3.4)	9 (15.0)	4 (14.3)	2 (8.3)

TXA = tranexamic acid

Data are number (%) unless stated otherwise.

*Patients with early hematoma calculated from all patients; patients with early hematoma excluded from other secondary postoperative outcome registrations.

†Patients with early or late hematoma, infection or wound rupture excluded from seroma registrations.

Table S4 Centre A. Primary and secondary outcome measures stratified for type of surgery – crude data

	All cases		SM/SNB		AC	
Primary outcome	**TXA (n=77)**	**Placebo (n=77)**	**TXA (n=52)**	**Placebo (n=56)**	**TXA (n=25)**	**Placebo (n=21)**
Drain production first 24h (ml)						
Mean (SD)	119 (68)	157 (121)	89 (48)	131 (117)	182 (60)	223 (107)
95% CI	104 to 135	130 to 185	75 to 102	101 to 164	156 to 207	175 to 272
Secondary outcomes*	**TXA (n=77)**	**Placebo (n=70)**	**TXA (n=52)**	**Placebo (n=53)**	**TXA (n=25)**	**Placebo (n=17)**
Early hematoma*	0 (0.0)	7 (9.1)	0 (0.0)	3 (5.4)	0 (0.0)	4 (19.0)
Total drain production (ml)						
Mean (SD)	181 (211)	230 (268)	97 (68)	143 (98)	355 (290)	501 (418)
95% CI	133 to 229	166 to 294	79 to 116	116 to 170	236 to 475	286 to 716
Days with drain (d)						
Mean (SD)	1.4 (0.9)	1.8 (1.1)	1.1 (0.3)	1.4 (0.6)	2.1 (1.4)	2.8 (1.6)
95% CI	1.2 to 1.6	1.5 to 2.0	1.0 to 1,2	1.3 to 1.6	1.5 to 2.6	2.0 to 3.7
Drain removed at 24h	59 (76.6)	37 (52.9)	47 (90.4)	34 (64.2)	12 (48.0)	3 (17.6)
Drain volume last 24h before removal (ml)						
Mean (SD)	103 (56)	101 (52)	82 (43)	88 (41)	147 (56)	141 (64)
95% CI	91 to 116	89 to 114	71 to 94	77 to 100	124 to 170	108 to 174
Late hematoma	3 (3.9)	2 (2.9)	2 (3.8)	2 (3.8)	1 (4.0)	0 (0.0)
Postoperative infection/wound rupture	8 (10.4)	7 (10.0)	3 (5.8)	4 (7.5)	5 (20.0)	3 (17.6)
Thromboembolic event	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Seroma†	**TXA (n=66)**	**Placebo (n=61)**	**TXA (n=47)**	**Placebo (n=47)**	**TXA (n=19)**	**Placebo (n=14)**
Number of seroma aspirations						
Mean (SD)	3.4 (4.0)	2.9 (3.4)	2.0 (1.8)	2.5 (2.9)	6.7 (5.8)	4.4 (4.5)
95% CI	2.4 to 4.4	2.1 to 3.8	1.5 to 2.6	1.6 to 3.4	3.9 to 9.5	1.8 to 6.9
Cumulative seroma volume (ml)						
Mean (SD)	739 (983)	502 (770)	342 (386)	324 (437)	1723 (1295)	1102 (1250)
95% CI	498 to 981	305 to 700	229 to 455	195 to 452	1099 to 2347	381 to 1824
Seroma aspiration was needed	53 (80.3)	43 (70.5)	34 (72.3)	33 (70.2)	19 (100.0)	10 (71.4)
≥5 seroma aspirations	17 (25.8)	16 (26.2)	6 (12.8)	10 (21.3)	11 (57.9)	6 (42.9)
Cumulative seroma ≥500 ml	28 (42.2)	17 (27.9)	11 (23.4)	10 (21.3)	17 (89.5)	7 (50.0)
Chronic seroma	5 (7.6)	10 (16.4)	2 (4.3)	9 (19.1)	3 (15.8)	1 (7.1)

TXA = tranexamic acid

Data are number (%) unless stated otherwise.

*Patients with early hematoma calculated from all patients; patients with early hematoma excluded from other secondary postoperative outcome registrations.

†Patients with early or late hematoma, infection or wound rupture excluded from seroma registrations.

Table S5 Centre B. Primary and secondary outcome measures stratified for type of surgery – crude data

	All Cases		SM/SNB		AC	
Primary outcome	**TXA (n=24)**	**Placebo (n=24)**	**TXA (n=15)**	**Placebo (n=14)**	**TXA (n=9)**	**Placebo (n=10)**
Drain production first 24h (ml)						
Mean (SD)	81 (55)	102 (66)	55 (31)	57 (28)	125 (60)	166 (49)
95% CI	58 to 105	74 to 130	38 to 72	41 to 73	79 to 171	131 to 200
Secondary outcomes*	**TXA (n=23)**	**Placebo (n=24)**	**TXA (n=14)**	**Placebo (n=14)**	**TXA (n=9)**	**Placebo (n=10)**
Early hematoma*	1 (4.2)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)
Total drain production (ml)						
Mean (SD)	218 (264)	168 (137)	98 (114)	80 (77)	403 (326)	290 (102)
95% CI	104 to 332	110 to 225	33 to 164	35 to 124	153 to 654	217 to 364
Days with drain (d)						
Mean (SD)	2.5 (1.6)	2.0 (1.2)	1.9 (1.0)	1.4 (0.6)	3.6 (1.7)	3.0 (1.2)
95% CI	1.9 to 3.2	1.5 to 2.6	1.3 to 2.5	1.0 to 1.7	2.2 to 4.9	2.2 to 3.8
Drain removed at 24h	6 (26.1)	10 (41.7)	5 (35.7)	10 (71.4)	1 (11.1)	0 (0.0)
Drain volume last 24h before removal (ml)						
Mean (SD)	60 (47)	62 (47)	39 (20)	47 (23)	92 (58)	83 (63)
95% CI	40 to 80	42 to 81	27 to 51	33 to 60	47 to 137	38 to 127
Late hematoma	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Postoperative infection/wound rupture	2 (8.7)	1 (4.2)	2 (14.3)	1 (7.1)	0 (0.0)	0 (0.0)
Thromboembolic event	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Seroma†	**TXA (n=21)**	**Placebo (n=23)**	**TXA (n=12)**	**Placebo (n=13)**	**TXA (n=9)**	**Placebo (n=10)**
Number of seroma aspirations						
Mean (SD)	2.7 (4.1)	1.6 (2.0)	1.7 (1.1)	1.4 (1.4)	4.1 (6.1)	1.8 (2.6)
95% CI	0.8 to 4.6	0.7 to 2.4	1.0 to 2.3	0.5 to 2.2	0.0 to 8.8	0.0 to 3.7
Cumulative seroma volume (ml)						
Mean (SD)	525 (936)	234 (349)	265 (307)	194 (254)	872 (1350)	286 (455)
95% CI	99 to 951	83 to 385	69 to 460	41 to 347	0 to 1910	0 to 611
Seroma aspiration was needed	16 (76.2)	13 (56.5)	10 (83.3)	8 (61.5)	6 (66.6)	5 (50.0)
≥5 seroma aspirations	2 (9.5)	1 (4.3)	0 (0.0)	0 (0.0)	2 (22.2)	1 (10.0)
Cumulative seroma ≥500 ml	6 (28.6)	3 (13.0)	2 (16.7)	1 (7.7)	4 (44.4)	2 (20.0)
Chronic seroma	1 (4.8)	1 (4.3)	0 (0.0)	0 (0.0)	1 (11.1)	1 (10.0)

TXA = tranexamic acid
Data are number (%) unless stated otherwise.
*Patients with early hematoma calculated from all patients; patients with early hematoma excluded from other secondary postoperative outcome registrations.
†Patients with early or late hematoma, infection or wound rupture excluded from seroma registrations.

Table S6 Independent effect of patient variables as main effect in a general linear model on primary outcome

Patient variables	**Drain production first 24h Effect size (95% CI)*, partial Eta^{2} †, *P***
Tranexamic acid	-31.8% (-51.0 to -15), 0.077, *P*<0.001
Axillary clearance	80.2% (52.8 to 112.3), 0.206, *P* <0.001
Recruited at Centre A	57.1% (33.1 to 85.3), 0.131, *P* <0.001
Wound surface (cm^{2})‡	Partial Eta^{2} 0.107, *P* <0.001
Operated by senior surgeon	-16.6% (-34.6 to -1.1 %), 0.023, *P*= 0.035
Perioperative anticoagulation	10.8% (-6.7 to 31.1), 0.008, *P*= 0.226
Neoadjuvant treatment	-6.3% (-27.5 to 12.5), 0.002, *P*= 0.496
Irradiated tissue	-22.0% (-50.7 to 1.2), 0.018, *P*= 0.064
Active smoker	-3.2% (-24.9 to 12.1), 0.001, *P*= 0.738
Age‡	Partial Eta^{2} 0.001, *P*= 0.701

* Ratio between ln mean as percentage difference (95% CI).
† R squared for all above variables = 0.510
‡ Only Eta^{2} presented as effect size for continous variables

Table S7. Subgroup analyses. Adjusted primary and secondary outcomes according to type of surgery and study centre

Outcome	AC (n=65) vs. SM/SNB (n=137)	Centre A (n=154) vs. Centre B (n=48)
Primary outcome		
Drain production first 24h*†	83.7% (57.3 to 114) P<0.001	60.3% (36.1 to 88.9) P<0.001
Secondary outcomes‡		
Early hematoma‡§	2.89 (0.57 to 14.6) P=0.19	2.68 (0.31 to 23.4) P=0.373
Total drain production*†	169%, (116 to 234) P<0.001	33.5% (6.5 to 67.4) P=0.013
Days with drain*	64.2% (43.0 to 88.5) P<0.001	-29.8% (-49.2 to -13.0) P<0.001
Drain removed at 24 h‡	0.17 (0.08 to 0.38) P<0.001	3.90 (1.74 to 8.74) P=0.001
Drain volume last 24 h before removal*	60.5% (36.6 to 88.7) P<0.001	97.4% (67.7 to 132) P<0.001
Late hematoma/ postoperative infection/ wound rupture‡	1.01 (0.36 to 2.80) P=0.989	2.52 (0.70 to 9.05) P=0.157
Seroma¶		
Seroma aspiration needed§	1.25 (0.55 to 2.84) P=0.593	1.76 (0.81 to 3.83) P=0.154
≥5 seroma aspirations§	4.05 (1.67 to 9.85) P=0.002	8.81 (2.33 to 33.3) P=0.001
Cumulative seroma ≥500 ml§	5.61 (2.45 to 12.8) P<0.001	4.24 (1.60 to 11.3) P=0.004
Chronic seroma§	1.06 (0.33 to 3.42) P=0.929	3.59 (0.76 to 17.1) P=0.108

TXA = tranexamic acid, AC = axillary clearance, SM/SNB = simple mastectomy/sentinel node biopsy

*Ratio between ln mean as percentage difference comparing group I to group II (95% CI). Univariate general linear model adjusted for drug application, type of surgery, study centre and wound surface area as appropriate.

† Additionally adjusted for surgeon seniority

‡ Cases with early hematoma calculated from all patients; patients with early hematoma excluded from other secondary postoperative outcome registrations.

§ Odds ratio comparing group I to group II (95% CI). Logistic regression model adjusted for type of surgery, study centre and wound surface area as appropriate.

¶ Patients with early or late hematoma, infection or wound rupture excluded from seroma registrations.

Paper III

OPEN

Serum Concentrations and Pharmacokinetics of Tranexamic Acid after Two Means of Topical Administration in Massive Weight Loss Skin-Reducing Surgery

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Background: Topical administration of tranexamic acid to reduce bleeding is receiving increasing attention, as it is inexpensive, simple, and possibly beneficial in most surgery. Concerns regarding potential systemic adverse effects such as thromboembolic events and seizures may prevent general use of tranexamic acid. Although serum concentrations after topical application are assumed to be low, proper pharmacokinetic studies of tranexamic acid after topical application are lacking.

Methods: The authors have investigated systemic absorption of tranexamic acid after two means of topical administration in patients undergoing abdominoplasty after massive weight loss: a bolus of 200 ml of 5 mg/ml into the wound cavity versus moistening the wound surface with 20 ml of 25 mg/ml. Twelve patients were recruited in each group. Serum concentrations achieved were compared with those after administration of 1 g as an intravenous bolus to arthroplasty patients. Serial blood samples for tranexamic acid analysis were obtained for up to 24 hours.

Results: After intravenous administration, the peak serum concentration was 66.1 ± 13.0 μg/ml after 6 ± 2 minutes. Peak serum concentration after topical moistening was 5.2 ± 2.6 μg/ml after 80 ± 33 minutes, and in the topical bolus group, it was 4.9 ± 1.8 μg/ml after 359 ± 70 minutes. Topical moistening resulted in homogenous and predictable absorption across the individuals included, whereas topical bolus administration caused variable and unpredictable serum concentrations.

Conclusion: Topical administration of tranexamic acid in patients undergoing abdominoplasty results in low serum concentrations, which are highly unlikely to cause systemic effects. (*Plast. Reconstr. Surg.* 143: 1169e, 2019.)

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The antifibrinolytic drug tranexamic acid is routinely used for blood conservation in surgery with high risk of significant bleeding.[1] Tranexamic acid prevents clot breakdown by inhibiting the activation of plasminogen to plasmin, and intravenous use reduces bleeding and

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transfusion needs by approximately one-third.[2] Fear of unrecognized adverse effects has so far limited routine use of intravenous tranexamic acid to high-risk surgery.

A drug that prevents bleeding may also promote thrombosis.[3] However, no increased risk of vascular occlusive events has been shown after intravenous use[4–8] and this worry may be unwarranted. In contrast, increasing attention is given to reports of a tranexamic acid–associated dose-dependent increased risk of nonischemic convulsive seizures, particularly in cardiac surgery.[9–13]

In vitro studies suggest that the minimum plasma concentration that significantly inhibits fibrinolysis is approximately 5 μg/ml in children and 10 μg/ml in adults.[14–16] In clinical practice, doses vary greatly, and some regimens advocate doses causing plasma levels above 150 μg/ml.[17–20] Tranexamic acid passes the blood-brain barrier and results in cerebrospinal fluid concentrations of approximately 10% of the plasma concentrations. Tranexamic acid may cause central nervous system hyperexcitability by blocking the action of the inhibitory neurotransmitters gamma-aminobutyric acid and glycine,[21–23] and a cerebrospinal fluid concentration of 15 μg/ml has been postulated as a threshold value for a potentially excitatory effect.[22–24]

Topical application of tranexamic acid can provide adequate concentrations in the wound with a low systemic concentration and thus lessen the risk of systemic adverse events. Topical application is receiving increasing attention, as it is inexpensive and simple, and may reduce bleeding from all surgical surfaces.[25] Large studies from joint replacement surgery have confirmed that topical use of tranexamic acid reduces blood loss at least as well as intravenous administration.[26–30] Studies from cardiac and thoracic surgery are fewer, smaller, and not unambiguous.[31–38] Studies on topical use of tranexamic acid from other surgical areas have so far been scarce.[39–46]

Topical use of tranexamic acid in surgery consists mostly of administration as a bolus into a confined space or by adding it to the irrigation fluid.[26] Moistening a wound surface can be performed with a small volume with a high drug concentration,[46] whereas irrigation or local bolus administration needs larger volumes with lower drug concentrations.[26] The lowest tranexamic acid concentration that can be administered in a solution and still have a topical hemostatic effect is unknown, but concentrations below 5 mg/ml have been shown to be effective.[37,38,47] In oral and dental surgery, a high-concentration mouthwash (48 mg/ml) has been reported but is not commercially available.[43,48,49] Only a few studies have measured systemic tranexamic acid concentrations after topical use in surgery, and then mostly at a single time point, rendering peak levels uncertain and precluding a complete pharmacokinetic analysis.[33,41,50,51]

The aim of this study was to investigate the degree of systemic absorption after two means of topical routine prophylactic application in patients having large wound surface areas: (1) moistening the wound surface before closure with 20 ml of tranexamic acid 25 mg/ml[46,52] or (2) instilling a bolus of 200 ml tranexamic acid 5 mg/ml into the wound cavity retrogradely by means of drains after closure. We also compared the systemic tranexamic acid concentrations achieved by these two methods with standard intravenous prophylactic administration of 1 g of tranexamic acid in hip replacement surgery.

PATIENTS AND METHODS

Patients older than 18 years undergoing skin-reducing abdominoplasty after massive weight loss were consecutively recruited from two plastic surgical clinics in Trondheim, Norway. St. Olav's University Hospital routinely moistens the wound surfaces with 20 ml of 25 mg/ml tranexamic acid (the topical moistening group), based on a previous study from our group showing the efficacy of this method.[46] The application is demonstrated in a video.[52] Twenty milliliters is a sufficient volume to moisten even large wounds, and 25 mg/ml is unlikely to be toxic.[53,54] Aleris Medical Center instills a bolus of 200 ml of 5 mg/ml tranexamic acid mixed with local anesthesia into the wound cavity retrogradely by means of the drains after wound closure (the topical bolus group). This concentration is lower than in the topical moistening group, but 5 mg/ml has had effect in published studies.[37,47] Both clinics practice prophylactic topical tranexamic acid in all surgery, but abdominoplasties have the largest wound surfaces, which would allow for maximum absorption and thus constitute a good model for a pharmacokinetic study. Patients undergoing hip replacement surgery and routinely receiving 1 g tranexamic acid intravenously constituted the reference group (the intravenous bolus group) and were consecutively recruited from the Department of Orthopedics at St. Olav's University Hospital.

Patients were not eligible for inclusion if they (1) were pregnant or nursing, (2) had a known allergy to tranexamic acid, (3) had a known history of a thromboembolic event, or (4) had an

estimated glomerular filtration rate less than 60 ml/minute. Twelve patients were recruited in each of the three groups. The Regional Committee for Medical and Health Research Ethics in Mid Norway and the Norwegian Medicines Agency approved the study. Written informed consent was obtained from all participants.

Interventions

Age, sex, height, body weight, body mass index, serum creatinine concentration, and estimated glomerular filtration rate were registered for all participants. The weight of the resected tissue was registered for the abdominoplasty groups and the maximum width and length of the wound were measured to allow calculation of an elliptical wound surface area (in square centimeters) as $\pi \times (\text{length}/2) \times (\text{width}/2)$.

In the topical moistening group, the wound surface was moistened with 20 ml of tranexamic acid 25 mg/ml (total dose, 500 mg) after completion of hemostasis and directly before wound closure, with no further swabbing of the wound. Drains were activated after completion of the wound closure, which was at least 45 minutes after tranexamic acid application. The dose of 500 mg is half of that given in the other two groups. However, 20 ml is enough to moisten even larger surfaces,[52] and doubling the volume would only cause more spill without increasing the absorbed dose. Doubling the concentration was not done, as potential local toxic effects of 50 mg/ml are not yet clarified, and 25 mg/ml has proven efficient.[46] In the topical bolus group, 200 ml of tranexamic acid 5 mg/ml (total dose, 1 g) was instilled into the wound cavity by means of the drains after wound closure. Drains were clamped for 1 hour thereafter. In the intravenous bolus group, 1 g tranexamic acid diluted in 100 mg 0.9% sodium chloride was administered intravenous immediately before surgery.

Blood samples for the analysis of tranexamic acid were obtained before drug administration, and after 10 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, and 6 hours. The intravenous bolus group also had a sample taken at 5 minutes, whereas the two topical groups had an additional sample taken the next morning. In the topical bolus group, the last eight patients had an additional sample obtained after 8 hours, as early analyses suggested that the peak serum concentration of tranexamic acid in this group could take place later than 6 hours. All blood samples were centrifuged at 2000 relative centrifugal force for 10 minutes within 15 to 30 minutes after sampling. Thereafter, serum was pipetted off, transferred to polypropylene tubes, and stored at –80°C until analysis.

Analysis of Tranexamic Acid in Serum

Tranexamic acid concentrations in serum were determined by an ultra-high performance liquid chromatography tandem mass spectrometry method specifically developed for sensitive and precise analysis of low tranexamic acid concentrations. (**See Appendix, Supplemental Digital Content 1**, for details of the analysis of tranexamic acid in serum, ***http://links.lww.com/PRS/D466***.)

Pharmacokinetic Analysis

Maximum measured peak serum concentration and the times to achieve these concentrations were obtained directly from the measured values. Other pharmacokinetic variables were calculated using the pharmacokinetic program package Kinetica, version 5.0 (ThermoFisher Scientific, Waltham, Mass.).

Area under the time–serum concentration curve was calculated using a mixed log-linear model with extrapolation to infinity. Clearance (Cl) was calculated as dose per area under the time–serum concentration curve. By applying a noncompartment model, the parameter estimate describing the decrease of the log-concentration (λ_z) was calculated using the best-fit log-linear regression line of the samples representing the elimination phase. The elimination half-life was calculated as $\ln 2/\lambda_z$. Volume of distribution was calculated as Cl/λ_z. Mean residence time was calculated as area under the serum concentration-time product versus time curve from zero to infinity/area under the time–serum concentration curve.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Version 25 (IBM Corp., Armonk, N.Y.). Descriptive data are presented as mean ± 1 SD or median (interquartile range) as appropriate. Categorical variables were compared using Fisher's exact test, and continuous variables were compared using an independent samples *t* test. Associations between continuous variables were analyzed using the Pearson correlation coefficient. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Patient characteristics are summarized in Table 1. There were no significant differences between the two topical groups with regard to age,

Table 1. Demographic Characteristics and Pharmacokinetic Data for 36 Patients Receiving Routine Prophylactic Tranexamic Acid When Undergoing Hip Arthroplasty (Intravenous Bolus Group) and Abdominoplasty (Topical Bolus Group and Topical Moistening Group)

	Intravenous Bolus Group (1 g TXA)	Topical Bolus Group (1 g TXA)	Topical Moistening Group (500 mg TXA)
Patients			
No.	12	12	12
Male-to-female ratio	6:6	1:11	2:10
Age, yr			
Mean ± SD	62 ± 11	41 ± 11	43 ± 13
Range	45–81	25–63	22–68
Body weight, kg			
Mean ± SD	82.3 ± 17.4	74.9 ± 10.3	73.8 ± 7.3
Range	54–107	59–95	60–83
BMI, kg/m^2			
Mean ± SD	27.2 ± 5.4	25.7 ± 2.4	25.9 ± 2.7
Range	16.9–34.9	20.7–31.0	22.0–30.5
eGFR, ml/min			
Mean ± SD	98.1 ± 16.9	122.4 ± 14.7	113.1 ± 16.3
Range	74–120	103–153	85–136
Wound area, cm^2			
Mean ± SD	—	1091 ± 388	879 ± 383
Range	—	491–1802	346–1571
Pannus weight, g			
Mean ± SD	—	1420 ± 623	1630 ± 756
Range	—	635–2495	581–3065
Mean C_{max} ± SD, μg/ml	66.1 ± 13.0	4.9 ± 1.8	5.2 ± 2.6
Mean t_{max} ± SD, min	6.2 ± 2.2	359 ± 70	80 ± 33
$t_{1/2}$, min			
Mean ± SD	114 ± 12		253 ± 32
Median (IQR)		500 (415–823)*	
AUC, (μg/ml) × hr			
Mean ± SD	99.1 ± 20.0		31.3 ± 9.7
Median (IQR)		92.6 (63.7–130.8)*	
Clearance, ml/min			
Mean ± SD	174 ± 33		292 ± 96†
Median (IQR)		181 (129–263)*†	
MRT, min			
Mean ± SD	151 ± 19		377 ± 57
Median (IQR)		902 (768–1312)*	
Volume of distribution, liters			
Mean ± SD	28.5 ± 5.0		107.6 ± 38.5
Median (IQR)		186.6 (131.4–206.9)*	

TXA, tranexamic acid; BMI, body mass index; eGFR, estimated glomerular filtration rate; C_{max}, maximum (peak) serum concentration; t_{max}, time to maximum concentration; $t_{1/2}$, elimination half-life; IQR, interquartile range; AUC, area under the concentration-time curve; MRT, mean residence time.

*Median value (interquartile range) given instead of mean ± SD because the distribution was extremely skewed, with four subjects having improbably high values (improbably low values for clearance).

†Apparent clearance (i.e., Cl/F, where F is the fraction absorbed).

sex, body mass index, estimated glomerular filtration rate, weight of resected tissue, and wound surface area. Patients in the intravenous group were significantly older ($p < 0.001$), had significantly lower estimated glomerular filtration rate ($p = 0.002$), and had a more homogenous male-to-female ratio than the two topical groups combined ($p = 0.036$).

Pharmacokinetic data in the three groups are summarized in Table 1. Average serum concentrations over time are presented in Figure 1, with exact concentrations at identical time points shown in Supplemental Digital Content 2. (**See Table, Supplemental Digital Content 2**, which shows serum concentrations of tranexamic acid at comparable selected sampling times in the intravenous bolus group, the topical bolus group, and the topical moistening group. Data are presented as means ± SD. All concentrations are in micrograms per milliliter, ***http://links.lww.com/PRS/D467***.) Peak serum concentration was considerably lower and occurred later in the two topical groups than in the intravenous bolus group (Table 1). Elimination half-life and mean residence time were also longer in the two topical groups than in the intravenous bolus group (Table 1).

Serum concentration over time data for each patient are presented. [**See Figure, Supplemental Digital Content 3**, which shows individual values for serum concentration (in micrograms per

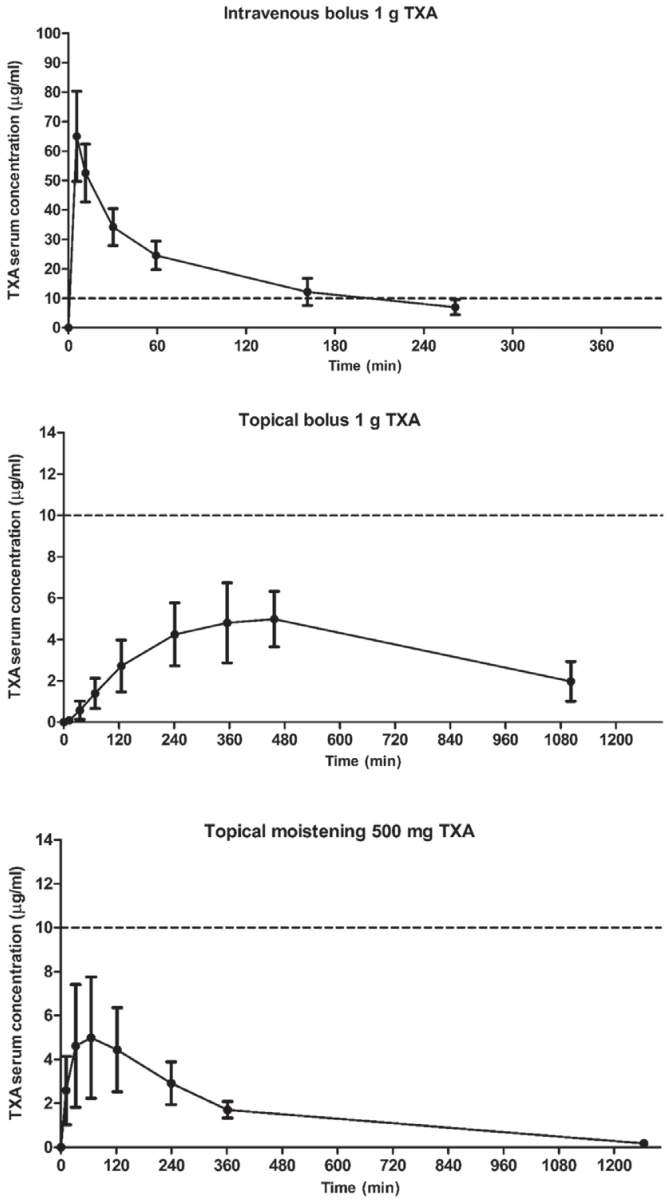

Fig. 1. Mean serum concentration versus time (minutes) after (*above*) intravenous bolus administration of 1 g of tranexamic acid (*TXA*); (*center*) topical bolus administration of 1 g of tranexamic acid; or (*below*) topical moistening with 500 mg of tranexamic acid. *Error bars* = 1 SD. The *dotted line* represents a concentration of 10 µg/ml, which is considered a threshold value for inhibition of fibrinolysis in adults. Individual patient curves are presented in Supplemental Digital Content 3 through 5.

milliliter) over time (in minutes) in 12 patients receiving intravenous bolus administration of 1 g of tranexamic acid (*TXA*), ***http://links.lww.com/PRS/D468***. **See Figure, Supplemental Digital Content 4**, which shows individual values for serum concentration (in micrograms per milliliter) over time (in minutes) in 12 patients receiving topical bolus administration of 1 g of tranexamic acid (*TXA*), ***http://links.lww.com/PRS/D469***. **See Figure, Supplemental Digital Content 5**, which shows individual values for serum concentration (in micrograms per milliliter) over time (in minutes) in 12 patients receiving topical moistening with 500 mg of tranexamic acid (*TXA*), ***http://links.lww.com/PRS/D470***.] When using a serum concentration of 10 μg/ml as a threshold for clinical antifibrinolytic effect in adults, intravenous bolus administration maintained above-threshold values for at least 150 minutes in all patients. In contrast, serum levels above 10 μg/ml were not seen in any patients in the topical bolus group and in only one patient in the topical moistening group.

Peak serum concentration was inversely correlated to body weight in the intravenous bolus group ($r = -0.695$; $p = 0.012$). In the topical moistening group, a similar albeit nonsignificant inverse correlation was seen ($r = -0.454$; $p = 0.138$), whereas no correlation was seen in the topical bolus group ($r = 0.130$; $p = 0.688$). Peak serum concentration was not correlated to wound surface area in either the topical bolus group ($r = -0.278$; $p = 0.382$) or the moistening group ($r = -0.219$; $p = 0.494$).

Adverse events were registered for the topical groups, as topical administration is still off-label. There was one postoperative hematoma in the topical moistening group that was managed conservatively, and one postoperative wound infection requiring antibiotics in the topical bolus group. There were no cases of thromboembolic events in either group.

DISCUSSION

This study demonstrates that moistening a large wound surface with a 25-mg/ml tranexamic acid solution, or instilling a bolus of 200 ml tranexamic acid 5 mg/ml into a large wound cavity, results in very low serum tranexamic acid levels compared to an intravenous bolus of 1 g of tranexamic acid. The mode of administration clearly accounts for the differences between the intravenous bolus group and the two topical groups. Although patients in the intravenous bolus group were on average 20 years older than those in the topical groups and had significantly lower estimated glomerular filtration rate, group interdiversity should not influence the general descriptive observations of this study.

The tranexamic acid concentration needed to inhibit fibrinolysis in vitro starts at approximately 10 μg/ml in adults and approximately 5 μg/ml in children.[15,16] Fibrinolysis is inhibited by more than 90 percent at tranexamic acid concentrations of approximately 20 μg/ml, and a concentration of 100 μg/ml provides a 98 percent inhibition.[14] In our study, topical application gave a mean peak serum concentration of 4.9 μg/ml in the bolus group and 5.2 μg/ml in the moistening group. The systemic antifibrinolytic effect should therefore be negligible. It would therefore appear safe to use these topical methods also in patients with increased risk of thromboembolic events[55] or at the donor sites for free flaps. However, we have not found any published studies on the effect of topical application directly onto microvascular anastomoses, and we have personally not used topical tranexamic acid at recipient sites.

Tranexamic acid passes the blood-brain barrier, reaching a concentration in cerebrospinal fluid of approximately 10% of that in plasma, although the degree of passage may vary considerably.[22,24] A plasma level of 5 μg/ml after topical application may thus cause a concentration of approximately 0.5 to 1 μg/ml in the brain.[24] As a cerebrospinal fluid concentration of at least 15 μg/ml has been necessary in experimental settings to increase the excitatory potential of tranexamic acid,[22] it is highly unlikely that a concentration of approximately 0.5 to 1 μg/ml may precipitate seizures. However, caution may be warranted should topical solutions come in direct contact with the central nervous system. Studies from topical use in spine surgery have not addressed this issue,[56] and the possibility of seizures is not common knowledge outside of the cardiac surgery community.[8] Any topical use in neurosurgery should be discouraged, as accidental intrathecal administration in humans[57–60] and direct topical application to the central nervous system in animal studies[61–63] have caused seizures.

Our findings after intravenous administration of 1 g of tranexamic acid are in accordance with earlier pharmacokinetic data.[64–67] Concentrations remained above 10 μg/ml for approximately 2.5 hours, which was well beyond the end of surgery in all patients.

The topical bolus group presented heterogeneous results both for the total amount of

absorbed drug and for its elimination (Fig. 1, *center*) (**see Figure, Supplemental Digital Content 4**, ***http://links.lww.com/PRS/D469***). Interindividual differences regarding the extent to which the drains actually eliminated the instilled fluids, patient mobility to stir up and distribute fluids, and wound cavity topography with nooks and crevices where fluid deposits reside are all factors that may add to the heterogeneity of this group. In four subjects in this group, the absorption was particularly low and irregular during the approximately 20 hours we followed them with serum concentrations measurements. Consequently, the area under the time–serum concentration curve calculations were uncertain because of a considerable degree of extrapolation; also, the elimination half-life, clearance, mean residence time, and volume of distribution values were correspondingly affected. We therefore present median values for these variables in this group in Table 1.

In the topical moistening group, tranexamic acid was smeared manually onto the wound surface. Moistening the entire wound surface was thus ensured under visual supervision, which may be beneficial for large wounds. A film is left on the wound surface and surplus volume is left to spill. We used a volume of 20 ml tranexamic acid 25 mg/ml (i.e., the total administered dose was 500 mg, which is half of the dose given to the other two groups). Abdominoplasties create large wounds, but 20 ml is still enough to moisten even larger surfaces.[52] Doubling the volume would only cause more spill without increasing the absorbed dose. We chose not to double the drug concentration for this pharmacokinetic study, as our published routine method has shown that a concentration of 25 mg/ml[46] is sufficient for an adequate clinical effect and because potential local toxic effects of higher concentrations are not yet clarified. We assume that a doubling of the drug concentration would have caused a doubling of the serum concentration, as demonstrated by Wong et al.,[51] who reported that an equal volume (100-ml) bolus of either 15 or 30 mg/ml tranexamic acid intraarticularly after knee arthroplasty resulted in serum concentrations of 4.5 μg/ml versus 8.5 μg/ml.

We crudely estimated the true net dose administered (i.e., the absorbed dose) in the topical moistening group by comparing the area under the time–serum concentration curve values in this group with the area under the time–serum concentration curve values in the intravenous bolus group, assuming that the true clearance in the two groups was the same. Mean net administered dose in the moistening group could then be estimated to be 316 ± 98 mg. Drains were not activated until at least 45 minutes after application, as closing of abdominoplasties takes time. Much of the absorption had presumably occurred at drain activation, and as the drug is applied as an evenly distributed film, little can be expected to have escaped through the drains, as time to maximum concentration had taken place already at 80 ± 33 minutes. Elimination half-life was 253 ± 32 minutes and mean residence time was 377 ± 57 minutes, with a small interindividual variability. Elimination is thus slower than after intravenous bolus administration, and also somewhat slower but comparable to the elimination reported after intramuscular injection.[68] Drug applied as a film would be expected to be quickly absorbed because of its short diffusion distance, with correspondingly little drug acting as a depot within the wound cavity. In contrast, the prolonged elimination is probably attributable to a certain extent of tissue drug deposition (e.g., subcutaneously). Unabsorbed film will be diluted by wound effusions, and whether concentrations lower than 25 mg/ml may be effective in a moistening technique is not known.

Systemic absorption of topically applied drugs is a product of concentration, contact surface area, volume, and time.[69] In our topical moistening group, neither the absorbed dose (as measured by the area under the time–serum concentration curve) nor the peak serum concentration was related to the wound surface area. One may speculate whether microstructural topographic differences or tissue vascularization may affect absorption and contact area to a larger extent than the surface area.

This study has some limitations, but also some strengths, that should be acknowledged. As intravenous tranexamic acid is not used routinely for bleeding prophylaxis in abdominoplasties and this was a descriptive study of methods already used for routine prophylaxis, we had to choose a completely different patient group (hip arthroplasties) to describe pharmacokinetics after intravenous use, with resulting differences in age, estimated glomerular filtration rate, and sex distribution.[70,71] Group interdiversity would, however, not be expected to significantly influence the general descriptive observations of this study. According to standard methodology, we have derived pharmacokinetic data from serum concentrations, but topical administration also allows for various nonbiological routes

of elimination (e.g., through the drains and into absorbent materials). We did not collect fluids from these alternative external pathways, and thus the true amount of absorbed drug is uncertain. In the topical moistening group, a collection and analysis of all absorbing material in the operating field could have been of value, whereas in the bolus group, both drain fluid analysis and not least prolonged blood sampling would have given more accurate results. Blood sampling beyond 24 hours was, however, not practically feasible in our routine surgery setting. It could also be considered a weakness that we have included only 12 patients in each group; however, such a number is generally regarded sufficient to provide a representative pharmacokinetic picture. Nevertheless, the topical bolus group could have benefited from a larger population because of the heterogeneity of the data in this group.

Strengths of the study include the frequent and timely blood sampling from the subjects (with the possible exception of the topical bolus group), allowing us to estimate reliable pharmacokinetic data. The sensitive and precise analytical method developed to accurately describe the low serum tranexamic acid levels expected from topical administration is also a significant strength. Finally, we consider it being a strength that we have studied patients with very large surgical wounds; thus, our study most likely represents a "worst case" scenario regarding drug absorption after topical administration.

Topical use of tranexamic acid is becoming widespread but is still off-label. The optimum dose and mode of administration for topical use of tranexamic acid are uncertain, and more efficacy studies are needed. Moistening of the wound surface before closure under visual and manual control ensures that a homogenous film of drug is applied to the entire wound surface. Pharmacokinetics in the topical moistening group was homogenous and predictable, and thus this mode of drug administration can be considered standardized and reproducible. When instilling a topical bolus into a closed wound cavity, the volume of the bolus must be adjusted to the size of the cavity. In large wound cavities, the bolus may reside in various locations, and contact with the entire wound surface is not ensured. This is reflected by the unpredictable and highly variable pharmacokinetics we observed in the topical bolus group. In patients with large wound cavities, we would thus advocate the use of topical moistening of the wound surface rather than topical bolus instillation.

CONCLUSIONS

In patients undergoing abdominoplasty, topical application of tranexamic acid—either with moistening with 20 ml of 25 mg/ml solution or by administration of a bolus of 200 ml of 5 mg/ml into the wound cavity—resulted in mean maximum (peak) serum concentration values of approximately 5 μg/ml, which is below the 10-μg/ml limit considered to cause any systemic antifibrinolytic effect in adults. Moreover, these concentrations are much lower than those being associated with a possible risk of seizures.

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Appendix, Supplemental Digital Content 1

Analysis of tranexamic acid in serum

Reference standard of TXA and the internal standard TXA-$^{13}C_2$,^{15}N were purchased from Toronto Research Chemicals (Toronto, Canada). For the preparation of spiked standards and quality controls, blank human serum was collected from healthy medication-free blood donors.

After thawing, automatic sample preparation was performed on Hamilton Microlab STAR, pipetting robot (Hamilton, Bonaduz, Switzerland). Aliquots of 50 µl standard, quality control, or patient sample in addition to internal standard (50 µl), were pipetted onto an Ostro^{TM} 96-well plate (Ostro Protein Precipitation & Phospholipid Removal Plate, 25 mg, Waters, Milford, MA, USA). Ice-cold acetonitrile with formic acid (1% v/v, 800 µl) was added and mixed with the sample for protein precipitation. A positive pressure unit (Positive pressure processor-96, Waters, Taunton, MA, USA) was used to facilitate the filtration of the samples in order to reduce the content of phospholipids in the eluates. The eluates were collected in 2 ml sample collecting well plate (96-well Square collection plate, Waters) and sealed with cap-mat square plugs (silicone/PTFE-treated preslit, Waters).

TXA concentrations in serum were determined by an ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method. A Waters Acquity UPLC I-class FTN system (Waters, Milford, MA, USA) equipped with Acquity UPLC HSS T3 (2.1 mm × 100 mm, 1.8 µm) column was used for chromatographic separation. Mobile phase consisted of 0.1 % formic acid in water (A) and acetonitrile (B) with a flow rate of 0.6 ml/min. The gradient run started from 1% to 20% B during the first 1.2 min and thereafter from 20%

to 90% B from 1.2 min to 1.5 min, until it was kept constant at 90% B from 1.5 to 1.9 min. From 1.91 min the composition was reset to the initial condition. Injection volume was set to 0.2 µl, and total run time was 2.5 min. Column temperature was kept at 50 °C, and autosampler temperature was set to 10 °C. A Xevo TQ-S tandem–quadrupole tandem mass spectrometry (Waters, Manchester, UK) equipped with a Z-spray electrospray interface. Positive electrospray ionization was performed in the multiple reaction monitoring (MRM) mode. The capillary voltage was set to 1.0 kV, the source block temperature was 120 °C and desolvation gas (nitrogen) was heated to 650 °C and delivered with a flow rate of 1000 L/h. Mass transitions were *m/z* 158.2 > 123.0 (cone voltage: 40 V, collision energy: 8 eV) for TXA and *m/z* 161.2 > 125.0 for the internal standard TXA-$^{13}C_2$,^{15}N (cone voltage: 40 V, collision energy: 8 eV).

The method was validated according to the US Food and Drug Administration guidelines[72]. The limit of quantification was 0.10 µg/ml and the method was linear at least up to 200 µg/ml. Recoveries were 90-97% at concentrations of 1.0 and 150 µg/ml, and between-day coefficients of variations were < 1.6% at concentrations of 0.25, 1.0 and 150 µg/ml.

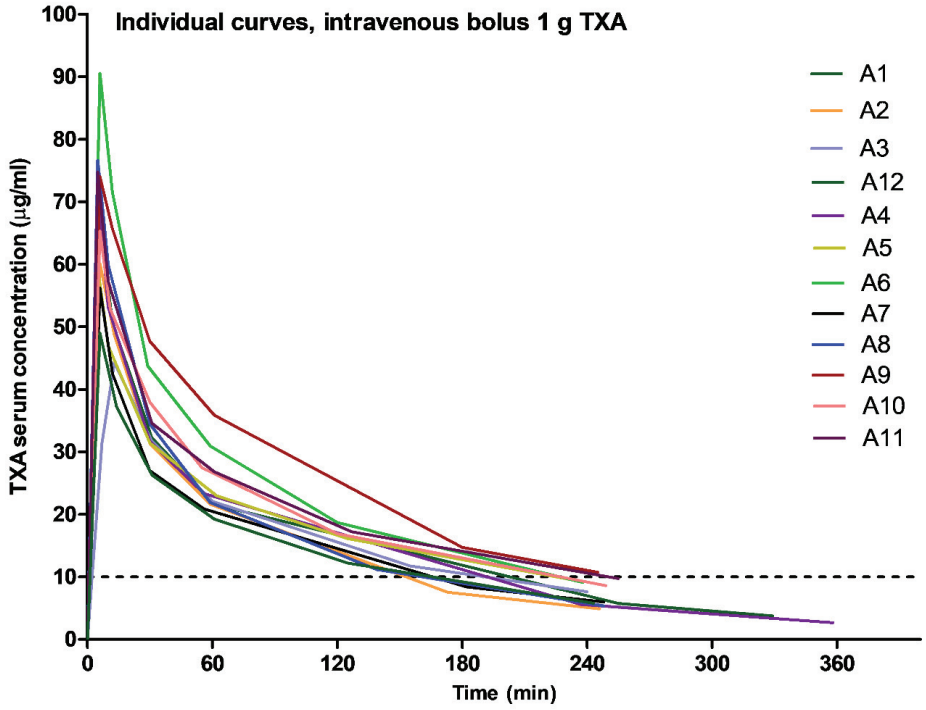
Table, Supplemental Digital Content 2. Serum concentrations of tranexamic acid at comparable selected sampling times in the intravenous bolus group, topical bolus group and the topical moistening group. Data are presented as means ± standard deviations. All concentrations are in µg/ml.

	Time since administration of tranexamic acid					
	30 minutes	1 hour	2 hours	4 hours	6 hours	Last sample[1]
Intravenous bolus group	34.2 ± 6.3	24.2 ± 4.9	12.2 ± 4.6	6.97 ± 2.58	— [2]	— [2]
Topical bolus group	0.58 ± 0.44	1.39 ± 0.73	2.72 ± 1.26	4.25 ± 1.52	4.81 ± 1.93	1.97 ± 1.34
Topical moistening group	4.58 ± 2.85	4.94 ± 2.84	4.38 ± 2.00	2.93 ± 0.96	1.75 ± 0.39	0.18 ± 0.07

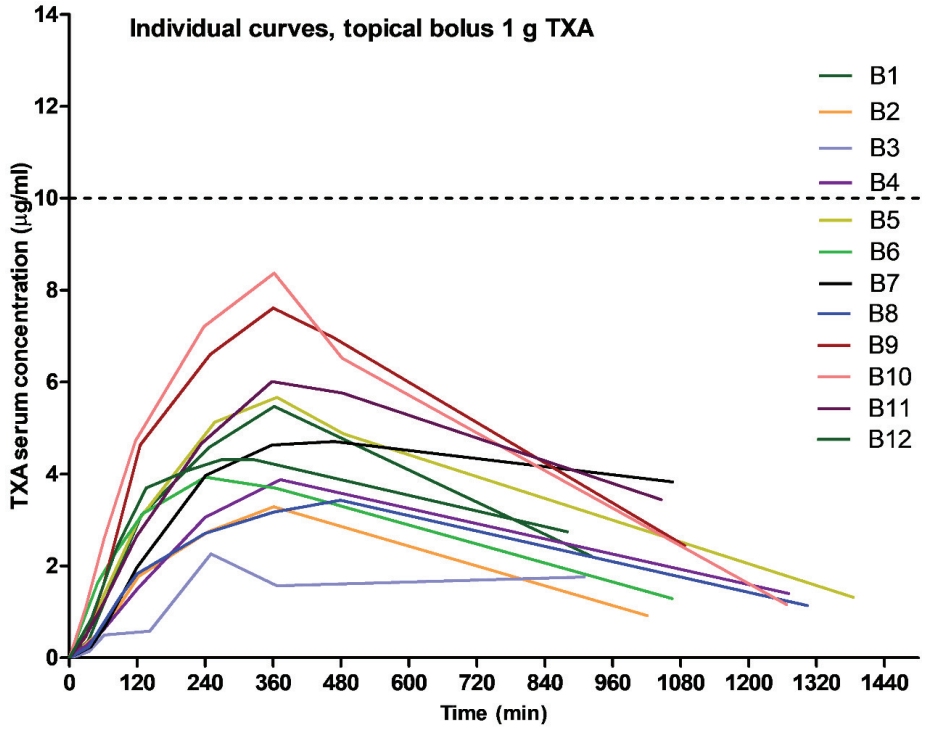
[1] Mean time from administration to sampling was 18.4 hours in the topical bolus group and 21.1 hours in the topical moistening group

[2] No more samples taken in the intravenous bolus group after 4 hours

Figure, Supplemental Digital Content 3



Figure, Supplemental Digital Content 4



Figure, Supplemental Digital Content 5