Monitoring of fibrinolytic system activity with plasminogen, D-dimers and FDP in primary total knee arthroplasty (TKA) after topical, intravenous or combined administration of tranexamic acid

Jiri Lostaka, Jiri Galioa, Ludek Slavikb, Jana Zapletalovac, Lubos Balaz

Aim. We assessed various ways of tranexamic acid (TXA) administration on the fibrinolytic system. Blood loss, transfusions, drainage and haematoma were secondary outcomes.

Methods. In this prospective study, we examined 100 patients undergoing primary total knee arthroplasty (TKA) between June and November 2018. Patients were randomly assigned to 4 groups according to the following TXA regimens: 1) loading dose 15 mg TXA/kg single intravenous administration applied at initiation of anesthesia (IV1); 2) loading dose 15 mg TXA/kg + additional dose 15 mg TXA/kg 6 h after the first application of TXA (IV2); 3) IV1 regime in combination with a local wash of 2 g of TXA in 50 mL of saline (COMB); 4) topical administration of 2 g of TXA in 50 mL of saline (TOP).

Results. Systemic fibrinolysis interference was insignificant in all of the regimens; we did not detect significant differences between IV1, IV2 and COMB in the monitored parameters within the elapsed time after the TKA; IV regimes had the lowest total drainage blood loss; the lowest blood loss was associated with the IV1 and IV2 regimens (IV1, IV2 < COMB < TOP); the lowest incidence of haematomas was in patients treated with TXA topically (i.e., in COMB + TOP).

Conclusion. The largest antifibrinolytic effect was associated with intravenous administration of TXA. In terms of blood loss, intravenously administered TXA can interfere with the processes associated with the formation of the fibrin plug more efficiently than the simple washing of wound surfaces with TXA.

Key words: tranexamic acid, total knee arthroplasty, topical application, intravenous administration, combined administration, plasminogen, D-dimers, FDP, blood loss

INTRODUCTION

Tranexamic acid (TXA) is a synthetic amino acid derivative of lysine that inhibits the conversion of plasminogen to plasmin by blocking the binding site of plasminogen to a fibrin molecule. At higher concentrations, TXA can also directly inhibit plasmin activity. It is also believed that TXA has an anti-inflammatory effect (inhibition of plasmin-mediated complement, monocyte and neutrophil inhibition). Compared with other antifibrinolytics, the effect of TXA is more potentiated by factor X (ref.4).

TXA has gradually become a routine part of perioperative care in primary total arthroplasty of the hips and knees. Therefore, the clinical utility of this intervention is unquestionable and is supported by several meta-analyses of RCTs (randomized clinical trials) for primary hips and knees. TXA can be administered by the intravenous route, orally or topically into the joint. Most studies were performed with systemic administration of TXA (ref.1). More recently, TXA has been combined with intra-articular (topical) and intravenous administration. Topical administration is justified by an effort to transfer the maximum effect to the target area and to avoid systemic effects on the fibrinolytic system. Excessive interference with the mechanism of fibrinolysis may lead to an increased risk of intravascular closures, for example, in the form of thromboembolic disease (VTE), a dreaded complication of joint replacement surgery.

The aim of our study was to evaluate the effect of 4 regimens of TXA administration on systemic fibrinolysis. Diagnosis of hyperfibrinolysis (HF) is not easy as there are currently no specific tests that allow for its assessment, but are available. In our study, we measured HF according to plasma levels in combination with determination of HF products, such as D-dimers (DDIMs) and fibrin-
fibrinogen degradation products (FDP). These tests are sensitive enough for monitoring within the time available in all coagulation laboratories.

MATERIAL AND METHODS

Patients
In this prospective study, we examined a total of 100 consecutive patients indicated for primary TKA at our clinic. They were assigned to any of the four study protocol groups based on the method of TXA administration. Each group comprised 25 patients. The baseline criteria for inclusion in the study were normal pre-operative blood count (haemoglobin, thrombocytes) and blood coagulation (INR, Quick, aPTT) parameters. Patients with any history of a blood clotting disorder, of VTE, or who had a more severe kidney disease and/or suffered from seizures were excluded. The patient groups displayed the same basic characteristics (Table 1). Prior to enrollment, patients signed a specific informed consent form. The Ethical Committee for Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc approved this study in accordance with the Helsinki Declaration (registration number 38/19).

Data Collection
The data collection was performed prospectively according to a previously agreed protocol. Medical data was collected by the physician during patient hospitalisation.

Perioperative regime
Patient preparation for TKA surgery started on the day of admission, i.e., the day before surgery. In terms of VTE prevention, we applied either low molecular weight heparin (Fraxiparine, Glaxosmithkline), which we administered subcutaneously for the first time 12 h before surgery at the dose recommended by the manufacturer, or the oral anticoagulants; IKDC – International Knee Documentation Committee; ASA – American Society of Anesthesiologists; IV1 – intravenous administration – single dose; IV2 – intravenous administration – two doses; COMB – combination of intravenous and topical administration; TOP – only topical administration; TXA – tranexamic acid.

Table 1. Comparison of TXA groups in patient demographic and clinical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>IV1 (n=25)</th>
<th>IV2 (n=25)</th>
<th>COMB (n=25)</th>
<th>TOP (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary/secondary</td>
<td>23/2 (92%/8%)</td>
<td>23/2 (92%/8%)</td>
<td>21/4 (84%/16%)</td>
<td>21/4 (84%/16%)</td>
<td>0.744</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>8/17 (32%/68%)</td>
<td>10/15 (40%/60%)</td>
<td>13/12 (52%/48%)</td>
<td>13/12 (52%/48%)</td>
<td>0.402</td>
</tr>
<tr>
<td>Average age (years)</td>
<td>71.2±7.3</td>
<td>68.6±6.4</td>
<td>67.9±7.2</td>
<td>70.1±7.6</td>
<td>0.348</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>31.2 (25.2–41.9)</td>
<td>30.7 (21.3–45.1)</td>
<td>32.0 (21.3–45.1)</td>
<td>31.2 (24.8–44.1)</td>
<td>0.884</td>
</tr>
<tr>
<td>ASA score (I/II/III/IV)</td>
<td>0/23/0/2</td>
<td>0/21/0/4</td>
<td>2/17/3/3</td>
<td>0/19/0/6</td>
<td>0.087</td>
</tr>
<tr>
<td>Anticoagulation prophylaxis</td>
<td>17/8 (68%/32%)</td>
<td>16/9 (64%/36%)</td>
<td>14/11 (56%/44%)</td>
<td>20/5 (80%/20%)</td>
<td>0.369</td>
</tr>
<tr>
<td>LMWH vs. DOAC</td>
<td>10/12/3</td>
<td>9/14/2</td>
<td>11/12/2</td>
<td>10/15/0</td>
<td>0.617</td>
</tr>
</tbody>
</table>

n – number of patients; P – significance value, <0.05 was considered statistically significant; LMWH – low molecular weight heparin; DOAC – direct oral anticoagulants; IKDC – International Knee Documentation Committee; ASA – American Society of Anesthesiologists; IV1 – intravenous administration – single dose; IV2 – intravenous administration – two doses; COMB – combination of intravenous and topical administration; TOP – only topical administration; TXA – tranexamic acid.

Operation procedure and implant
The surgery was performed under general or spinal anesthesia. For all patients, we operated from the middle skin incision and medial parapatellar approach. Bleeding was continuously stopped during TKA with electrocoagulation. A tourniquet was only applied upon cementing (usually 10–15 min). All operations were performed by experienced surgeons. We solely used cemented posterior cruciate retaining implants, respectively posterior-stabilised implants (PS version).

Study design
Each group had a precisely defined TXA protocol and dosing schedule. The first group (IV1) included patients who were administered TXA via a single intravenous dose (15 mg TXA/kg) at initiation of anaesthesia. In the second group of patients (IV2), TXA was administered in two intravenous doses (15 mg TXA/kg), at the initiation of anaesthesia and 6 hours after the start of surgery. The third group of patients (TOP) was administered TXA topically by rinsing with a diluted solution containing 2 g of TXA in 50 mL of saline. In the fourth group (COMB), TXA was administered in combination, the first 15 mg TXA/kg intravenously at the initiation of anaesthesia, and the second dose was topically rinsed with 2 g of TXA in 50 mL of saline at the end of the operation. For all patients, we left the drain closed for 1 hour after surgery. The TXA protocols are listed in Table 2.

The primary objective of the study was to determine how the different methods of TXA administration influence the systemic parameters of fibrinolysis. A secondary objective was to compare which of the tested regimens resulted in the lowest postoperative blood loss, respectively to the lowest consumption of blood transfusions and complication rates.
**Table 2. TXA administration protocols.**

<table>
<thead>
<tr>
<th>Operation room</th>
<th>Intensive care unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOP</strong> Only topical (intraarticular) administration</td>
<td>topical administration 2 g TXA + 50 mL saline solution applied by surgeon after insertion of original inlay</td>
</tr>
<tr>
<td><strong>IV1</strong> Intravenous administration single dose</td>
<td>single intravenous administration (15 mg/kg TXA) when initiating anesthesia applied by anesthesiologist</td>
</tr>
<tr>
<td><strong>IV2</strong> Intravenous administration two doses</td>
<td>first dose of intravenous administration 15 mg/kg TXA when initiating anesthesia applied by anesthesiologist</td>
</tr>
<tr>
<td><strong>COMB</strong> Combination of intravenous and topical administration</td>
<td>first dose intravenous administration (15 mg/kg TXA) when initiating anesthesia applied by anesthesiologist</td>
</tr>
</tbody>
</table>

TXA – tranexamic acid

**Monitoring parameters**
For each patient, we took the plasminogen, D-dimers, FDP and blood count according to the protocol. The first sample was performed by an anaesthesiologist at the start of anaesthesia, just before applying TXA. Other samples were taken 3, 6 and 12 h after the start of surgery.

**Blood loss including hidden blood loss**
We recorded the amount of blood loss during TKA and postoperative drainage and, respectively, the number of blood transfusions. Hidden blood loss was calculated according to a formula that includes peroperative and postoperative loss based on gender and patient weight15.

**Haemoglobin, haematocrit**
The blood count (haemoglobin, haematocrit, erythrocytes, platelets) was routinely examined on the Sysmex XN 3000 analyser (Sysmex, Kobe, Japan) at the intervals listed above, additionally on the 1st and 2nd postoperative days and, in some patients, also on the 3rd postoperative day. By having pre-operative baseline values, it was possible to determine the decrease in haemoglobin (the difference between pre-operative and postoperative value) induced by surgery.

**Fibrinolysis monitoring**
Plasminogen (Plg) was detected by the chromogenic method. Plg is measured by its specific activation upon the addition of excess streptokinase and fibrinogen without plasminogen. The plasminogen-streptokinase complex has a plasmin-like activity that specifically cleaves a plasmid-specific substrate SPm41 releasing a characteristic para-nitroaniline stain (pNA) detected at 405 nm. The rate of increase in absorbance is directly proportional to plasma plasminogen16-18.

D-dimer is formed by the cleavage of cross-linked fibrin fibres by the action of plasmin. D-dimer is detected by a suspension of polystyrene latex particles of uniform size coated with the F(ab’)2 monoclonal antibody fragment highly specific for the D-Dimer domain. The degree of agglutination is directly proportional to the concentration of D-dimer in the sample and is determined by measuring the decrease in transmitted light caused by aggregates (turbidimetric immunoassay)19-21.

FDP is detected with a suspension of polystyrene latex particles of uniform size coated with a monoclonal antibody highly specific for the D fragment included in fibrin/fibrinogen soluble derivatives. The degree of agglutination is directly proportional to the concentration of FDP in the sample and is determined by measuring the decrease of transmitted light at the wavelength of 671 nm due to aggregates (turbidimetric immunoassay)22, 23.

**Haematoma incidence, wound secretion after the 4th postoperative day**
We monitored the surgical wound healing and the presence and localisation of the haematoma. Major wound disturbances that persisted after the 4th postoperative day were recorded.

**Statistical analysis**
The distribution of quantitative data was verified by the Shapiro-Wilk’s normality test. Data that had a normal distribution was presented using mean and standard devia-
Table 3. Level of D-dimers and fibrin degradation products 3, 6 and 12 h after the start of surgery.

<table>
<thead>
<tr>
<th></th>
<th>D-dimers</th>
<th>FDP</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IV1</td>
<td>IV2</td>
</tr>
<tr>
<td>preoperative</td>
<td>537 (157–15,239)</td>
<td>402 (139–888)</td>
</tr>
<tr>
<td>3 h after the start of surgery</td>
<td>1,638 (376–6,780)</td>
<td>1,409 (411–5,516)</td>
</tr>
<tr>
<td>6 h after the start of surgery</td>
<td>3,897 (592–16,249)</td>
<td>3,610 (799–13,814)</td>
</tr>
<tr>
<td>12 h after the start of surgery</td>
<td>4,970 (836–26,270)</td>
<td>1,925 (599–13,159)</td>
</tr>
</tbody>
</table>

$P$ = significance value Kruskal-Wallis test, median (min–max); IV1 – intravenous administration – single dose; IV2 – intravenous administration – two doses; COMB – combination of intravenous and topical administration; TOP – only topical administration; TKA – total knee arthroplasty; FDP – fibrin degradation products

Fig. 1. Comparison of the level of D-dimers for each TXA group after 3, 6 and 12 h after the start of the surgery.

TXA – tranexamic acid; TKA – total knee arthroplasty

tion, and variance analysis (ANOVA) was used to verify differences between the groups. If the data did not have a normal distribution, it was described using median, minimum and maximum values. Comparison of the groups was performed by the non-parametric Kruskal-Wallis test and post-hoc Dunn test. Qualitative data was described using absolute and relative frequencies and analysed with Fisher’s exact test. The dependence between the quantitative parameters was assessed using Spearman’s correlation analysis. The Mann-Whitney U test was used to analyse the dependence between measured quantitative parameters and sex, type of arthrosis or selected VTE prevention. The IBM SPSS Statistics version 22 software was used for statistical analysis. All tests were performed at a statistical significance level of 0.05.
Table 4. Comparison of perioperative blood loss between patient TXA groups.

<table>
<thead>
<tr>
<th></th>
<th>IV1</th>
<th>IV2</th>
<th>COMB</th>
<th>TOP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total blood loss to drains (mL)</td>
<td>300 (0–1,370)</td>
<td>350 (0–1,000)</td>
<td>650 (0–1,500)</td>
<td>670 (0–1,070)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hidden blood loss (mL)</td>
<td>228 (28–619)</td>
<td>203 (13–754)</td>
<td>328 (9–690)</td>
<td>349 (84–596)</td>
<td>0.007</td>
</tr>
<tr>
<td>Blood loss during TKA</td>
<td>300 (200–500)</td>
<td>300 (200–800)</td>
<td>300 (200–700)</td>
<td>300 (100–650)</td>
<td>0.959</td>
</tr>
<tr>
<td>Total blood loss (mL), (peroper. + drainage)</td>
<td>600 (200–1,720)</td>
<td>700 (200–1,540)</td>
<td>900 (300–1,900)</td>
<td>970 (200–1,570)</td>
<td>0.009</td>
</tr>
<tr>
<td>Total blood loss to drains (mL) and hidden blood loss</td>
<td>510 (0–1,839)</td>
<td>523 (114–1,507)</td>
<td>859 (120–2,024)</td>
<td>995 (84–1,666)</td>
<td>0.003</td>
</tr>
<tr>
<td>Total blood loss (mL), (peroper. + drainage) and hidden blood loss</td>
<td>810 (300–2,339)</td>
<td>752 (326–2,294)</td>
<td>1,159 (420–2,424)</td>
<td>1,290 (284–2,166)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

P – significance value, median (min-max); IV1 – intravenous administration – single dose; IV2 – intravenous administration – two doses; COMB – combination of intravenous and topical administration; TOP – only topical administration; TKA – total knee arthroplasty

**RESULTS**

**Primary study objective – evaluation of the antifibrinolytic effect of TXA**

The pre-operative values of plasminogen, D-dimers and FDP corresponded to variability within the physiological standard (although in one patient we captured HF pre-operatively).

**Postoperative level of FDP and D-dimers**

In the course of surgery, the level of fibrinolysis fission products is increased due to tissue damage during surgery. In the first 6 hours after the start of surgery, we observed significantly lower levels of fibrin cleavage products (D-dimers, FDP) in protocols where TXA was administered intravenously or in combination with topical administration (IV1, IV2, COMB) versus topical TXA, which had the least effect on systemic inhibition of fibrin...
Fig. 3. Comparison of postoperative level of plasminogen at 3, 6, and 12 h after the start of the surgery.

Fig. 4. Range of blood loss for each TXA group.
BL – blood loss; TXA – tranexamic acid; IV1 – intravenous administration – single dose; IV2 – intravenous administration – two doses; Comb – combination of intravenous and topical administration; Topic – only topical administration
nalysis in all monitored parameters. Table 3 and Fig. 1 and 2 show the basic characteristics and comparison of the individual parameters at each recorded perioperative time.

Plasminogen

A postoperative plasminogen decrease after intravenous administration of TXA (including combined) occurred over time at each measurement, whereas in topical administration the decrease was only 6 and 12 hours after the start of surgery (Fig. 3). Nevertheless, the fibrinolytic system was never depleted.

Pre-operative plasminogen, respectively 3, 6 and 12 hours after the start of surgery, weakly correlated with age ($r=0.201$ to $0.275$), while pre-operative plasminogen weakly correlated with BMI ($r=0.258$). The level of pre-operative D-dimers weakly correlated with age ($r=0.225$). Conversely, we did not prove the effect of chosen VTE prevention or smoking at the time of surgical intervention.

Secondary objectives of the study

Blood loss including hidden blood loss

From the point of view of the amount of postoperative blood loss, a significant difference was confirmed between the observed patient groups ($P=0.002$). Patients with intravenous TXA (IV2, IV1) had the lowest total drainage blood loss compared to patients with TXA topical administration ($P=0.024$ or $P=0.026$). Intravenous administration (IV1, IV2) also significantly reduced the amount of hidden blood loss compared to topical administration alone. A significant difference in the amount of blood loss was observed 6 hours after the start of surgery ($P=0.001$) at the earliest. After 12 h, the difference in blood loss was even more pronounced ($P<0.0001$). When compared to the individual groups in the study, the lowest blood loss was achieved with IV1, IV2 $<$ COMB $<$ TOP regimens. Blood loss during TKA did not differ among the TXA regimens ($P=0.959$). A summary of the determined results is given in Table 4 and Fig. 4.

Consumption of blood transfusions

Although the differences in blood loss between the tested TXA regimens were significant, we did not find any differences in blood transfusion consumption ($P=0.410$).

Decrease in haemoglobin levels

The lowest decrease in post-operative haemoglobin levels was for combined and topical administration of TXA. Conversely, according to the value of haematocrit or platelets, the preferred regimen of TXA administration could not be identified as most beneficial.

3. Complications

There were no differences in the parameters of haematoma, wound secretion, limb swelling, early postoperative revision, or length of hospitalisation among the monitored groups.

DISCUSSION

Our study first describes the effect of different administration of TXA on the plasma levels of plasminogen, D-dimers and FDP in patients undergoing TKA surgery. From the patient’s point of view, it is most important that the treatment or prevention strategies work well, while also being safe at the same time. The first part of the claim is well documented when using TXA in the primary TKA. However, some concerns remain about the unsolicited systemic use of antifibrinolytic agents. In our study, we found that no intervention significantly interfered with processes associated with systemic fibrinolysis, although D-dimers and FDPs increased over the first 12 hours after the start of surgery and culminated around 6 hours after the start of surgery. The lowest blood loss was found in intravenous TXA regimens, which also had lower drainage. On the contrary, we did not notice differences in blood transfusion consumption.

The effect of TXA varies with its route of administration. The advantage of intravenous TXA administration is its distribution to the extracellular and intracellular space. However, this is at the cost of a higher systemic dose that might at least potentially induce VTE in some patients. A recent meta-analysis, which was aimed, among other things, at determining the safety profile of systemic and topical administration of TXA, did not reveal a higher risk of VTE in either of the analysed modes of administration. No available evaluated study showed a higher incidence of VTE compared to placebo and routine surgical haemostasis. Intravenous administration of TXA did not increase the risk of VTE, neither in patients who had already experienced this complication. Similarly, administration of TXA does not increase 30-day mortality. The explanation will probably be comprehensive. Apparently, in this case, there is a strong "balancing" effect on the area-wide prevention of VTE, which is now recommended after implantation of TKA. However, it is also important that we did not detect significant interference with the fibrinolytic system from the fibrinolysis markers point of view.

The TXA effect should depend on the dose and duration of TXA administration. Therefore, with the intravenous administration of two doses of TXA, we should expect a deeper antifibrinolytic effect with a clear impact on the overall size of blood loss, the size of hidden blood loss, or the decrease in haemoglobin levels. Although a recent meta-analysis does not report a profound effect of multiple TXA administration, randomised clinical trials clearly demonstrated the clinical utility of multiple intravenous TXA administration. As of the date of writing, only a few pharmacokinetic studies have been undertaken to assess the impact of TXA on the antifibrinolytic system in detail. In one recent study with oral TXA (ref.3), the plasma levels of TXA after oral administration achieved the threshold for the haemostatic effect (10 mg/L) at 10, 14, 18 and 22 h after surgery, and for all of the examined modes (i.e., 1, 2, 3 or 4 doses). A similar arrangement as in our study was also used by Zhang et al., who observed...
Fibrinolysis is a physiological and highly regulated response of the organism to the formation of fibrin deposits occurring during vascular wall injury. The principle of fibrinolysis is the cleavage of the fibrin network, which is the basic building block of the blood clot, by the action of the enzyme plasmin. Plasmin works as a serine protease cleaving high molecular weight fibrin polymers to fragments of varying sizes. Fibrinolysis products, either DDIM (specific fibrin fibre cleavage product) or FDP (non-specific fibrinogen and fibrin fibre cleavage products) provide us with information about the residual activity of plasmin when inhibiting with TXA. These markers may be affected by a number of other factors, ranging from basal fibrinogen levels, but also by the extent and duration of surgery. However, their change over time is already significant for the HF course in the postoperative period.

Plasminogen level monitoring is an important parameter of fibrinolysis activity blocked by TXA administration. In case of insufficient inhibition of fibrinolysis, plasma plasminogen levels decrease as a result of its conversion to plasmin, which is undesirable. Small changes in plasma levels indicate, among others, the safe effect of TXA administration. Godier et al. evaluated the in vitro plasminogen activator (t-PA) and TXA effect on blood coagulation and fibrinolysis. They state that TXA in the cascade does not affect plasmin activation, but rather inhibits fibrinolysis by protecting fibrinogen from fibrinogenolysis. Tests such as the determination of plasmin-antiplasmin complexes or alpha 2-antiplasmin assays are time-consuming and difficult to perform in everyday clinical work. The same applies to the euglobulin lysis period, long regarded as a gold standard, but also time-consuming and prone to error. In viscoelastic tests such as thromboelastometry or thrombelastography (Rote TEG), on the other hand, the HF can be detected, but with a significantly lower sensitivity compared with DDIM and FDP, and only when the level of plasmin-antiplasmin complex is very high or the alpha 2-antiplasmin level is very low.

The main effect of TXA administration is a reduction in blood loss, which should automatically lead to a less frequent indication of blood transfusions. Regarding the primary effect of TXA intervention, the recent meta-analysis shows the lowest risk of blood transfusion in combined TXA administration compared to intravenous, IV and purely local p.o. We failed to prove a significant reduction in the consumption of blood transfusions when comparing the study groups. In spite of all standardisation attempts, the indication of blood transfusion is still subject to some variability in physician decision making, and it is therefore not easy to link it with preventive strategies. Although our study was not primarily designed to demonstrate the main effect of TXA administration, we were able to detect a reduction in blood loss, which only illustrates the strength of the intervention being evaluated. Similarly, our study was not primarily focused on finding complications potentially related to TXA administration. Here we found the smallest incidence of complications with intravenous TXA regimens and a combined mode. In this case, the results correspond to large studies, respectively meta-analyses.
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REFERENCES