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WHAT'S NEW?

For the first time the impact of routine administration of protamine sulfate (PS) in patients undergoing transcatheter aortic valve implantation was assessed in a randomized controlled trial (RCT).

In this first, albeit relatively small RCT, routine administration of PS did not significantly decrease the rate of major and life-threatening bleedings.

ABSTRACT

Background: Bleeding complications after transcatheter aortic valve implantation (TAVI) negatively affect the post-procedural prognosis. Routine use of protamine sulfate (PS) to reverse unfractionated heparin after TAVI was never assessed in a randomized controlled trial.

Aims: The aim of this study was to assess the impact of PS on bleeding complications after TAVI.

Methods: Between December 2016 and July 2020 311 patients qualified to TAVI in one academic center were screened. Patients that met the inclusion criteria were randomized to either PS or normal saline administration at the moment of optimal valve deployment. Baseline, procedural and follow-up data up to 30 days were collected and analyzed. The primary endpoint (PE) was a composite of life-threatening and major bleeding according to Valve Academic Research Consortium within 48 hours after the procedure.

Results: Overall, 100 patients (48 males, median age 82 years) met the inclusion criteria and were included in the study. Forty-seven subjects (47%) were randomized to PS. The primary endpoint occurred in the 29% of the study population. Despite a numerically lower rates of PE in patients randomized to PS, a statistical significance was not reached (21% in the PS group and 36% in the placebo group; odds ratio [OR], 0.48; 95% confidence intervals [CI] 0.2–1.2; *P* = 0.11). There were no significant differences in secondary endpoints.

Conclusions: Routine protamine sulfate administration did not significantly decrease the rate of major and life-threatening bleeding complications after TAVI. Larger studies are required to assess the impact of routine PS use.

Key words: aortic, implantation, protamine, sulfate, transcatheter, valve

INTRODUCTION

Transcatheter aortic valve implantation (TAVI) is an increasingly popular treatment method of patients with severe, symptomatic aortic stenosis (AS). In a plethora of studies either a clear benefit or non-inferiority was demonstrated in comparison to gold standard — surgical aortic valve replacement — across almost all spectrums of AS patients [1]. Despite the fact, that many aspects of the antithrombotic treatment before and after TAVI have been studied, routine use of protamine sulfate (PS) to reverse the effect of the unfractionated heparin (UFH) was never included in those analyses.

Based on an expert consensus [2], UFH should be administered in every patient and should be reversed with PS after transapical and transfemoral TAVI except for transfemoral cases with minimal bleeding risk. The clinical practice, however, differs between centers [3, 4]. The impact of PS on bleeding and thromboembolic complications is unknown and reports of pro-thrombotic effect of the PS have been published in different clinical settings [5–7]. Hemorrhagic complications (at least major bleedings according to Valve Academic Research

Consortium [VARC] criteria [8]) increase mortality after TAVI [9] and are relatively frequent ranging from 4.7% up to 77% [10–12]. No randomized trials assessing the influence of PS on bleeding rates after TAVI have been published to date. In order to comply with the rule of thumb — "when in doubt, randomize" — a clinical, placebo-controlled trial is required in order to properly assess the impact of protamine sulfate administration.

METHODS

Trial design and funding

The protamine sulfate during transcatheter aortic valve implantation (PS TAVI) is a single-center, single-blind randomized placebo-controlled trial in which routine PS administration to reverse UFH was compared to placebo. The study was investigator-initiated and did not receive any funding from the industry. Informed consent was obtained from all participating patients and local ethics committee granted permission for the study (approval number KB/212/2016). The study protocol is available on ClinicalTrials.gov (NCT02974660). Study design is presented on Figure 1.

Patients, randomization and procedures

The study aimed to include patients with severe, symptomatic aortic stenosis (aortic valve area [AVA] <1.0 cm^2 or indexed valve area less than 0.6 cm^2/m^2 or mean gradient >40 mm Hg or maximum jet velocity >4.0 m/s or velocity ratio <0.25), qualified by the Heart Team to a transfemoral TAVI with a planned application of pre-close devices such as Prostar® or Proglide®. The exclusion criteria were lack of informed consent, participation in another clinical trial and known allergy to protamine sulfate.

Both mechanically- and self-expandable aortic valve prostheses of the second generation were used. In all cases transfemoral access with at least two pre-close devices was applied. The procedures were performed in hybrid operating rooms under general anesthesia or local anesthesia with conscious sedation. After obtaining the vascular access, all patients received UFH at the dose of 100 IU/kg with the target activated clotting time (ACT) of 250–300 seconds. At the moment of the optimal valve implantation, eligible patients were randomly assigned using the envelope method to either protamine sulfate or normal saline. The PS was administered in a slow bolus at the dose of 1 mg per 100 IU of unfractionated heparin administered within the last 30 minutes plus 0.5 mg per 100 IU of the UFH administered earlier. The successful reversal of heparin was confirmed by ACT measurements at baseline, after UFH boluses, before and after PS administration.

The type and number of preclose devices was noted as well as potential issues with the closure, including extravasation of the contrast in the final femoral angiography, device malfunction, need for balloon angioplasty, stent implantation or emergent surgical cut-down.

In terms of antithrombotic treatment before and after TAVI, patients without indications to chronic oral anticoagulation (OAC) were given loading doses of 300 mg of aspirin and clopidogrel within 24 hours before TAVI, and then continued 75 mg daily after the procedure. In patients requiring chronic OAC, the treatment was stopped 2–3 days before the procedure in order to obtain international normalized ratio <2 in case of vitamin K antagonists (VKA) and 1–2 days before the procedure depending on the renal function in case of non-vitamin K antagonists. After TAVI the oral anticoagulation was restarted as soon as deemed safe, with additional bridging with low-molecular weight heparin in patients receiving VKA.

Endpoints and definitions

The primary endpoint of the study was a composite of life-threatening and major bleeding complications according to VARC-2 at 48 hours after the procedure. The secondary endpoints were major and minor bleedings according to VARC-2 at 48 hours after the procedure, all-cause mortality at 30 days, drop in hemoglobin concentration 48 hours after the procedure, the length of the hospitalization (the time from the index procedure to discharge) and thromboembolic events (stroke, transient ischemic attack, myocardial infaction) within 48 hours after the procedure. Coronary artery disease was defined as the presence of at least one lesion >70% in the epicardial coronary vessel >1.5 mm (>50% for left main), history of myocardial infarction, previous percutaneous coronary intervention or coronary artery bypass grafting. Successful closure of the access artery was defined as obtaining proper haemostasis with no residual bleed, without device malfunction or a need for prolonged balloon inflation or covered stent implantation. Access-site and access-related vascular injury (ASARVI) was defined according to a modified classification by Sedaghat et al. [13]: type I, blush or minimal extravasation; type II, moderate extravasation (<5 mm); type III, major extravasation (>5 mm) including vessel perforation/rupture; and type IV, vessel dissection or occlusion.

Statistical analysis

An estimation was done based on the major and life-threatening bleeding rates from the historic material of the center (28%), that 100 patients are required to have a 90% chance of detecting a significant decrease in the primary outcome occurrence from 28% in the placebo group to 5% in the PS group. The primary analysis was performed in the intention-to-treat population.

Continuous variables, expressed as median and interquartile range (IQR), were compared between the study and control groups using Mann–Whitney U-test. Shapiro-Wilk test was used to confirm or reject normal distribution of each continuous variable. Categorical variables, expressed as counts and percentages, were compared using Chi-square test or Fisher's exact test, as appropriate.

An uni- and multivariable backwards likelihood ratio logistic regression model was used to identify predictors of the primary and secondary endpoints. Variables from the univariate analysis (with a *P* value ≤0.20 difference) were included into multivariable analysis. Results are presented as odds ratio (OR) with 95% confidence intervals (CI).

All probability values reported are 2-sided and a value <0.05 was considered to be significant. All data were processed using the SPSS software, version 22 (IBM SPSS Statistics, New York, US).

RESULTS

Population

Of the 311 consecutive patients screened between December 2016 and July 2020 in one academic center, 85 (27%) underwent TAVI via an other-than-transfemoral or transfemoral with surgical cut-down access, 78 (25%) participated in other clinical trials and 48 (15%) did not consent to participation in the study. Study flow-chart is presented on Figure 2. Overall, one hundred patients were included in the study. The median age was 82 years (IQR 77–85), there were 48 males (48%), almost 90% of patients had hypertension, 43% — diabetes and approximately one-third (36%) was in New York Heart Association (NYHA) class III or IV. Median logistic EuroSCORE was 10.5 (IQR 8–16). All the procedures were performed via a transfemoral access and in all cases a pre-close system was used. Detailed baseline data is shown in Table 1.

Protamine sulfate administration

Forty-seven subjects (47%) were randomized to protamine sulfate and 56 patients (56%) have received PS (cross-over: 9%; in all cases the reason for cross-over was due to operators' decision). Median PS dose was 35 mg or 0.5 mg per 100 IU of UFH. There were no major differences between the PS and placebo group, except for presence of moderate or severe mitral regurgitation (49% vs 30%, PS and placebo respectively, $P = 0.07$) as well as pre- and post-dilatation (pre-dilatation: PS — 45%, placebo — 68%, $P = 0.03$; post-dilation: PS — 26%, placebo — 46%, $P = 0.04$).

Primary endpoint

The primary composite endpoint of VARC-defined major and life-threatening bleeding was observed in 29 patients (29% of the study population, 21% of the PS cohort and 36% of the control group, *P* = 0.13). Major bleeding occurred in 19 patients (19%, 13% of those randomized to PS and 25% of the control group, *P* = 0.2). One disabling stroke (1%) and 2 transient ischaemic attacks (2%) were reported. The 30-day all-cause mortality was 5%. Detailed list of study endpoints is presented in Table 1.

In a multivariable analysis of the primary endpoint occurrence, only serum creatinine (OR, 2.93 per 1 mg/dl increment; CI, 0.97–8.8, *P* = 0.06) has shown a trend towards statistical significance, while the remaining parameters included in the model: female gender (OR, 2.21; CI, 0.86–5.66, *P* = 0.1) and randomization to protamine sulfate administration (OR, 0.49; CI 0.92–1.2, *P* = 0.13) did not reach significance (Table 2).

Protamine sulfate and secondary endpoints and the per-protocol analysis

Impact of randomization to PS on VARC-defined major bleeding (OR, 0.45; CI, 0.2–1.3, *P* = 0.14, Table 3), any bleeding (OR, 0.55; CI 0.2–1.27, *P* = 0.16) as well as the remaining study endpoints also did not reach statistical significance (Table 4). Results of the per-protocol analysis are presented in the Supplementary material, *Tables S1*, *S2* and *S3*.

DISCUSSION

Despite long-lasting presence of protamine sulfate in the pharmacological arsenal of peri-procedural drugs in the field of interventional cardiology, it has never been studied in a randomized fashion in the setting of TAVI. Both bleeding and thromboembolic complications may potentially arise from PS administration, with the first being a result of potential rebound anticoagulation due to PS short half-life (7 minutes as compared to UFH's 60–90 minutes), while the latter occurring due to possible rebound thrombosis after sudden UFH reversal [14]. In a study assessing UFH reversal with PS after carotid endarterectomy, a trend towards thrombosis and stroke was reported [5].

To the best of our knowledge, our study reports the results of the first ever randomized, clinical, placebo-controlled trial evaluating the impact of routine PS administration after TAVI. The trial design aimed to assess the impact of PS in the setting reflecting the majority of TAVI procedures performed worldwide — via a transfemoral access with a pre-close device.

Despite a numerically lower rate of VARC-defined life-threatening and major haemorrhagic complications in patients randomized to protamine sulfate, the bleeding reduction did not reach statistical significance. Similarly, there were no differences in terms of stroke or TIA occurrence between the PS and the placebo group, however the number of thromboembolic events was low in the study population.

Only 2 previously published papers focused on protamine sulfate administration after TAVI. In a recently published retrospective analysis of our own material (186 patients undergoing transfemoral TAVI, 44% via surgical cut-down, 21% received PS at operators' discretion) PS administration did not decrease the rate of bleeding complications [15]. Conversely, in a much larger (873 patients), single-center, prospective observational study, in which 677 patients undergoing TAVI received PS, protamine administration resulted in a significantly lower rates of life-threatening and major bleeding complications while not increasing the occurrence of stroke and myocardial infarction [16]. In said study, however, the PS administration was not randomized and left at operators' discretion in the initial phase of the study, whereas towards the end of the trial all patients received protamine, potentially introducing a selection bias as well as a confounding bias secondary to improvements in vascular access technics over time.

The reported rate of VARC-defined life-threatening and major bleeding complication (29%) remains high but is in line with the previously published results from large real-life populations of TAVI patients [4, 14, 17] — in a meta-analysis of 3519 patients, the rate of haemorrhagic sequelae ranged from 27% to 77% [12]. The tendency to suffer from bleeding complications is multifactorial in this elderly, often frail population with numerous comorbidities. Apart from obvious bleeding risks associated with the primary and secondary access sites, such as the diameter of the femoral and iliac arteries, the delivery sheath profile and the quality of the puncture and the closure, additional blood loss may arise either from gastrointestinal and urinary tracts [12] or from acquired coagulopathies, such as acquired von Willebrand factor abnormalities and heparin-induced thrombocytopenia [18]. The disproportionate rate of bleeding complications and ASARVI can potentially be explained by a blood loss occurring throughout the procedure, despite a successful haemostasis visualized during final angiography of the femoral access.

In terms of primary endpoint predictors, only a negative impact of serum creatinine was close to reach statistical significance. Renal function-dependant increase in bleeding complications after TAVI is consistent with previous reports and may be a result of an impaired metabolism of a variety of antithrombotic drugs administered before, during and after TAVI. In a study

assessing the importance of ACT-guided heparin administration in 362 patients, baseline GFR was an independent predictor of 30-day bleeding with an odds ratio of 0.96 [19].

Lack of statistical significance precludes drawing unequivocal conclusions in regard to usefulness of routine PS administration after TAVI. Perhaps, a larger, multi-center trial would provide a clear answer whether the rich historical past of protamine sulfate can translate to a great future in the TAVI world.

Limitations

Despite the obvious advantages of the randomized placebo-controlled trial, our study has a number of limitations. First and most importantly, the small sample size precluded obtaining statistically significant differences between the groups. Secondly, the cross-over rate was almost 9% and concerned patients randomized to placebo, who ended up receiving protamine. Thirdly, the endpoints were not independently adjudicated and the intervention was not blinded. On the other hand, only patients qualified to a transfemoral procedure with a pre-close device were included. This eliminated the potential bias of surgeon-dependent haemostasis present in cut-down approaches.

CONCLUSIONS

Routine protamine sulfate administration did not significantly decrease the rate of major and life-threatening bleeding complications after TAVI. Larger studies are required to assess the impact of routine PS use.

Contribution statement

Each author has contributed significantly to the submitted work. As the contribution of each co-author is concerned, their authorship include the following: 1) conception and design or analysis and interpretation of data, or both; 2) drafting of the manuscript or revising it critically for important intellectual content; 3) final approval of the manuscript submitted.

Declarations:

Funding: No funding to be reported.

Ethics approval: The study was approved by the local bioethics committee.

Consent to participate: All subject signed an informed consent prior to enrollment in the study.

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Table 1. Baseline and procedural characteristics of the study population along with endpoint rates

	Total (n = 100)	**Protamine sulfate (n = 47, 47%)**	**Placebo (n = 53, 53%)**	***P*-value**
Demographics				
Female gender, n (%)	52 (52)	25 (53)	27 (51)	0.84
Age, years, median [IQR]	81.7 [77–85]	81.8 [77–85]	81 [75–86]	0.63
Baseline characteristics				
BMI, kg/m^2, median [IQR]	26.6 [23–28]	26 [23–29]	26.6 [23–28]	0.78
BSA, m^2, median [IQR]	1.79 [1.7–1.9]	1.79 [1.7–1.9]	1.78 [1.7–1.9]	0.84
logEuroSCORE, %, median [IQR]	10.5 [7.9–16.3]	10.5 [7.8–16.3]	10.4 [8–16.9]	0.75
Hypertension, n (%)	87 (87)	41 (87)	46 (87)	1
Diabetes, n (%)	43 (43)	24 (51)	19 (36)	0.16
GFR <30 ml/min, n (%)	9 (9)	4 (9)	5 (9)	1
History of bleeding, n (%)	10 (10)	6 (13)	4 (8)	0.51
Coronary artery disease, n (%)	75 (75)	37 (79)	38 (72)	0.49
Prior cardiac surgery, n (%)	12 (12)	7 (15)	5 (9)	0.54

History of stroke/TIA, n (%)	12 (12)	4 (9)	8 (15)	0.37
Atrial fibrillation, n (%)	44 (44)	20 (43)	24 (45)	0.84
COPD, n (%)	16 (16)	9 (19)	7 (13)	0.59
Prior pacemaker implantation, n (%)	24 (24)	8 (17)	16 (30)	0.16
Oral anticoagulation, n (%)	46 (46)	22 (47)	24 (45)	1
NYHA class 3-4, n (%)	36 (36)	16 (34)	20 (38)	0.84
LVEF, %, median [IQR]	58 [53–63]	60 [55–64]	58 [53–62]	0.4
LVEF <30%, n (%)	10 (10)	6 (13)	4 (8)	0.51
Mean AV pressure gradient, mm Hg, median [IQR]	42 [34.5–50]	42 [38–50]	42 [34–50]	0.81
Aortic valve area, cm^2/m^2, median [IQR]	0.7 [0.6–0.8]	0.7 [0.6–0.8]	0.74 [0.6–0.8]	0.17
Moderate or severe MR, n (%)	39 (39)	23 (49)	16 (30)	0.07
Serum creatinine, mg/dl, median [IQR]	1.22 [1–1.5]	1.22 [1.0–1.5]	1.21 [1–1.5]	0.97
Hemoglobin, g/dL, median [IQR]	12.5 [11.4–13.6]	12.6 [11–13.6]	12.4 [11.5–13.6]	0.9
Procedural and post-precedural data				
General anaesthesia, n (%)	5 (5)	3 (6)	2 (4)	0.66
Other than femoral access, n (%)	0 (0)	0 (0)	0 (0)	1
Self-expandable prosthesis, n (%)	92 (92)	42 (89)	50 (94)	0.47
Delivery system profile >16 French, n (%)	54 (54)	24 (51)	30 (57)	0.69
Predilation, n (%)	57 (57)	21 (45)	36 (68)	0.03
Postdilation, n (%)	36 (36)	12 (26)	24 (46)	0.04
Closure device, n (%)	100 (100)	47 (100)	53 (100)	1

Number of closure devices >2, n (%)	16 (16)	9 (19)	7 (13)	0.59
Successful closure, n (%)	91 (91)	43 (92)	48 (91)	1
ASARVI ≥3, n (%)	6 (6)	3 (6.4)	3 (5.7)	1
Need for peripheral angioplasty, n (%)	15 (15)	7 (15)	8 (15)	1
UFH, IU 10^3, median [IQR]	7 [6–8]	7 [6–8]	7 [6–8]	0.83
UFH/kg, IU, median [IQR]	100 [86–117]	100 [87–117]	100 [86–117]	0.9
LVEF, %, median [IQR]	60 [52–65]	60 [55–65]	57 [51–62]	0.17
Mean AV pressure gradient, mm Hg, median [IQR]	8 [6–10]	8.5 [5.8–11]	8 [6–10]	0.72
Aortic valve area, cm^2/m^2, median [IQR]	1.9 [1.8–2.1]	1.9 [1.7–2]	1.9 [1.8–2.1]	0.52
Serum creatinine, mg/dl, median [IQR]	1.28 [1–1.5]	1.27 [1–1.5]	1.3 [1–1.6]	0.68
Hemoglobin, g/dL, median [IQR]	9.8 [8.8–10.9]	9.8 [8.8–11.1]	9.8 [8.8–10.8]	0.83
Protamine sulfate				
Randomized to protamine, n (%)	47 (47)	–	–	–
Received protamine, n (%)	56 (56)	–	–	–
Protamine dose, mg, median [IQR]	–	35 [25–50]	–	–
Protamine dose per 100 IU of UFH, mg, median [IQR]	–	0.5 [0.4–0.6]	–	–
Endpoints				
30-day all-cause mortality, n (%)	5 (5)	3 (6)	2 (4)	0.66
Life threatening bleeding, n (%)	10 (10)	4 (9)	6 (11)	0.75

Major bleeding, n (%)	19 (19)	6 (13)	13 (25)	0.20
Minor bleeding, n (%)	8 (8)	4 (9)	4 (8)	1
Need for transfusion, n (%)	26 (26)	10 (21)	16 (30)	0.37
TIA, n (%)	2 (2)	0	2 (4)	0.5
Disabling stroke, n (%)	1 (1)	0 (0)	1 (2)	1
Need for permanent pacemaker, n (%)	24 (24)	11 (23)	13 (25)	1
Moderate or severe PVL, n (%)	16 (16)	6 (13)	10 (19)	0.43
Length of stay, days, median [IQR]	8 [6–15]	7 [6–15]	9 [6–14.5]	0.25
Any bleeding, n (%)	37 (37)	14 (30)	23 (43)	0.21
Major + life threatening bleeding , n (%)	29 (29)	10 (21)	19 (36)	0.13
Major + minor bleeding, n (%)	27 (27)	10 (21)	17 (32)	0.26

Abbreviations: AR, aortic regurgitation; ASARVI, access-site or access-related vascular injury; AV, aortic valve; BMI, body mass index; BSA, body surface area; COPD, chronic obstructive pulmonary disease; GFR, glomerular filtration rate; IQR, interquartile range; LVEF, left ventricle ejection fraction; MR, mitral regurgiation; NYHA, New York Heart Association; PS, protamine sulfate; TIA, transient ischemic attack; UFH, unfractionated heparin

Table 2. Uni- and multivariable logistic regression analysis of the composite of VARC-defined major and life-threatening bleeding occurrence

	Univariate			**Multivariable**		
	OR	**CI**	**P-value**	**OR**	**CI**	**P-value**
Demographics						
Female gender	1.78	0.74–4.31	0.2	2.21	0.86–5.66	0.1
Age per 1 year	0.99	0.93–1.06	0.83			
Baseline characteristics						
BMI per kg/m^2	0.95	0.84–1.07	0.38			
Hypertension	1.42	0.36–5.59	0.62			
Diabetes	0.61	0.25–1.49	0.27			
GFR <30 ml/min	3.49	0.87–14.1	0.08	1.84	0.31–10.8	0.5
History of bleeding	0.58	0.12–2.9	0.51			
History of stroke/TIA	0.8	0.2–3.17	0.75			
Oral anticoagulation	0.77	0.32–1.84	0.55			
Serum creatinine per 1 mg/dl	2.35	0.89–6.21	0.09	2.93	0.97–8.79	0.06
Hemoglobin per 1 g/dl	1.1	0.86–1.4	0.45			
Procedural data						
General anaesthesia	1.68	0.27–10.6	0.58			
Delivery system profile >16 French	0.88	0.37–2.0.9	0.77			

Number of closure devices >2	2.19	0.73–6.59	0.16	2.27	0.68–7.5	0.18
Successful closure	0.47	0.12–1.91	0.29			
ASARVI ≥3	2.62	0.5–13.8	0.26			
Need for peripheral angioplasty	0.87	0.25–3	0.83			
UFH/kg per 1 IU	1	0.99–1.02	0.77			
Randomized to protamine	0.48	0.2–1.19	0.11	0.49	0.92–1.2	0.13
Received protamine	0.64	0.27–1.54	0.32			

Abbreviations: AR, aortic regurgitation; ASARVI, access-site or access-related vascular injury; AV, aortic valve; BMI, body mass index; BSA, body surface area; CI, confidence intervals; COPD, chronic obstructive pulmonary disease; GFR, glomerular filtration rate; LVEF, left ventricle ejection fraction; MR, mitral regurgiation; NYHA, New York Heart Association; OR, odds ratio; PS, protamine sulfate; TIA, transient ischemic attack; UFH, unfractionated heparin

Table 3. Uni- and multivariable logistic regression analysis of VARC-defined major bleeding occurrence

	Univariate			**Multivariable**		
	OR	**CI**	**P-value**	**OR**	**CI**	**P-value**
Demographics						
Female gender	1.76	0.63–4.92	0.28	1.77	0.51–5.14	0.29
Age per 1 year	2	0.9–1.1	0.82			
Baseline characteristics						
BMI per kg/m^2	1.01	0.9–1.1	0.92			
Hypertension	0.75	0.2–3	0.69			
Diabetes	0.73	0.26–2	0.55			
GFR <30 ml/min	2.3	0.5–10.4	0.26	2.6	0.57–12	0.22
History of bleeding						
History of stroke/TIA						
Oral anticoagulation	1.1	0.4–2.9	0.89			
Serum creatinine per 1 mg/dl	1.5	0.6–4	0.39			
Hemoglobin per 1 g/dl	1.02	0.8–1.4	0.87			
Procedural data						
General anaesthesia	3.1	0.5–19.8	0.24	3.6	0.53–24.5	0.19
Delivery system profile >16 French	0.55	0.2–1.5	0.31			
Number of closure devices >2	1.5	0.4–5.4	0.51			
Successful closure	0.8	0.2–4.2	0.8			
ASARVI ≥3	0.84	0.1–7.7	0.88			
Need for peripheral angioplasty	0.62	0.1–3	0.55			

UFH/kg per 1 IU	1.01	0.99–1.03	0.58			
Randomized to protamine	0.45	0.2–1.3	0.14	0.45	0.16–1.3	0.14
Received protamine	0.5	0.2–1.4	0.18			

Abbreviations: AR, aortic regurgitation; ASARVI, access-site or access-related vascular injury; AV, aortic valve; BMI, body mass index; BSA, body surface area; CI, confidence intervals; COPD, chronic obstructive pulmonary disease; GFR, glomerular filtration rate; LVEF, left ventricle ejection fraction; MR, mitral regurgiation; NYHA, New York Heart Association; OR, odds ratio; PS, protamine sulfate; TIA, transient ischemic attack; UFH, unfractionated heparin

Table 4. Impact of protamine sulfate administration on study endpoints occurrence

	OR	**CI**	***P*-value**
30-day all-cause mortality, n (%)	1.8	0.3–10.8	0.55
Life threatening bleeding, n (%)	0.73	0.2–2.76	0.64
Major bleeding, n (%)	0.45	0.2–1.3	0.14
Minor bleeding, n (%)	1.14	0.3–4.8	0.86
Need for transfusion, n (%)	0.63	0.3–1.56	0.31
TIA, n (%)	–	–	–
Disabling stroke, n (%)	–	–	–
Any bleeding, n (%)	0.55	0.2–1.27	0.16
Major + life threatening bleeding , n (%)	0.48	0.2–1.19	0.11
Major + minor bleeding, n (%)	0.57	0.2–1.42	0.23

*at 48 hours;

Abbreviations: CI, confidence interval; OR, odds ratio; TIA, transient ischemic attack

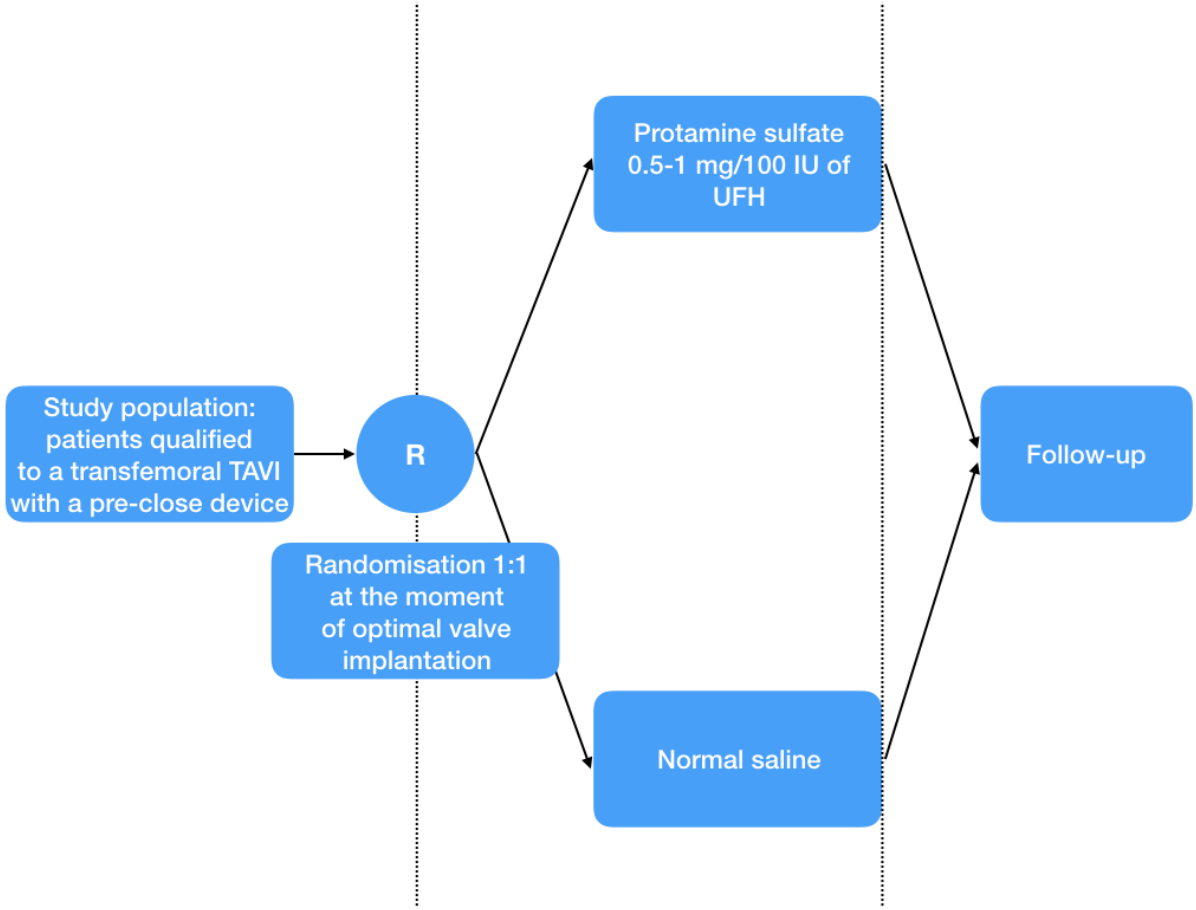

Figure 1. Study design

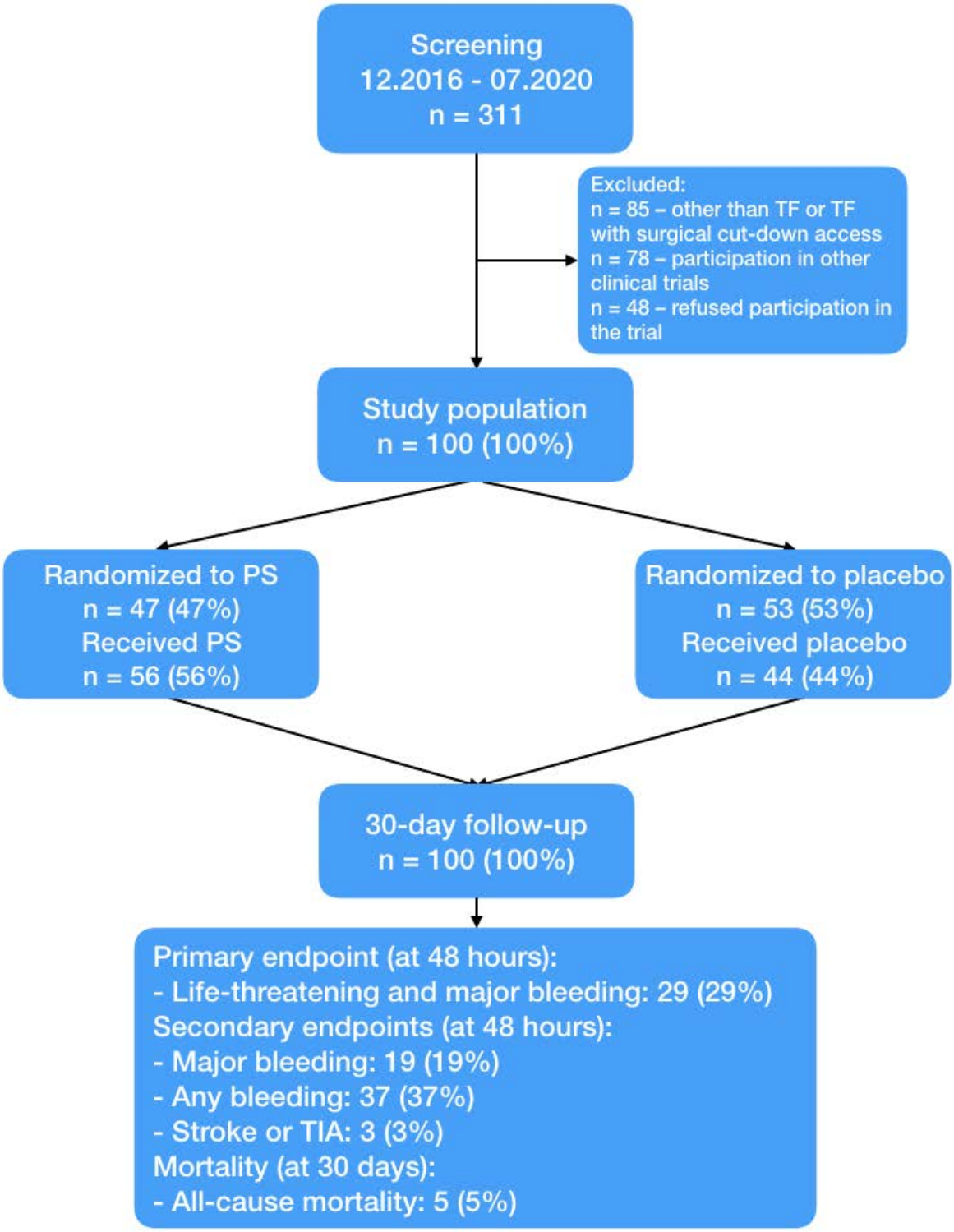

Figure 2. Study flowchart