Does acetylsalicylic acid reduce the mortality of patients admitted to an Intensive Care Unit

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A randomized, double-blind, placebo-controlled, parallel group trial in the Intensive Care Unit

Prinicipal Investigator: Bernd Jilma, Ao. Univ. Prof. Dr. med.

Email: bernd.jilma@meduniwien.ac.at

Währinger Gürtel 18-20

1090 Vienna

Austria

Tel: +43 1 40400 29810

Fax: +43 1 40400 29980

Participating Departments Department of Internal Medicine I, II, III

Department of Clinical Pharmacology

Department of Pathology

Sponsor: Medical University of Vienna

Währinger Gürtel 18-20, 1090 Vienna, Austria

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Study Synopsis

Title	Does acetylsalicylic acid (ASA) reduce the mortality of patients admitted to an intensive care unit (ICU)
Clinical Study Phase	Phase III
Project Number	ASA-MORT, EUDRA-CT#: 2012-002235-29
Study objective(s)	To investigate whether ASA-treatment reduces the mortality of a mixed medical ICU patient population; *Primary objective*: To investigate the 28-/90-day mortality of patients treated with ASA vs. placebo; *Secondary objectives*: To examine the ICU mortality of patients admitted to an ICU treated with ASA or placebo; To examine whether ASA treatment reduces the incidence of thromboembolic events; To evaluate the pharmacokinetics (PK) and the pharmacodynamics (PD) of ASA in the intensive care unit; To evaluate safety of ASA treatment assessed by post-mortem examinations (PME) with special regards to bleeding incidences;
Test drugs	intravenous ASA
Name of active ingredients	Acetylsalicylic Acid
Dose(s)	100mg
Duration of treatment	once daily for entire ICU stay
Background treatment	any concomitant treatment is allowed, the study will not interfere with standard care of patients

Rationale for the study	In a retrospective study ASA use was associated with a substantial reduction in mortality in a medical ICU population (Winning *et al.*, 2010). A stepwise logistic regression model suggested an odds ratio of 0.26 (confidence interval (CI): 0.13-0.53) in ASA treated patients (Losche *et al.*, 2012). Also animal models of sepsis demonstrated that ASA reduces mortality (Halushka *et al.*, 1981). Further ASA in certain populations reduces the risk for thromboembolic complications (Landolfi *et al.*, 2004; Thomas, 2000).
Main criteria for inclusion	-patients admitted to an ICU -patients naïve to ASA
Study design	randomized, placebo-controlled, double-blind interventional trial

METHODOLOGY

Primary variable	28-day, 90-day mortality
Secondary variables	• ICU mortality • Thromboembolic events • Bleeding incidences • PK/PD evaluation • Post-mortem examination
Plan for statistical analysis	Demographic and baseline data: • Quantitative descriptive statistics Primary variables: • Kaplan Meier estimates will be plotted • Chi²-Test for primary variable

	Secondary variables: • Comparative (treatment vs. placebo) • Cox-Regression Model including major prognostic factors
Safety assessment	Standard monitoring at the ICU
Benefit/risk assessment	Patients included in the trial will receive placebo or 100mg intravenous (i.v.) ASA. Any concomitant treatment is allowed and the trial activities will not interfere with standard care of the patients. The main side effects of ASA treatment are gastrointestinal bleeding incidences. ASA will be infused to lower adverse effects in the gastrointestinal system. Moreover, routine treatment of patients with proton pump inhibitors should reduce this risk. Patients will be closely monitored and checked for bleeding and will be withdrawn from the trial if major bleeding occurs. Winning et al. did not observe increased risk for ICU patients treated with ASA (Winning *et al.*, 2010). We hypothesize that ASA may reduce mortality of critically ill patients. Therefore, we consider the benefits to outweigh the potential risks of the trial.

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1. Background

Platelets play a central part in homeostasis and thrombosis as the primary effector cells, but they are also key cells in the regulation of the immunological response to various stressors. After activation, platelets release their granules which store different inflammatory mediators that induce coagulation, recruit further platelets, activate complement, attract neutrophils and leukocytes and regulate the vascular tone (Katz *et al.*, 2011; Peerschke *et al.*, 2008). Platelets activated by systemic inflammation and infection, may also contribute to the development of multiple organ failure (Gawaz *et al.*, 1995; Losche *et al.*, 2012). Thus, various platelet parameters have been established as clinical predictors of cardiovascular events and mortality, even in healthy volunteers (Sharma *et al.*, 2011). In critical illness a drop in platelet counts predicts a higher mortality (Arevalo-Lorido *et al.*, 2011; Baughman *et al.*, 1993; Tekbas *et al.*, 2011; Vanderschueren *et al.*, 2000).

Acetylsalicylic acid is a commonly used drug to reduce platelet activation by irreversibly inhibiting the enzyme cyclooxygenase (Paez Espinosa *et al.*, 2012). It has been demonstrated that the effects of ASA are dose dependent (Mitchell *et al.*, 1993). ASA is relatively selective for the inhibition of the enzyme cyclooxygenase-1 (COX-1). At higher doses ASA will also inhibit the inducible isoform cyclooxygenase-2 (COX-2) (Mitchell *et al.*, 1993). COX-1 is mainly found in platelets and metabolizes arachidonic acid to Thromboxane A2 (TXA2), which is a potent vasoconstrictor and has proaggregatory effects. On the other hand, COX-2 is an enzyme mainly found in the endothelium that metabolizes AA to prostacyclin (PGI2), which has anti-aggregatory and vasodilatory effects (Moncada *et al.*, 1978). The aim of low-dose therapy with ASA is to inhibit the effects of TXA2 but to maintain the effects of PGI2. The American Heart Association recommends the daily use of aspirin for all patients with cardiovascular disease for secondary prevention, unless contraindicated, at doses ranging from 71-326mg (Smith *et al.*, 2006). However, there are considerable interindividual differences in the PK/PD response to ASA treatment. “ASA resistance” or “high on treatment platelet reactivity” are at least partly explained by a worse PK profile, occur less frequently at higher doses and can be overcome by using higher doses (Neubauer *et al.*, 2011; Zimmermann *et al.*, 2008).

Rationale for the trial: Intake of anti-platelet drugs before hospitalization was associated with a substantial decrease in mortality in a mixed intensive care unit patient population. In non-surgical patients an odds ratio of 0.26 (CI 0.13-0.53) was found (Winning *et al.*, 2010). A stepwise logistic regression model suggested an odds ratio of 0.2 (CI 0.12-0.35) for critically ill patients, and an odds ratio of 0.55 (CI 0.38-0.81) for patients suffering from severe sepsis or septic shock (Losche *et al.*, 2012). Patients with community acquired pneumonia who took ASA before hospitalization were less likely to be admitted to the ICU, although they were significantly older than patients without ASA (9% vs. 26%). Additionally hospital stay was significantly shorter in the group of patients taking ASA

(Winning *et al.*, 2009). Animal studies also demonstrate that ASA reduces the mortality of mice in experimentally induced septic shock (Halushka *et al.*, 1981; Losche *et al.*, 2012).

Hypothesis: We hypothesize that ASA reduces mortality in a mixed medical ICU population.

We furthermore hypothesize that ASA leads to a reduction of the risk of suffering thromboembolic complications.

2. Study objectives

The aim of the study is to investigate potential beneficial effects of ASA on mortality of a mixed medical ICU patient population. Moreover, potential beneficial effects of ASA on the risk of suffering thromboembolic complications will be examined. Therefore patients will be randomized to receive either 100mg i.v. ASA or a placebo.

2.1. Primary end-point

Evaluation of 28- and 90-day-mortality of patients treated with ASA and patients in the control group receiving 0.9% sodium-chloride as a placebo.

2.2. Secondary end-points

- Evaluation of the ICU- mortality
- Evaluation of the PK/PD of ASA
- Incidence of thromboembolic events (symptomatic deep venous thrombosis (DVT), pulmonary embolism (PE), myocardial infarction (MCI), stroke)
- Incidence of major bleeding
- PMEs investigating the occurrence of major bleeding and thromboembolic events

3. Treatments

Commercially available products will be stored according to their product information. The doses used in this trial are commonly used.

- Acetylsalicylic acid 100mg i.v.
- 0.9% sodium-chloride i.v.

4. Methods

4.1.Study design

A randomized, double-blind, placebo controlled trial will be conducted in ASA *naïve* patients to investigate potential beneficial effects of ASA on the mortality of medical patients admitted to an ICU.

Patients will be randomized to receive 100mg ASA i.v. or 0.9% sodium-chloride i.v. as a placebo once daily. Patients will be included in the study as soon as possible but at least within 60 hours after their admittance to the ICU.

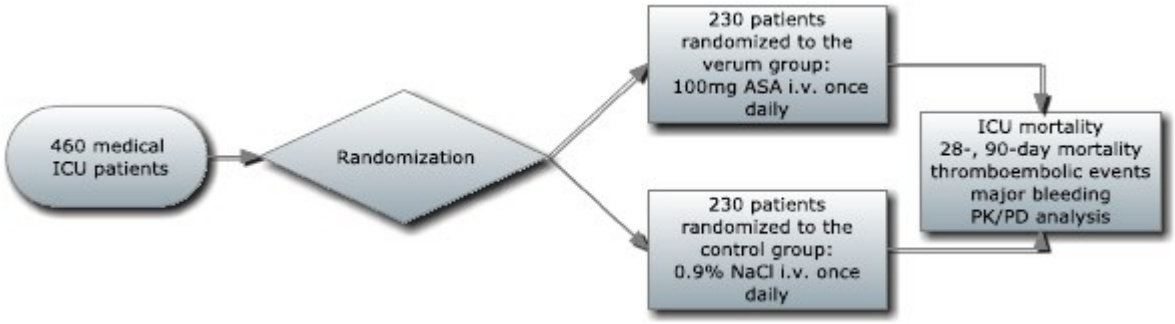
A total of 460 patients will be included in the trial (2 groups, n=230). This sample size is based on the results of a study conducted by Winning et al. examining the effects of ASA in a mixed medical ICU patient population (Winning *et al.*, 2010). As explained below (4.8.1), we performed some adaptions.

The primary variable is 28/90-day-mortality which is objectively measurable. 28-day and 90-day mortality are commonly used end-points in ICU trials and recommended by the specific guidelines of EMEA for sepsis (EMEA, 2006). 28-day and 90-day mortality will be assessed by chart review and/or telephone survey and/or review of the register of deaths. Moreover, ICU-mortality will be assessed.

PK/PD analyses will be performed to assess the efficacy of ASA treatment. We will furthermore compare data from this study with another study conducted in patients pretreated with ASA admitted to an ICU. PK/PD will be assessed on the first day of ASA treatment and analyses may be repeated during the ICU stay.

Daily physical examinations are part of the standard care of patients in ICUs. All patients included in the study will be checked daily for possible thromboembolic events or bleeding incidences. PME will be carried out for all patients included in the study who die in the course of the ICU stay. Special interest will be paid to thromboembolic events and bleeding incidences during these examinations.

4.1.1. Flow-chart



4.1.2. Rationale for the trial design

We chose to perform this trial as a randomized, double-blind, placebo-controlled trial. Winning et al. has shown potential beneficial effects of ASA treatment on mixed medical ICU patients. Although, this study was a retrospective, observational study (Winning *et al.*, 2010), this hypothesis is also supported by animal studies (Halushka *et al.*, 1981; Losche *et al.*, 2012). To our knowledge no prospective, randomized trial exists to test this hypothesis in humans.

ASA treatment reduces the incidence of thromboembolic events in patients with polycythemia vera and in patients undergoing hip surgery (Landolfi *et al.*, 2004; Thomas, 2000). Therefore we want to assess whether ASA reduces the risk of thromboembolic complications in critically ill patients. In addition to standard care, which includes a physical examination of each patient every day and laboratory control of coagulation parameters, PMEs will be performed to assess the incidence of thromboembolic events.

PK/PD analyses will be performed on the first day of treatment and may facultatively be repeated. We hypothesize that the responsiveness of patients to ASA treatment varies with the clinical condition of patients. We plan to perform another trial investigating the effects of anti-platelet drugs in non-naïve patients of ICUs.

4.1.3. Benefit/Risk Assessment

The main risk of ASA treatment is the occurrence of bleeding incidences, especially in the gastrointestinal system, allergic reactions and asthma. However, we chose to infuse ASA to reduce the risk of gastrointestinal bleeding. Moreover, routine treatment with proton pump inhibitors should reduce this risk further. Allergic reactions are possible, but generally occur seldom. The first dose will therefore be given with great care and special attention will be given to allergic reactions. However, patients with known allergies to ASA will be excluded from the trial. The trial will not otherwise interfere with standard care of the patient. Concomitant treatment will be recorded, but is

permitted. All actively bleeding patients and all patients with a planned surgical intervention will not be included in the trial. Therefore we consider the risks of the trial to be acceptable.

The studies by Winning et al. suggest a reduction of mortality for ASA treated patients admitted to ICU patients and animal studies support this hypothesis (Halushka *et al.*, 1981; Losche *et al.*, 2012; Winning *et al.*, 2010; Winning *et al.*, 2009). Moreover, ASA might reduce the risk of thromboembolic events in patients admitted to the ICU. Therefore, we consider the potential benefits to outweigh the possible risks of this trial.

4.2.Study population

All patients participating in the study are ASA *naïve*. The trial is conducted to investigate whether administration of ASA reduces the mortality in a mixed medical ICU population. Therefore we choose open inclusion criteria.

4.2.1. Inclusion criteria

- ≥18 years of age
- Admittance to an ICU

4.2.2. Exclusion criteria

- Known allergy or intolerance to ASA
- Recent surgery or planned surgery
- Active bleeding
- Known coagulation disorders
- Discretion of the physician
- Terminal illness (anticipated life expectancy <3months; e.g. due to cancer)
- Platelet count <20000
- Recent gastrointestinal bleeding
- Recent ulcera
- Pregnancy

4.2.3. Premature discontinuation

Inclusion and exclusion criteria will be checked before the first trial related activity. Patients will be withdrawn from the trial in case of a planned surgery, in case of major bleeding or at the discretion of the treating physician. All patients who are conscious and able to understand the full nature of the study will be informed about their inclusion and informed consent will be sought. Participation of the

trial is voluntary and patients may discontinue at their free will. Patients, who at the moment of study inclusion, are unconscious or unable to understand the nature and purpose of the study will be informed about their participation as soon as possible.

4.3.Randomization

A stratified randomization procedure will be used. Age, SAPS III score will be used as stratification factors, because they are well known predictors of mortality, and were independently associated with mortality in Winning's study. Furthermore prophylactic and therapeutic anti-coagulation will be used as stratification factors. A minimization procedure using software available in the web may be used:

https://www.meduniwien.ac.at/randomizer/web/login.php

Allocation concealment will be performed either by using opaque sealed randomization envelopes, which are opened only after the patient has been found eligible for the trial, or the randomization program.

4.4.Medication

Patients will be randomized to receive either 100mg i.v. ASA or 0.9% sodium-chloride as a placebo.

The most relevant possible side-effects of aspirin include the following:

- Gastrointestinal bleeding
- Asthma
- Thrombocytopenia
- Renal and hepatic impairment
- Dizziness
- Tinnitus
- Rarely, allergic reactions

No side effects are expected by the infusion of 0.9% sodium-chloride.

4.4.1. Packaging and labeling of trial medication

The final packaging size as well as labels will be defined in a note-to-file.

Labels will contain at a minimum the following information:

- Study number

- Product name
- Fill volume or dose
- Mode of administration

Product will be stored as declared in the product information. Study medication will be prepared by trained nurses or pharmacists.

4.4.2. Drug dispensing and accountability

An investigational medicinal product (=IMP) log will be kept current by the site, detailing the dates and quantities of IMP administered to each subject. This documentation will be available to the CRA to verify drug accountability during the study.

4.4.3. Blinding of medication

Blinding of medication will be ensured by dissolution of ASA and preparation of identical syringes/infusion bottles by a study nurse and/or a pharmacist.

Medication will be labeled as “Placebo (NaCl 0.9%)/ASA 100mg i.v.”.

4.5.Trial procedures

Patients included in the trial will be randomized to ASA treatment or to the control group receiving sodium-chloride. They will receive the trial drugs once daily. Inclusion in the trial will be performed as soon as possible and latest within 60 hours after their admittance to the ICU.

Beside the administration of the trial drugs we will not interfere with standard care of the patients. Any concomitant treatment is allowed.

On the first day of treatment PK/PD will be measured and may be repeated once (5-8 days later) during the ICU stay. Therefore blood samples will be drawn at the baseline as well as two hours thereafter.

The primary end-point is the 28-/90-day mortality which we will assess by chart review, telephone survey or the register of deaths. However, ICU- mortality will be assessed by chart review.

Post-mortem examination will be conducted by the department of pathology and special attention will be paid to major bleeding incidences and thromboembolic events.

4.5.1. Pharmacodynamic analysis

The efficacy of ASA will be assessed by whole blood impedance aggregometry (MEA, multiple electrode aggregometry) and by thromboxane B2 (TXB2), the stable metabolite of TXA2. A commercially available enzyme immune assay will be used to measure TXB2. Whole blood impedance aggregometry will be performed using arachidonic acid to induce aggregation. Both tests examine ASA specific effects on platelets (Kuliczkowski *et al.*, 2009). Low-dose ASA is thought to reduce TXA2 levels by inhibiting COX-1, but not to affect levels of PGI2. PGI2 is unstable and therefore the stable metabolite 6-Keto-PGF1-alpha is usually measured. We will use commercially available enzyme immune assays for assessment (Gao *et al.*, 2009; Gryglewski, 2008). Platelet function under high shear rates will be analyzed by the PFA-100 (platelet function analyzer). Closure time of an aperture in a membrane coated with either collagen or epinephrine (CEPI) or collagen and adenosine diphosphate (CADP) will be measured in whole blood. The use of the PFA-100 can not only identify patients at a high risk for ACS, but help in monitoring therapy with antiplatelet drugs as well (Derhaschnig *et al.*, 2009).

4.5.2. Pharmacokinetics

The population of interest are patients admitted to an ICU. A relatively large amount of blood and numerous blood samples would be needed to perform a thorough analysis of PK. In order to keep the amount of blood volume small, we will only perform a minimal analysis. Blood samples will be drawn at the baseline and 2h after administration. PK will be assessed on the first day and may be repeated once during the ICU stay. We will compare the PK profiles of this population with data from another study including non-ASA-naïve patients admitted to an ICU.

The PK of aspirin will be determined by plasma ASA and/or salicylate levels in the local central laboratory (Hobl et al., manuscript in preparation).

4.6.Trial schedule

4.6.1. Screening

All patients admitted to the ICUs (Dept. of Internal Medicine I-III) will be screened for in- and exclusion criteria. All patients who meet the criteria and are ASA naïve will be included in this trial and randomized to one of the groups. Patients included to the trial must receive the first dose of the trial drugs within 60 hours after their admittance.

4.6.2. Interventional phase

All patients randomized will receive the treatment by investigators blinded to the trial drugs. Special attention will be paid to the first administration of the trial drug because of possible allergic reactions. Patients will receive the trial drug every day in the course of their ICU stay.

4.6.3. PK/PD assessment

All patients randomized will be included in the PK/PD assessment. Blood samples will be drawn before and 2h after administration of the study drug. We may repeat the analyses once during the ICU stay. Analyses will be performed by blinded laboratory personnel. The investigators and the treating physicians will remain blinded.

4.6.4. Follow-up visit

We plan to assess 28-day and 90-day mortality. Therefore we will perform chart-reviews, telephone survey or check the register of deaths.

4.6.5. Post-mortem examination

All patients who die during their ICU stay will be examined by the department of pathology. Special attention will be paid to major bleeding incidences and to thromboembolic events (PE, MCI, DVT, stroke).

4.6.6. Safety assessment

Patients admitted to an ICU are closely monitored on a routine basis.

The simplified acute physiology score (SAPS III) will be recorded for each patient (Moreno *et al.*, 2005).

Concomitant treatment of each patient will be recorded.

4.6.6.1. Major bleeding

To define major bleeding we will use the criteria from the TIMI-Triton-38 study (Wiviott *et al.*, 2007). Major bleeding will be defined as bleeding incidences that lead to a decrease in hemoglobin of ≥5g/dL (each unit of packed red blood cells or whole blood transfused will count as 1g of hemoglobin), or a ≥15% absolute decrease in hematocrit (each unit of packed red blood cells or whole blood transfused will count as 3% points) or it is intracranial (confirmed by computer tomography).

All bleeding incidences that do not meet these criteria will therefore be identified as minor bleeding.

4.6.7. Blood sampling and preparation for PK/PD analysis

Blood samples will be collected from an appropriate forearm vein, an existing central venous catheter or an arterial catheter at the baseline and 2 hours after trial drug administration, after discarding the first 5 ml.

A total volume of 39 ml blood will be collected for the PK/PD analysis of one trial day.

For PK analysis Sodium-Fluoride/K3EDTA tubes will be used.

For PD assessment hirudin (MEA), buffered sodium-citrate (3.8%) (PFA-100) and serum tubes (TXB 2, 6-Keto-PGF1-alpha) will be used.

Actual sampling times for each patient will be recorded in the individual CRFs.

Preparation for PD analysis:

Hirudin (MEA), buffered sodium-citrate (3.8%) (PFA-100) and serum tubes will be used for the PD analysis. For the PFA-100, tubes will be gently inverted (3-4 times). Tubes will be stored <4 hours at ambivalent temperature (16-26˚C9) until analysis. For the Multiplate system, tubes will be gently inverted (3-4 times). Blood will not be frozen, refrigerated or preheated. Analysis of blood samples should be performed within the period of 0.5-3 hours after blood collection. Thromboxane and 6-Keto-PGF1-alpha measurements will be conducted according to the user manual of the commercially purchased TXB2 enzyme immune assay.

4.6.7.1. Labeling of samples

Each sample tube will be clearly and unequivocally identified by a label resistant to the storage temperature and reporting:

Trial ID ASA-MORT

Subject ID number XX

Time ID day X / hours XX

4.7.Evaluation Parameters

4.7.1. Study outcome parameters

4.7.1.1. Primary variable

28-/90-day mortality between the two trial groups, ASA vs. placebo

4.7.1.2. Secondary variables

- ICU-mortality
- ASA-/salicylate-serum concentrations for PK assessment
- Serum TXB2 levels and 6-keto-PGF1-alpha levels
- Area under the aggregation curve (AUC) (MEA): arachidonic acid induced aggregation
- Thromboembolic events (PE, MCI, DVT, stroke)
- Closure time (s) (PFA-100®) CEPI-CT, CADP-CT, or Innovance CT
- Major bleeding
- PME for major bleeding and thromboembolic events (PE, MCI, DVT, stroke)

4.7.1.3. Tertiary variables

Laboratory analyses

4.8.Statistical methods

4.8.1. Sample size calculation and statistical power considerations

We based our considerations for the sample size on the study conducted by Winning et al. (Winning *et al.*, 2010).

Winning et al. observed that intake of anti-platelet therapy in ICU patients of internal medicine wards was associated with a substantial decrease in mortality: HR of 0.26 (CI 0.13-0.53) in a logistic regression model and a HR of 0.36 (95% CI: 0.18-0.75) in a Chi2 test.

The current mortality rate in the placebo group at our ICUs is 20% and will probably be lower than the 30% in Winning's study.

To be more conservative, we will use the upper 95% confidence interval of the logistic regression model for the effect size, i.e. a 47% reduction in mortality. A sample size of 230 patients in each group will allow us to define the following confidence intervals when the relative risk (RR) is 0.5 (95% CI 0.31-0.79) and when the RR is 0.65 (95% CI 0.43-0.99).

Based on these data we plan an interim analysis after half of the patients (n=230).

Purpose of the interim analysis will be a re-examination of the assumptions of the mortality rate in the placebo group and re-adjustment of the sample size if the mortality rate is lower than 20% in the placebo group.

4.8.2. Stopping rules

The trial will be stopped at the time of the interim analysis if aspirin shows outstanding efficacy (>60% reduction) in mortality.

4.8.3. Feasibility of the trial

The trial will be mainly conducted in two ICUs. Approximately 500 patients are admitted to these ICUs per year. Even if approximately half of the patients will be on prior aspirin therapy before being admitted to the ICU, the remaining half will be sufficient to theoretically allow recruitment within the time frame of 2 years. However, it is planned to recruit the patient population over a period of 2.5 years accounting for the possibility that not all remaining patients may be eligible for the trial

4.8.4. Statistics of the outcome

Kaplan Meier estimates will be plotted. A Chi^2 Test will compare treatment and placebo group with regards to the primary outcome variable. A 2-tailed p-value of <0.05 in the adjusted model will be considered significant.

4.8.5. Regression Model calculation

A Cox regression model including major prognostic factors (age, SAPS III score, therapeutic vs. prophylactic anticoagulation) will be calculated for mortality data (ICU-mortality, 28-day mortality and 90-day mortality).

4.8.6. Analysis of demographic characteristics

Quantitative demographic and baseline data will be summarized using quantitative descriptive statistics such as mean, standard deviation, median, quartiles and minimum/maximum values.

4.8.7. Analysis of PK/PD outcome parameters

Comparisons between treatment groups will be performed by non-parametric tests (given their robustness when assumptions of normal distribution do not apply). Comparisons within subject will be performed by the Friedmann ANOVA, and Wilcoxon test. A 2-sided p-value of 0.05 will be considered significant.

4.8.8. Analysis of occurrence of major bleeding and thromboembolic events

Comparisons between groups will be performed by Chi2-test. A two-sided p-value of 0.05 will be considered significant.

4.9. Adverse events

4.9.1. Definitions

4.9.1.1. Adverse Event (AE)

An AE is any undesirable medical event occurring to a subject in a clinical trial, whether or not considered related to the trial product(s). This includes events not seen at baseline or worsened if present at baseline. The following should not be recorded as AEs, if recorded at screening (on Screening Form or CRF):

Pre-planned procedure, unless the condition for which the procedure was planned has worsened since baseline.

Pre-existing conditions found as a result of screening procedures.

4.9.1.2. Serious Adverse Event (SAE)

An SAE is any adverse experience that results in any of the following outcomes:

- death
- a life-threatening experience
- in-patient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the health of the subject or patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

*The term life-threatening in the definition of serious adverse event refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death if it was more severe.

4.9.1.3. Non-Serious Adverse Event

A non-serious adverse event is any AE, which doesn't fulfill the definition of an SAE.

4.9.1.4. Definition of Adverse Reaction (AR)

An AR is a noxious and unintended response to a study drug irrespective of the dose administered. All ARs are judged by either the reporting Investigator or the principal investigator as having a reasonable causal relationship to the study drug. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship. An

unexpected adverse reaction is an AR, the nature or severity of which is not consistent with the information available on the study drug (i.e. the current Investigator's Brochure).

4.9.1.5. Severity Assessment Definition

- **Mild:** Transient symptoms, no interference with the subject's daily activities
- **Moderate:** Marked symptoms, moderate interference with the subject's daily activities
- **Severe:** Considerable interference with the subject's daily activities, unacceptable

4.9.1.6. Relationship to Trial Drug Assessment Definitions

- **Definitely:** Temporal relationship to the administration of the study drug and course following a known reaction pattern
- **Probably:** Good reasons and sufficient documentation to assume a causal relationship
- **Possible:** A causal relationship is conceivable and cannot be dismissed
- **Not Related**: No temporal relationship to the administration of the drug or other factors have caused the event

For reporting purposes, the categories "definitely", "probably" , "possibly" will be summarized as "suspected" Adverse Reactions.

4.9.1.7. Outcome Categories and Definitions

- **Recovered**
- **Stabilized:** An AE is stabilized when, according to the investigator, the subject is in a clinically stable condition. This term should only be used for chronic conditions and for a given subject only when he/she has completed the protocol.
- **Recovered with sequelae:** As a result of the SAE, the subject suffered persistent and significant disability/incapacity (e.g. became blind, deaf, paralyzed). Any AE recovered with sequelae should be rated as an SAE.
- **Ongoing at final examination**
- **Died**

4.9.2. Collection, Recording and Reporting of Adverse Events

All events meeting the definition of an adverse event must be collected and reported from the first trial-related activity after the subject signs the informed consent until the end of the ICU stay.
At each contact to the site (visit or telephone, excluding safety visits, where the subject is not seeing the investigator or his staff (e.g. visits to the laboratory)), the subject must be asked about adverse

events. All adverse events, either observed by the investigator or reported by the subject, must be recorded by the investigator and evaluated.
The subjects will be asked a general question “How are you?” as well as a specific question “Have you experienced any problems since the last contact?”
The investigator should record the diagnosis, if available. If no diagnosis is available the investigator should record each sign and symptom as individual Adverse Events.
The investigator must record all adverse events on the standard Adverse Event Form. One single Adverse Event Form/line must be used per adverse event from start to resolution. For serious adverse events, the Serious Adverse Event Supplementary pages must also be completed.
The investigator must report *all SAEs* in the first place to the sponsor by direct contact, telephone, e-mail or fax and receiving suitable confirmation of receipt.
Reporting time-frame for the Investigator is 24 h or at the latest the following working day. The Monitor must be informed accordingly.

4.9.3. Follow-up of Adverse Events

During and following a subject’s participation in a clinical trial, the investigator/institution will ensure that adequate medical care is provided to the subject for any adverse events, including clinically significant laboratory values related to the trial. The investigator/institution will inform the subject when medical care is needed for adverse event(s) of which the investigator becomes aware. The active post-treatment follow-up period will end with the end of the ICU stay.
All serious adverse events must be followed until the subject has recovered, stabilized, recovered with sequelae or died.

4.9.4. Suspected Unexpected Serious Adverse Reaction (SUSAR)

All suspected adverse reactions related to a study drug which occur in the concerned study, and that are both unexpected and serious are classified as suspected unexpected serious aderse reactions (SUSARs).

4.9.5. Reporting of SUSARs

SUSARs will be expedited by the principal investigator to health authorities and the IRB. SUSARs must be reported as soon as possible, but at least within 7 days in case of a life-threatening reaction or in case of death. All other SUSARs must be reported within 15 days.

4.10. Monitoring Procedure and Audits

The purpose of monitoring visits is to make sure that the CRFs are completed correctly, the protocol is adhered to, to monitor drug accountability, and to collect completed pages of the CRFs. The Monitor must be available for discussions by telephone.

The monitor must be given direct access to source documents (original documents, data and records). Direct access includes permission to examine, analyze, verify and reproduce any record(s) and report(s) that are important to evaluation of the clinical trial. Source data will be available for all data in the CRFs, including all laboratory results.

An audit of the study may be conducted in the course of the study.

4.11. Data Management

CRF entries and corrections will only be performed by trained study site staff authorized by the investigator.

All visit data need to be recorded in the database as soon as possible after each visit. The investigator or investigator's authorized staff must ensure that all information derived from source documents should be consistent with the source information.

Deviations from the protocol will be documented. A major protocol violation is defined as a violation that may impact subject safety, affect the integrity of study data and/or affect subject's willingness to participate in the study. A minor deviation is a violation that does not impact subject safety, compromise the integrity of study data and/or affect subject's willingness to participate in the study.

Corrections may be requested as follows:

Investigators' responses are checked as they are entered and are rejected if they do not fulfill quality criteria. A message will specify the type of error or syntax error and assist in its correction.

If required, the monitor can ask for information to be corrected during study-site visits. Manual checks will identify clinical data discrepancies for resolution. Corresponding queries will be sent and the site will be informed about new issues to be resolved online.

All discrepancies will be solved on-line directly by the investigator or authorized staff. Once all the Data Quality Control steps have been performed, the database will be locked and the records will be released for reporting and statistical evaluation.

4.12. Ethics

The trial will be conducted in accordance with the protocol, ICH-GCP guidelines and the Declaration of Helsinki for biomedical research involving human subjects.

4.12.1. Informed Consent Form of Trial Subjects (AMG §38)

In obtaining and documenting informed consent, the investigator must comply with the applicable regulatory requirement(s) and adhere to ICH guideline for GCP and the requirements in the Declaration of Helsinki.
Prior to any trial-related activity, the investigator or an authorized physician must give the subject oral and written information about the trial in a form that the subject can read and understand.
A voluntary, signed and dated Informed Consent Form will be obtained from the subject prior to any trial-related activity. The subject must have consented to participate after the nature, scope and possible consequences of the clinical trial have been explained in a form understandable to him.
The written informed consent must be signed by the person who conducted the informed consent.
Since this trial is conducted in the ICU, at least part of the patients will be unconscious or compromised in their ability to understand the purpose and nature of the trial. As soon as patients recover and are able to understand the purpose and nature of the trial, they will be informed about their inclusion and informed consent will be sought.
If information becomes available that may be relevant to the subject's willingness to continue participating in the trial, the investigator must inform the subject in a timely manner, and a revised written informed consent must be obtained.

4.12.2. Independent Ethics Committees (AMG §§40, 41)

Prior to commencement of the trial, the protocol, any amendments, Subject Information/Informed Consent Form, any other written information to be provided to the subject, subject recruitment procedures (e.g. advertisements), if any, investigator's Brochure (IB), package insert (if marketed product), information about payments and compensation available to subjects if not mentioned in the subject information, the investigator's current CV and/or other documentation evidencing qualifications, and other documents as required by the local Independent Ethics Committee (IEC) should be submitted. The submission letter should clearly identify (by including version no. and/or date of the document) which documents have been submitted to the IRB/IEC. Written approval/favorable opinion must be obtained from the IEC prior to commencement of the trial.
During the trial, the investigator must promptly report the following to the IEC: Updates to IB, unexpected SAEs where a causal relationship cannot be ruled out, amendments to the protocol, notes of administrative changes, deviations to the protocol implemented to eliminate immediate hazards to the trial subjects, new information that may affect adversely the safety of the subjects or the conduct of the trial, annually written summaries of the trial status, and other documents as required by the local IEC.

Amendments must not be implemented before approval/favorable opinion, unless necessary to eliminate immediate hazards to the subjects.

4.12.3. Regulatory Authorities

The Regulatory Authorities will receive the protocol, amendments, reports on SAEs, and the Final Report according to EU Directive and national regulations.

4.12.4. Insurance (AMG § 32(2))

All subjects participating in this interventional trial will be insured through the Department of Clinical Pharmacology, Vienna University Hospital. The insurance is contracted with the **ZÜRICH Versicherungs-AG**, 1010 Wien, Schwarzenbergplatz 15, Policy number **07229622-2**.

4.12.5. Confidentiality

All subject names will be kept secret in the investigator's files.

4.12.6. Premature Termination of the Trial

The investigator may decide to stop the trial or part of the trial at any time, but agreement on procedures to be followed must be obtained. If a trial is prematurely terminated or suspended, the investigator should promptly inform the subjects and assure appropriate therapy and follow-up. Furthermore, the investigator should promptly inform the IRB/IEC and provide a detailed written explanation. The pertinent regulatory authorities should be informed according to national regulations.

4.12.7. Retention of Clinical Trial Documentation

Subject notes must be kept for the maximum time period as permitted by the hospital, institution or private practice. Other source documents and the investigator's trial file must be retained for at least 15 years or longer in accordance with local regulation.

The investigator must agree to archive the documentation pertaining to the trial in an archive after completion or discontinuation of the trial, if not otherwise notified. If any modifications become necessary or desirable, these will be documented in writing; major changes require the approval of all investigators and the ethics

5. List of abbreviations

AA = Arachidonic Acid

ASA = Acetylsalicylic Acid

AUC = Area Under the Curve

CI = Confidence Interval

COX-1/-2 = Cyclooxygenase -1/-2

DVT = Deep Venous Thrombosis

ICU = Intensive Care Unit

IMP = Investigational Medicinal Product

i.v. = intravenous

MCI = Myocardial Infarction

MEA = Multiple Electrode Aggregometry

PD = Pharmacodynamic

PE = Pulmonary Embolism

PGI-2 = Prostacyclin

PK = Pharmacokinetic

PME = Post-Mortem Examination

TXA-2 = Thromboxane A2

TXB-2 = Thromboxane B2

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