Study protocol for a randomized, triple-blind, placebo controlled phase II trial of aspirin as prophylactic therapy for thrombotic events in patients with End-Stage Renal Disease

Abbreviations:

ADP: Adenosine diphosphate; AIDS: Acquired Immune Deficiency Syndrome; DCC: Data Coordinating Center; CKD: Chronic Kidney Disease; DSMB: Data and Safety Monitoring Board; ESRD: End-Stage Renal Disease; FDA: Food and Drug Administration; GFR: Glomerular Filtration Rate; IRB: Institutional Review Board; ITT: Intention-to-Treat analysis; MAR: Missing data At Random; MRI: Magnetic Resonance Imaging; NIH: National Institutes of Health; REDCap: Research Electronic Data Capture management system; RTT: Renal Replacement Therapy; TIMI: Thrombolysis In Myocardial Infarction.

Paper metrics:

Abstract: 324 words (max 450 words)
Keywords: 10 (3-10 keywords or short phrases)
Text: 3473 words (no limit)
Tables and Figures: 2 Figures
References: 25 (no limit)
ABSTRACT

Background: End-Stage Renal Disease (ESRD) is the last stage of Chronic Kidney Disease, mainly caused by type-2 Diabetes Mellitus and characterized by an increased mortality risk related to cardiovascular disease. Low-dose aspirin (acetylsalicylic acid) seems to effectively prevent cardiovascular events in ESRD patients. However, the number of interventional studies in this population remains limited and the mechanisms of aspirin-related bleeding remain poorly understood. Aspirin’s efficacy and safety seem to modulate by type-2 Diabetes Mellitus and platelet hyper-reactivity.

Objectives: Assess aspirin’s prophylactic efficacy and safety in ESRD patients on hemodialysis and their modulation by type-2 Diabetes Mellitus and platelet hyper-reactivity.

Results: We developed a protocol for a phase II randomized, single-center, placebo-controlled, triple-blind, superiority clinical trial in order to assess aspirin prophylactic efficacy and safety in ESRD patients on hemodialysis. It follows the ethical principles of the Declaration of Helsinki of the World Medical Association. Participants will be randomized (1:1 ratio) in 2 arms and orally receive acetylsalicylic acid 100mg/day or placebo, for 12 months. An intent-to-treat (ITT) statistical analysis will be performed. The primary composite outcome (12-month incidence of thrombotic events [cardiac death, nonfatal myocardial infarction, nonfatal stroke, arteriovenous fistula thrombosis] and TIMI major bleeding) will be analyzed with Kaplan Meier curves and a log-rank test to compare treatment arms. Cox proportional hazards regression will be used to adjust for covariates, if appropriate. As secondary outcomes, the same components of the primary outcome will be assessed in a subgroup analysis after patients’ stratification for the presence versus absence of type-2 Diabetes Mellitus and platelet hyper-reactivity. The presence of TIMI minor bleeding will be compared between groups and tested with a Chi-square test.

Conclusions: We provide a protocol for a randomized controlled trial to evaluate aspirin’s prophylactic efficacy for thrombotic events and safety in ESRD patients. When conducted, such a study would further our understanding of the mechanism of aspirin-related bleeding, and would help identify best-responders and patients with a higher risk of adverse events.
KEYWORDS

- Randomized controlled trial
- Phase 2
- End-Stage Renal Disease
- Aspirin
- Prophylactic efficacy
- Thrombotic events
- Safety
- Bleeding
- Platelet reactivity
- Type 2 Diabetes Mellitus
INTRODUCTION

Chronic Kidney Disease (CKD) is a pathology defined by a decrease in Glomerular Filtration Rate (GFR) below 60 mL/min/1.73m$^2$ or by the presence of kidney damage for at least three months (1). It affects 200 million people worldwide and the main risk factor for this disease is Diabetes Mellitus (2-4). The life expectancy of CKD patients decreases progressively with the severity of kidney impairment (5). The most severe stage is called End-Stage Renal Disease (ESRD) and refers to patients ongoing Renal Replacement Therapy (RRT) or for whom the GFR is lower than 15 mL/min/1.73m$^2$ (1). The high risk of mortality observed in ESRD patients is mainly due to cardiovascular events, which is 10-20 fold higher in these patients when compared to non-CKD subjects, and is significantly increased by the presence of Diabetes Mellitus (4;6;7). In order to prevent cardiovascular events, ESRD patients receive anti-coagulant or anti-platelet therapy (8;9). The benefits of low doses (75-100 mg/day) of aspirin (acetylsalicylic acid), an anti-platelet agent, as prophylactic drug for some specific types of cardiovascular events (atherosclerotic and ischemic events) in CKD and ESRD patients have been reported in several observational and interventional studies (10-14).

However, there is a lack of information about the prophylactic efficacy of aspirin for all types of thrombotic events that ESRD patients may develop. There is also a gap of knowledge concerning the safety profile of aspirin in ESRD. Concerning the risk of aspirin-related bleeding, there is some discrepancy between the results of observational and interventional studies. Indeed, an increased risk of bleeding has been reported in some observational studies (15;16). However, all interventional studies performed on ESRD patients showed that low doses of aspirin are not associated with an increase in major bleeding in dialysis patients, even if an increased risk of minor bleeds (e.g. gastro-intestinal bleeding) seem to happen (10;11;13;14). This discrepancy may be explained by the fact that the mechanism of aspirin-related bleeding adverse events is not yet fully understood. Some authors suggest that the prophylactic efficacy of aspirin and the risk of bleeding related to this drug may be influenced by a phenomenon of platelet hyper-reactivity (17). However, further research is necessary to establish the real impact of platelet hyper-reactivity on the aspirin safety profile in ESRD patients.
Finally, we designed a randomized, triple-blind, placebo controlled phase II trial to confirm the efficacy of aspirin as prophylactic therapy for thrombotic events in ESRD patients and to assess the safety profile of this anti-platelet agent in this specific population. For that purpose, a primary composite outcome will be used to address the global benefit of aspirin in terms of prophylactic efficacy and safety. This outcome will include (i) the assessment of aspirin prophylactic efficacy for all types of thrombotic events that ESRD patients may develop, namely nonfatal stroke, non-fatal myocardial infarction, arteriovenous fistula thrombosis and cardiac mortality, and (ii) the assessment of aspirin-related major bleeding events using TIMI criteria (18-20). As for secondary analysis, we will investigate aspirin-related minor bleeding and the impact of type 2 Diabetes Mellitus and platelet hyper-reactivity on the prophylactic efficacy and safety profile of aspirin in ESRD patients, as well as performing a subgroup analysis for these two covariates. Our hypothesis is that aspirin is superior to placebo as prophylactic therapy for thrombotic events in ESRD patients, without increasing the risk of major bleeding.
METHODS AND ANALYSIS

Study design
We will perform a phase II randomized, single-center, placebo-controlled, triple-blind, superiority clinical trial with 1:1 allocation to receive either 100 mg of acetylsalicylic acid/day (oral pills) or placebo. Randomization will be stratified based on two factors: (i) the presence versus absence of type 2 Diabetes Mellitus, and (ii) the presence versus absence of a platelet hyper-reactivity. The study will start after the approval of the Institutional Review Board (IRB) and will follow the ethical principles of the Declaration of Helsinki of the World Medical Association. The study design is illustrated in Figure 1.

Adult patients from hemodialysis units will undergo screening and, if eligible, will receive an invitation to join the trial. All participants will provide written informed consent before enrollment. Recruitment will last for 12 months. One venous blood sample will be collected prior to randomization in order to assess platelet reactivity profile, a stratification factor.

Randomized patients will receive either acetylsalicylic acid or placebo daily *per os* for 12 months.

The primary outcome is presence of composite cardiac events at twelve months. Outcomes will be assessed in every patient immediately after randomization (week 0) and during every visit (at weeks 13, 26, 39, 52). After the end of the experimental phase, patients will be followed up for 6 more months. The total length of the study will be 30 months.

Setting and Recruitment strategy
The trial involves patients on chronic intermittent hemodialysis and will be conducted in a great dialysis center in Belo Horizonte - Brazil, called Associação Evangélica Beneficente de Minas Gerais. This center entails 4 different dialysis units, with over 2,000 patients on renal replacement therapy. The service’s admission rate to hemodialysis is 50 patients per month. Therefore, we expect to include 8-10 patients every week, completing our recruitment goal in 9 months. Despite that, we planned a recruitment period of 12 months in case of difficulties.
The first step of the recruitment strategy is to identify eligible patients. This entails active search in medical records, clinician invitation letters along with personal approach and internal advertisement for patients who are willing to participate. Secondly, the study team will approach eligible patients to invite for the trial. At this point, every aspect of the study will be explained and all participants who accept to participate must provide written informed consent.

**Eligibility criteria**

The study population consists of men or women 18 years of age or more, who started chronic intermittent hemodialysis in the previous 3 months.

Exclusion criteria include any contraindications to acetylsalicylic acid, concurrent treatment with anticoagulants or platelet aggregation inhibitors, and pregnancy or lactation. In addition, life-threatening conditions other than renal or vascular disease will also be excluded from the trial: all types of cancer, liver disease, AIDS or severe lung disease. End-stage renal disease due to glomerulopathy has a different pattern of mortality (21), which precludes its inclusion in this trial. Moreover, patients on other modalities of renal replacement therapy will also be excluded.

**Randomization**

Patients will be allocated to one of the two study groups based on a computed-generated blocked randomization of variable block sizes (4, 6 and 8), with stratification by two factors (Type 2 Diabetes Mellitus and platelet hyper-reactivity as explained previously). The software used is available at www.randomization.com and the study pharmacists will coordinate the randomization plan.

**Interventions**

The intervention investigated is an enteric-coated oral pill containing 100 mg of acetylsalicylic acid, which will be administered once daily, after lunch, over 12 months. The comparator will be a placebo tablet with the same characteristics as the acetylsalicylic acid pills. Both arms will have the same administration schedule and will start the treatment on the day after the randomization process (day 0).
Adherence

Participants will receive training about the importance of taking the drug accordingly, which will be done during every hemodialysis session (10 minutes educative video focused on compliance to the intervention that the patient can view on a numeric tablet as often as desired).

In order to ensure proper compliance, the study patients will be approached monthly by the study team to receive oral reminder sessions and advices. During these visits, patients’ compliance will be assessed by direct questioning and by counting of returned tablets (pills will be provided in a monthly basis). Good adherence will be defined as taking at least 80% of the prescribed daily dose and attending 100% of the visits.

In the case of patient withdrawal, the study coordinator will contact the participant in order to know the reasons for the withdrawal and record them for the subsequent analysis of missing data and the interpretation of the results.

Blinding

The trial is triple-blinded to the study allocation and intervention: participants, enrolling and treating physicians, as well as data analysts. Blinding will be assured by the use of a placebo drug, which will be of the same use, color, size, shape and taste as the active drug, and will last until the end of the study. The head of the pharmacy will determine the study allocation and randomization and will be the only person to know the identity of the drugs delivered. A questionnaire will be used to assess the effectiveness of the participant’s blinding (22).

Emergency unblinding

In exceptional circumstances, unblinding may happen if knowledge of the actual treatment is essential for further management of the patient. In case of severe adverse events, investigators should discuss unblinding with a Medical Advisor from the DSMB who is not involved with the trial within 24 hours. Unblinding will take place immediately after the decision is made, easily done by a 24-hour emergency phone service provided by the head of pharmacy.
Outcomes

The primary outcome aims to assess the incidence of the composite of events: cardiac death, nonfatal myocardial infarction, nonfatal stroke, arteriovenous fistula thrombosis, and TIMI major bleeding (20) at twelve months of treatment. The secondary outcomes entail the same components of the primary outcome, in addition to the assessment of the risk of TIMI minor bleeding and to a subgroup analysis of all outcomes for patients stratified based on the presence versus absence of Type 2 Diabetes Mellitus and platelet hyper-reactivity.

According to the TIMI criteria, major bleeding is defined as the presence of any intracranial bleeding (excluding micro-hemorrhages <10 mm evident only on gradient-echo MRI) or fatal bleeding (bleeding that directly results in death within 7 days) or Clinically overt signs of hemorrhage associated with a drop in hemoglobin of ≥5 g/dL or a ≥15% absolute decrease in hematocrit (20). Minor bleeding is defined as clinically overt with hemoglobin drop of 3 to <5 g/dL (20).

The profile of platelet reactivity will be determined by Multiple Electrode Aggregometry, using a Multiplate® analyzer (Dynabyte GmBH, Munich, Germany). This method was chosen because it currently is the most efficient assay that can be used to assess platelet reactivity in humans (23). The Multiplate will measure the platelet activity (defined as aggregation capability after activation with Adenosine diphosphate [ADP]) as an area under the curve reported in area units over time (AU*min). Platelet hyper-reactivity is defined based on the manufacturer’s recommendations and published data, with a cut-off of 50 AU*min for ADP-induced aggregation (24).

Outcomes assessment

A multidisciplinary team, composed of a blinded nephrologist, a blinded cardiologist and a blinded nurse, will follow participants and will be responsible to review medical records and identify outcomes and endpoints from the study.

A medical appointment will be scheduled for weeks 0, 13, 26, 39 and 52, where the multidisciplinary team will assess every component of the primary and secondary outcomes described before and report them to the blinded data analyst, as well as to
the Data Coordinating Center (DCC). At each visit, a venous blood sample (5 mL) will be collected in order to assess the hematocrit, concentration of hemoglobin, and the platelet hyper-reactivity profile.

Participants will be followed by an extra 6 months after the end of the administration of the interventions to assess further adverse events. For that purpose, the multidisciplinary team will approach them monthly during the hemodialysis sessions.

The schedule and procedures of patients’ visits to the participating center (recruitment, hemodialysis sessions, and monthly measurements of outcomes) are summarized in Figure 2.

**Data management**

For quality control, patient records (source document) will be stored in the participating site, and the original data will be available to be monitored by the DCC. It is the responsibility of the investigator to keep, maintain and provide, the documents audited by IRB, Sponsor, NIH or the FDA when necessary.

After the study data is collected, the patient identification will be encoded, and only the investigator will have access to this information, in accordance with Good Clinical Practice and Helsinki declaration of patient confidence (24). The collected data will be entered electronically in a Research Electronic Data Capture management system (REDCap). This database is a cloud-based system, and it will have a backup in a hard-disk in the DCC. The dataset will be encrypted, in order to guarantee data’s safety and confidence.

**Preventing missing data**

We intend to minimize participants-related missing data by providing educative information and reminder sessions. An adherence check will be done by counting returned tablets as stated above.

Regarding data collection and storage, the study team will be trained to master all key aspects of the protocol, namely: (i) methodology, forms and tools that must be used for the collection, entry, monitoring and editing of data, (ii) appropriated
methods to communicate among investigators and among investigators and participants, and (iii) importance of reporting data as close to real-time as possible during the course of the study.

**Data and safety monitoring plan**
A Data and Safety Monitoring Board (DSMB) is planned to oversight the trial. It consists of an independent Nephrologist and Cardiologist (adverse events may happen mainly in those fields) together with an independent statistician. Furthermore, based upon federal regulations, an ethicist may be included. According to our inclusion and exclusion criteria, no vulnerable population is targeted.

**Sample size calculation**
Sample size for the trial was calculated based on estimates for the rate of events in the primary composite outcome over 1 year: TIMI major bleeding (placebo 1% vs aspirin 2.5% ([11])), cardiovascular events (Placebo 3.0% vs aspirin 2.5% ([10])), and Fistula Thrombosis (Placebo 19% vs aspirin 8% ([25])); for a total one-year event rate of 23% in the placebo group and 13% in the aspirin group. A sample size of 318 patients (159 per arm) provides 80% power to detect a difference between groups of this magnitude (corresponding to a hazard ratio of 0.53), using a two-sided log-rank test and alpha=0.05 (PASS 14 Power Analysis and Sample Size Software (2015). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass). The sample size for the trial was increased to 342 patients (171 per arm) to account for estimated attrition of 10%.

**Statistical analysis plan**
Statistical analysis will be performed including all randomized patients according to their assigned treatment group (i.e., intention-to-treat). The software used will be Stata 14 (StataCorp, College Station, Texas, USA). All testing will be two-sided with statistical significance defined as p<0.05. The primary composite outcome (first time-to-event of thrombotic events and TIMI major bleeding) will be analyzed with Kaplan Meier curves and a log-rank test to detect difference between the groups. Cox
proportional hazards regression may be used to adjust for relevant covariates, if appropriate (e.g., for any unbalanced baseline characteristics that may occur by chance). Similarly, secondary outcomes will be analyzed with Kaplan Meier curve and log-rank test for all events. The same analysis plan will be conducted for the following groups based on their stratified classification: Type 2 Diabetes Mellitus and platelet hyper-reactivity. The proportion of patients with TIMI Minor bleeding in each group will be compared with a Chi-square test. Multiple imputation will be used to correct for missing data.

**IRB SUBMISSION**

Prior to recruitment of study subjects, the full study protocol will be submitted to the local ethics committee for evaluation and approval.

**REGISTRATION**

The trial will be registered on [www.clinicaltrials.gov](http://www.clinicaltrials.gov).
DISCUSSION

End-Stage Renal Disease affects 2 million people worldwide, and the number of diagnosed patients increase every year at a rate of 5-7% per year (21). Hemodialysis is started on most part of these patients as the treatment modality of Renal Replacement Therapy (21). ESRD has a high mortality rate, especially if the cause of Chronic Kidney Disease is type 2 Diabetes Mellitus or Hypertension, and comprises cardiovascular disease as the main cause of death (4;6;7;21). Some studies demonstrate mixed results regarding the use of thrombotic prevention for cardiovascular disease and arteriovenous fistula thrombosis in hemodialysis patients (10;11;13;14). Moreover, there is also an uncertainty about the safety profile of antiplatelet blockage in this population (10;11;13;14).

Finding a safe preventive measure for thrombotic events for ESRD patients on hemodialysis is of utmost importance, as it would reduce the number of fistula thrombosis and cardiovascular events, having a direct impact on morbidity and mortality rates. Thus, this clinical trial will provide data on the safety of antiplatelet blockade with acetyl-salicylic acid, which is a possible preventive measure for thrombosis, as well as assessing the impact of the intervention in hemodialysis patients. Moreover, it will yield essential information to foster further interventional trials, as well as redefining international guidelines on the prevention of thrombotic disease on ESRD patients on hemodialysis.

The main strength of the trial is its study design, which includes allocation concealment, randomization, triple-blinding and the use of a control group, in order to reduce possible biases. In addition, stratification will balance two variables that have a higher impact on the outcome: type 2 Diabetes Mellitus and platelet hyper-reactivity. The latter will provide preliminary evidence on the possible prediction of complications related to the interventional drug, as well as its indication based on a biomarker. Above all, the study protocol is feasible and potentially comfortable for patients, as the study team will approach participants during their hemodialysis sessions. Likewise, all visits and training will be conducted after them as well.
Potential limitations of the study protocol include the difficulty in interpreting a composite outcome. The use of a composite primary outcome is supported by the fact that major bleeding rates are very rare, thus requiring a large sample size and lengthy study duration that would render the trial unfeasible. We address this issue by adding efficacy outcomes related to thrombosis to major bleeding outcomes. In order to clarify interpretation of the primary composite outcome, they are individualized in the secondary analysis. Regarding the study population, peritoneal dialysis patients will not be included as most of them do not have arteriovenous fistula and, hence, are not at risk for fistula thrombosis. Furthermore, glomerular disease patients who developed ESRD will not be included, as they show a different pattern of morbidity and mortality (21). Another limitation is in case we do not meet the planned recruitment time, as it affects study power and validity. For that matter, we allow 12 more weeks for recruitment than initially planned. Lastly, despite the simplicity of the drug administration, adherence is always a potential problem, which will be dealt with by identification of non-adherent patients and systematic training.

Finally, the study protocol will provide essential evidence to foster further clinical research on preventive measures of thrombotic events in hemodialysis patients. Future research is needed to provide information about the impact of preventive antiplatelet blockage on mortality and thrombotic events in these patients. Moreover, the study of potential biomarkers to identify patients who would benefit the most with the intervention is also required and may have a direct effect on drug prescription and control of adverse events. Therefore, identifying the potential safety and effectiveness of acetyl-salicylic acid for that matter will improve morbidity and mortality, lowering the burden of such a severe disease for these patients, as well as giving them a chance for a better and longer life.
AUTHORS’ CONTRIBUTIONS:
A.F.G., C.K.P.F., F.F., N.M.V. and T.L.C. wrote the manuscript. A.F.G., C.K.P.F., D.A.S., E.E.B., J.L.B., N.M.V., P.G.M., R.W., T.L.C., W.E.O. developed the study protocol. All authors approved the final version of the manuscript.

FUNDING:
This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. N.M.V. is a scientific collaborator from the Belgian Scientific Institute of Public Health (WIV-ISP) and is supported by the Belgian National Institute for Health and Disability Insurance (RIZIV-INAMI; grant W4043.0100.8)

COMPETING INTERESTS:
None to declare.
The authors followed the International Committee of Medical Journals Editors (ICMJE) form to declare potential conflicts of interest. All authors approved the final version of the manuscript and concur with its submission.

ACKNOWLEDGMENT:
We would like to thank our colleagues who contributed to the early development of this project: Juan Carlos Aldana, Rafael Barreto Silva, Daniella Caputo Dorta, Marcelo Costa, Caio de Assis Moura Tavares, Luís Gustavo de Mil-Homens e Vinagre, Maria Analayi Estudillo, Dayani Galato, Dolores Gonzales Fabra, Eman Hassan Satti Elsayed, Lorna Marisolva Galleguilos, Bruno Silva, Carina Vorisek, Yiling Yang and Chen Xhi Zian. We would also like to thank Pr. Felipe Fregni (Harvard T.H. Chan School of Public Health and Harvard Medical School [Laboratory of Neuromodulation and Center for Clinical Research Learning, Department of Physical Medicine and Rehabilitation, Spaulding Rehabilitation Hospital], Boston, MA) and Pr. Ben Min-Woo Illigens (Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA), Karla Mëtte Waldrich Tauil, Ana Rita Simoes Martins,
Christian Acosta Villegas for their support and valuable suggestions on this study design.

Reference List


