Aspirin and clonidine in non-cardiac surgery: Acute kidney injury substudy protocol of the Perioperative Ischemic Evaluation (POISE) 2 randomised controlled trial

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ABSTRACT

Introduction: POISE-2 is an international 2 x 2 factorial randomised controlled trial of low-dose aspirin versus placebo and low-dose clonidine versus placebo in patients who undergo non-cardiac surgery. Peri-operative aspirin (and possibly clonidine) may reduce the risk of post-operative acute kidney injury (AKI).

Methods and analysis: After receipt of grant funding, serial post-operative serum creatinine measurements began to be recorded in consecutive patients enrolled at substudy participating centres. With respect to the study schedule the last of over 6500 substudy patients from 82 centres in 21 countries were randomised in December 2013. The authors will use logistic regression to estimate the adjusted odds ratio of AKI following surgery (compared to the pre-operative serum creatinine value, a post-operative increase ≥ 26.5 µmol/L in the 2 days following surgery or an increase of ≥ 50% in the 7 days following surgery) comparing each intervention to placebo, and will report the adjusted relative risk reduction. Alternate definitions of AKI will also be considered, as will the outcome of AKI in subgroups defined by the presence of pre-operative chronic kidney disease and pre-operative chronic aspirin use. At the time of randomisation, a subpopulation agreed to a single measurement of serum creatinine between 3 and 12 months after surgery, and the authors will examine intervention effects on this outcome.

Ethics and dissemination: The authors were competitively awarded a grant from the Canadian Institutes of Health Research for this POISE-2 AKI substudy. Ethics approval was obtained for additional kidney data collection in consecutive patients enrolled at participating centres, which first began for patients enrolled after Jan 2011. In patients who provided consent, the remaining longer term serum creatinine data will be collected throughout 2014. The results of this study will be reported no later than 2015.

Clinical Trial Registration Number: NCT01082874

ARTICLE SUMMARY

Article focus

- POISE-2 is a large international 2 x 2 factorial randomised controlled trial of low-dose aspirin versus placebo and low-dose clonidine versus placebo in patients at risk of a peri-operative cardiac event who undergo non-cardiac surgery
- Compared to placebo, we hypothesize that use of peri-operative aspirin (and potentially peri-operative clonidine) will reduce the risk of post-operative AKI.
- Secondary analyses will examine whether results are consistent across alternate definitions of AKI, whether effects are similar in subgroups with pre-operative chronic disease and pre-operative chronic aspirin use, and the impact of the intervention on longer term kidney function.

Key messages

- Presented is this pre-specified POISE-2 AKI analytic protocol.
- Data and analysis will be completed in 2015.
- If aspirin or clonidine reduces the risk of AKI, the finding may support the rationale to test these interventions in other settings to prevent AKI.

Strengths and limitations of this study

- International recruitment of over 6500 patients across 82 centres in 21 countries will provide generalizable estimates of the treatment effects on AKI.
- The kidney data collection schedule in this large international trial is feasible and efficient. Additional studies of the effects of aspirin in the surgical setting can consider multiple measures of kidney function over time, both before and far after AKI, both before and far after AKI, examine trajectories of kidney function loss, and new markers of kidney function or injury.

BACKGROUND

Worldwide an estimate of 200 million adults have major non-cardiac surgery each year.[1] About 10% of patients develop acute kidney injury (AKI) (defined by an acute rise in serum creatinine of 50% or more or an acute rise of 26 µmol/L or more), and 0.5% receive acute dialysis.[2] In other words, worldwide there are several million annual cases of AKI attributable to major non-cardiac surgery, with about a million cases receiving acute dialysis. When AKI occurs (versus when it does not) it is associated with increased mortality, longer hospital length of stay, and higher healthcare costs.[3-5] Treatments to prevent the onset of AKI or its complications remain elusive.[6]

The major mechanism of peri-operative AKI is impaired kidney perfusion and ischaemia. Activation of inflammatory mediators, adhesion molecules, platelets and thromboxane are thought to be involved in the injury (Figure 1).[7;8]

In the surgical setting, aspirin prevents platelet aggregation and may improve glomerular blood flow. In Appendix 1 we provide three lines of evidence to support the hypothesis that peri-operative aspirin use reduces the risk of post-operative AKI.[7-15] The pathways where aspirin may mitigate AKI are presented in Figure 1.[16]

We are conducting the Peri-operative Ischemia Evaluation-2 trial (acronym POISE-2). The methods of this large, international 2 x 2 factorial randomised placebo controlled trial are described elsewhere (available from the authors upon request).[17] In brief, adults at moderate and high risk for post-operative cardiac events are randomly allocated to receive either aspirin or matching placebo, and clonidine or matching placebo. Eligible surgeries are those where a patient is expected to stay for at least one night in the hospital after surgery. The aspirin intervention is 100 mg tablets of aspirin or matching placebo, where patients take 2 tablets orally 2 to 4 hours prior to surgery, and then take 1 tablet daily for either 7 or 30 days (depending on whether they were taking aspirin chronically prior to surgery or not, respectively). The clonidine intervention consists of 0.2 mg of oral clonidine or matching placebo given 2 to 4 hours prior to surgery and at the same time a transdermal clonidine or placebo patch is applied for 72 hours, providing clonidine at 0.2 mg/day. Allocation is done by a central randomisation service and is stratified by centre. Patients, health care providers, data collectors, and outcome adjudicators are blinded to treatment allocation. This trial is primarily funded by the Canadian Institutes of Health Research. With respect to the study schedule, the last of 10,000 patients from 140 centres in 23 countries was randomised in December 2013. The primary outcome is a composite of 30-day all-cause mortality or nonfatal myocardial infarction. The secondary outcome is a composite of 30-day all-cause mortality, nonfatal myocardial infarction, and nonfatal stroke.

In addition to the primary outcome, POISE-2 is uniquely positioned to determine the effects of peri-operative aspirin (and clonidine) on AKI, to consider whether treatment effects on AKI differ in those with pre-operative chronic kidney disease (CKD) and pre-operative chronic aspirin use, and to investigate the treatment effects on longer term kidney function, based on a single measurement taken 3 months to 1 year after surgery. We were awarded an additional grant from the Canadian Institutes of Health Research to examine these issues in a POISE-2 AKI substudy. The AKI questions detailed in the grant are presented below and are followed by our pre-specified analytic plan.

Primary question

1. In patients undergoing non-cardiac surgery, does the use of aspirin at the time of surgery compared with placebo alter the risk of post-operative AKI? Is the treatment effect (the observed relative risk reduction) similar across alternate definitions of AKI? Hypothesis: Peri-operative aspirin versus placebo at the time of surgery will reduce the risk of post-operative AKI, and the treatment effect will appear similar across alternate definitions of AKI.

Secondary questions

2. Does the observed relative risk reduction of aspirin versus placebo on post-operative AKI differ in patients with and without pre-operative CKD? Hypothesis: The relative risk reduction will be greater in patients with pre-operative CKD than those without CKD (as we demonstrated in another setting - a large international randomised trial of coronary artery bypass surgery performed either with or without a bypass pump).

3. Does the observed relative risk reduction of aspirin versus placebo on post-operative AKI differ in patients who were chronically taking aspirin before surgery, compared to those had aspirin initiated at the time of surgery? Hypothesis: The relative risk reduction will be greater in the aspirin continuation stratum, then the aspiring initiation stratum, as there may be an increased risk of thrombosis after aspirin discontinuation (as observed in some human studies recognising the biology is complicated).[18]

4. Does peri-operative aspirin versus placebo alter longer term kidney function (indicated by a single measure of serum creatinine 3 to 12 months after surgery)? Hypothesis: We will be unable to demonstrate a better outcome with peri-operative aspirin versus placebo due to limitations in the measurement techniques, or the limited impact of any observed AKI risk reduction on longer term kidney function.

Other questions

5. In patients undergoing non-cardiac surgery, does the use of peri-operative clonidine at the time of surgery compared with placebo alter the risk of post-operative AKI? Hypothesis: Peri-operative clonidine versus placebo at the time of surgery will reduce the risk of post-operative AKI (a notion supported by some animal and human studies,[19-24] recognising an elevated risk of AKI could also manifest from clonidine induced hypotension).

POISE-2 DATA COLLECTION AND ANALYTIC PLAN

POISE-2 enrollment began in July, 2010, and enrollment in the POISE-2 AKI substudy began in Jan 2011. To refine the analytic plan we reviewed POISE-2 data as of April 2013 (when data reconciliation was ongoing and without any knowledge of patient allocation to the assigned treatments).

POISE-2 AKI substudy data collection

A pre-operative serum creatinine value is measured within 6 weeks of surgery. The date of surgery (and not the date of randomisation) is used to identify the start of follow-up in this protocol; the median (interquartile range (IQR)) number of days between the date of randomisation and the date of surgery is 0 (0 to 0; 95th percentile 1 day)). After receipt of grant funding to support this substudy, serial peri-operative serum creatinine data began to be recorded in consecutive patients enrolled at AKI substudy participating centres (earliest centre to start this process began in Jan, 2011). All centres were encouraged to record a serum creatinine value post-operative day 1, day 2 and day 3 in consecutively enrolled patients (provided the patient was not discharged from hospital). This will help reduce concerns about an ascertainment bias related to AKI (i.e. where aspirin versus placebo alters the incidence of another event such as myocardial infarction or bleeding, which in turn influences the likelihood of serum creatinine measurement). We also record all other serum creatinine measurements done as a part of routine care (and their dates) during the hospital stay. At the time of final analysis we will examine the number of measurements by treatment group (and the post-operative days of these measurements), to confirm there is no differential ascertainment of AKI in the two treatment groups.

The median hospital length of stay in POISE-2 is 4 days (IQR 3 to 7 days). The proportion of patients who die in the operating room or in the 48 hours after surgery, that may result in no serum creatinine measurement, is expected to be <1% of patients. Less than 10% of patients are expected to have a missing peak serum creatinine value during the hospital stay, and in such cases, in the absence of receipt of acute dialysis, we will carry forward the pre-randomisation serum creatinine value for all analyses (which means the patient will not have developed AKI; see analysis section). Urine output data is however not collected in POISE-2 given difficulties with accurate measurement in the setting of international data collection (and thus a low urine output is not used to define AKI in POISE-2). Receipt of new dialysis for kidney failure is recorded at hospital discharge and at 30 days after surgery.

Longer term kidney function measurement: In participating centres, at the time of randomisation patients were invited to enroll in a substudy to record a single longer term serum creatinine value, anytime between 3 and 12 months after surgery. A measurement done at a time when a patient is not acutely ill is valid.

The additional serum creatinine collection (particularly the longer term creatinine measurement) was added to the patient consent requests and received ethical approval at each of the AKI substudy participating sites.

Patient selection

All patients enrolled in the POISE-2 AKI substudy at the time of randomisation will be included in the final analysis except for the following three reasons: (1) Those with end-stage renal disease prior to randomisation (expected <2% of patients), as the assessment of AKI is no longer relevant (estimated glomerular filtration rate (eGFR) < 15 mL/min per 1.73 m^2 as determined by the CKD-Epi equation[25], receipt of chronic dialysis, or a baseline serum creatinine > 327 µmol/L (the last exclusion also enables retained patients to have their new-onset AKI staged according to most recent guidelines[26])), (2) Those missing a pre-randomisation serum creatinine value, or missing age or sex (expected in < 3% of

patients) as this is needed to define baseline CKD or subsequent AKI, (3) those who never underwent surgery (expected in < 2% of patients) as they won't have the opportunity to have any post-operative serum creatinine measurements. We expect to enroll over 6500 eligible patients in the POISE-2 AKI substudy, with a subpopulation of at least 3000 patients who, at the time of randomisation, agree to the longer-term creatinine measurement.

Intention to treat

The intention to treat principle will guide all primary analyses, irrespective of whether there is a deviation from the randomly allocated therapy. Currently, < 1.5% of patients in POISE-2 did not receive the study medication (neither aspirin nor clonidine), 0.1% of patients received non-study aspirin within 24 hours prior to surgery, and 5.2% received non-study aspirin in the first 3 days after surgery. At the time of final analysis these results will be reported by treatment group.

Primary Definition of Acute Kidney Injury

We will use a mixed effects logistic regression model to obtain an estimate of the odds ratio of AKI comparing aspirin to placebo (after testing model assumptions; Appendix 2). While logistic regression models will be used for hypothesis testing, at the time of final analysis we will preferentially report relative risk reductions of aspirin relative to placebo as this metric is easier to interpret by a clinical audience (and hence we use relative risk reduction nomenclature throughout this protocol appreciating the logistic regression model produces an odds ratio; the method to derive a relative risk reduction from an odds ratio is presented in Appendix 3).[27]

AKI will be primarily defined as per recent guidelines, as any of the following 2 criteria: (1) an increase in serum creatinine ≥ 26.5 µmol/L within 48 hours of surgery, or (2) increase in serum creatinine ≥50% from baseline within 7 days of surgery.[26] For the primary analysis we will treat centre as a random effect, and adjust for the following baseline characteristics [28]: age (per year), sex, presence of cardiovascular disease (any of coronary artery disease, peripheral vascular disease, or stroke), presence of diabetes, pre-operative eGFR category (>60 ml/min/1.73m² versus ≤60 ml/min/1.73m²), a history of smoking within 2 years of surgery, receipt of urgent or emergency surgery (about 8% of POISE-2 participants), type of surgery (major vascular surgery, major thoracic surgery, other surgery), chronic use of aspirin therapy, and use of the following medications in each of two periods prior to randomisation (7 days to < 6 hours prior to surgery, and ≤ 6 hours prior to surgery): COX-2 inhibitor / non-steroidal anti-inflammatory drugs / non-COX-2 inhibitor, a statin, an ACE inhibitor / angiotensin receptor blocker / direct renin inhibitor, and use of another anti-hypertensive agent (any of the following: a rate controlling calcium channel blocker, dihydropyridine calcium channel blocker or a beta-blocker). We will also adjust for the random allocation of clonidine (clonidine vs. placebo). In patients who underwent surgery but are missing a post-operative serum creatinine value (expected < 10% of patients), we will impute in its place the pre-randomisation serum creatinine value which should provide a more conservative estimate of the intervention effect than the alternative of removing such patients. We expect <0.5% data will be missing for each variable, and if missing we will consider the condition to be absent. We will report the 95% confidence interval (CI) of our estimates, and a two-tailed p-value of

≤0.05 will be considered statistically significant. With a sample of at least 6500 patients we will have over 80% power to detect at least a 20% relative risk reduction in AKI with aspirin versus placebo should it exist (anticipated incidence of AKI of ~ 12% in the placebo group, two-tailed α 0.05, χ^2 test; Table 1). For completeness and interpretation, in the setting of a significant adjusted relative risk reduction we will also report the unadjusted relative risk reduction (with 95% CI) and the unadjusted absolute risk difference (with 95% CI). A significant unadjusted absolute risk difference will also be expressed as the 'number needed to treat (NNT)' (1/absolute risk difference; a measure which indicates how many patients need to receive peri-operative aspirin to prevent one patient from developing AKI who otherwise would develop AKI if they receive placebo; a lower number indicating a greater benefit of aspirin). The 95% CI of the NNT is the inverse of the Wald CI for the absolute risk difference.[29;30]

Alternate Definitions of Acute Kidney Injury

To determine how robust the AKI results are, we will examine the effect of aspirin versus placebo on alternate definitions of AKI. A two-tailed p value ≤0.05 will be considered statistically significant in these analyses if results are concordant with the primary results. The statistical power to detect a 20% relative risk reduction in each of these outcomes is also presented in Table 1. As seen there is inadequate statistical power to detect a clinically important difference in some outcomes (such as stage 2 AKI); these outcomes will be reported given their clinical significance and we will visually compare the point estimates and 95% CI of the relative risk reduction for each definition of AKI.

- AKI or death. A composite outcome of either the primary AKI definition or death within 48 hours of surgery. This is to account for potential impact early deaths may have on the ascertainment of AKI.
- AKI for at least 2 days. Defined by evidence of a ≥50% or a ≥ 26.5 µmol/L increase in post-operative serum creatinine from the pre-operative value, evident on at least 2 different days within 7 days of surgery. While the magnitude of the peak change in serum creatinine defines the stage of AKI in recent guidelines, a longer duration of AKI is also associated with poorer outcomes.[31]
- Stage 2 AKI or more: Defined as any of the following three criteria: (1) post-operative percent increase in serum creatinine ≥100% from the pre-operative value within 7 days of surgery, (2) increase in post-operative serum creatinine to an absolute value ≥ 353.6 µmol/L within 7 days, or (3) receipt of acute dialysis within 30 days.

In addition to dichotomous outcomes, we will use a linear regression model to compare the two groups in the outcome of percent change in serum creatinine ((peak post-operative serum creatinine (within 7 days of surgery) – pre-operative serum creatinine)/pre-operative serum creatinine), adjusting for the variables described in the primary outcome analysis (assuming model assumptions are not violated; testing presented in Appendix 2). We will report the result as the average difference in percent change in serum creatinine between the two treatment groups with 95% CI.

Subgroup analyses: presence of pre-operative CKD and chronic aspirin use

We will use an interaction term in a mixed effects logistic regression model where centre is treated as a random effect to determine if the adjusted odds ratio of AKI comparing aspirin to placebo differs in those with and without CKD (assuming model assumptions are not violated, testing presented in

Appendix 2). We will interpret a two-tailed p value ≤0.05 as statistically significant. With 6500 patients we will have over 80% power to detect a 45% lower odds ratio of AKI with treatment for patients with and without CKD (an estimate derived from our simulations; in another recent large trial of coronary artery bypass graft surgery performed with and without a bypass pump, the observed percent decrease in the odds ratio of AKI was 40% lower in patients with CKD compared to those without CKD (p-value for interaction 0.01). Similar to techniques used for the CKD subgroup, we will examine subgroups defined by the presence of pre-operative chronic aspirin use (which was a stratification factor used in the randomisation – aspirin initiation and aspirin continuation strata). Similarly, we will have over 80% power to detect a 40% lower odds ratio of AKI with treatment for patients in the continuation stratum compared to the initiation stratum. An increased risk of thrombosis after aspirin discontinuation has been observed in some human studies, recognising the biology is complicated.[18]

Additional Analyses

Longer term kidney function loss after surgery: This analysis will focus on those patients who, at the time of surgery, consent to a single serum creatinine measurement between 3 and 12 months after surgery. For our main analysis, we will use linear regression adjusting for prior listed covariates to compare the absolute change in eGFR between the aspirin and placebo groups, reporting the mean and 95% CI (assuming model assumptions are not violated, Appendix 2). We will carry forward the pre-randomisation serum creatinine value for patients with a missing longer term value after surgery (for reasons of death or missing measurement) and will impute an eGFR value of 5 mL/min/1.73 m^2 for the long term measurement for any patient who developed end-stage renal disease (≥3 months of continuous dialysis), or who died shortly after receipt of acute dialysis for severe AKI. A sample of 3000 patients will allow a 5 mL/min per 1.73 m^2 or more difference to be detected between the two groups with over 80% statistical power (two-tailed alpha 0.05, independent samples t-test, expected standard deviation 40). As observed in the setting of another international trial of coronary artery bypass surgery performed with and without a cardiopulmonary bypass pump, we do not expect to demonstrate peri-operative aspirin versus placebo alters this outcome, even in the situation where we demonstrate aspirin versus placebo reduces the risk of post-operative AKI (which may relate to our study methods, or the limited impact of any observed AKI risk reduction on longer term kidney function).

Post-operative Cardiac Events: Acute cardiac events are inextricably linked to AKI events and both events often occur together in the non-operative setting. In the setting were aspirin versus placebo reduces the risk of AKI, we will also examine cardiac events in the analysis, and report how frequently both co-occur.

A higher risk of AKI with aspirin. Although we hypothesize that aspirin use will prevent peri-operative AKI, our analysis may also elucidate AKI harm from aspirin if it exists. Although nephrotoxicity from non-steroidal anti-inflammatory agents is well appreciated (through the inhibition of intra-renal prostaglandin), aspirin doses of 100 mg/day, as used in POISE-2, is unlikely to be nephrotoxic.[32] However, bleeding with aspirin is an important concern which can cause hypotension and AKI.[7;33] Similarly, blood transfusions may directly predispose to AKI, possibly through some hemolysis.[34] If we

observe aspirin versus placebo increases the risk of AKI, then we will then examine bleeding events and AKI and report how frequently both co-occur.

Exclusion of major urological surgeries: POISE-2 records surgical types in categories, and one of these categories is major urological or gynecological surgery (about 13% of patients enrolled in POISE-2). This category includes the procedure of nephrectomy. Because post-operative changes in serum creatinine in the context of partial or complete nephrectomy can occur for reasons other than AKI, in an additional analysis we will exclude this category of surgery to confirm the results on the remaining sample are no different than the primary results.

Effects of Clonidine on AKI: While our pre-specified hypotheses focus on aspirin, it is conceivable clonidine may reduce the risk of AKI as supported by prior animal and human studies.[19-24] The analytic techniques for the assessment of clonidine on AKI are as specified for aspirin, with only minor adjustments (i.e. adjustment for the random allocation to aspirin, and no prespecified subgroup analysis for chronic aspirin use). If a greater risk of AKI is observed with clonidine versus placebo, then the blood pressure data collected in POISE-2 will be reviewed to determine if this potentially explains the observed effect.

RECOGNIZED LIMITATIONS

There are some limitations to our protocol.

Errors with serum creatinine as a measure of kidney function: In POISE-2 the pre-operative serum creatinine is only recorded once in the 6 weeks prior to surgery (where patients may be undergoing elective, urgent or emergent surgery). It would be preferable to have at least two baseline serum creatinine values on all POISE-2 patients, separated by at least 3 months, to more accurately define the presence of CKD. Also, in POISE-2 there is no knowledge of potential serum creatinine changes close to the time of surgery (particularly in the case of urgent / emergent surgery; 8% of POISE-2 patients) which may mean the pre-operative serum creatinine value is not in a steady state. All of these considerations increase 'noise' related to the baseline serum creatinine measurement, and reduce the ability to detect post-operative changes in the serum creatinine – to partly address this we are adjusting for a definition of urgent / emergent surgery in the primary ANCOVA models. As well, in an additional analysis we will exclude patients undergoing urgent / elective surgery to confirm the findings are robust in remaining patients undergoing elective surgery.

Worldwide, over the last decade, there have been efforts to better standardize the serum creatinine assay. It seems likely (although not documented in POISE-2) that most pre-operative and post-operative serum creatinine measurements prior to hospital discharge would be done in the same laboratory, and most measurements are done within two months of each other. This reduces concerns about inter-laboratory and intra-laboratory variability (drift) in the serum creatinine measurement. However, the same cannot be said for the longer term serum creatinine measurement. As with the pre-operative value, multiple serial measures of serum creatinine over the year (and longer) were desired, but not possible to obtain in POISE-2. Also POISE-2 has no measures of baseline or follow-up proteinuria, which are now featured in more recent CKD staging systems.[35] However, the randomisation and analysis of this

substudy is stratified by centre, which should result in similar measurement errors within a centre for the aspirin and placebo groups.

Limited statistical power for the most clinically important kidney outcomes. Stage III AKI with receipt of acute dialysis, and long-term end-stage renal disease are the most clinically important kidney outcomes. However, there will be too few events for adequate statistical power to detect these outcomes in POISE-2. We focus on mild to moderate AKI (defined by thresholds of changes in serum creatinine). While mild to moderate AKI is the outcome used in virtually all prior AKI prevention trials, we recognize that it is a surrogate outcome that may not directly impact how a patient feels, functions or survives. Similarly, the longer term outcome of between-group difference in change in eGFR is also a surrogate outcome, and concerns about its validity as a measure of kidney disease progression have been raised.[36] For this reason the overall POISE-2 trial results (which focus on non-kidney outcomes) rather than the results of this AKI protocol, should be the primary information used to inform decisions about whether the tested interventions should be adopted as a standard in surgical care.

CONCLUSIONS

Strengths of this POISE-2 AKI protocol and the overall POISE-2 trial are generalizable estimates derived from patients recruited in an international context using rigorous randomised trial methodology (e.g. concealed allocation, placebo-controlled trial, blinded central adjudication of outcomes). In this report, we have judiciously pre-specified the main questions and analytic protocol that will be used to test relevant AKI hypotheses in the POISE-2 AKI substudy. We have done so to minimise the chance of spurious post-hoc assertions of effect, so that the AKI results from this large international are robust and believable.

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Contributorship: All the authors contributed to the conception and design, acquisition of data, and analysis and interpretation of data; drafting the protocol or revising it critically for important intellectual content and final approval of the version to be published.

Figure legend:

Figure 1: Mechanism for Development of Acute Kidney Injury in Major Non-Cardiac Surgery: Role of Aspirin

Table 1. Incidence of Acute Kidney Injury in POISE-2 and associated Statistical Power to detect an Intervention Effect.

	All patients (n=4880) *	Patients with a pre-operative eGFR >60 ml/min/1.73 m² (n=3690)	Patients with a pre-operative eGFR ≤60 ml/min/1.73 m² (n=1190)	Statistical Power to detect a 20% Relative Risk Reduction (6500 patients) ₸
AKI (Primary definition)	580 (11.9%)	398 (10.8%)	182 (15.3%)	88%
Alternate Definitions				
AKI or Death	583 (12.0%)	399 (10.8%)	184 (15.5%)	88%
AKI for at least 2 days	309 (6.3%)	190 (5.2%)	119 (10.0%)	57%
Stage 2 AKI or more	138 (2.8%)	97 (2.6%)	41 (3.5%)	32%

AKI, Acute kidney injury

*Number of POISE-2 AKI substudy patients randomised as of April 2013. By December 2013 expect over 6500 patients will be enrolled into the study.

₸ two-tailed α 0.05, χ^2 test, assumes that the incidence observed in all patients (n=4880), will be the incidence observed in the placebo group.

Reference List

(1) Weiser TG, Regenbogen SE, Thompson KD, et al. An estimation of the global volume of surgery: a modelling strategy based on available data. *Lancet* 2008; 372(9633):139-144.

(2) Abelha FJ, Botelho M, Fernandes V, et al. Determinants of postoperative acute kidney injury. *Crit Care* 2009; 13(3):R79.

(3) Chertow GM, Burdick E, Honour M, et al. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005; 16(11):3365-3370.

(4) Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. *Kidney Int* 2008; 73(5):538-546.

(5) Coca SG, Yusuf B, Shlipak MG, et al. Long-term risk of mortality and other adverse outcomes after acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009; 53(6):961-973.

(6) Landoni G, Bove T, Szekely A, et al. Reducing mortality in acute kidney injury patients: systematic review and international web-based survey. *J Cardiothorac Vasc Anesth* 2013; 27(6):1384-1398.

(7) Gerrah R, Izhar U. Beneficial effect of aspirin on renal function post-cardiopulmonary bypass. *Asian Cardiovasc Thorac Ann* 2003; 11(4):304-308.

(8) Gerrah R, Ehrlich S, Tshori S, et al. Beneficial effect of aspirin on renal function in patients with renal insufficiency postcardiac surgery. *J Cardiovasc Surg (Torino)* 2004; 45(6):545-550.

(9) Mangano DT. Aspirin and mortality from coronary bypass surgery. *N Engl J Med* 2002; 347(17):1309-1317.

(10) Serhan CN, Arita M, Hong S, et al. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. *Lipids* 2004; 39(11):1125-1132.

(11) Serhan CN, Gotlinger K, Hong S, et al. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their aspirin-triggered endogenous epimers: an overview of their protective roles in catabasis. *Prostaglandins Other Lipid Mediat* 2004; 73(3-4):155-172.

(12) Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* 2008; 8(5):349-361.

(13) Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol* 2007; 25:101-137.

(14) Duffield JS, Hong S, Vaidya VS, et al. Resolvin D series and protectin D1 mitigate acute kidney injury. *J Immunol* 2006; 177(9):5902-5911.

(15) Cao L, Young N, Liu H, et al. Preoperative aspirin use and outcomes in cardiac surgery patients. *Ann Surg* 2012; 255(2):399-404.

(16) Rosner MH, Okusa MD. Acute kidney injury associated with cardiac surgery. *Clin J Am Soc Nephrol* 2006; 1(1):19-32.

(17) Devereaux PJ. http://clinicaltrials.gov/ct2/show/NCT01082874?term=POISE-2&rank=1. *last accessed January 16 2014* 2014.

(18) Doutremepuich C, Aguejouf O, Desplat V, et al. Aspirin discontinuation syndromes: clinical implications of basic research studies. *Am J Cardiovasc Drugs* 2013; 13(6):377-384.

(19) Kulka PJ, Tryba M, Zenz M. Preoperative alpha2-adrenergic receptor agonists prevent the deterioration of renal function after cardiac surgery: results of a randomized, controlled trial. *Crit Care Med* 1996; 24(6):947-952.

(20) Myles PS, Hunt JO, Holdgaard HO, et al. Clonidine and cardiac surgery: haemodynamic and metabolic effects, myocardial ischaemia and recovery. *Anaesth Intensive Care* 1999; 27(2):137-147.

(21) Billings FT, Chen SW, Kim M, et al. alpha2-Adrenergic agonists protect against radiocontrast-induced nephropathy in mice. *Am J Physiol Renal Physiol* 2008; 295(3):F741-F748.

(22) Eknoyan G, Dobyan DC, Senekjian HO, et al. Protective effect of oral clonidine in the prophylaxis and therapy of mercuric chloride--induced acute renal failure in the rat. *J Lab Clin Med* 1983; 102(5):699-713.

(23) Solez K, Racusen LC, Whelton A. Glomerular epithelial cell changes in early postischemic acute renal failure in rabbits and man. *Am J Pathol* 1981; 103(2):163-173.

(24) Ideura T, Solez K, Heptinstall RH. The effect of clonidine on tubular obstruction in postischemic acute renal failure in the rabbit demonstrated by microradiography and microdissection. *Am J Pathol* 1980; 98(1):123-150.

(25) Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150(9):604-612.

(26) Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guidelines for acute kidney injury. *Kidney Int* 2012; 2:1-138.

(27) Austin PC. Absolute risk reductions, relative risks, relative risk reductions, and numbers needed to treat can be obtained from a logistic regression model. *J Clin Epidemiol* 2010; 63(1):2-6.

(28) Kahan BC, Morris TP. Adjusting for multiple prognostic factors in the analysis of randomised trials. *BMC Med Res Methodol* 2013; 13:99.

(29) Altman DG. Confidence intervals for the number needed to treat. *BMJ* 1998; 317(7168):1309-1312.

(30) Bender R. Calculating confidence intervals for the number needed to treat. *Control Clin Trials* 2001; 22(2):102-110.

(31) Brown JR, Kramer RS, Coca SG, et al. Duration of acute kidney injury impacts long-term survival after cardiac surgery. *Ann Thorac Surg* 2010; 90(4):1142-1148.

(32) Juhlin T, Jonsson BA, Hoglund P. Renal effects of aspirin are clearly dose-dependent and are of clinical importance from a dose of 160 mg. *Eur J Heart Fail* 2008; 10(9):892-898.

(33) Acedillo RR, Shah M, Devereaux PJ, et al. The risk of perioperative bleeding in patients with chronic kidney disease: a systematic review and meta-analysis. *Ann Surg* 2013; 258(6):901-913.

(34) Vellinga S, Verbrugghe W, De PR, et al. Identification of modifiable risk factors for acute kidney injury after cardiac surgery. *Neth J Med* 2012; 70(10):450-454.

(35) Stevens PE, Levin A. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med* 2013; 158(11):825-830.

(36) Gaspari F, Ruggenenti P, Porrini E, et al. The GFR and GFR decline cannot be accurately estimated in type 2 diabetics. *Kidney Int* 2013; 84(1):164-173.

Appendix 1. Rationale why aspirin would prevent AKI

First, the cardiovascular benefits of aspirin are well known (Mangano D.). Aspirin use is being tested in POISE-2 because it may mitigate peri-operative cardiac events, as aspirin inhibits platelet aggregation (Gerrah R.). As mentioned, acute cardiac events are inextricably linked to AKI events; both frequently co-occur in the non-operative setting.(Newsome B) Thus, preventing acute cardiac events may also prevent AKI.

Second, an emerging basic science literature supports a protective effect of aspirin on AKI. In the last decade, scientists have discovered that the kidneys produce a novel family of endogenous anti-inflammatory lipid mediators (lipoxin, resolvin, protectin) in response to ischemia reperfusion injury (Serhan C.). Administration of resolvin and protectin to mice before ischemia reduced AKI (Duffield J.).Similarly, administration of these mediators 10 minutes after ischemia reperfusion also mitigated AKI. Importantly, production of these beneficial mediators is enhanced by aspirin (Figure 1).

Third, although there is no evidence from randomised controlled trials, four prospective human cohort studies suggest peri-operative aspirin use prevents AKI.

In the *first study*, 94 consecutive patients with chronic kidney disease (serum creatinine ≥ 133 µmol/L) underwent cardiac surgery (Gerrah R). Patients were divided into 2 groups: those who received aspirin (100 mg) until the day of the operation (n = 46) and those who either never took aspirin or whose aspirin was discontinued electively at least 7 days before surgery (n = 48). The baseline characteristics in the 2 groups were almost identical (age ~ 68 years, ~ 75% male, ~ 42% diabetic, baseline serum creatinine 248 umol/L, baseline creatinine clearance 31 mL/min). The mean serum creatinine was significantly lower 2 days after surgery in those who took pre-operative aspirin compared with those who did not (247 µmol/L (sd 141) vs. 327 µmol/L (sd 141), p = 0.001) as was the serum creatinine at the time of hospital discharge (p < 0.001). Similar results were seen in creatinine clearance and 24-hour urine output. The number of acute dialysis events was lower among patients who took aspirin (5 vs. 9 events). The authors concluded: “Thromboxane has an important role in the pathophysiology of kidney injury, and increased thromboxane levels correlate with drops in kidney function. Thromboxane is a very potent vasoconstricting agent, and is at least partially responsible for the kidney injury. Furthermore, aspirin is an anti-aggregating agent and can reduce platelet clumps, resulting in improved glomerular blood flow. Aspirin can also decrease the risk of microembolization in the rich vascular bed of the kidney”. To support this assertion, the authors published related studies where they measured thromboxane levels in the same setting. Thomboxane levels were lower in the urine of those who took pre-operative aspirin compared with those who did not (p < 0.001).

In the *second study,* 5022 patients in 70 centres who survived for 48 hours after cardiac surgery were prospectively enrolled in a cohort study (Mangano D.). Some patients received aspirin (ranging from 80 mg to 650 mg / day) within 48 hours of surgery (n = 2999), while others did not (n = 2023). The characteristics of these two groups of patients were similar (median age ~ 64 years, 21% women, 30% diabetic, 67% hypertensive, 52% a history of myocardial infarction). There were fewer AKI events in aspirin users (1.8% vs. 4.9%, p < 0.001) and fewer patients required acute dialysis (0.9% vs. 3.4%, p < 0.001) [AKI defined by a serum creatinine of at least 177 µmol/L accompanied by an increase of at least 62 µmol/L from the pre-operative value]. The authors concluded: “Aspirin had a broad effect, substantially mitigating both fatal and nonfatal damage not only to the heart, but also to the brain and kidneys. These findings suggest that the platelet has a fundamental role in orchestrating the ischemic response to reperfusion injury by multiple organs in surgery”. The authors go on to present three ancillary analyses to support this hypothesis.

The *third study* was recently reported in abstract form at an anesthesia meeting (Longhui C.). A total of 1148 patients undergoing elective cardiac surgery were divided into two groups: 288 patients who took aspirin within 5 days preceding surgery, and 860 patients who did not. Baseline characteristics were similar in both groups. The incidence of AKI (undefined) was significantly lower in the aspirin group (2.6% vs. 5.2%, p = 0.03) as was the need for post-operative dialysis (0.8% vs. 3.1%).

In the *fourth study* 2868 patients undergoing cardiac surgery in 2 tertiary hospitals were divided into 2 groups: those taking (n = 1923) or not taking (n = 945) aspirin within 5 days proceeding surgery. The propensity scores adjusted and multivariate logistic regression showed that preoperative aspiring therapy (vs non aspirin) significantly reduced the risk of postoperative renal failure (3.7% vs. 7.1%, odds ratio 0.384, 95% CI 0.254 – 0.579) and receipt of dialysis (1.9% vs. 3.6%, odds ratio 0.441, 95% CI 0.254 to 0.579).

Appendix 1 references

Mangano DT. Aspirin and mortality from coronary bypass surgery. *N Engl J Med* 2002; 347(17):1309-1317.

Gerrah R, Izhar U. Beneficial effect of aspirin on renal function post-cardiopulmonary bypass. *Asian Cardiovasc Thorac Ann* 2003; 11(4):304-308.

Gerrah R, Ehrlich S, Tshori S, Sahar G. Beneficial effect of aspirin on renal function in patients with renal insufficiency postcardiac surgery. *J Cardiovasc Surg (Torino)* 2004; 45(6):545-550.

Newsome BB, Warnock DG, McClellan WM, Herzog CA, Kiefe CI, Eggers PW et al. Long-term risk of mortality and end-stage renal disease among the elderly after small increases in serum creatinine level during hospitalization for acute myocardial infarction. *Arch Intern Med* 2008; 168(6):609-616.

Duffield JS, Hong S, Vaidya VS, Lu Y, Fredman G, Serhan CN et al. Resolvin D series and protectin D1 mitigate acute kidney injury. *J Immunol* 2006; 177(9):5902-5911.

Serhan CN, Arita M, Hong S, Gotlinger K. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. *Lipids* 2004; 39(11):1125-1132.

Serhan CN, Gotlinger K, Hong S, Arita M. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their aspirin-triggered endogenous epimers: an overview of their protective roles in catabasis. *Prostaglandins Other Lipid Mediat* 2004; 73(3-4):155-172.

Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* 2008; 8(5):349-361.

Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol* 2007; 25:101-137.

Longhui C, Nishanthi K, Scott S, Zvi G, Jian-Zhong S: Does Preoperative Aspirin Reduce Cardiocerebral Ischemic Events in Cardiac Surgery Patients? [Abstract]. *Annual Meeting of the American Society Anesthesiologists* 2010

Cao L, Young N, Liu H, Silvestry S, Sun W, Zhao N et al. Preoperative aspirin use and outcomes in cardiac surgery patients. *Ann Surg* 2012; 255(2):399-404.

Appendix 2. Testing statistical model assumptions.

To test the assumptions of the logistic regression model we will use the following steps: 1) visual assessment of the plot of residuals versus predicted values to assess model fit and residual trends; 2) a Hosmer-Lemeshow test to assess the goodness of fit of the logistic regression model (where a Hosmer-Lemeshow test p-value <0.05 indicates a poor fit). If the logistic regression assumptions are not appropriate, (1) to obtain estimates of the adjusted and stratified odds ratios we will use nonparametric ANCOVA with logit transformation; (2) to obtain estimates of the adjusted relative risk of aspirin versus placebo on AKI, we will use nonparametric analysis of covariance (ANCOVA) with log transformation. If the logistic regression assumptions are not appropriate, to test for interaction, we will test whether the (unadjusted) log relative risks for each subgroup are equal, assuming the distribution of the difference in log relative risks is Normal.

To test the assumptions of the linear regression model we will use the following steps: 1) visual assessment of the normal probability plot of residuals to assess whether residuals are normally distributed; 2) visual assessment of the plot of residuals versus predicted values to assess model fit and homoskedasticity of residuals; 3) the Durbin-Watson test statistic to test for autocorrelation of residuals when data are ordered by randomisation date (significant autocorrelation is detected if the test p-value is <0.05; Cook's D statistic to detect outlying observations (where we will investigate a Cook's D > |2| as influential). If the residuals are non-normal or heteroskedastic, rather than a linear regression model we will use a non-parametric analysis of covariance with covariates to test whether the mean response values are equal between the groups. We expect no significant effect of time on responses since the study accrual period is less than four years. If there are influential observations we will exclude them in sensitivity analysis, comparing the output in our main result.

Appendix 2 references

http://support.sas.com/documentation/cdl/en/etsug/60372/HTML/default/viewer.htm#etsug_autoreg_sect024.htm. (website) 2012.

Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996 Feb 28;15(4):361-87

Bewick V, Cheek L, Ball J. Statistics review 14: Logistic regression. Crit Care 2005; 9(1):112-118

Altman DG, Bland JM. Interaction revisited: the difference between two estimates. BMJ 2003; 326(7382): 219

Koch GG, Tangen CM, Jung JW, Amara IA. Issues for covariance analysis of dichotomous and ordered categorical data from randomized clinical trials and non-parametric strategies for addressing them. Statistics in Medicine 1998; 17:1863-1892

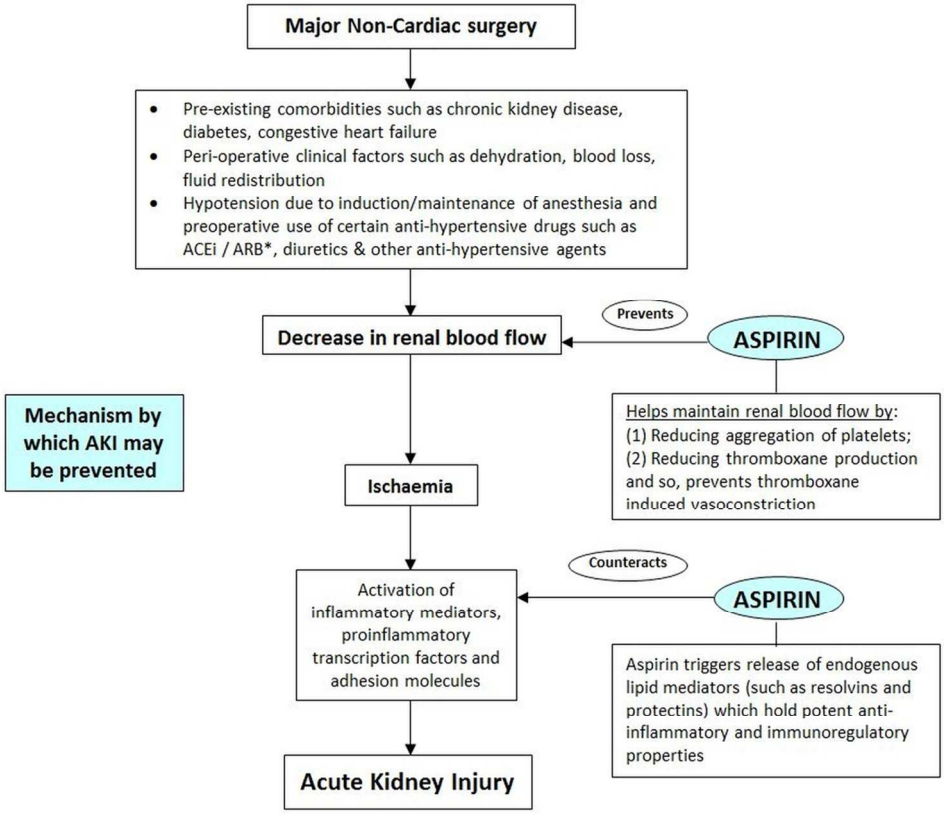
Zink RC, Koch GG. NParCov3: A SAS/IML Macro for Nonparametric Randomization-Based Analysis of Covariance. Journal of Statistical Software 2012; 50(3)

Appendix 3. Obtaining estimates of relative risk from a logistic regression model

The probability of the outcome (AKI) will be estimated for each patient twice using parameter estimates from the mixed effects logistic regression adjusted for covariates: once given that the patient was treated (i.e. received aspirin) and once given that the patient received placebo. The average probability of outcome given all patients were treated is calculated, and the average probability of outcome given all patients were not treated is calculated. The ratio of these two averages is the adjusted relative risk of outcome; the difference in probabilities divided by the estimated probability of outcome given all patients were not treated times 100% is the adjusted relative risk reduction. We will use bootstrap methods to obtain a confidence interval for the relative risk reduction: (1) we will draw a random sample with replacement from the original sample of the same size as the sample, (2) for each bootstrap sample we will compute the adjusted relative risk reduction, (3) we will repeat the process 1,000 times, with the 2.5^{th} and 97.5^{th} percentiles of the resulting boostrap relative risk reduction distribution corresponding to the 95% confidence interval for the adjusted relative risk reduction.

Appendix 3 reference

Austin PC. Absolute risk reductions, relative risks, relative risk reductions, and numbers needed to treat can be obtained from a logistic regression model. J Clin Epi 2010; 63(1):2-6



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