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