Appendix: CORONARY Kidney Substudy Analytic Protocol

Testing the assumptions of the linear regression model

We will test the assumptions of the linear regression model using the following steps: 1) visual assessment of a 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 homoscedasticity of residuals; 3) the Durbin-Watson statistic to test for autocorrelation of residuals when data are ordered by randomization date (significant autocorrelation is detected if the test p-value is <0.05;[1]; 4) Cook's D statistics to detect outlying observations (where we will investigate a Cook's D $>|2|$ as influential.[2] If the residuals are non-normal or heteroscedastic, rather than a linear regression model we will use a non-parametric analysis of covariance with covariates to test whether the median response values are equal between the two groups. Since the study accrual period is only four years we expect no significant effect of time on the responses. If there are influential observations we will exclude them in sensitivity analysis, comparing the output to our main result.

Testing the assumptions of the logistic regression model

We will test the logistic regression model assumptions using the following steps:1) visual assessment of the plot of residuals versus predicted values to assess model fit and residual trends ; 2) for each observation we will compute the generalized difference of fits (GDFFITS) (a measure of how influential they are; if this measure is $\geq c*(k/(n-d))^{1/2}$ then the observation will be considered influential, where c is an arbitrary constant, k is the number of regression parameters, n is the number of observations (patients) and d is the number of observations that have been deleted;[3]; 3) 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 [4]). If the logistic regression model does not provide a good fit, we will derive the odds ratio from the Mantel-Haenszel method, stratified by centre and pre-operative CKD.[5]

Bootstrapping method to derive the 95% confidence interval for the relative risk

The bootstrapping method involves the following steps: 1) we will draw a random sample of 4700 patients with replacement after the selection of each patient from the original sample of 4700 patients, 2) for each bootstrap sample, we will compute the adjusted relative risk, 3) we will repeat this process 2,000 times, with the 2.5^{th} and 97.5^{th} percentiles of the resulting bootstrap relative risk distribution corresponding with the 95% confidence interval for the adjusted relative risk.

Rank based assessment accounting for competing event of death

We appreciate if there is a differential impact of the intervention on 1-year mortality across the surgical groups this may introduce informatively missing 1-year eGFR values. To consider this issue we will perform a rank based procedure as follows: patients who die before 12 months will be ranked from lowest (indicating the poorest outcome) to the highest on the basis of survival time after surgery before death. Similarly, surviving patients will next be ranked from lowest to highest on the basis of survival time from surgery to the onset of ESRD (for patients who transition from acute dialysis to chronic dialysis care, this will be defined by 90 days after receipt of the first acute dialysis treatment). Finally patients who survive 12 months will be ranked on the basis of unfavourable to favourable 1-year percent change in eGFR. For patients who did not die or develop ESRD but have a missing 1-year serum creatinine measurement, we

will right-censor 1- year after surgery and impute a value of zero percent change in eGFR. We will compare ranks between the treatment groups using a log-rank test and calculate hazard ratios and 95% confidence intervals with the use of Cox proportional-hazards regression.[6] We will report the rate of missing 1-year eGFR values for both surgical groups for patients who did not die before 12 months.

Test of mediation: AKI as a percent change in serum creatinine

Methods to test for a significant mediation effect in the setting where the mediating and response variables can be modeled using linear regression include Baron and Kenny's causal steps , Sobel first-order test , PRODCLIN, percentile bootstrap, and bias corrected bootstrap.[7-9] We will use the PRODCLIN percentile bootstrap method to estimate the percentage of the total effect of surgery type (off-pump vs. on-pump CABG) on percent change in eGFR at 1- year (eGFR measured at 1- year compared to pre-operative eGFR) that is mediated by peri-operative percent change in serum creatinine [(peak post-operative serum creatinine – pre-randomization serum creatinine)/pre-randomization serum creatinine].[10]

Let X denote the binary variable indicating surgery type where X=1 for off-pump surgery and 0 for on-pump surgery (on-pump surgery is the referent surgery type). Let M denote percent change in serum creatinine. The linear regression model relating M to X is

$$E(M|X;\alpha_0, \alpha) = \alpha_0 + \alpha X \text{ . (Model 1)}$$

Then α represents the average difference in percent change in peri-operative serum creatinine comparing patients receiving off-pump surgery to those receiving on-pump surgery. Let Y denote percent change in eGFR [(post-operative eGFR measured 1-year after surgery - pre-randomization eGFR)/pre-randomization eGFR]. The linear regression model relating Y to X and M is

$$E(Y|X, M; \tau_0, \tau^*, \beta) = \tau_0^* + \tau^* X + \beta M \text{ . (Model 2)}$$

Then τ^* represents the direct effect of surgery type on percent change in eGFR (i.e. the effect that is not mediated by peri-operative percent change in serum creatinine), and β represents the effect of the percent change in peri-operative serum creatinine on percent change in eGFR at 1- year, controlling for surgery type. Note that the model relating X to Y is

$$E(Y|X; \tau_0, \tau^*, \beta) = \tau_0 + \tau X \text{ , (Model 3)}$$

where τ represents the total effect of surgery type on percent change in 1-year eGFR.

To detect mediation using the PRODCLIN method, we will perform the following steps:

1) Fit the data to obtain an estimate of α from (Model 1), $\hat{\alpha}$. Let $\hat{\sigma}_\alpha$ denote the standard error of $\hat{\alpha}$.
2) Fit the data to obtain an estimate of β from (Model 2), $\hat{\beta}$. Let $\hat{\sigma}_\beta$ denote the standard error of $\hat{\beta}$.
3) The product of the estimated coefficients $\hat{\alpha}$ and $\hat{\beta}$ is the indirect effect of X on Y. This product is not asymptotically normally distributed therefore a method to create confidence intervals based on the distribution of $\alpha\beta$ will be used. To create a confidence interval for $\hat{\alpha}\hat{\beta}$, we will undertake the following steps:
    a. Calculate $z_\alpha = \hat{\alpha}/\hat{\sigma}_\alpha$ and $z_\beta = \hat{\beta}/\hat{\sigma}_\beta$.
    b. Obtain critical values from the PRODCLIN Fortran program (http://www.public.asu.edu/~davidpm/ripl/Prodclin/) using z_α and z_β and the type I error rate equal to 0.05.

c. Let $\delta_{\substack{lower \\ upper}} = \dfrac{critical\ value - \dfrac{\hat{\alpha}\hat{\beta}}{\hat{\sigma}_{\alpha}\hat{\sigma}_{\beta}}}{\sqrt{\dfrac{\hat{\alpha}^2}{\hat{\sigma}_{\alpha}^2} + \dfrac{\hat{\beta}^2}{\hat{\sigma}_{\beta}^2} + 1}}$.

d. The limits $= \hat{\alpha}\hat{\beta} \mp \delta_{\substack{lower \\ upper}} \times \hat{\sigma}_{\alpha\beta}$, where $\hat{\sigma}_{\alpha\beta} = \sqrt{\hat{\alpha}^2\hat{\sigma}_{\beta}^2 + \hat{\beta}^2\hat{\sigma}_{\alpha}^2}$, are the lower and upper confidence limits respectively of the indirect effect of surgery type on 1-year percent change in eGFR. If the confidence interval does not contain zero, then the indirect effect is significant and we will conclude that some or all of the effect of surgery type on 1-year eGFR is mediated by the percent change in peri-operative serum creatinine caused by surgery type.[9]

If the indirect effect is significantly different from zero, the proportion of the total effect of surgery type on 1-year percent change in eGFR that is mediated through percent change in peri-operative serum creatinine, given by $\dfrac{\hat{\alpha}\hat{\beta}}{\hat{\tau}}$, will be reported. To do this, we will use (Model 3) to fit the data in order to obtain an estimate of τ, $\hat{\tau}$. We will report the bootstrap confidence interval for this measure. To do this, and expected 2,000 bootstrap samples of size n will be taken from the original sample of size n with replacement, and for each bootstrap sample, we will calculate the value. We then use bootstrap estimates corresponding to the ω/2 and the 1 - ω/2 percentiles of the bootstrap distribution to create a 100(1 – ω)% confidence interval, where ω is the type I error rate.

We will use previously described techniques to impute the peri-operative and 1-year serum creatinine value in cases where it is missing (expect this to be done in a minimal number of cases). Similarly, covariates that have been previously described will be used in the three linear regression models (steps (1) and (2)) to improve statistical efficiency.

The Sobel mediation method is similar to, but more conservative than, the PRODCLIN mediation method.[9] If a trial is adequately powered to detect a significant indirect effect using the Sobel mediation procedure, we will then assume there is adequate power to detect a significant effect using the PRODCLIN method. Presented in the table below are empirical power calculations for the Sobel mediation method for different true values of α and β from (Model 1) and (Model 2) respectively.

Power calculations for Sobel mediation tests

		β		
		-0.5	**-1**	**-1.5**
α	**-1**	10%	6%	7%
	-3	26%	30%	31%
	-5	58%	59%	59%
	-7	87%	87%	88%
	-9	97%	99%	99%

Results presented for given α and β from (Model 1) and (Model 2) respectively, for τ^{*}=3 from (Model 2), standard deviation of M|X = 50, standard deviation of Y|X,M = 50, and type I error rate=0.05. Number of subjects per simulated dataset is 2,000 and the number of datasets simulated per α, β combination is 300. In this table, if α=-3 then the percent change in peri-operative serum creatinine is 3 units lower than in the on-pump surgery group. If

β=-1, then a one unit increase in percent change in post-operative serum creatinine is associated with a 1% decrease in eGFR at 1-year, adjusted for surgery type.

In the event that the linear regression model assumptions do not hold for (Model 1), (Model 2) or (Model 3), we will use non-parametric methods as described below for binary variables to investigate mediation.

In the event that a significant subgroup by pre-operative eGFR categories ($\leq$60 mL/min per 1.73m^2 vs. >60 mL/min per 1.73m^2) effect is detected in prior analyses, we will conduct two mediation analyses, one for each subgroup. We note that power to detect significant mediation will be compromised if there is a significant subgroup effect.

Test of mediation: categorized AKI

Let X denote surgery type as before. Let M indicate post-operative AKI defined by a 50% increase in serum creatinine or more. Let Y indicate a 1-year decrement in eGFR of at least 20% compared to the pre-operative value. We define a direct effect as the effect of X on Y that is not mediated by M, and define an indirect effect as the effect of X on Y that is mediated by M.

Definition of values used to compute direct effects, indirect effects and total effects when X, M and Y are binary.

	X	**M**	**Y**	**P(Y=1|x, m) = g_{xm}**	**P(M=1|x) = h_x**
n_1	0	0	0	$n_2/(n_1+n_2) = g_{00}$	$(n_3+n_4)/(n_1+n_2+n_3+n_4) = h_0$
n_2	0	0	1		
n_3	0	1	0	$n_4/(n_3+n_4) = g_{01}$	
n_4	0	1	1		
n_5	1	0	0	$n_6/(n_5+n_6) = g_{10}$	$(n_7+n_8)/(n_5+n_6+n_7+n_8) = h_1$
n_6	1	0	1		
n_7	1	1	0	$n_8/(n_7+n_8) = g_{11}$	
n_8	1	1	1		

The direct effect (DE), the indirect effect (IE), and the total effect (TE) are given by:

$DE = (g_{10} - g_{00})(1 - h_0) + (g_{11} - g_{01})\, h_1$

$IE = (h_1 - h_0)(g_{01} - g_{00})$

$TE = g_{11}h_1 + g_{10}(1-h_1) - [g_{01}h_0 + g_{00}(1-h_0)]$

The mediated effect (ME) is given by

$$ME = (1-(DE/TE))*100\%.$$

The mediated effect is the percentage of the total effect that is owed to mediation [11]. We will obtain a confidence interval for the mediated effect using the percentile bootstrap method. We will take 2,000 bootstrap samples of size n from the original sample of size n with replacement, and for each bootstrap sample, the value ME is calculated. We will use bootstrap estimates corresponding to the $\omega/2$ and the $1 - \omega/2$ percentiles of the bootstrap distribution to create a $100(1 - \omega)\%$ confidence interval, where ω is the type I error rate.[9] We will report the confidence interval and we will conclude that significant mediation is detected if the confidence interval does not contain zero.

Appendix References

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