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Circulating biomarkers are not asasociated with endoleaks after endovascular repair of abdominal aortic aneurysms

Joseph V. Moxon,[1,2] Eugene Ng,[3] Sharon M. Lazzaroni,[1] Margaret Boult,[4] Ramesh Velu,[3] Robert A. Fitridge,[4] Jonathan Golledge[1,2,3*]

1. The Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, Townsville, QLD, Australia.

2. The Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, QLD, Australia.

3. The Department of Vascular and Endovascular Surgery, the Townsville Hospital, Townsville, QLD, Australia,

4. Discipline of Surgery, The University of Adelaide, The Queen Elizabeth Hospital, Adelaide, SA, Australia.

* To whom correspondence should be addressed:

Email: jonathan.golledge@jcu.edu.au; Phone: +61 (0)7 4781 4130; Fax: +61 (0)7 4725 8250

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ABSTRACT

Objective: Endoleak is a common complication of endovascular repair (EVAR) for abdominal aortic aneurysm (AAA), but can only be detected through prolonged follow-up with repeated aortic imaging. This study examined the potential for circulating matrix metalloproteinase-9 (MMP9), osteoprotegerin (OPG), D-dimer, homocysteine (HCY) and C-reactive protein (CRP) to act as diagnostic markers for endoleak in AAA patients undergoing elective EVAR.

Methods: Linear mixed effects models were constructed to assess differences in AAA diameter after EVAR, between groups of patients who did, and did not develop endoleak during follow-up, adjusting for potential confounders. Circulating MMP9, OPG, D-dimer, HCY and CRP concentrations were measured in pre- and post-operative plasma samples. The association of these markers with endoleak diagnosis was assessed using linear mixed effects adjusted as above. The potential for each marker to diagnose endoleak was assessed using receiver operator characteristic (ROC) curves.

Results: Seventy-five patients were included in the current study, 24 of whom developed an endoleak during follow-up. Patients with an endoleak had significantly large AAA sac diameters than those that did not have an endoleak. None of the assessed markers showed a significant association with endoleak. This was confirmed through ROC curve analyses indicating poor diagnostic ability for all markers.

Conclusions: Circulating concentrations of MMP9, OPG, D-dimer, HCY and CRP were not associated with endoleak in patients undergoing EVAR in this study.

INTRODUCTION

Abdominal aortic aneurysm (AAA) affects ~2% of men over the age of 65 years, and is a leading cause of mortality in the elderly [1-5]. The main current treatment for AAA is endovascular repair (EVAR). This involves the endovascular placement of stent grafts to isolate the AAA wall from the main aortic blood flow [6]. Despite low perioperative morbidity and mortality, the durability of EVAR is of concern as a high proportion of patients have continued perfusion of the AAA sac or endoleak [7-9]. Endoleak is the most common complication of EVAR, and may occur due to incomplete seal of the proximal or distal ends of the graft (type I endoleak), reverse flow through collateral arteries (type II endoleak), or stent defects (types III-V) [10, 11]). Patients undergoing EVAR require long-term monitoring involving computed tomography and/or ultrasound to detect endoleak [12]. This has several disadvantages including repeated exposure of the patient to ionising radiation, and the requirement for specialist infrastructure and trained staff which negatively impacts on the cost-effectiveness of EVAR [10].

It has been suggested that the current disadvantages associated with imaging-based monitoring may be overcome through the discovery of blood-borne markers to diagnose endoleak, which may ultimately reduce the need for post-EVAR imaging [10]. This is based on the theory that successful EVAR will place a physical barrier between the aneurysmal wall and the bloodstream, thereby reducing the circulating concentrations of AAA-secreted proteins. Continued perfusion of the AAA sac due to endoleak would therefore be reflected by persistent elevations or spikes in the circulating concentration of AAA biomarkers during follow-up. Aortic inflammation, excessive extracellular matrix remodeling and thrombosis are implicated in AAA pathogenesis, suggesting that circulating markers of these processes

may be useful in diagnosing endoleak [4, 13-15]. To date, relatively few studies have specifically investigated the association of blood-borne markers with the presence of endoleak, and the potential value of a blood marker-based approach for EVAR surveillance remains unclear. The aim of the current study was therefore to assess the association of circulating concentrations of 3 putative biomarkers previously associated with AAA presence (matrix metalloproteinase-9 [MMP9], osteoprotegerin [OPG], and D-dimer), and two routinely assessed blood parameters (homocysteine [HCY] and C-reactive protein [CRP]) with endoleak, in a cohort of patients undergoing elective EVAR.

METHODS

Patient recruitment and follow-up: This study analysed a subset of patients recruited to the Australian EVAR outcomes modelling trial which has been described in detail in previous publications [16-18]. For the purposes of this study, patients undergoing EVAR were followed prospectively to monitor outcome. To be eligible for inclusion in the current study, patients were required to have i) undergone elective EVAR to repair an AAA; ii) received at least 1 infra-renal aortic computed tomography angiogram pre, and post-EVAR; and iii) provided a fasting blood sample pre-, and at least 3 months post-EVAR. All patients provided written informed consent upon recruitment, and the study was conducted under institutional ethics approval in accordance with the guidelines of the Declaration of Helskini. Follow-up was conducted according to institutional guidelines. All patients underwent imaging at 1 and/or 6 months after EVAR, followed by repeated scans at 12, 24 and 36 months.

Diagnosis of endoleak: This was performed through assessment of computed tomographic angiography as a contrast blush inside the AAA sac after EVAR. To be included in the

current study this endoleak needed to have been confirmed on at least two occasions during follow-up.

Data collection and definitions used: Characteristics collected from each patients included age at the time of operation (referred to as age), sex, history of smoking, hypertension, diabetes mellitus, ischaemic heart disease (IHD) and prescribed medications. Height and weight were measured and were used to calculate body mass index (BMI; calculated as weight in kg/ height in metres^2). For the purposes of this study patients were classified as having never or ever smoked. Diabetes mellitus and hypertension were defined by a history of diagnosis or treatment for these conditions. Serum lipids (total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides), HCY, CRP and creatinine were measured in hospital pathology laboratories using previously described methods [19].

Assessment of circulating AAA biomarkers: Commercial ELISAs were used to measure plasma concentrations of MM9, OPG (R&D Systems, both using plasma collected in EDTA-coated tubes) and D-dimer (Technozym, using plasma collected in Sodium citrate-coated tubes) according to the manufacturer's directions. We have previously used these kits to analyse clinical samples with excellent reproducibility [20-22].

Statistical analyses: Demographic differences between patient groups were compared by univariate statistics using the SPSS software package (version 23.0, IBM, Armonk, NY, USA). Continuous demographic variables were compared using the Mann-Whitney U test and were presented as median and inter-quartile range. Nominal variables were presented as

count and percent, and were compared using the Chi-squared test. Longitudinal comparisons to assess changes in AAA diameter and circulating biomarker concentrations were performed using random-intercept linear mixed effects models using the freely available R statistical package as previously described [23]. Initially this was assessed in unadjusted analyses including, endoleak presence and time as fixed effects, and variation between individual patients as a random effect (unadjusted models). Time was treated as a factorial variable in models assessing AAA diameter as images were acquired at set intervals post-EVAR. For models assessing biomarker concentrations, time was considered a continuous variable owing to variations in blood collection intervals following EVAR. For these analyses MMP-9, D-dimer, OPG and CRP concentrations were log-transformed to conform to model assumptions. We then assessed the association of biomarkers with endoleak presence in multivariable linear mixed effects models including age, prescription for statins and hypertension as additional fixed effects, based on observations of significant differences for these variables between groups on univariate comparisons (adjusted models). Model fit was assessed by examining the distribution of residuals using qq-normal plots, and scatter plots of the fitted values vs standardized residuals. No issues with residual distributions were observed for the models reported. To minimize the potential for over-parameterisation, goodness of fit for each model was assessed using Akaike's second order Information Criterion (AICc), whereby lower scores denote better model fit. The potential for the assessed blood markers to diagnose endoleak was further examined using receiver operator characteristic (ROC) curves generated for the unadjusted and adjusted models. For these analyses, post-operative plasma concentrations of each marker were used. The difference in AUC of the unadjusted and adjusted analyses was assessed using DeLong's test for paired ROC curves. The additive benefit of considering each of the biomarkers in diagnosing endoleak was assessed in sensitivity analyses, which comparing the AUC of each adjusted model to that of a base

model which included the relevant covariates (age, hypertension and statin prescription), but omitted the blood marker.

For all analyses, p-values <0.05 were considered statistically significant.

Sample size calculation: Sample sizes for the current study were calculated based on previous data from studies assessing the association of circulating MMP9 concentrations with endoleak diagnosis. Findings from our recent meta-analysis indicated that circulating concentrations of MMP9 would be markedly higher in the endoleak patients than the controls [10]. One of the largest studies in this field was conducted by Sangiorgi *et al.* who reported that circulating MMP9 concentrations were 40.2 (±20.9) ng/mL in patients with endoleak, and 23.6 (±10.4) in non-endoleak controls [24]. Assuming similar data in the current study, sample size calculations suggested this difference could be detected with 80% power by including 13 cases and 25 controls (effect size 1.01, 2-tailed alpha 0.05). Final participant numbers in the current study exceeded these predicted sample sizes, and we therefore considered this investigation to be appropriately powered.

RESULTS

Pre-operative characteristics of patients who did, and did not have endoleak within 6 months of EVAR

A total of 75 patients undergoing EVAR fulfilled the inclusion criteria for the current analysis. The majority (86.7%) of patients received aorto-bi-iliac stents, the remainder received aorto-uni-iliac grafts (6.7%), or fenestrated devices (5.3%). Stent type was not reported for 1 patient. During follow-up, 24 (32.0%) patients were identified to have an endoleak. Of these, 2 (8.3%), were type-I endoleaks, 20 (83.3%), were type-II endoleaks, and

1 patient developed both type-I and type-III endoleaks. The cause of endoleak could not be ascertained for 1 patient. Endoleak was not associated with the type of stent used. Compared to those who did not develop endoleak, patients who developed endoleak were older (P=.023), were more likely to be prescribed statins, with concomitantly lower circulating LDL-C concentrations (both P=.036), and had higher circulating HCY concentrations prior to operation (Table I). The prevalence of hypertension was also higher in the endoleak group, which although not statistically significant (P=.051), was considered a confounding variable in subsequent analyses.

Comparing changes in post-EVAR AAA diameter in groups of patients who did and did not develop endoleak

Figure 1 shows AAA diameter measured over time for patients who did, and did not develop endoleak during follow-up. Linear mixed effects modelling demonstrated significant differences in changes in AAA diameter between the groups over the follow-up period (P<.001; Figure 1 and Supplementary File I). More specifically, modelling analyses demonstrated that AAA diameters were similar between groups at the time of EVAR, and 1 month thereafter, but were significantly larger in the endoleak group at 6 months after EVAR and for the remainder of follow-up.

Testing the associations of circulating markers with endoleak presence

Circulating concentrations of D-dimer, MMP9, OPG, HCY and CRP were measured in fasting blood samples collected from all patients at recruitment (pre-EVAR), and during follow-up (post-EVAR; Table II). Plasma D-dimer concentrations increased in all patients after EVAR (P<.001 for patients who did and did not suffer endoleak). Similarly, a trend towards increased plasma concentrations of HCY and OPG after EVAR was also observed

for both groups, however significance was only observed for patients who did not develop endoleak. No differences in pre- and post-operative plasma concentrations of MMP9 and CRP were observed for either group. Temporal changes in the plasma concentrations of the assessed markers were compared between patient groups using linear mixed effects models. No significant association of any of the assessed blood markers with endoleak was observed during follow-up, evidenced by the absence of robust interactions between time and endoleak status in the models (Table II and Supplementary File II).

Investigating the potential for the assessed biomarkers to diagnose endoleak

The ability for post-operative concentrations for each of the assessed markers to identify patients suffering endoleak was investigated using ROC curves (Table III). The AUC for each assessed marker was low when assessed alone (range 0.469-0.624), but markedly increased when considered in conjunction with age, statin use and hypertension (adjusted models; range of AUC for adjusted model: 0.760-0.844; P-value for improvement in AUC <.050 for all markers except D-dimer). Sensitivity analyses, however, demonstrated that this increase in AUC was largely attributable to consideration of the covariates, rather than the assessed biomarker (Table III).

DISCUSSION

The current study investigated the association of several markers for AAA with endoleak diagnosis in a cohort of prospectively followed patients undergoing EVAR. AAA sac diameters of the patients who developed endoleak were significantly higher than those who had successful EVAR, however, none of the examined biomarkers were associated with endoleak in this patient population.

MMP9 is a zinc-dependent gelatinase which has been implicated in AAA development and progression through proteolytic degradation of the aortic extracellular matrix [10]. Several prior investigations have assessed the relationship between circulating MMP9 concentrations and endoleak presence, although the significance and extent of the reported associations vary between studies [24-28]. A recent meta-analysis of these studies identified a significant positive association of plasma MMP9 concentration with endoleak presence [10]. The findings of the meta-analysis are contradicted by those of the current study as no relationship between endoleak diagnosis and circulating MMP9 concentration was observed. The reasons for this discrepancy may in part be related to differences in populations studied or different handling of blood samples and assessment methods. Monaco *et al.* for example specifically investigated endoleaks following EVAR for descending thoracic aortic aneurysm, compared to AAA included in the current study, and differences in disease pathophysiology may complicate direct comparison of their findings and ours [25]. Sample sizes used in the current study were larger than those of the previous reports which assessed MMP9 in AAA patients with endoleak [24, 26-28], suggesting greater analytical power, although further studies employing large patient cohorts are needed to more definitively assess the association of circulating MMP9 concentrations with endoleak presence.

Numerous reports have suggested that circulating D-dimer concentrations are elevated in patients with AAA [20, 29-31], attributable in part to the formation of a large non-occlusive thrombus within the aneurysmal sac [32]. Surprisingly, the association of plasma D-dimer concentration with endoleak has only been directly examined in a single study which reported that circulating D-dimer concentrations were significantly higher in patients suffering type 1 endoleak (compared to non-endoleak controls), and were highly diagnostic for type 1 endoleak following ROC analysis [33]. Importantly, sample sizes in this previous study were

extremely small (n=4 for the type 1 endoleak group), making it hard to draw firm conclusions from the presented data. Indeed, these findings are challenged by that of a larger study suggesting that post-EVAR plasma fibrinogen degradation product concentrations (of which D-dimer is a component) are lower in patients with endoleak than those without [34]. Data from the current study contrast with both of these reports. We observed no association of plasma D-dimer concentration with endoleak, but did note significant increases in circulating D-dimer titres for both groups following EVAR. The reasons for this remain unclear, although previous studies have independently reported an increase in circulating D-dimer concentration following EVAR [35-38]. This is arguably due to thrombosis of the AAA sac around the stent graft, although some studies suggest that D-dimer titres return to basal levels within 1 month [35], whereas others report an elevation which persists for up to 6 months [36-38]. The median time to post-operative blood collection for the current study was ~8 months for the whole cohort. This therefore suggests a prolonged elevation in circulating D-dimer concentration following EVAR, which therefore limits the ability for this marker to diagnose endoleak.

OPG is a member of the tumour necrosis factor receptor super-family, [39], and we and others have reported a positive association of circulating OPG concentration with AAA presence [21, 40, 41]. To our knowledge, this is the first study to directly assess the association of OPG with endoleak presence. Our data identified a post-operative increase in median plasma OPG concentration in the patients who did not develop endoleak, however, no significant inter-group difference was observed during follow-up. This is further supported by the secondary ROC analyses which indicated low potential for OPG to act as a biomarker for endoleak in the current cohort. The reasons for the observed post-operative increase in plasma OPG in the no endoleak group remain unclear. Two surgical investigations have

reported significant post-operative increases in circulating OPG concentration for AAA patients undergoing aneurysm repair [41, 42].

The final two markers assessed (CRP and HCY) are commonly measured as part of patient care, and the availability of pre- and post-operative measurements for these markers provided the opportunity to assess their association with endoleak. CRP is an acute phase protein which is used to assess systemic inflammation, and has been suggested to be associated with AAA presence in a number of studies (discussed in [13, 15]). To our knowledge, the relationship between CRP and endoleak has not been directly assessed, although prior data suggest that CRP concentrations may naturally increase in patients following EVAR [34]. De Haro and colleagues recently reported a positive association between AAA sac expansion after EVAR and elevations in CRP in a large patient series, although they indicated that none of these patients had demonstrable endoleaks [43]. Interpreting the findings of their study is difficult for two reasons. Firstly, De Haro *et al.* report post-operative AAA sac expansion in 63% (n=120) of their patients which appears unusually high. Secondly, the extent of annual AAA sac expansion appeared relatively low (cut-off for 'fast' expanders defined as >5.7%), which may be within the range of measurement error for most imaging modalities, complicating patient stratification [44, 45]. In contrast we observed no increase in post-operative CRP concentrations for any of our patients, and further investigations into the association of CRP with endoleak status are warranted to validate this finding.

Univariate analyses demonstrated that preoperative HCY levels were markedly higher in patients who subsequently developed endoleak, however, no association of this protein with endoleak presence was observed during follow-up. No other papers have directly investigated

the association of HCY concentration with endoleak, and it is therefore not possible to compare this finding with other sources. However, our observations tentatively suggest that HCY might not directly influence endoleak susceptibility, but may be a surrogate marker for other factors which contribute to post-operative risk. HCY is a surrogate marker of atherosclerotic severity; moreover, a higher proportion of patients who developed endoleak were prescribed statins [46, 47]. Taken together, these findings suggest that the extent of atherosclerosis may have been more severe in the patients who developed endoleak. Thus, it is tempting to speculate that a higher atherosclerotic burden may have compromised stent placement and seal during EVAR leading to eventual endoleak, although data to specifically test this hypothesis were not available.

The findings of the current study should be considered in light of its limitations. Firstly, the number of participants was relatively low, although the current cohort is larger than most of the studies which have previously published in this area. Moreover, sample sizes included in these analyses exceeded those suggested by *a priori* sample size calculations suggesting that the study was adequately powered to detect previously suggested differences between groups. Secondly, owing to small sample sizes, we were underpowered to assess the association of each marker with specific endoleak sub-types, or compare the expression of circulating markers from patients with endoleak in whom AAAs continued to expand, with those whose AAA did not increase in size. The majority of patients assessed here had type-II endoleaks which may result from reperfusion of the AAA sac with blood at a relatively low pressure (discussed by [48, 49]), possibly limiting the potential for AAA-secreted markers to enter the bloodstream. It is therefore possible that circulating concentrations of the assessed biomarkers may be more markedly elevated in patients suffering higher-pressure endoleaks although targeted studies are needed to explore this further. Thirdly, some baseline

differences in key characteristics were observed between the groups, however, the impact of this was mitigated by performing multivariable analyses. Finally, the timing of collection of the post-operative blood samples differed between patients, and this may have contributed to the observed negative findings, however, the impact of this was minimized in several ways. Firstly our prior meta-analysis demonstrated that circulating MMP9 concentrations were significantly higher in patients with endoleak compared to those who did not, in samples collected 3 months after EVAR (consistently reported across multiple studies) [10]. Accordingly, we analysed post-operative blood samples which were collected at least 3 months after EVAR to maximize the chances of detecting a difference between groups. In addition, we ensured that all patients had an endoleak at the time the post-operative samples were collected, and the analysed blood samples were therefore representative of the phenotype of interest. Moreover, time was included as a continuous variable in our linear mixed effects analyses. Model diagnostics demonstrated that inter-patient differences in follow-up were handled appropriately in our longitudinal analyses.

Conclusions

In summary, the current study assessed the potential for circulating concentrations of MMP9, OPG, D-dimer, CRP and HCY to act as diagnostic markers for endoleak. None of the assessed markers showed any association with endoleak status, however circulating D-dimer concentrations were markedly increased in all patients following EVAR. Collectively these findings suggest that the assessed markers have little potential to influence current post-EVAR monitoring practices.

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Table I – Comparison of pre-operative characteristics between groups of AAA patients who did and did not develop endoleak following EVAR

Characteristic	Whole cohort (n=75)	No endoleak (n=51)	Endoleak (n=24)	P-value
Age (y)	74.2 (67.3-80.1)	72.8 (66.1-78.7)	78.4 (73.2-80.6)	**.023**
Male sex	67 (89.3%)	47 (92.2%)	20 (90.9%)	.248
BMI	27.1 (24.7-29.7)	26.5 (24.7-30.6)	27.4 (24.8-29.0)	.955
AAA diameter (mm)	55.0 (52.0-61.0)	55.0 (52.0-61.0)	54.0 (51.0-59.5)	.446
Months from EVAR to biomarker blood sampling (months)	8.3 (7.2-10.3)	8.4 (7.2-10.3)	8.1 (7.3-10.5)	.896
Stent type [1]:				
Aorto-bi-iliac	65 (86.7%)	46 (90.2%)	19 (79.2%)	.272
Aorto-uni-iliac crossover	5 (6.7%)	2 (3.9%)	3 (12.5%)	
Fenestrated	4 (5.3%)	2 (3.9%)	2 (8.3%)	
History of:				
Ever smoking	59 (78.7%)	43 (84.3%)	16 (66.7%)	.082
Hypertension	62 (82.7%) [1]	39 [1] (76.5%)	23 (95.8%)	.051
Diabetes	13 (17.3%) [1]	9 (17.6%) [1]	4 (16.7%)	.888
IHD	34 (45.3%)	21 (41.2%)	13 (54.2%)	.292
Prescription for:				
Beta blockers	30 (40.0%)	21 (41.2%)	9 (37.5%)	.762
Statins	50 (66.7%)	30 (58.8%)	20 (83.3%)	**.036**
Warfarin	7 (9.3%) [2]	7 (13.7%) [1]	0 (0.0%) [1]	.059
Circulating concentrations of:				
Total cholesterol (mmol/L)	3.8 (3.3-4.6) [11]	3.8 (3.3-4.6) [9]	3.5 (3.2-4.5) [2]	.288
HDL-C (mmol/L)	1.0 (0.8-1.3) [11]	1.0 (0.8-1.2) [9]	1.1 (0.9-1.3) [2]	.143
LDL-C (mmol/L)	2.0 (1.6-2.6) [11]	2.3 (1.8-2.7) [9]	1.9 (1.5-2.2) [2]	**.036**
Triglycerides (mmol/L)	1.3 (1.0-1.9) [11]	1.4 (1.0-1.8) [9]	1.3 (1.0-2.1) [2]	.932
C-reactive protein (mg/L)	2.2 (1.1-5.8) [15]	2.4 (1.1-8.5) [13]	2.0 (1.7-4.7) [2]	.994
Homocysteine (mol/L)	12.0 (10.0-16.3) [13]	12.0 (10.0-14.0) [10]	16.0 (11.3-19.2) [3]	**.020**
Creatinine (μmol/L)	88.5 (72.8-107.0) [1]	89.0 (73.0-102.0)	88.0 (72.0-114.0) [1]	.833

IHD: Ischaemic heart disease; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol. Numbers in square brackets refer to number of missing data points. Bold text denotes statistically significant differences between the groups.

Table II – Comparison of circulating marker concentrations

Plasma marker (ng/mL)	Comparisons within groups (Wilcoxon matched-pairs signed rank test)						Comparison between groups (linear mixed effects models)	
	No endoleak group (n=51)			Endoleak groups (n=24)				
	Pre-op	Post-op	P-value	Pre-op	Post-op	P-value	Unadjusted P-value	Adjusted P-value
MMP9	87.5 (66.4-144.1)	88.6 (64.7-130.8)	.632	77.3 (51.7-116.3)	91.3 (66.2-111.5)	.768	.949	.997
D-dimer	272.4 (141.9-394.1)	401.3 (246.4-674.8)	<.001	272.4 (189.8-435.8)	568.1 (347.9-951.3)	<.001	.382	.312
OPG	1.1 (0.9-1.4)	1.2 (0.9-1.5)	.020	1.2 (1.0-1.6)	1.3 (1.0-1.5)	.938	.080	.090
HCY	12.0 (10.0-14.0) [10]	13.0 (11.3-16.3) [17]	<.001[a]	16.0 (11.3-19.2) [3]	16.1 (10.5-23.0) [5]	.086[b]	.576	.551
CRP	2.4 (1.1-8.5) [13]	2.2 (1.3-4.2) [15]	.927[c]	2.0 (1.7-4.7) [2]	2.9 (1.0-7.3) [5]	.346[d]	.449	.448

Circulating concentrations of each marker are shown as median and inter-quartile range. Numbers in square brackets denote number of missing datapoints.

Linear mixed effects p-values relate to the interaction between time and endoleak status. Unadjusted p-values relate to models including the assessed blood marker as the sole covariate. Adjusted p-values related to models incorporating the blood marker, age at the time of operation, prescription for statins and history of hypertension.

[a] Relates to comparisons of 33 pairs; [b] Relates to comparisons of 18 pairs; [c] Relates to comparisons of 32 pairs; [d] Relates to comparisons of 18 pairs

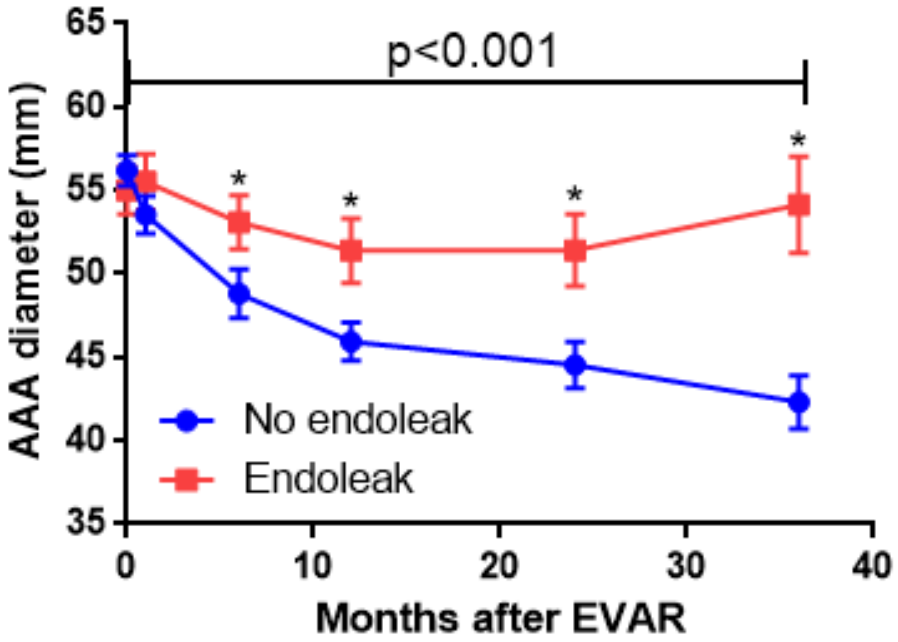
Table III – ROC curve analyses assessing the ability of post-operatively measured markers to diagnose endoleak

Blood marker	Unadjusted model		Adjusted model[a]		Comparison between Adjusted and unadjusted (P-value)	Comparison with base model[b] (P-value)
	AUC	95% CI	AUC	95% CI		
MMP9	0.469	0.331-0.607	0.760	0.642-0.878	.002	.857
OPG	0.523	0.378-0.669	0.771	0.657-0.885	.007	.561
D-dimer	0.624	0.489-0.760	0.761	0.644-0.878	.137	.808
C-reactive protein	0.538	0.366-0.710	0.844	0.738-0.951	.003	.288
Homocysteine	0.590	0.708-0.772	0.841	0.726-0.955	<.001	.324

[a] Adjusted model comprises the circulating marker, age at operation, history of hypertension and prescription for statins based on differences observed between groups upon recruitment (see Table I).

[b] Base model comprises age at operation, history of hypertension and prescription for statins. P-values relate to the comparison of adjusted blood marker model to the base model as assessed using DeLong's test for matched ROC curves. The base model has an AUC of 0.758 (95% CI 0.640-0.875).

A



B

	Number of observations					
Time (months)	**0**	**1**	**6**	**12**	**24**	**36**
No endoleak	51	43	42	45	43	31
Endoleak	24	23	23	23	17	16

Figure 1. A) Comparisons of AAA diameter following EVAR in groups of patients who did (red squares) and did not (blue circles) develop endoleak during follow-up. Data are shown as mean and standard error for each group. P value refers to the overall difference of AAA diameter between groups during follow-up as assessed by linear mixed effects modelling. Asterisks show significant differences between groups for each time point evidenced by p-values <0.05 within the linear mixed effects model. **B)** Details of the number of patients included for each assessed timepoint.

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Circulating biomarkers are not asasociated with endoleaks after endovascular repair of abdominal aortic aneurysms

SUPPLEMENTARY MATERIAL – DETAILED OUTPUT OF LINEAR MIXED EFFECTS MODELS

Contents:

Supplementary file 1 – Output from linear mixed effects analyses detailing changes in AAA diameter during follow-up.

Supplementary file 2 – Output from linear mixed effects analyses detailing changes in circulating MMP9, D-dimer, OPG, HCY and CRP concentrations in patients who do or do not have endoleak.

Preface

Linear mixed effects models were created and run using the publically available R software package - data below show the raw output for all models. For each mode, the variable of interest was assessed in an unadjusted model, or an adjusted model incorporating covariates selected based on baseline differences identified between cohorts (age at operation, hypertension and statin use). In unadjusted analyses, endoleak presence and time are considered as fixed effects, and variation between patients was considered a random effect. In adjusted models, age at operation, hypertension and statin use were treated as additional fixed effects.

Supplementary file 1 – Output from linear mixed effects analyses detailing changes in AAA diameter during follow-up.

Note – for models assessing changes in AAA diameter, time is treated as a factorial variable.

Unadjusted model

```
Follow.up<-as.factor(AAA.endoleak$Time)
Ever_Endoleak<-as.factor(AAA.endoleak$Endoleak_ever.)
Ever.endoleak.lme<-lme(AAA_size~Endoleak_ever*Follow.up, random = ~1|TrialI
D,
                       data=AAA.endoleak, na.action='na.omit')


summary(Ever.endoleak.lme)

## Linear mixed-effects model fit by REML
##  Data: AAA.endoleak
##       AIC      BIC    logLik
##   2509.95 2564.702 -1240.975
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept) Residual
## StdDev:     6.37118 5.386626
##
## Fixed effects: AAA_size ~ Endoleak_ever * Follow.up
##                               Value Std.Error  DF   t-value p-value
## (Intercept)                56.19216  1.168271 296  48.09858  0.0000
## Endoleak_ever              -1.19216  2.065230  73  -0.57725  0.5655
## Follow.up1                 -3.06864  1.125313 296  -2.72692  0.0068
## Follow.up6                 -7.06895  1.132866 296  -6.23988  0.0000
## Follow.up12               -10.20119  1.109673 296  -9.19297  0.0000
## Follow.up24               -12.11384  1.126065 296 -10.75767  0.0000
## Follow.up36               -14.19999  1.257173 296 -11.29517  0.0000
## Endoleak_ever:Follow.up1    3.33535  1.935500 296   1.72325  0.0859
## Endoleak_ever:Follow.up6    5.10752  1.940400 296   2.63220  0.0089
## Endoleak_ever:Follow.up12   6.74182  1.926948 296   3.49870  0.0005
## Endoleak_ever:Follow.up24   8.61569  2.069545 296   4.16308  0.0000
## Endoleak_ever:Follow.up36  14.64031  2.172873 296   6.73776  0.0000
##  Correlation:
##                           (Intr) Endlk_ Fllw.1 Fllw.6 Fll.12 Fll.24 Fll.36
## Endoleak_ever             -0.566
## Follow.up1                -0.433  0.245
## Follow.up6                -0.430  0.243  0.442
## Follow.up12               -0.439  0.248  0.456  0.452
## Follow.up24               -0.432  0.245  0.448  0.441  0.459
## Follow.up36               -0.387  0.219  0.409  0.393  0.414  0.416
## Endoleak_ever:Follow.up1   0.252 -0.445 -0.581 -0.257 -0.265 -0.261 -0.238
## Endoleak_ever:Follow.up6   0.251 -0.444 -0.258 -0.584 -0.264 -0.258 -0.230
## Endoleak_ever:Follow.up12  0.253 -0.447 -0.262 -0.260 -0.576 -0.264 -0.238
## Endoleak_ever:Follow.up24  0.235 -0.416 -0.244 -0.240 -0.250 -0.544 -0.226
## Endoleak_ever:Follow.up36  0.224 -0.396 -0.237 -0.227 -0.240 -0.241 -0.579
```

```
##                            En_:F.1 E_:F.6 E_:F.12 E_:F.2
## Endoleak_ever
## Follow.up1
## Follow.up6
## Follow.up12
## Follow.up24
## Follow.up36
## Endoleak_ever:Follow.up1
## Endoleak_ever:Follow.up6   0.472
## Endoleak_ever:Follow.up12  0.477   0.475
## Endoleak_ever:Follow.up24  0.442   0.444  0.446
## Endoleak_ever:Follow.up36  0.424   0.418  0.429   0.408
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.72574871 -0.51937789 -0.03171875  0.53679700  3.39708439
##
## Number of Observations: 381
## Number of Groups: 75
```

```
anova(Ever.endoleak.lme)
```

```
##                          numDF denDF  F-value p-value
## (Intercept)                  1   296 4121.846  <.0001
## Endoleak_ever                1    73    7.355  0.0083
## Follow.up                    5   296   33.976  <.0001
## Endoleak_ever:Follow.up      5   296   10.389  <.0001
```

Adjusted model

```
Ever.endoleak.lme1<-
lme(AAA_size~Endoleak_ever*Follow.up+Age_at_op_years+Cohypertension+
                           MedStatin, random = ~1|TrialID,
                       data=AAA.endoleak, na.action='na.omit')
```

```
summary(Ever.endoleak.lme1)
```

```
## Linear mixed-effects model fit by REML
##  Data: AAA.endoleak
##        AIC      BIC    logLik
##   2471.384 2537.495 -1218.692
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept) Residual
## StdDev:    5.971898 5.414953
##
## Fixed effects: AAA_size ~ Endoleak_ever * Follow.up + Age_at_op_years + Cohyper
tension +      MedStatin
##                               Value Std.Error  DF    t-value p-value
## (Intercept)                38.34689  7.348002 292   5.218682  0.0000
## Endoleak_ever              -2.19600  2.132744  69  -1.029660  0.3068
## Follow.up1                 -3.15792  1.143786 292  -2.760939  0.0061
## Follow.up6                 -7.17229  1.151647 292  -6.227859  0.0000
## Follow.up12               -10.17645  1.127399 292  -9.026485  0.0000
## Follow.up24               -12.16963  1.144412 292 -10.633956  0.0000
## Follow.up36               -14.23125  1.267337 292 -11.229253  0.0000
```

```
## Age_at_op_years             0.25035  0.100456  69   2.492099  0.0151
## Cohypertension              2.05910  2.182972  69   0.943255  0.3488
## MedStatin                  -2.16379  1.725444  69  -1.254050  0.2141
## Endoleak_ever:Follow.up1    3.42969  1.952965 292   1.756146  0.0801
## Endoleak_ever:Follow.up6    5.23332  1.958164 292   2.672564  0.0080
## Endoleak_ever:Follow.up12   6.72466  1.943886 292   3.459388  0.0006
## Endoleak_ever:Follow.up24   8.70875  2.086851 292   4.173153  0.0000
## Endoleak_ever:Follow.up36  14.65747  2.185869 292   6.705556  0.0000
##  Correlation:
##                            (Intr) Endlk_ Fllw.1 Fllw.6 Fll.12 Fll.24 Fll.36
## Endoleak_ever               0.048
## Follow.up1                 -0.067  0.240
## Follow.up6                 -0.077  0.234  0.441
## Follow.up12                -0.076  0.243  0.455  0.451
## Follow.up24                -0.064  0.242  0.447  0.440  0.458
## Follow.up36                -0.064  0.221  0.412  0.396  0.417  0.418
## Age_at_op_years            -0.895 -0.254  0.000  0.012  0.005 -0.007 -0.004
## Cohypertension             -0.167  0.156 -0.015 -0.007 -0.003  0.005 -0.007
## MedStatin                  -0.082  0.194  0.010 -0.006  0.004 -0.001  0.024
## Endoleak_ever:Follow.up1    0.040 -0.434 -0.586 -0.258 -0.266 -0.262 -0.241
## Endoleak_ever:Follow.up6    0.043 -0.429 -0.259 -0.588 -0.265 -0.259 -0.233
## Endoleak_ever:Follow.up12   0.042 -0.437 -0.264 -0.262 -0.580 -0.266 -0.242
## Endoleak_ever:Follow.up24   0.026 -0.407 -0.245 -0.241 -0.251 -0.548 -0.229
## Endoleak_ever:Follow.up36   0.035 -0.392 -0.239 -0.229 -0.242 -0.243 -0.580
##                            Ag_t__ Chyprt MdSttn En_:F.1 E_:F.6 E_:F.12
## Endoleak_ever
## Follow.up1
## Follow.up6
## Follow.up12
## Follow.up24
## Follow.up36
## Age_at_op_years
## Cohypertension             -0.116
## MedStatin                  -0.165 -0.241
## Endoleak_ever:Follow.up1    0.000  0.009 -0.007
## Endoleak_ever:Follow.up6   -0.007  0.012  0.000  0.471
## Endoleak_ever:Follow.up12  -0.001  0.001 -0.003  0.476   0.475
## Endoleak_ever:Follow.up24   0.010  0.002  0.003  0.442   0.444  0.445
## Endoleak_ever:Follow.up36   0.006  0.000 -0.016  0.425   0.419  0.430
##                            E_:F.2
## Endoleak_ever
## Follow.up1
## Follow.up6
## Follow.up12
## Follow.up24
## Follow.up36
## Age_at_op_years
## Cohypertension
## MedStatin
## Endoleak_ever:Follow.up1
## Endoleak_ever:Follow.up6
## Endoleak_ever:Follow.up12
## Endoleak_ever:Follow.up24
## Endoleak_ever:Follow.up36   0.409
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -2.7502797 -0.5174926 -0.0340882  0.5393181  3.3461924
##
```

```
## Number of Observations: 376
## Number of Groups: 74

anova(Ever.endoleak.lme1)

##                           numDF denDF  F-value p-value
## (Intercept)                   1   292 4511.285  <.0001
## Endoleak_ever                 1    69    9.214  0.0034
## Follow.up                     5   292   32.992  <.0001
## Age_at_op_years               1    69    5.800  0.0187
## Cohypertension                1    69    0.492  0.4855
## MedStatin                     1    69    1.376  0.2449
## Endoleak_ever:Follow.up       5   292   10.260  <.0001
```

Supplementary file 2 – Output from linear mixed effects analyses detailing changes in circulating MMP9, D-dimer, OPG, HCY and CRP concentrations in patients who do or do not have endoleak.

Note – for biomarker assessments, time is treated as a continuous variable. Plasma concentrations of MMP9, D-dimer, OPG and CRP required log-transformation to conform to model assumptions.

Assessing MMP9 – Unadjusted analysis

```
MMP9.endoleak.lme2<-lme(log.MMP9~Endoleak_ever*Time, random = ~1|TrialID,
                        data=Endoleak.biomarkers, na.action='na.omit')
summary(MMP9.endoleak.lme2)
```

```
## Linear mixed-effects model fit by REML
##  Data: Endoleak.biomarkers
##        AIC      BIC    logLik
##   298.2529 316.1545 -143.1264
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept)  Residual
## StdDev:   0.2485381 0.5454957
##
## Fixed effects: log.MMP9 ~ Endoleak_ever * Time
##                         Value  Std.Error DF  t-value p-value
## (Intercept)         4.615262 0.08112459 73 56.89104  0.0000
## Endoleak_ever      -0.105131 0.14145520 73 -0.74321  0.4597
## Time               -0.006587 0.01118334 73 -0.58903  0.5577
## Endoleak_ever:Time -0.001170 0.01821423 73 -0.06424  0.9490
##  Correlation:
##                    (Intr) Endlk_ Time
## Endoleak_ever      -0.574
## Time               -0.610  0.350
## Endoleak_ever:Time  0.375 -0.596 -0.614
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -1.79715393 -0.71086624 -0.08778804  0.48268375  3.17565319
##
## Number of Observations: 150
## Number of Groups: 75
```

```
anova(MMP9.endoleak.lme2)
```

```
##                    numDF denDF  F-value p-value
## (Intercept)            1    73 7374.306  <.0001
## Endoleak_ever          1    73    0.986  0.3241
## Time                   1    73    0.634  0.4285
## Endoleak_ever:Time     1    73    0.004  0.9490
```

Assessing MMP9 – Adjusted analysis

```
MMP9.endoleak.lme3<-lme(log.MMP9~Endoleak_ever*Time+
                           Age_at_op_years+Cohypertension+MedStatin,
                        random = ~1|TrialID,
                        data=Endoleak.biomarkers, na.action='na.omit')
summary(MMP9.endoleak.lme3)
```

```
## Linear mixed-effects model fit by REML
##  Data: Endoleak.biomarkers
##        AIC      BIC    logLik
##   309.7779 336.3167 -145.8889
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept)  Residual
## StdDev:   0.2502074 0.5443314
##
## Fixed effects: log.MMP9 ~ Endoleak_ever * Time + Age_at_op_years + Cohypertensi
on +      MedStatin
##                         Value Std.Error DF   t-value p-value
## (Intercept)          5.555515 0.5222474 72 10.637708  0.0000
## Endoleak_ever       -0.114621 0.1518215 69 -0.754973  0.4528
## Time                -0.007602 0.0112789 72 -0.674014  0.5025
## Age_at_op_years     -0.009319 0.0071551 69 -1.302411  0.1971
## Cohypertension      -0.122852 0.1552174 69 -0.791482  0.4314
## MedStatin           -0.075535 0.1224800 69 -0.616714  0.5395
## Endoleak_ever:Time   0.000076 0.0182563 72  0.004158  0.9967
##  Correlation:
##                     (Intr) Endlk_ Time   Ag_t__ Chyprt MdSttn
## Endoleak_ever        0.058
## Time                -0.061  0.332
## Age_at_op_years     -0.895 -0.268 -0.029
## Cohypertension      -0.165  0.152 -0.015 -0.117
## MedStatin           -0.086  0.204  0.000 -0.161 -0.240
## Endoleak_ever:Time   0.037 -0.563 -0.618  0.026  0.009 -0.020
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -1.6531018 -0.7331094 -0.1393002  0.4695892  3.1790086
##
## Number of Observations: 148
## Number of Groups: 74
```

```
anova(MMP9.endoleak.lme3)
```

```
##                     numDF denDF  F-value p-value
## (Intercept)             1    72 7272.622  <.0001
## Endoleak_ever           1    69    1.020  0.3162
## Time                    1    72    0.839  0.3628
## Age_at_op_years         1    69    2.558  0.1143
## Cohypertension          1    69    0.936  0.3366
## MedStatin               1    69    0.380  0.5394
## Endoleak_ever:Time      1    72    0.000  0.9967
```

Assessing D-dimer – Unadjusted analysis

```
log.D.dimer<-log(Endoleak.biomarkers$D_Dimer)

D.dimer.endoleak.lme2<-lme(log.D.dimer~Endoleak_ever*Time, random =
~1|TrialID,
                           data=Endoleak.biomarkers, na.action='na.omit')
```

```
anova(D.dimer.endoleak.lme2)

##                    numDF denDF  F-value p-value
## (Intercept)            1    73 6144.364  <.0001
## Endoleak_ever          1    73    1.332  0.2523
## Time                   1    73   42.283  <.0001
## Endoleak_ever:Time     1    73    0.773  0.3822

summary(D.dimer.endoleak.lme2)

## Linear mixed-effects model fit by REML
##  Data: Endoleak.biomarkers
##       AIC      BIC   logLik
##   338.348 356.2496 -163.174
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept)  Residual
## StdDev:   0.5335491 0.5114108
##
## Fixed effects: log.D.dimer ~ Endoleak_ever * Time
##                        Value  Std.Error DF  t-value p-value
## (Intercept)        5.556647 0.10189977 73 54.53051  0.0000
## Endoleak_ever      0.095389 0.17897951 73  0.53296  0.5957
## Time               0.049320 0.01068168 73  4.61727  0.0000
## Endoleak_ever:Time 0.015398 0.01751529 73  0.87913  0.3822
##  Correlation:
##                    (Intr) Endlk_ Time
## Endoleak_ever      -0.569
## Time               -0.464  0.264
## Endoleak_ever:Time  0.283 -0.453 -0.610
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.72021982 -0.45478083  0.08205852  0.43413852  2.33561858
##
## Number of Observations: 150
## Number of Groups: 75
```

Assessing D-dimer – Adjusted analysis

```
D.dimer.endoleak.lme3<-lme(log.D.dimer~Endoleak_ever*Time+
                             Age_at_op_years+Cohypertension+MedStatin,
                           random = ~1|TrialID,
                           data=Endoleak.biomarkers, na.action='na.omit')
summary(D.dimer.endoleak.lme3)

## Linear mixed-effects model fit by REML
##  Data: Endoleak.biomarkers
##        AIC      BIC    logLik
```

```
##   335.9594 362.4982 -158.9797
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept)  Residual
## StdDev:   0.4700461 0.5111707
##
## Fixed effects: log.D.dimer ~ Endoleak_ever * Time + Age_at_op_years + Cohyperte
nsion +      MedStatin
##                          Value Std.Error DF   t-value p-value
## (Intercept)          3.0946397 0.6739732 72  4.591636  0.0000
## Endoleak_ever       -0.0274158 0.1816015 69 -0.150967  0.8804
## Time                 0.0470473 0.0107595 72  4.372612  0.0000
## Age_at_op_years      0.0308385 0.0092396 69  3.337646  0.0014
## Cohypertension       0.3876755 0.2004709 69  1.933824  0.0572
## MedStatin           -0.1615298 0.1581752 69 -1.021208  0.3107
## Endoleak_ever:Time   0.0178353 0.0175122 72  1.018449  0.3119
##  Correlation:
##                     (Intr) Endlk_ Time   Ag_t__ Chyprt MdSttn
## Endoleak_ever        0.073
## Time                -0.045  0.265
## Age_at_op_years     -0.897 -0.282 -0.022
## Cohypertension      -0.165  0.166 -0.011 -0.118
## MedStatin           -0.086  0.215  0.000 -0.161 -0.240
## Endoleak_ever:Time   0.027 -0.452 -0.614  0.019  0.007 -0.015
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.82448143 -0.49911525  0.06057422  0.47402564  2.07675651
##
## Number of Observations: 148
## Number of Groups: 74
```

```
anova(D.dimer.endoleak.lme3)
```

```
##                     numDF denDF  F-value p-value
## (Intercept)             1    72 7180.976  <.0001
## Endoleak_ever           1    69    1.409  0.2394
## Time                    1    72   41.041  <.0001
## Age_at_op_years         1    69   12.455  0.0007
## Cohypertension          1    69    3.015  0.0869
## MedStatin               1    69    1.013  0.3178
## Endoleak_ever:Time      1    72    1.037  0.3119
```

Assessing OPG – Unadjusted analysis

```
OPG.endoleak.lme2<-lme(log.OPG~Endoleak_ever*Time, random = ~1|TrialID,
                       data=Endoleak.biomarkers, na.action='na.omit')
summary(OPG.endoleak.lme2)
```

```
## Linear mixed-effects model fit by REML
##  Data: Endoleak.biomarkers
##        AIC      BIC    logLik
##   69.51017 87.41181 -28.75509
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept)  Residual
## StdDev:   0.3022596 0.1618153
```

```
##
## Fixed effects: log.OPG ~ Endoleak_ever * Time
##                          Value  Std.Error DF   t-value p-value
## (Intercept)         0.10931784 0.04771058 73  2.291270  0.0248
## Endoleak_ever       0.08683655 0.08411967 73  1.032298  0.3053
## Time                0.00786169 0.00341123 73  2.304650  0.0240
## Endoleak_ever:Time -0.00997165 0.00561339 73 -1.776406  0.0798
##  Correlation:
##                    (Intr) Endlk_ Time
## Endoleak_ever      -0.567
## Time               -0.317  0.180
## Endoleak_ever:Time  0.192 -0.309 -0.608
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.91816923 -0.37808975  0.01168734  0.43301771  2.64550101
##
## Number of Observations: 150
## Number of Groups: 75
anova(OPG.endoleak.lme2)
##                    numDF denDF   F-value p-value
## (Intercept)            1    73 17.828597  0.0001
## Endoleak_ever          1    73  0.275718  0.6011
## Time                   1    73  2.379810  0.1272
## Endoleak_ever:Time     1    73  3.155617  0.0798
```

Assessing OPG – Adjusted analysis

```
OPG.endoleak.lme3<-lme(log.OPG~Endoleak_ever*Time+
                         Age_at_op_years+Cohypertension+MedStatin,
                       random = ~1|TrialID,
                       data=Endoleak.biomarkers, na.action='na.omit')
summary(OPG.endoleak.lme3)
```

```
## Linear mixed-effects model fit by REML
##  Data: Endoleak.biomarkers
##        AIC      BIC    logLik
##   64.96082 91.49965 -23.48041
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept)  Residual
## StdDev:   0.2491271 0.1629221
##
## Fixed effects: log.OPG ~ Endoleak_ever * Time + Age_at_op_years + Cohypertensio
n +      MedStatin
##                          Value  Std.Error DF   t-value p-value
## (Intercept)        -1.5224839 0.31181534 72 -4.882646  0.0000
## Endoleak_ever      -0.0333694 0.07953020 69 -0.419582  0.6761
## Time                0.0078236 0.00346206 72  2.259800  0.0269
## Age_at_op_years     0.0235770 0.00427639 69  5.513294  0.0000
## Cohypertension      0.0386338 0.09279411 69  0.416339  0.6785
## MedStatin          -0.0858677 0.07321208 69 -1.172862  0.2449
## Endoleak_ever:Time -0.0097160 0.00565460 72 -1.718243  0.0901
##  Correlation:
##                    (Intr) Endlk_ Time   Ag_t__ Chyprt MdSttn
## Endoleak_ever       0.083
## Time               -0.031  0.194
```

```
## Age_at_op_years    -0.898 -0.294 -0.015
## Cohypertension     -0.166  0.177 -0.008 -0.118
## MedStatin          -0.086  0.223  0.000 -0.161 -0.240
## Endoleak_ever:Time  0.019 -0.333 -0.612  0.013  0.005 -0.010
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -4.1402564 -0.4182389 -0.0190227  0.4608153  2.3881732
##
## Number of Observations: 148
## Number of Groups: 74
```

```
anova(OPG.endoleak.lme3)
```

```
##                    numDF denDF   F-value p-value
## (Intercept)            1    72 25.364107  <.0001
## Endoleak_ever          1    69  0.305344  0.5823
## Time                   1    72  2.512075  0.1174
## Age_at_op_years        1    69 30.387226  <.0001
## Cohypertension         1    69  0.020374  0.8869
## MedStatin              1    69  1.417806  0.2378
## Endoleak_ever:Time     1    72  2.952360  0.0901
```

Assessing HCY – Unadjusted analysis

```
HCY.endoleak.lme<-lme(Homocysteine~Endoleak_ever*Time,
                      random = ~1|TrialID,
                      data=Endoleak.HCY.CRP, na.action='na.omit')
summary(HCY.endoleak.lme)
```

```
## Linear mixed-effects model fit by REML
##  Data: Endoleak.HCY.CRP
##       AIC      BIC   logLik
##   650.442 666.6992 -319.221
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept) Residual
## StdDev:     4.45459 2.163362
##
## Fixed effects: Homocysteine ~ Endoleak_ever * Time
##                         Value Std.Error DF   t-value p-value
## (Intercept)        12.598839 0.7673516 62 16.418599  0.0000
## Endoleak_ever       2.746764 1.3122313 62  2.093201  0.0404
## Time                0.297727 0.0877642 49  3.392349  0.0014
## Endoleak_ever:Time -0.083237 0.1478505 49 -0.562980  0.5760
##  Correlation:
##                    (Intr) Endlk_ Time
## Endoleak_ever      -0.585
## Time               -0.290  0.170
## Endoleak_ever:Time  0.172 -0.301 -0.594
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -1.95743721 -0.43049917 -0.07213615  0.40915278  3.22599474
##
## Number of Observations: 115
## Number of Groups: 64
```

```
anova(HCY.endoleak.lme)
```

```
##                     numDF denDF  F-value p-value
## (Intercept)             1    62 573.9631  <.0001
## Endoleak_ever           1    62   4.2546  0.0433
## Time                    1    49  14.4407  0.0004
## Endoleak_ever:Time      1    49   0.3169  0.5760
```

Assessing HCY – Adjusted analysis

```
HCY.endoleak.lme2<-lme(Homocysteine~Endoleak_ever*Time+
                           Age_at_op_years+Cohypertension+MedStatin,
                       random = ~1|TrialID,
                       data=Endoleak.HCY.CRP, na.action='na.omit')
summary(HCY.endoleak.lme2)
## Linear mixed-effects model fit by REML
##  Data: Endoleak.HCY.CRP
##        AIC      BIC    logLik
##   644.1483 668.2037 -313.0741
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept) Residual
## StdDev:    4.406285  2.16745
##
## Fixed effects: Homocysteine ~ Endoleak_ever * Time + Age_at_op_years +
Cohypertension +      MedStatin
##                          Value Std.Error DF   t-value p-value
## (Intercept)          1.1485622  5.613852 58  0.204594  0.8386
## Endoleak_ever        2.3929173  1.483982 58  1.612498  0.1123
## Time                 0.2973717  0.088014 49  3.378686  0.0014
## Age_at_op_years      0.1342701  0.079315 58  1.692880  0.0958
## Cohypertension       0.9076661  1.922006 58  0.472249  0.6385
## MedStatin            0.5515177  1.415911 58  0.389514  0.6983
## Endoleak_ever:Time -0.0891014  0.148213 49 -0.601170  0.5505
##  Correlation:
##                     (Intr) Endlk_ Time   Ag_t__ Chyprt MdSttn
## Endoleak_ever        0.089
## Time                -0.054  0.155
## Age_at_op_years     -0.883 -0.346  0.008
## Cohypertension      -0.142  0.241  0.011 -0.169
## MedStatin           -0.072  0.248  0.000 -0.169 -0.284
## Endoleak_ever:Time   0.052 -0.263 -0.594 -0.027 -0.002 -0.001
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.08233939 -0.40934734 -0.04892402  0.39039878  3.09229883
##
## Number of Observations: 114
## Number of Groups: 63
```

```
anova(HCY.endoleak.lme2)
```

```
##                     numDF denDF  F-value p-value
## (Intercept)             1    58 582.5927  <.0001
## Endoleak_ever           1    58   3.9859  0.0506
## Time                    1    49  14.1568  0.0004
```

```
## Age_at_op_years        1    58   3.8444  0.0547
## Cohypertension         1    58   0.3684  0.5463
## MedStatin              1    58   0.1514  0.6986
## Endoleak_ever:Time     1    49   0.3614  0.5505
```

Assessing CRP – Unadjusted analysis

```
log.CRP<-log(Endoleak.HCY.CRP$CRP)
CRP.endoleak.lme2<-lme(log.CRP~Endoleak_ever*Time,
                       random = ~1|TrialID,
                       data=Endoleak.HCY.CRP, na.action='na.omit')
summary(CRP.endoleak.lme)
## Linear mixed-effects model fit by REML
##  Data: Endoleak.HCY.CRP
##        AIC      BIC    logLik
##   1009.726 1025.929 -498.8632
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept) Residual
## StdDev: 0.007650407 20.56505
##
## Fixed effects: CRP ~ Endoleak_ever * Time
##                        Value Std.Error DF    t-value p-value
## (Intercept)        10.281316  3.336091 62  3.0818450  0.0031
## Endoleak_ever      -6.249043  5.509373 62 -1.1342566  0.2611
## Time               -1.025775  0.797171 48 -1.2867696  0.2043
## Endoleak_ever:Time  1.030766  1.349862 48  0.7636086  0.4488
##  Correlation:
##                    (Intr) Endlk_ Time
## Endoleak_ever      -0.606
## Time               -0.697  0.422
## Endoleak_ever:Time  0.412 -0.680 -0.591
##
## Standardized Within-Group Residuals:
##          Min           Q1          Med           Q3          Max
## -0.480977017 -0.184889404 -0.103411639 -0.002512321 10.051942390
##
## Number of Observations: 114
## Number of Groups: 64
anova(CRP.endoleak.lme)
##                    numDF denDF   F-value p-value
## (Intercept)            1    62 10.194615  0.0022
## Endoleak_ever          1    62  0.645054  0.4250
## Time                   1    48  1.072699  0.3055
## Endoleak_ever:Time     1    48  0.583098  0.4488
```

Assessing CRP – Adjusted analysis

```
CRP.endoleak.lme3<-lme(log.CRP~Endoleak_ever*Time+
                           Age_at_op_years+Cohypertension+MedStatin,
                       random = ~1|TrialID,
                       data=Endoleak.HCY.CRP, na.action='na.omit')
## Linear mixed-effects model fit by REML
##  Data: Endoleak.HCY.CRP
##        AIC      BIC    logLik
```

```
##   368.8461 392.8171 -175.4231
##
## Random effects:
##  Formula: ~1 | TrialID
##         (Intercept)  Residual
## StdDev:   0.7011283 0.8748673
##
## Fixed effects: log.CRP ~ Endoleak_ever * Time + Age_at_op_years +
Cohypertension +      MedStatin
##                         Value Std.Error DF    t-value p-value
## (Intercept)          1.6976831 1.1664713 58  1.4554007  0.1509
## Endoleak_ever       -0.0712491 0.3346794 58 -0.2128877  0.8322
## Time                -0.0296947 0.0350143 48 -0.8480748  0.4006
## Age_at_op_years     -0.0119860 0.0163173 58 -0.7345560  0.4656
## Cohypertension      -0.0037226 0.3948664 58 -0.0094276  0.9925
## MedStatin            0.1987483 0.2880057 58  0.6900846  0.4929
## Endoleak_ever:Time   0.0450457 0.0588788 48  0.7650592  0.4480
##  Correlation:
##                     (Intr) Endlk_ Time   Ag_t__ Chyprt MdSttn
## Endoleak_ever        0.061
## Time                -0.104  0.319
## Age_at_op_years     -0.880 -0.318 -0.001
## Cohypertension      -0.143  0.228  0.014 -0.172
## MedStatin           -0.094  0.223  0.029 -0.147 -0.273
## Endoleak_ever:Time   0.104 -0.473 -0.595 -0.044  0.004 -0.026
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -2.2626440 -0.4962737 -0.1164732  0.3893526  3.8035212
##
## Number of Observations: 113
## Number of Groups: 63
anova(CRP.endoleak.lme3)
##                     numDF denDF  F-value p-value
## (Intercept)             1    58 68.57790  <.0001
## Endoleak_ever           1    58  0.05982  0.8076
## Time                    1    48  0.27171  0.6046
## Age_at_op_years         1    58  0.33129  0.5671
## Cohypertension          1    58  0.03550  0.8512
## MedStatin               1    58  0.50414  0.4805
## Endoleak_ever:Time      1    48  0.58532  0.4480
```