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Correspondence

Utility of Plasma Matrix Metalloproteinase-9 as a Possible Diagnostic Marker of Endoleak Post Endovascular Aneurysm Repair

Dear Editor,

In a recent article published in the European Journal of Vascular and Endovascular Surgery, Hellentahl et al. provided further evidence that plasma biomarkers, such as matrix metalloproteinase-9 (MMP-9), may be a valuable adjunctive diagnostic tool for predicting the presence of an endoleak post endovascular aneurysm repair (EVAR).[1]

Although EVAR compares favourably to open surgical abdominal aortic aneurysm (AAA) repair in terms of short term mortality and morbidity, the potential longer term complications mandate the lifelong follow up post procedure. The cost involved with the surveillance program, and the inconvenience for the patients to partake in a lifelong surveillance, remain a significant issue.[2] Therefore, the discovery of potential diagnostic plasma biomarker(s) for the purpose of endoleak detection would represent a breakthrough in current standard clinical practice; it would also underpin the ethos of surgical translational research.

In our search for potential plasma biomarkers for clinical applications, it is critical to examine the pre-analytical factors which may affect quantitative measurements of the biomarkers. MMP-9 is known to be affected by several pre-analytical issues, including the kind of sample matrix collected or the type of anti-coagulants used for the preparation of plasma. Blood components such as white blood cells and platelets are sources of MMP-9 and may release MMP-9 ex-vivo during the coagulation process. It has been shown that serum levels of MMP-9 are higher than that measured in paired plasma, and that higher levels of MMP-9 are measured in EDTA plasma compared to paired lithium heparin plasma.[3,4]

Given the pre-analytical influence on the measured MMP-9 levels, it would be worthwhile for research publications, such as the report by Hellentahl et al., to include the sample preparation protocol as part of the methods section. This will allow for comparisons between studies, and to guide potential clinical applications in the future.

Acknowledgement

Regent Lee is a Lumley Surgical Research Fellow and Foundation of Surgery Research Scholar with the Royal Australasian College of Surgeons.

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Re: Utility of Plasma Matrix Metalloproteinase-9 as a Possible Diagnostic Marker of Endoleak Post Endovascular Aneurysm Repair

Dear Sir,

We would like to thank Lee and Handa for their interest in and commentary on our report. We share their views on the influence of pre-analytical processing on the measured MMP-9 levels. Moreover, we support the necessity of inclusion of the sample preparation protocol for comparisons between studies. However, since the nature of this article was a short report, we were unable to report details of the materials and methods. We therefore gladly provide the details on the pre-analytical processing of our samples and assay protocol in the present reply.

Blood Collection

Venous blood was drawn via an antecubital vein puncture and collected in EDTA buffered (K2E 7.2 mg) vacutainer® for plasma. Exactly

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30 min after collection, blood was centrifuged (15 min, 3000 g, 4 °C) and multiple aliquots were stored at exactly 1 h after collection at −80 °C pending analysis. As demonstrated by others, concentration and activity of MMP is stable during long term storage at −80 °C.[1]

Assays

Plasma levels of MMP- 2, -9 and TIMP-1 were determined by means of a commercially available enzyme linked immunosorbent assay (GE Healthcare/lifesciences, Upssala, Sweden) according to the manufacturer's guidelines and were determined in duplicate. Duplicates with less than 10% within assay variation in concentration were accepted and means were determined for further analysis. The between-assay variation (7% for MMP-2, 10% for both MMP-9 and TIMP-1) was determined by the manufacturer.

Yours faithfully,

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Factors Affecting Amputation-free Survival Rates in Critical Limb Ischemia

First of all, we would like to congratulate the authors upon their study investigating the factors affecting amputation-free survival in the patients with critical limb ischemia (CLI).[1] Cilostazol is not mentioned among the medical treatment protocols provided to the patients in the article, whereas there are a number of studies reporting lower amputation rates upon cilostazol administration in the patients with CLI who had undergone endovascular intervention.[2,3] Moreover, cilostazol administration was also documented to pull down the amputation rates even in the cases with CLI in whom no intervention was undertaken.[4,5] Therefore, we believe the amputation-free survival rates emerging in the present study would be more accurate if an evaluation is made by taking into consideration that cilostazol is not used in the study. Likewise, we also noted in the study that extra-anatomic by-pass (EAB) procedure is implemented in 3 out of 15 patients undergoing revascularization due to ischemic pain. If EAB was undertaken in these patients during the first surgical intervention, what was the actual reason behind? If EAB procedure was preferred in these patients owing to the presence of previous surgical operation, do you think the previous history of vascular surgery had an impact on the amputation rates? We believe that evaluation of the present study would be comprehended much more thoroughly by keeping all the aforementioned issues in mind.

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Response to Letter to the Editor: Factors Affecting Amputation-Free Survival Rates in Critical Limb Ischemia

We appreciate the comments concerning our article on amputation-free survival in patients with CLI.

We collected our data in 2004 and 2005. At that time, cilostazol was not yet approved for treatment of patients with PAD, in Germany. Therefore, we do not have any data concerning the influence of cilostazol on amputation-free survival in our study population. Nevertheless, we agree with the authors that cilostazol might h-
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