Reviewer's report

Title: Serum levels of matrix metalloproteinases -1,-2,-3 and -9 in thoracic aortic diseases and acute myocardial ischemia

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Reviewer: Paschalis Tossios

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The purpose of this study was to evaluate serum levels of MMP1, MMP2, MMP3 and MMP9 in different cardiovascular pathologies. The study population consisted of patients with acute aortic dissection (n=34), chronic aortic dissection (n=18), thoracic aortic aneurysm (n=18), acute coronary syndrome (n=18), and healthy individuals as controls (n=15). The authors conclude, that measurement of serum MMP levels can be used as a biochemical indicator of aortic disease or myocardial ischemia.

This observational clinical study addresses an important area of aortic research, namely, how to diagnose or monitor aortic pathologies by monitoring MMPs. Major strength of this study includes the relatively big number of patients. The authors have to be congratulated for their huge efforts to study these patients. The manuscript would be enhanced, however, if the authors would address more thoroughly some of the following questions:

Critique:

1. It is now becoming recognized that alterations in a specific extracellular matrix proteolytic cascade involving the MMPs and their endogenous inhibitors occur in cardiovascular disease status such as abdominal aortic aneurysms (AAA) as well as in thoracic aortic aneurysms (TAA). Because of changes on the cellular level are reflected in body fluids, determination of MMPs in blood have been recommended as noninvasive tools in the diagnosis and monitoring of several disease.

To date, most of our knowledge of the pathobiology has not been a product of direct study, but rather an extension of studies regarding AAA, beginning with the first report of upregulated MMP activity in aneurysm tissue. Work by Hovespian et al (JVIR 2000) has demonstrated that circulating plasma levels of MMP9 correlate directly to aortic wall levels of MMP9 in AAA disease. For human TAA, recent work from the Yale Group has demonstrated, for the first time, that there is a correlation between tissue levels of MMP9 and circulating levels of this enzyme (Botta, ELEFTERIADIS, Koullias, MMPs in aortic aneurysm and dissection, from Acute aortic disease, Edited by ELEFTERIADIS). This pilot study involved ten patients with TAA who underwent elective operative repair of ascending TAA. No further investigations have been published to date examining this issue.

Blood measurements of MMP9 are solely based on these data. It is mandatory that the authors explain this concept of laboratory testing within the manuscript.
Thus far, for MMP1, MMP2 and MMP3 no concordance between circulating and tissue levels in TAA has been demonstrated. What was the rationale for their measurement?

2. Study population consisted of 34 patients with acute aortic dissection. Please define according to the Stanford classification.

3. Study population consisted of 18 patients with chronic aortic dissection. Please define according to the Stanford classification. In addition, a definition of chronic aneurysm should be given.

4. Study population consisted of 13 patients with acute coronary syndrome. The authors should replace this term by STEMI throughout the manuscript, as all patients suffered an acute ST-segment elevation myocardial infarction (AMI).

5. The authors should mention that approval for the study was obtained from the institutional review board, and informed consent was granted by each patient and normal subjects.

6. Is TAA formation the focus of the paper or patients with STEMI per se? A large body of data exists with regard to MMPs in patients with stable and unstable angina, acute coronary syndrome and AMI. The present study does not provide novel additional information on this topic. The focus of the manuscript needs to be redirected so that its conclusions relate primarily only to TAA. Otherwise, the authors have to perform a subgroup analysis with patients suffering from an acute type A aortic dissection with and without involvement of the coronaries going clinically ahead with an AMI. The latter patients could then be compared with the group of STEMI.

7. The authors should provide information on treatment regimen after the diagnosis has been established. How many patients were treated interventionally or surgically in acute and chronic type A and B dissection?

8. The authors should report briefly on their operative technique: Did the authors perform aortic valve sparing techniques? How many extended aortic arch and/or descending procedures were necessary in TAA or dissections? How was their cerebral protection management? What did their interventional strategy look like?

9. How many hours after onset of symptoms in patients with dissection and in patients with STEMI were the blood samples collected?

10. The authors performed perioperative measurements up to 24 h after aortic root replacement. The authors should comment on the fact that, as recently reported, CABG but not OPCAB leads to a 700-900-fold increase of plasma MMP9 levels (Sokal, JTCVS 2009). The scientific value of this short-term analysis after the use of extracorporeal circulation, cardiac and possibly circulatory arrest is questionable, thus the authors should not hesitate to delete their data if interpretation is not conclusive.

11. It is not clear how long the time interval was between blood sample collection
12. Blood sampling: Although assays for an ever-increasing number of MMPs are now commercially available, the preanalytical impact of blood collection methods needs careful evaluation to limit technical pitfalls that may lead to misinterpretation. Therefore, the authors have to give a more accurate and detailed information on blood sampling. Were venous blood samples collected in plastic tubes with (serum+) or without additives (serum-) as coagulation accelerator? The name of the manufacturer’s bioanalyzer (company) has to be mentioned. Was total serum MMP (active and pro-MMP) measured? The sensitivity of the test has to be given.

13. The role of serum or plasma collection procedures has been very recently defined by Mannello (Arterioscler Thromb Vasc Biol 2008) and Jung et al (Clinical Chemistry, 2008). These data are of paramount importance when using these substances as comparisons. The present study by Karapanagiotidis is the first to determine MMP in serum, whereas only two other studies exist showing plasma MMP in patients with involvement of the thoracic aorta. Sangiorgi et al (J Cardiovasc Med, 2006) studied plasma MMP2 and MMP9 in patients with acute type A (n=9) and type B (n=4) aortic dissection (control, n=10). Monaco et al (JTCVS, 2007) examined plasma MMP9 and MMP3 in 32 patients with descending thoracic aortic aneurysms who underwent endovascular repair (controls, n=25 healthy volunteers). In both studies, preoperative plasma MMP levels of the patients were 2-fold to 3-fold higher than those in the controls. This is in contrast to the present study by Karapanagiotidis that needs further discussion in the manuscript.

14. In healthy adults MMPs are characterized by higher concentrations in serum than in plasma samples, particularly for MMP1 and MMP9, which also show 2- to 4-fold higher concentrations in serum+ than in serum- samples. The authors have be aware of these discrepancies and to use those values (Jung et al) as reference to compare serum samples of healthy controls. The manuscript would also benefit from some discussion of this problematic issue.

15. The present study does not have the power to show any positive correlation to gender or sex, due to the fact that the study groups were not age- and sex matched. It is not suprising that younger aged female volunteers (3 males, 12 females) showed lower MMP levels than elder male patients (57 males, 13 females). Many other reports studying bigger collectives could not show any significant correlation so far. Thus, these sentences, Table 3 and figure 1 have to be removed from the manuscript.

16. MMP levels are presented in table 1. Figure 2 and 3 is repetitive and of no additional value and has to be removed.

17. Table 2 has to be restructured. Intra- and inter-groups differences have to be given with p-values. Healthy volunteers do not represent patients, so this term has to deleted.
18. Conclusions have to be strictly referred to the main findings of this study. Despite the currently available knowledge base, the authors should remove all enthusiasmus of this kind. At present, the MMP levels are followed as part of research efforts. Laboraties do not offer MMP assays routinely as a clinical test. If a preective utility for TAA disease should be developed, there would then be impetus for conversion to a clinically available testing modality.

19. Limitations of the study have to be added.

20. References: Some references are too old. They have to be replaced by more recently published papers on MMP research. The reference list and particularly the pagiation has to be corrected according to the Journals´s guidelines.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests