A pilot study on the predictive role of CD105 in the development of abdominal aortic aneurysms

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Abstract:

Introduction: The progress and the risk factors of an abdominal aortic aneurysm (AAA) is well described in the literature. The exact pathophysiological mechanism that triggers the development of an AAA in some individuals and lies behind the gradual dilatation of the aorta and the consequent rupture still remain unknown. A connection is known to exist between AAA and CD105 through the TGF-βR pathway. Our aim is to study the potential predictive role of CD105 in aortic dilatation and the development of AAA in an animal model using porcine pancreatase under pressure.

Methods & Materials: Thirty-two wild-type male Wistar rats were recruited, weighted and then equally distributed in the study and control groups. In the study group, animals were subjected to laparotomy under general anesthesia and their aortas were perfused with Type I porcine pancreatic elastase (PPE) under hydrostatic pressure. The perfusion time was defined as T0. In a similar manner, the aortas of the control group were perfused with natural saline. On day 7 (T7) and day 14 (T14), each animal underwent additional laparotomies, the aortic diameters were measured and blood samples were drawn. CD105 serum levels were quantified using the ELISA technique and CD105 concentrations were calculated. Matrix metalloproteinase 9 (MMP9) serum concentrations were also measured and compared to CD105 concentrations.

Results: After the intervention, significantly higher levels of CD105 were recorded in the study group at T14. MMP9 levels were significantly higher in this group at both T7 and T14. CD105 could potentially act as biomarkers of the development of AAA.

Conclusions: CD105 has a prognostic value for the reconstruction of the vessel, but it doesn't reflect the destruction of the aortic well as MMP9 does.

Keywords: aneurysm, rupture, animal model, aorta, biomarker, endoglin, metalloprotease, MMP

INTRODUCTION

Abdominal aortic aneurysm (AAA) is a potentially lethal disease affecting a significant percentage of males over the age of 65 and especially those individuals in this age and gender group who are smokers.[1-3] Aortic aneurysm is defined as aortic diameter growth more than 50% compared to the expected normal diameter for the same segment of the aorta. The development of AAA is a continuous process frequently leading to aortic rupture, a life-threatening event that is still linked to significant morbidity and mortality.[4] According to the existing guidelines, AAA repair is necessary only if the AAA is large (≥55mm), symptomatic, or rapidly growing by ≥10mm annually.[5] Based on the current guidelines, asymptomatic aneurysms smaller than 55mm should be subjected to annual follow-up imaging, but not to elective repair.[6,7] Despite what the international guidelines recommend and the existence of screening programs, a debate regarding the cost-effectiveness and technical or logistical feasibility to screen smaller aneurysms exists. In some countries there is a clear benefit from small AAA screening, while others do not see this benefit.[8-10] This gap could be potentially be filled-in by the use of financially and logistically easier to deploy blood biomarkers providing a prognosis of future aortic dilatation. The World Health Organization defines a biomarker as any structure, substance, or process that can be measured in the human body and influences or predicts the incidence of outcome or disease.[11] A number of biomarkers has been suggested, but no biomarker has been validated to enter clinical practice to date.[12-15] Matrix Metalloproteinase 9 (MMP9) is already established as a biomarker for the progressive destruction of the aortic wall elements (elas-

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tin and collagen fibers).[2,16-19] In this study, we use MMP9 as an indirect sign of aortic wall destruction, in order to examine the role of CD105. CD105 or endoglin is a homodimeric transmembrane protein that has a similar structure in humans, rodents and pigs. Through the Tissue Growth Factor-β receptors (TGF-βR) there is a connection between AAA and CD105 and this potential biomarker is also linked to neoangiogenesis. The aim of this study is to report on the potential predictive role of CD105 in aortic diameter growth and the development of AAA in an animal model

METHODS & MATERIALS

This animal model protocol was approved by the General Directorate of Veterinary Services, National Bioethics Commission, according to Greek legislation regarding ethical experimental procedure, in compliance with the European Law (European Economic Community Directive 86/609) the Hellenic National Law (Act 2015/1992) and in conformance with the European Convention on the protection of vertebrate animals used for experimental or other scientific purposes. All animal research also complied with the *Animal Research: Reporting of In Vivo Experiments* (ARRIVE) guidelines.[20] The study was performed at the Laboratory for Experimental Surgery and Surgical Research "N.S. Christeas"; recognized by the European Union (reference number EL 25 BIO 005). Handling and care of the animals were in accordance with the National guidelines for Ethical Animal Research and the Principles of Replacement, Reduction and Refinement (3Rs).[7] All animals were housed in a specially prepared pathogen-free environment with ad libitum access to water and food. Lighting conditions mimicked the daily variation of light in nature. All efforts possible were made to minimize the suffering of animals.

The murine model used in this study is a variant of the experimental *in vivo* model of aortic aneurysm development with PPE infusion under hydrostatic pressure, initially developed by Anidjar et al.[21] Our modified experimental protocol has been previously published in details.[22]

Thirty-two wild-type male Wistar rats were recruited, weighted and then equally distributed in two groups - study and control groups. In the study group, animals were subjected to laparotomy under general anesthesia and their aortas were perfused for 5-15 minutes with a solution of 4.5 U/mL Type I porcine pancreatic elastase (PPE) under hydrostatic pressure of 100mmHg. The perfusion aimed in dilating the aorta by a further 50% of its original diameter. The perfusion time was defined as T0. In a similar manner, the aortas of the control group were perfused with natural saline 0.9% solution. On day 7 (T7) and day 14 (T14), each animal underwent additional laparotomies aiming in measuring the aortic diameter and blood sampling for future analysis. CD105 serum levels were quantified using the ELISA technique (CD105 ELISA Kit, # E02E0186, BlueGene, China) and CD105 concentrations are reported as μg/L. We evaluated the correlation between CD105 and the development and progression of an AAA within a period of time of 14 days.

At the same intervals, the levels of MMP9 were simultaneously measured in the serum samples of the rats of both groups. As mentioned earlier, MMP9 is a biomarker that is linked to the progression of AAA and the continuous dilatation of the infrarenal aorta.

Statistical analysis

All continuous variables are expressed as mean ± standard deviation (SD). The distributions normality was assessed using Kolmogorov-Smirnov's test and graphical methods. Between-group comparisons were performed using student's T-Test and Mann-Whitney U test, where appropriate. Comparisons between multiple time points were performed using repeated ANOVA and Friedman's test with Wilcoxon's Signed Ranks test. Pearson's correlation coefficient and Spearma's rho were calculated in order to examine relationships between variables. Differences were considered significant if the null hypothesis could be rejected with >95% confidence (two-sided $p<0.05$).

RESULTS

Not significant change of the animals' weights was demonstrated within each group at different time points (437.4±55.2 vs 429.1±65.9 at T0, 457.4±55.3 vs 415.1±62.5 at T7, 468±50.4 vs 416.4.1±67.4 at T14; $p>0.05$ for all).

At T0, there was no significant difference in the diameter of the infrarenal aorta between the two groups: 0.94±0.1mm and 0.909±0.08mm for the study and the control groups, respectively (Figure 1). Similarly, at T7, there was no significant difference between the two groups: 2.51±0.4mm and 1.13±0.1mm for the study and the control groups, respectively. At T14, the mean aortic diameter of the study group was significantly higher compared to the control group: 3.04±0.45 versus 1.17±0.12mm, respectively ($p<0.05$). In the study group, the aortic dilatation at T7 was significantly higher compared to T0, and at T14 was significantly higher compared to both T7 and T0 ($p<0.05$ for all in-group comparisons). The diameter of the infrarenal aorta of the control group did not grow significantly in any time point ($p>0.05$ for all in-group comparisons).

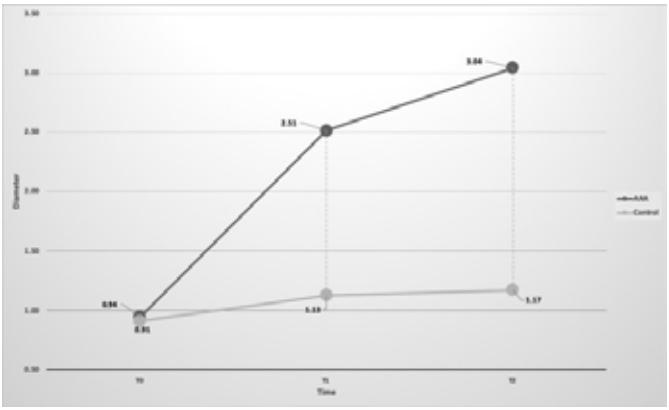

Figure 1. Diameter (in mm) of the infrarenal aorta for the study (AAA) and the control groups.

At T0 the concentrations of CD105 were 0.764±0.021 μg/l and 0.773±0.026 μg/l for the control and study groups, respectively (p>0.05) (Figure 2, Table 1). Similarly, at T7, the CD105 concentration of the two groups were not significantly different: 0.866±0.045 vs 0.816±0.016 for the control and study groups, respectively. At T14, the concentration of CD105 of the study group was significantly higher compared to the control group: 1.261±0.246 vs 0.815±0.031 (p<0.05). The CD105 concentration in the study group did not rise significantly between T0 and T7 (p>0.05). In the study group, the CD105 concentration at T14 was significantly higher compared to both T7 and T0 (p<0.05). The concentration of the control group did not rise significantly at any time point (p>0.05 for all in-group comparisons).

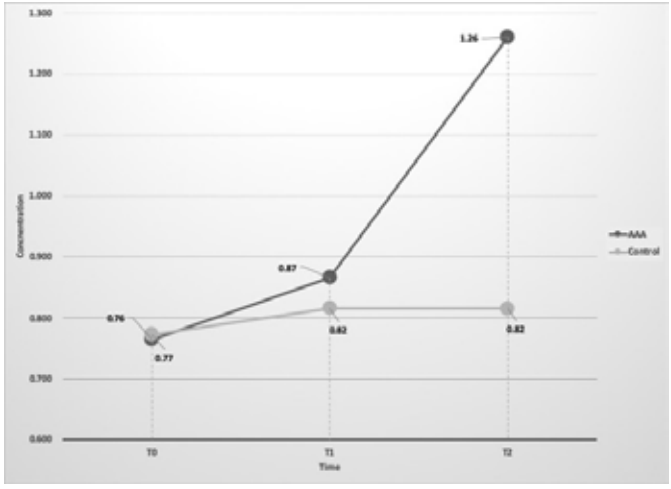

Figure 2. CD105 concentration (μg/L) of the study (AAA) and the control groups.

Time point	Study group		Control Group		p
	Concentration	SD	Concentration	SD	
T0	0.764	0.021	0.773	0.026	NS
T7	0.866	0.045	0.816	0.016	NS
T14	1.261	0.246	0.815	0.031	p<0.05

Table 1. CD105 concentrations (μg/L)

At T0, MMP9 concentrations were 149.385±62.387μg/l and 187.187±61.906μg/l for the study and control groups, respectively (p<0.05) (Figure 3, Table 2). These two concentrations were similar without any statistically significant difference. At T7, the MMP9 concentrations of the two groups were not significantly different: 208.306±52.669 vs 187.100±61.9 for the study and control groups, respectively. At T14, the concentration of MMP9 of the study group was significantly higher compared to the control group: 230.661±40.745 vs 187.091±61.89 (p<0.05). The concentration MMP9 in the study group did not rise significantly between T0 and T7 (p>0.05). At T14, the MMP9 concentration of the study group was significantly higher compared to both T7 and T0 (p<0.001). The concentration of the control group was almost constant at all time points (p>0.05 for all in-group comparisons).

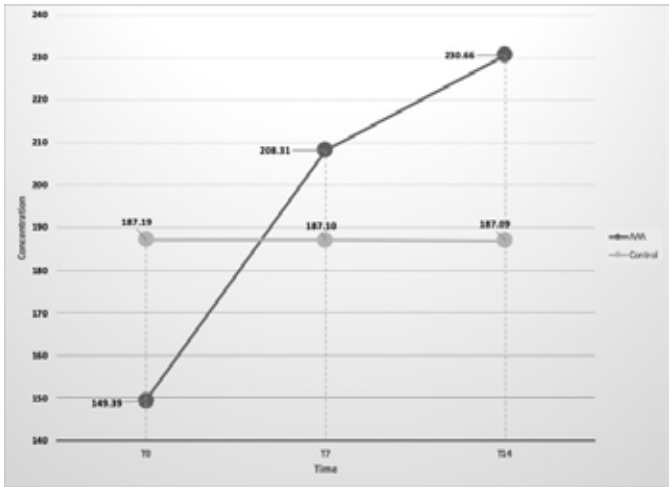

Figure 3. MMP9 concentrations of the study (AAA) and the control groups.

Time point	Study group		Control Group		p
	Concentration	SD	Concentration	SD	
T0	149.385	62.387	187.187	61.906	NS
T7	208.306	52.669	187.100	61.900	NS
T14	230.661	40.745	187.091	61.890	P<0.05

Table 2. Matrix MetalloProteinase 9 (MMP9) concentrations (μg/L)

DISCUSSION

CD105 or endoglin is a homodimeric transmembrane glucoprotein consisted of 633 aminoacids and with a molecular mass of 180kDa.[23-26] CD105 has short endocellular and transmembrane domain, while its extracellular domain is large.[23-25] The intracellular domain can be phosphorylated at multiple points.[24,27-29] CD105 has four points for N-glycozylation and one point for O-glycozylation, rich in serine and threonine.[24,30] Human CD105 has a similar aminoacid sequence to that of pigs and rodents with the exception of the extracellular domain that is significantly different.[31] Another significant difference of the human CD105 is the existence of the tripeptide RGD, which makes human endoglin unique.[30] The transmembrane and the cytoplasmic parts of CD105 demonstrate similarities to the β-glycane of the type III TGF-b receptor.[32] The human gene coding endoglin is located at chromosome 9q34->qter24 and is fourteen exons long.[33-35] Mutations of this gene usually lead to alterations of the extracellular domain.[36,37] Two isomorphs of CD105 exist: L- and S-CD105.[38] These two isoforms differ in the length of the cytoplasmic domain and their distribution in various tissues.[33] Apart its presence in human tissues, a soluble form of CD105 exists in human blood.[39] CD105 plays a major role in homeostasis, the development of blood vessels and the formation of the heart from gestational weeks four to eight.[40,41] In adult humans, CD105 is mainly located in the vascular endothelial and stromal cells, while it is also expressed in activated monocytes, differentiated macrophages, erythroid precursors, fibroblasts, melanocytes and dendritic cells in smaller quantities.[42-45] CD105 is significantly expressed in tissues with increased neo-angiogenesis, as a result of inflammation or neoplasia.[45] In normal endothelium, CD105 is

expressed along β-glycane, which is part of the TGF-β receptor.[32] CD105 is an auxiliary co-receptor of TGF-β, which is a pleiotropic cytokine regulating the differentiation, migration and coagulation of cells.[32,46,47] TGF-β also promotes inflammation and the release of angiogenetic factors by the inflammatory cells.[48,49]

Despite the fact that existing literature supports that MMPs play a significant role in the development of AAA, the predictive role of MMPs for AAA is not well established.[50] Some studies have demonstrated the strong correlation between MMP9 and the aortic dilatation.[18] Other studies also so the predictive value of MMPs for the development of AAA, the presence of persisting endoleak or the successful exclusion of an AAA.[16,51] In our study, MMP9 was employed as an indirect biomarker of aortic wall destruction in order to evaluate the effect of the hydrostatic pressure and PPE on the aortic wall elastic and collagen fibers.

As expected the selected animal model of PPE infusion under hydrostatic pressure was technically successful, producing AAA in all the animals of the study group. The efficiency of this animal model is well described in the existing literature since its first conception by the team of Anidjar.[21,52] The study group demonstrated a gradual dilatation of the infrarenal aorta at T7, but that did not reach a statistically significant difference compared to the control group until T14.

Concentrations of CD105 followed the trend of aortic dilatation. Initially at T0, CD105 concentration of the study group was at similar levels with the control group and at T7 it rose without reaching statistical significance. At T14, CD105 of the study group rose significantly compared to the control group and the T0 and T7 concentrations of the study group. MMP9 demonstrated a slightly different trend.

At T0, MMP9 concentration was similar for both groups. At T7, MMP9 of the study group rose significantly, that is before the aortic diameter changed significantly. At T14, the aortic dilatation as well as the levels of both CD105 and MMP9 rose significantly.

From these results, it is evident that MMP9 follows the destruction of the aortic wall elements and the consequent dilatation of the aorta. This is an expected process as MMP9 as PPE infusion under pressure acts by overstretching the aortic wall while there is chemical degradation of elastin fibers. These two mechanisms are reflected on the serum concentration of MMP9 in the study group. Despite that at T7 the aortic diameter is still not significantly increased (although a clear trend exists), MMP9 concentration is already significantly higher than at T0. The process of aortic wall destruction is already commenced, but the result is still to be observed. Simultaneously, the control group does not demonstrate any of these changes; no increase in diameter nor in MMP9 concentration.

At T14 all variables peak and their values are significantly higher compared to the in-group values at T7 and compared to the values of the control group. As described, CD105 plays a major role in the neo-angiogenesis and therefore to the reconstruction of damaged tissue. At T14, while the inflammation still persists and aortic aneurysm is already established, the organism tries to employ CD105 (amongst other biomarkers) to assist in the reconstruction of the vessel. As a result, CD105 could potentially have a prognostic value for the reconstruction of the vessel wall, but it doesn't reflect the destruction of the aortic wall.

All future studies should also pay attention on the threshold and time point at which CD105 starts to have a predictive role for AAA expansion. We chose a weekly interval between different points in time (T) based on our previous knowledge of "delayed" AAA formation using this animal model and in order to (a) allow the rats some time to recuperate in-between two laparotomies and (b) to maintain the same model with previous research on similar biomarkers.

Our study comes with two limitations: there was no power analysis and the cohort of the study was limited. Both weaknesses derive from the fact that this was a pilot study in order to initially evaluate whether CD105 would demonstrate the qualities of a valid biomarker for AAA development. A larger study should be completed in order to confirm or not the role of CD105 as a prognostic biomarker for the development of AAA or for process of aortic wall repair. Translation of our finding into humans could also represent a hazardous task, despite the molecular similarities between human and animal C105.

CONCLUSIONS

CD105 could have a prognostic value for the reconstruction of the vessel after an aneurysm is formed, but it doesn't reflect the destruction of the aortic wall as MMP9 does or the rate of aortic expansion.

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Conflict of interest: None

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