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ASCENDING AORTA ANEURYSM: BIOMOLECULAR AND BIOMECHANICAL FACTORS

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1. Summary of the project

Ascending aorta aneurysms may occur as a result of genetic factors that predispose to weakening of the aorta wall, biomechanical factors that increase wall or shear stress, and atherosclerosis. The main objective of the present study was to determine biomechanical, molecular and epigenetic mechanisms favouring ascending aorta dilation. From the coordinating centre (**Team 1**), patients with ascending aorta dilation with different aetiologies i.e. Marfan syndrome, bicuspid aortic valve and degenerative or hypertensive disease were studied. 4D-flow MRI was performed in all patients to analyse aortic distensibility, pulse wave velocity, wall shear stress and flow parameters. **Team 2** analysed a mouse model of syndromic and non-syndromic aortic disease to determine molecular mechanisms involved in ascending aortic dilation, and echo studies were conducted to quantify aortic distensibility and pulse wave velocity. Serum markers ADAMTS1, versican, aggrecan and TGF-Beta in patients were also analysed. **Team 3** defined epigenetic factors (DNA-methylation) related to biomechanical variables obtained by MRI. In vitro analysis was performed on biobank samples to establish flow-mediated epigenetic variance. The integration of biomechanical, biological and epigenetic data from patients and animals defined several mechanisms related to ascending aorta dilation.

2. Results of the project

The study aimed to gather significant information for improving the prognosis and management of aortic diseases. One of the main aims of the project was to define the biomechanical factors related to ascending aorta dilation. **Team 1** showed that proximal aorta longitudinal strain was independently related to the aortic root dilation rate and aortic events in addition to aortic root diameter, clinical risk factors, and demographic characteristics in Marfan syndrome patients (*Proximal aorta longitudinal strain predicts aortic root dilation rate and aortic events in Marfan syndrome. Eur Heart J. 2019 Jul 1;40(25):2047-2055*). Furthermore, descending aorta dissection has become one of the most significant clinical complications. Our study *"Decreased rotational flow and circumferential wall shear stress as early markers of descending aorta dilation in Marfan syndrome: a 4D flow CMR study. J Cardiovasc Magn Reson. 2019 Oct14;21(1):63*" showed that rotational flow is reduced in patients with Marfan

syndrome, even in the absence of aortic dilation, and is related to aortic stiffness, which provokes abnormal shear stress in the aortic wall. Rotational flow and circumferential wall shear stress were identified as early markers of descending aorta dilation in Marfan syndrome patients.

In order to differentiate biomechanics of the aorta, bicuspid, Marfan and degenerative aortic disease patients were studied by 4D-MRI, and aortic stiffness, pulse wave velocity, wall shear stress and flow parameters were assessed (*Influence of aortic dilation in the regional aortic stiffness of bicuspid aortic valve assessed by 4D-flow CMR: comparison with Marfan syndrome and degenerative aortic aneurysm. JACC cardiovascular imaging. 12(6) 1020-9, 2019. D1 doi: 10.1016/j.jcmg.2018.03.017).* In one study, several flow patterns related to valvular morphotype were observed in bicuspid aortic valve patients (*Aortic flow patterns and wall shear stress maps by 4D-flow MRI in the assessment of aortic dilatation in bicuspid aortic valve. J Cardiovasc Magn Reson. 2018, 20:28 D1. https://doi.org/10.1186/s12968-018-0451-1).* Patients with right-left fusion (RL-BAV) had an anterior flow direction and those with right non-coronary fusion (RN-BAV) presented flow near the posterior wall at the sino-tubular junction which shifted to the anterior-right wall in the distal ascending aorta. This flow distribution induces an increase in anterior aortic wall shear stress in RL-BAV patients, whereas RN-BAV morphology results in an increase in axial plane and circumferential wall shear stress in the middle and distal ascending aorta. These results may explain the different aortic dilation phenotypes observed in BAV. In addition to aortic diameters, assessment of the different wall shear stress components (axial and circumferential) and flow parameters may help to identify patients at higher risk of aortic dilation (*Increased rotational flow in the proximal aortic arch contributes to its dilation in bicuspid aortic valve disease. 2019. Eur Heart J Card Img. doi: 10.1093/ehjci/jez046, 27 February 2019*). Although echocardiography is the technique of choice for monitoring aortic root diameter, its measurement may be inaccurate when root asymmetry or RN-BAV are present *(Implications of asymmetry and valvular morphotype on echocardiographic measurements of aortic root in bicuspid aortic valve. Journal of the American Society of Echocardiography. 2019. doi: 10.1016 / j.echo.2018.08.004).* In these cases, an MRI or CT scan should be performed to confirm aortic diameters.

Team 2 stated in the original proposal that they expected this project to help elucidate the mechanisms related to aortic dilation in genetic-based diseases. These objectives were met. They were able to pinpoint key molecular players in the development of aortic dilation in Marfan syndrome patients. Their research demonstrated that Adamts1 substrates such as versican and the NO pathway are essential players in the development of the disease. Current understanding has allowed them to correlate the appearance of certain nitrated peptides and rise in cGMP levels with development of the disease, and they could thus act as biomarkers of the disease. **Team 3** combined global methylation analysis with in vitro studies of flow-sensitive methylation to identify biological processes associated with BAV aortopathy and the potential contribution of flow. Biopsies from non-dilated and dilated ascending aortas were collected from BAV (n=21) and tricuspid aortic valve (TAV) patients (n=23). Analysing the pattern of DNA methylation in non-dilated (ND) BAV and TAV ascending aortas they found 681 differentially methylated regions (DMRs), mapped to 894 genes (using GREAT, Stanford University, US). Pathway and ontology analyses were performed on genes with a methylation fold change of ±10% (n=540) to capture relevant biological processes of DMR-genes. Hallmark analysis showed oestrogen response to be enriched among differentially methylated (DM) genes, followed by TNF/NFKB signalling, hypoxia and epithelial mesenchymal transition (EMT). The KEGG pathway showed a similar signature. Interestingly, myosin light chain kinase (MYLK), a major RHOA kinase involved in stress fibre formation and cytoskeleton rearrangements, was also DM. They exposed endothelial cells (ECs) isolated from BAV and TAV to oscillatory (±12 dynes/cm2) or laminar (12 dynes/cm2) flow. They then identified the overlap among genes DM in BAV-ND and TAV-ND and those changing methylation in response to flow. This showed that BAV-ND methylation signature to be associated with oscillatory flow (P=0.01), specifically related to endocytosis (FDRq=1.5e-3) and actin cytoskeleton regulation (FDRq=2.8e-3). Next, they exposed primary BAV and TAV ECs to oscillatory flow and identified DMR genes and their expression levels. In total, 400 genes in BAV and 1251 in TAV changed expression in response to oscillatory flow (FDR10%). In BAV, 83% of genes were upregulated, compared to only 10% in TAV (KEGG pathway). In BAV, genes involved in WNT/f3-catenin and KRAS-signalling were upregulated. In flowed TAV ECs, the EMT/cancer profile was related to angiogenesis and inflammation. Furthermore, they analysed other epigenetic modulations associated with BAV. The miR-200 family was the highest ranked miRNA, and potentially hence having the strongest effect on the signalling network associated with BAV.

3. Relevance to potential future implications

This project provided important information and knowledge on fundamental aspects of aortic disease, such as the prognostic value of ascending aortic strain, and defined PWV determined by 4D-flow MRI as the most robust parameter in biomechanical aorta assessment. 4D-flow MRI studies determined the biomechanical characteristics of the proximal and distal segment of the ascending aorta, arch and descending aorta. The significance of our findings lies in differentiating the aortic mechanical properties of BAV and Marfan syndrome patients. The aorta of BAV patients has no intrinsic mechanical deficiency such as in Marfan syndrome. Thus, BAV patients do not appear to benefit from a different clinical management compared to those with tricuspid valve and are not comparable to those with Marfan syndrome. Aortic dilation exerts similar effects on the local aortic mechanical properties of BAV and TAV patients, which differ from those of Marfan. PWV showed the best association with AAo dilation in patients with BAV beyond clinical risk factors. The main advantages of PWV are that, unlike distensibility, it does not depend on any geometric or mechanical assumptions or local pressure, which cannot be measured non-invasively. This study provided some interesting data, such as the biphasic trend of pulse wave velocity in the ascending aorta when the diameter of the aorta reaches 50 mm, which suggests that aortic remodelling can no longer compensate for alterations in the aorta wall. This value is very close to that empirically suggested to indicate prophylactic surgery of the ascending aorta. Despite using the recommendations of the Guidelines to measure the aorta by echo and CRM, the maximum diameter of the aortic root shows acceptable agreement; however, in 39% of cases the differences were 3 mm and 5 mm in 15%. These differences were mainly related to the presence of root asymmetry and the BAV-RN morphotype.

The presence of root asymmetry or RN-BAV valvular morphotype is associated with underestimation of echocardiographic measurements and requires a CRM or CT scan to validate the larger diameter in three-dimensional techniques. Finally, mini-raphe has been shown to generate aortic flow disturbance and facilitate dilation of the ascending aorta.

Team 2 showed that the NO pathway in Marfan patients may be a clear target for therapy. This group currently has a patent in this regard (PCT/EP2016/082925, "*In*

*vitro method for identifying thoracic aortic aneurysms (TAA) in a subjec*t") which was recently licensed with the aim of conducting clinical trials to determine whether this approach can indeed constitute a beneficial tool for the treatment of TAAD. Similarly, ongoing efforts are expected to characterise molecules as mediators and biomarkers of the progress of MFS-associated aortopathy which in the mid-term may provide tools for monitoring this disease. MFS patients have a much greater risk of undergoing aortic dissection, an event that commonly leads to sudden death. They are in the process of establishing a model that connects all the mediators and precisely defining the relationship among them, which will be a long-term objective.

Team 3 was able to demonstrate that the response to oestrogen was methylation of different genes, followed by TNF / NFKB signalling, hypoxia and epithelial mesenchymal transition. An over-position of DM genes between BAV-ND and TAV-ND and genes changing methylation in response to flow has been identified. BAV-ND methylation was shown to be associated with oscillatory flow (P = 0.01), especially related to endocytosis (FDRq = 1.5e-3) and regulation of the actin cytoskeleton (FDRq = 2.8e-3). When BAV and TAV ECs were exposed to oscillatory flow, DMR genes and levels and expression were identified. A total of 400 genes in BAV and 1251 in TAV changed expression in response to oscillatory flow (FDR 10%). In GAB, 83% of genes were overregulated compared to only 10% in VAT (KEGG pathway). Notch target genes and Notch-related pathways showed endothelial cells of aneurysm patients to have dysregulated Notch/BMP/WNT pathways compared to donor cells. Wnt pathway activity was significantly high in patients' endothelial cells. Cells from patients had attenuated activation of DLL4, SNAILI, DKK1 and BMP2 in response to shear stress. The results indicate that the flow-response in BAV ECs involves hypomethylation and increased expression of WNT/P-catenin genes, as opposed to an angiogenic profile in TAV ECs. Therefore, the results of this project with its sub-studies have provided information of clinical significance that has led to a significant improvement in the use of imaging biomarkers as predictors of aortic complications beyond maximum aortic diameter and in increase understanding of the biomechanical, molecular and epigenetic mechanisms that promote dilation of the ascending aorta. These data will promote new studies on risk stratification of the disease with management implications and new therapeutic targets which will require a new drug trial to reduce aorta enlargement in these diseases.

4. Papers and proceedings

Peer-reviewed journal articles

Guala A, Teixido-Tura G (co-first author), Rodríguez-Palomares J, Ruiz-Muñoz A, Dux-Santoy L, Villalva N, Granato C, Galian L, Gutierrez L, Gonzalez-Alujas T, Sanchez V, Forteza A, García-Dorado D, Evangelista A. Proximal aorta longitudinal strain predicts aortic root dilation rate and aortic events in Marfan syndrome. *European Heart Journal* 2019; 40(25): 2047-2055.

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