CHAPTER

3B

Reply to Editorial: The aortic wall with bicuspid aortic valve: immature or prematurely ageing?

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Dear Editor:

We thank dr. Della Corte for his insightful comments and the opportunity to clarify a number of points from our work and you as an Editor to give us this opportunity. We agree with authors of the letter that the mechanisms driving aortic dilation in bicuspid aortic valve (BAV) and tricuspid aortic valve (TAV) are different but not explained as yet. Following that line, also based on the expertise of most of our authors, we hypothesized that a developmental background linking BAV and the accompanying aortic wall might provide new insights into the matter. Haemodynamic differences could still play a role in the developing pathobiology but might not be the primary insult. This approach led to different selection criteria for our patient population. We did not primarily take into account the presence of aortic stenosis/regurgitation but focused on non-dilation (< 45 mm) and dilation of the aortic wall within the BAV and TAV groups. Additional characteristics of our study population are presented in Table 1, which also provides answers to a number of questions with regard to the material studied. The aortic valve pathology in non- and dilated BAV and dilated TAV varied from either stenosis or no stenosis, with or without regurgitation. In dilated BAV and TAV in some cases only an ascending aorta replacement was performed as there was no aortic root pathology that needed surgical correction. All biopsy specimen were taken from the ascending aortic wall adjoining the surgical transverse aortotomy site. In case of post-mortem or transplantation material the tissue was taken at identical sites. The results of the Forte paper (1) and our publication (2) can in part be appreciated as complementary. Some differences need an additional explanation from our side as provided below. The described results in expression of alpha-smooth muscle actin in the Forte paper (1) and our study do indeed not correlate. This is most probably due to a difference of technique in which mRNA expression based on RT-PCR analysis of the vascular wall (1) was compared to our immunohistochemistry of the three vessel wall layers (2). In case of increased mRNA detection a lowered translation into protein cannot be excluded. Dr Della Corte is correct that almost absent smoothelin expression cannot be detected by immunohistochemistry, so we agree with their results that smoothelin expression is lowered, which correlates with an immature smooth muscle cell (SMC) phenotype (3, 4). With regard to the cellular composition of the media and intima of the aortic wall we do not postulate a few resident fibroblasts to

Characteristic	TA	TAD	BA	BAD
	N=11	N=12	N=17	N=19
Specimen obtained from	Post mortem, LUMC	During elective repair of the ascending aorta, LUMC	During stentless root replacement, collected when waste material became available from the proximal anastomosis from the LUMC and six biopsies from the EMC.	During elective repair of the ascending aorta, LUMC
Age (years)	64.5 ± 9.0	72.3 ± 11.2	55.8 ± 9.8	60.7 ± 7.8
Males (%)	54.5%	33.3%	70.1%	84.2%
Females (%)	45.5%	66.7%	29.4%	15.8%
Ascending aorta diameter (mean)	*	55.0 ± 10.7	36.5 ± 7.4**	52.7 ± 6.2
Commissure position			Unicuspid N=1 RCC/LCC*** N=8 RCC/NCC*** N=4 LCC/NCC*** N=1 Unknown N=3	RCC/LCC*** N=15 Unknown N=4

TA: non-dilated tricuspid aortic valve, TAD: dilated tricuspid aortic valve, BA: non-dilated bicuspid aortic valve, BAD: dilated bicuspid aortic valve, LUMC: Leiden University Medical Center, EMC: Erasmus Medical Center
* data unavailable, clinically defined as non-dilated by pathologist. ** data unavailable for 5 patients, clinically defined as non-dilated by pathologist. *** RCC/LCC: fusion of the right and left coronary cusp; RCC/NCC: fusion of the right and non coronary cusp, LCC/NCC: fusion of the left and non coronary cusp

be present that, under experimental development of thoracic aortic dilation, replace the disappearing SMC population by myofibroblasts as indicated by Jones et al (5) and adopted by the Della Corte group (1). In our studies it suffices to have a mature contractile SMC phenotype that can switch to a synthetic (immature) phenotype that expresses most of the markers also attributed to the myofibroblast phenotype, including the fibronectin splice variants (6). Our assumption is supported by less apoptosis (caspase-3) in the wall of the non-dilated as well as the dilated BAV (data unpublished). Apoptosis was however detected (not shown) in the media of the dilated TAV

and was in our study (2) linked to the increased expression of progerin. On this aspect we will have further comments when discussing the aspect of ageing. As described above, our study population was primarily not selected on the valve pathology or on the difference in architecture of the convex versus concave site. The fact that our group consisted of patients with a non- and dilated aortic wall, variable aortic valve pathology and commissure position, underlines that the observed differences between all BAVs and TAVS are intrinsic to the BAV wall morphology. This is further supported in a recently accepted paper (7) in which we did address the differences between the convex and concave aortic sites of patients with a dilated aortic wall, provided by our German co-authors that have adopted the selection technique as described before (8). We did not disclaim the earlier published differences (8, 9) in the structure of the wall and the expression of markers between the convex and concave site of the aorta. We performed, however, an additional analysis on these specimen with Transforming-Growth-Factorβ and endothelial nitric oxide and found no difference in expression between the convex and concave sites (7). Haemodynamic influences are therefore not excluded from having an important influence but according to our studies cannot be considered as the primary source for the observed differences in dilation formation between BAV and TAV.

On basis of the above observations and additional arguments provided below we cannot endorse the concluding remarks of Dr Della Corte: 'Functionally, one of the early signs of arterial ageing is impaired wall distensibility, which is known to occur in the BAV even before overt dilation. Thus, with the currently available data, an hypothesis of defective wall maturation is hardly sustainable, without ruling out the contribution of flow induced remodeling: a conceptually opposite theory of premature ageing of the aortic wall, prompted by altered biomechanical environment could be drawn as well'. In this respect the definition and detection of ageing of the vascular wall is an issue. Loss of aortic wall distensibility, based on the increase of collagen and loss of elastin is adapted by some as a measure of vascular wall ageing (5) and used not only for the TAV models but also for BAV (1). We have not specifically studied the collagen content but confirm the diminished elastin structure in BAV (2). Additionally we have introduced a new set of ageing markers being the balance between lamin A/C and progerin (2, 10). Even physiologic ageing with increasing age has been correlated with the increased expression of progerin (10-12). We are excited about the fact that in this way we could differentiate

between TAV and BAV, showing increased physiologic ageing in TAV based on an increase of progerin and accompanying apoptosis resulting in cytolytic necrosis, while this was lacking in BAV. The decrease of distensibility as described (1, 5) might still hold for the aortopathy in both BAV and TAV but should in our opinion not be translated as an indication of premature ageing in BAV. In our study we had the relatively unique opportunity to show that the structural immaturity was present in both non- and dilated BAVs independent of the degree and type of underlying aortic valve pathology.

In conclusion we demonstrated that a different mechanism underlies aortic dilation in BAV and TAV patients. We postulate that secondarily haemodynamic influences, additionally triggered by the specific BAV aortic valve pathology, might have a different impact on the immature BAV wall as compared to the ageing TAV aortic wall.

REFERENCES

(1) Forte A, Della CA, Grossi M, Bancone C, Provenzano R, Finicelli M, et al. Early cell changes and TGFbeta pathway alterations in the aortopathy associated with bicuspid aortic valve stenosis. Clin Sci (Lond) 2013;124:97-108.

(2) Grewal N, Gittenberger-de Groot AC, Poelmann RE, Klautz RJ, Lindeman JH, Goumans MJ, et al. Ascending aorta dilation in association with bicuspid aortic valve: A maturation defect of the aortic wall. J Thorac Cardiovasc Surg 2014;148(4):1583-90.

(3) Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 2004;84:767-801.

(4) DeRuiter MC, Rensen SS, Coolen GP, Hierck BP, Bergwerff M, Debie WM, et al. Smoothelin expression during chicken embryogenesis: detection of an embryonic isoform. Dev Dyn 2001;221:460-3.

(5) Jones JA, Beck C, Barbour JR, Zavadzkas JA, Mukherjee R, Spinale FG, et al. Alterations in aortic cellular constituents during thoracic aortic aneurysm development: myofibroblastmediated vascular remodeling. Am J Pathol 2009;175:1746-56.

(6) Slomp J, Gittenberger-de Groot AC, Glukhova MA, van Munsteren JC, Kock MM, Schwartz SM, et al. Differentiation, dedifferentiation, and apoptosis of smooth muscle cells during the development of the human ductus arteriosus. Arterioscler Thromb Vasc Biol 1997;17:1003-9.

(7) Grewal N, Gittenberger-de-Groot AC, DeRuiter MC, Klautz RJM, Poelmann RE, Duim SN, et al. Bicuspid aortic valve: phosphorylation of c-Kit and downstream targets are prognostic for future aortopathy. Eur J Card Thor Surg 2014;46(5):831-9.

(8) Cotrufo M, Della CA, De Santo LS, Quarto C, De FM, Romano G, et al. Different patterns of extracellular matrix protein expression in the convexity and the concavity of the dilated aorta with bicuspid aortic valve: preliminary results. J Thorac Cardiovasc Surg 2005;130:504-11.

(9) Mohamed SA, Noack F, Schoellermann K, Karluss A, Radtke A, Schult-Badusche D, et al. Elevation of matrix metalloproteinases in different areas of ascending aortic aneurysms in patients with bicuspid and tricuspid aortic valves. ScientificWorldJournal 2012;2012:806261.

(10) Bökenkamp R, Raz V, Venema A, DeRuiter MC, van MC, Olive M, et al. Differential temporal and spatial progerin expression during closure of the ductus arteriosus in neonates. PLoS One 2011;6:e23975.

(11) Olive M, Harten I, Mitchell R, Beers JK, Djabali K, Cao K, et al. Cardiovascular pathology in Hutchinson-Gilford progeria: correlation with the vascular pathology of aging. Arterioscler Thromb Vasc Biol 2010;30:2301-9.

(12) Ragnauth CD, Warren DT, Liu Y, McNair R, Tajsic T, Figg N, et al. Prelamin A acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. Circulation 2010;121:2200-10