Risk Stratification in Bicuspid Aortic Valve Aortopathy: Emerging Evidence and Future Perspectives

Alessandro Della Corte, MD, PhD, Hector I. Michelena, Angelo Citarella, Emiliano Votta, Filippo Piatti, Federica Lo Presti, Rasul Ashurov, Marilena Cipollaro, and Amalia Forte

Abstract: The current management of aortic dilatation associated with congenital bicuspid aortic valve (bicuspid aortic valve aortopathy) is based on dimensional parameters (diameter of the aneurysm, growth of the diameter over time) and few other criteria. The disease is however heterogeneous in terms of natural and clinical history and risk of acute complications, ie aortic dissection. Dimensional criteria are now admitted to have limited value as predictors of such complications. Thus, novel principles for risk stratification have been recently investigated, including phenotypic criteria, flow-related metrics, and circulating biomarkers. A systematization of the typical anatomoclinical forms that the aortopathy can assume has led to the identification of the more severe root phenotype, associated with higher risk of progression of the aneurysm and possible higher aortic dissection risk. Four-dimensional-flow magnetic resonance imaging studies are searching for potentially clinically significant metrics of flow derangement, based on the recognized association of local abnormal shear stress with wall pathology. Other research initiatives are addressing the question whether circulating molecules could predict the presence or, more importantly, the future development of aortopathy. The present review

Curr Probl Cardiol 2019;00:1–18
0146-2806/$ – see front matter
https://doi.org/10.1016/j.cpcardiol.2019.06.002

summarizes the latest progresses in the knowledge on risk stratification of bicuspid aortic valve aortopathy, focusing on critical aspects and debated points. (Curr Probl Cardiol 2019;00:1−18.)

Bicuspid aortic valve (BAV), the most common cardiac congenital malformation, is associated with increased risk of developing aortic vascular complications, usually in adulthood, including aortic aneurysm and dissection.[1] While aortic dilatation and aneurysm occur at a high rate (nearly 1% patient-years), aortic dissection represents a catastrophic but rarer complication (0.03% patient-years), however occurring at an 8- to 9-fold higher rate than in the general population.[2] Patients are usually submitted to elective surgery on the basis of "dimensional" criteria, ie the size and progression rate of aortic diameter,[3] to prevent the risk of acute aortic events, ie rupture and dissection. However, it is now understood that aortic diameter has a limited predictive value, as aortic events may occur when aortic diameters are <55 mm,[4] and even <45 mm[5] and thus, do not fall within the guidelines for elective thoracic aortic aneurysm surgery.[6] Consequently, "nondimensional" criteria for a more precise risk assessment and for a patient-tailored strategy are required.

Studies currently focusing on the identification of new criteria for risk stratification in BAV aortopathy are based on 3 research lines: clinical significance of aorta "phenotypes" [1], novel imaging techniques capable to describe and quantify flow-related mechanical stimuli,[7] and circulating molecules representing potential biomarkers of the presence or severity of the aortic wall disease.[8] The present review addresses these 3 lines of research underscoring both interesting applicative perspectives and current limitations.

Phenotypic Stratification

The current knowledge of BAV-related aortopathy is recognized to be scarce, especially in its pathophysiological and prognostic aspects.[9] In the recent past, research in this field has focused on the attempt to answer the pathogenetic question of whether BAV aortopathy is the result of a genetically-mediated disorder or a hemodynamically-driven phenomenon. Subsequently, evidence that aortic dilatation can assume different anatomical forms, each typically (though not exclusively) associated with peculiar clinical features, has appeared.[10-12] Consequently, due to the inconclusive results of the "genetically-mediated" vs "hemodynamically-driven" research approach, and the increasing awareness of the anatomoclinical

phenotypic heterogeneity of BAV aortopathy, that pathogenic dichotomic view has been recognized to be limitative. Moreover, the existence of 2 opposite views of the disease (ie as a genetically mediated "Marfan-like" disorder or as a more benign poststenotic dilatation) has caused confusing changes in subsequent surgical guideline recommendations[13] and remarkable differences in the principles and policies of management among different centers and surgeons.[14]

Today, the clinical course of BAV aortopathy, in terms of rapidity of progression, incidence of acute aortic events, and need for surgery, is recognized to vary considerably among different subsets of patients[15]: in some of them it assumes the form of an indolent chronic disease, potentially harmless to the patient's life expectancy, while in others it entails a considerable burden of acute complications, making case-by-case risk stratification a challenging clinical task. It is now believed that this prognostic heterogeneity results from a complex, multifactorial pathogenesis, in which different relative contributions are at play, including several possible genetic variants and varying flow-related mechanisms.[9,16] Assuming that the phenotypic heterogeneity is caused by the same pathogenetic heterogeneity, it has been suggested that defining the phenotype can be a tool to identify individual patients that will incur more severe forms of the disease, in a way to guide personalized surgical decision making.[17] Therefore, a number of studies have attempted to classify the phenotypic diversity of BAV aortopathy, and they can be summarized as: (1) studies based on echocardiographic assessment of the aortic shape, ie the relative dimensions among segments of the aorta: root, sinotubular junction, ascending tubular tract; either with[10] or without[11] adjunct consideration of the absolute dimensions; and (2) studies based on CT scan and/or MRI evaluations; including more classes to include description of involvement of the arch segment.[12,18] Some studies on the different phenotypes of aortic dilatation have searched for significant associations with the valve morphotypes (ie pattern of cusp fusion of the BAV, presence/absence of the raphe) and/or function (ie stenosis or regurgitation), others have looked also at the correlations with clinical features of the patient.[19] However, the only phenotypic classification that has been tested for possible prognostic predictive value is the one that distinguishes aortic dilatation of the more common ascending phenotype (maximal dilatation diameter located at the tubular tract beyond the sinotubular junction, usually associated with aortic stenosis or echocardiographically normofunctional valve, accounting for ~80% of BAV aortopathy), from a rarer root phenotype (maximal diameter at the sinuses of Valsalva level, associated with aortic regurgitation, representing 15%-20% of

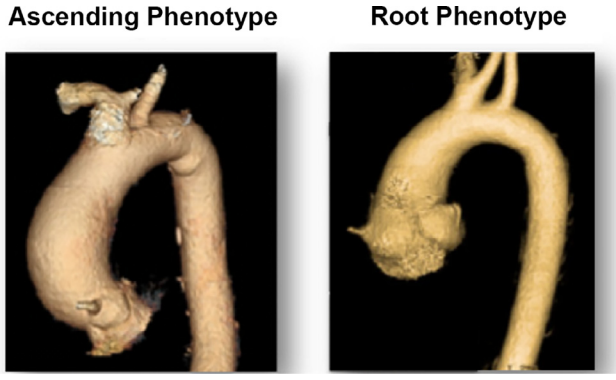

FIG 1. Reconstructions of CT scan studies of the thoracic aorta illustrating the anatomy of the 2 phenotypic forms of BAV aortopathy identified to have different clinical significance: the more frequent ascending phenotype (maximal diameter at the tubular ascending tract) and the less frequent root phenotype (maximal diameter at the sinuses of Valsalva), recognized to be a more severe form, in terms of progression of the dilatation and risk of acute aortic dissection.

BAV-associated dilatations – Fig 1): in a single-center study, the root phenotype was found to be the significant predictor, after correcting for initial diameter and other clinical factors, of a faster growth of the ascending aorta over a mean follow-up time of 4 years.[20] Other evidence has been accumulated showing that the root phenotype might represent a more severe form of aortopathy, possibly the one in which the genetic contribution to the pathogenesis is most important, compared to the ascending phenotype, whose course might be more significantly influenced by flow derangements. Moreover, the root phenotype has been found associated with acute aortic events in the postoperative follow-up of BAV patients who had undergone simple aortic valve (AV) replacement[21] as well as with potentially aortopathy-related genetic variants, both in the gene encoding fibrillin-1 in a small study[22] and within a panel of 20 candidate aortopathy genes in targeted genetic analysis.[23] Therefore, the root phenotype has been recently included among the adjunctive risk factors to consider when indicating earlier elective surgery for BAV aortopathy.[24]

Fluid-Dynamics-related Risk Markers

The search for quantitative scores suitable for stratifying the risk of BAV-related aortopathy has been focusing also on the BAV-associated deranged fluid dynamics, which are consistently characterized by asymmetrical accelerated systolic jets deflected toward localized portions of the aortic wall, which are at least partially dependent on BAV fusion

type.[25-31] The underlying hypothesis is that such flow derangements translate into altered localized mechanical shear stimuli on the aortic endothelium, and trigger or exacerbate chronic adverse remodeling of the wall through mechanotransduction. Therefore, current research is testing the quantification of these derangements through clinically available imaging modalities (Fig 2).

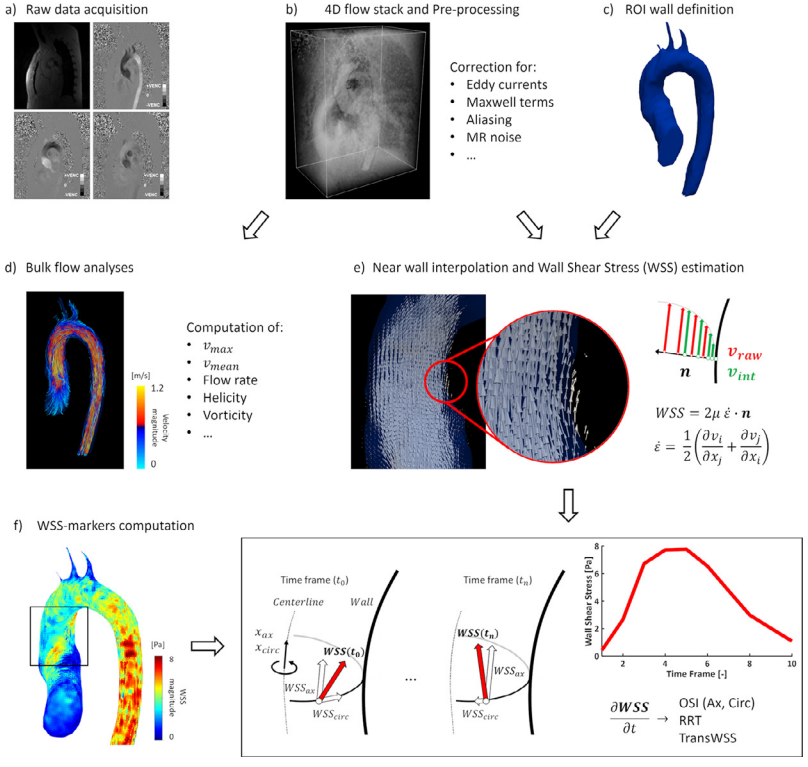

FIG 2. Fluid-dynamics-related risk markers. (a) 4D-flow acquisition consisting of magnitude and phase-contrast images encoding velocity along Head-Foot, Anterior-Posterior, and Right-Left directions throughout the cardiac cycle. (b) Volume rendering of the 3D stack of phase-contrast images to highlight their volumetric coverage. The 3D (or 3D + time) stack can be preprocessed to minimize MR-related artifacts and noise effects. (c) Definition of the aortic wall position from the 3D region of interest (ROI) extracted from the 4D-flow volume. (d) Visualization of 3D blood flow streamlines (color-coded by velocity magnitude) and examples of hemodynamic variables computed by postprocessing the aorta bulk-flow. (e) Visualization of near-ROI wall blood velocity and possible strategy of data filtering through interpolation (v_{int}) from raw velocity points (v_{raw}) to optimize the estimation of wall shear stresses (WSS) based on the computation of numerical velocity derivatives. (f) 3D WSS heat-map (color-coded by WSS magnitude) of the shear stimuli acting on the aorta wall (left panel); schematic description of WSS possible decomposition in a local frame of reference with respect to the aorta wall (axial, circumferential) and time-dependency along the cardiac cycle, allowing for the estimation of supplementary WSS-related hemodynamic markers.

In vivo studies based on 4-dimensional flow cardiac magnetic resonance (4D-flow) imaging have shown that in BAV patients, even with "normal" valve function, blood flow in the ascending aorta is characterized by increased *helicity*, and most frequently by a right-handed helical flow.[32,33] In addition, this pattern may change over time as AV function deteriorates.[34] Despite being a hallmark of BAV, blood flow helicity does not seem a useful clinical score, inasmuch as a feature of the bulk flow and not of the near-wall flow (ie the components of flow that closely interact with the aortic wall), whose features are purported to contribute to the BAV-related aortopathy pathogenesis.

Nonetheless, 4D-flow imaging has shown that the accelerated systolic jet locally impinges on the wall of the ascending aorta, thereby generating a wall shear stress (WSS) overload. In a pivotal study, Guzzardi and colleagues[35] recently suggested a potential tight link between *WSS anomalies* and aortic wall remodeling: they acquired preoperative 4D-flow imaging in candidates for elective aortic resection and mapped the aortic WSS distribution across peak systole. Postsurgery histology on ascending aorta samples retrieved from high and low WSS regions showed that the formers were systematically characterized by more pronounced dysregulation of the extracellular matrix and elastic fiber degeneration in comparison to the latter ones,[35] providing direct evidence of what had been previously suggested based on similar results.[36,37] More recently the same group[38] validated the association between locally increased WSS and decreased elastic fiber thickness, notably highlighting that this association was stronger in patients with AV stenosis.

This evidence was further confirmed by a combination of computational and in vitro modeling. In 2 studies, Atkins et al,[39,40] used fluid-structure interaction modeling to obtain the highly time- and space-resolved WSS patterns at the convexity and at concavity of the ascending aortic wall in BAV patients and in healthy controls. Initially physiologic porcine tissue from the corresponding aorta regions were then exposed in vitro to those WSS patterns through a bioreactor: by applying convexity-like WSS patterns to convexity specimens, a significant effect on expression of matrix metalloproteinases 2 and 9 was observed.

A number of studies investigated the BAV-related WSS alterations in vivo by postprocessing 4D-flow imaging data, typically through custom software. Despite the heterogeneity of study populations (nondilated young aortas[41] or frank aneurysms[38]), and of postprocessing software tools, all these studies detected significant BAV-related alterations in WSS patterns and peak/average values during systole with respect to healthy controls. Yet, depending on the specific algorithm implemented

in the processing software, WSS values computed for comparable subjects can range over 2 orders of magnitude,[42-45] suggesting that reproducibility and clinical applicability, strictly dependent on reliable reference values, still remain unresolved.

Also, with few exceptions (eg, Hope et al[42]), a direct correlation was not found between WSS magnitude increase and aortic growth, which is conventionally assumed as a marker of disease severity, given the lack of large and adequately long-term follow-up studies. Probably driven by that lack of correlation between WSS magnitude and clinical outcomes, the focus of 4D-flow studies has shifted progressively toward the quantification of finer features of WSS: peak magnitude, peak circumferential, and axial components, as well as indexes quantifying WSS time-dependency. A first insight was provided by Bissell et al,[32] reporting altered WSS values in the *circumferential component*, which accounted for up to 50% of the total amount of WSS overload, predominantly as a result of right-/left-handed abnormal flow rotations. Such features were later also observed by other authors[26,46] at different portions of the ascending aorta, ie, proximal, mid, and distal.

Recent studies have begun to focus on the *time-dependency* of WSS vectors, both through high-end in silico approaches[44] and processing of 4D-flow data.[45] In particular, Piatti and colleagues[45] quantified pronounced differences between WSS oscillations in young BAV patients with nondilated aortas as compared to age-matched healthy controls: these were particularly evident when analyzing time-dependent changes of both the magnitude of the WSS component transversal to the main flow direction and the orientation of the 3D WSS vector. Interestingly, non-negligible intersubject differences were found among BAV patients, suggesting a possible role of these features as improved markers of aortopathy progression.[45]

Circulating Biomarkers

The ideal prognostic circulating biomarker of BAV aortopathy should be of pathogenetic significance, reliably measurable in serum/plasma, with a significant concentration difference vs basal levels in healthy subjects, of proven relevance to the course of the disease (ie, capable of predicting progressive dilatation and/or acute aortic events), and ideally it should have no or loose correlation with aortic diameter, to provide additional information for prognostic stratification alongside this parameter.

The search for circulating biomarkers in the setting of BAV aortopathy currently represents a particularly lively research field. Among BAV

aortopathy biomarkers, a number of investigations have focused mainly on circulating *proteins* belonging to different pathways or on noncoding circulating *ribonucleic acid* (RNA) molecules, including microRNAs and long noncoding RNAs (lncRNAs). The background hypothesis is that dysregulated expression of those key molecules in the aorta could be associated with altered circulating levels of the same molecules: interestingly, the secretome produced during 24-hour in vitro incubation of aortic samples harvested from aortic graft replacement patients revealed marked differences between BAV and TAV patients.[47]

A synopsis of the above investigations is reported in Table 1. A negative correlation between alpha 1-antitrypsin, an abundant serine protease inhibitor able to protect tissues from enzymes of inflammatory cells, and aortic diameter has been identified in BAV patients by Kilickesmez et al,[48] with alpha 1-antitrypsin levels and age emerging as independent predictors of aortic dilatation.

Others focused on advanced glycation end products, a heterogeneous group of molecules playing an important role for the development and progression of cardiovascular disease mainly through the induction of oxidative stress and inflammation and triggering the release of a soluble receptor (sRAGE).[49] Interestingly, a study revealed that high levels of circulating sRAGE are associated with the presence of BAV and aortopathy and directly correlated with altered ascending aortic microstructure independently of aortic diameter.[50]

Drapisz et al[51] revealed that asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase responsible for nitric oxide production, could be found at increased plasmatic levels in nonstenotic BAV patients with dilated aorta, and that ADMA correlated positively with impairment of aortic elastic properties. Although that study concluded that plasma ADMA levels might prove of prognostic value, it failed to perform a follow-up study to validate this hypothesis.

Among the proteins investigated so far as potential biomarkers of BAV aortopathy, the Transforming Growth Factor-β (TGF-β) signaling pathway is attracting increasing attention, by virtue of its role in fibrosis, inflammation, cell proliferation and migration, and extracellular matrix remodeling, and in light of its involvement in aortopathy syndromes, such as Loeys-Dietz and Marfan syndromes.[52-54] A higher ratio between plasmatic TGF-β1 and the soluble form of its coreceptor endoglin (sENG) revealed to be indicative of a detrimental gene expression signature in the aorta and a propensity to aortopathy progression in BAV patients with aortic stenosis and nondilated ascending aorta over a 3-year follow-up, thus supporting its potential prognostic value.[55] This is so far

TABLE 1. Summary of the main studies focusing on the identification of potential early biomarkers of BAV aortopathy

Author(s)	Year of publication	Number of patients, study design	Aortic valve dysfunction	Biomarker(s) investigated	Correlations found
Forte et al	2017	101 BAV patients (2 subgroups: nondilated aorta vs *ascending phenotype* dilatation), 66 TAV patients, 32 heart transplant donors (aortic tissue), 32 control subjects	Stenosis	Circulating TGF-β1, sENG, SOD3, MMP-2, MMP-14, CTGF, TGF-β1/sENG ratio	TGF-β1/sENG ratio higher in nondilated BAV vs TAV and control groups, higher in BAV ascending phenotype dilatation. Correlation with *aortic diameter growth rate* in BAV patients with nondilated aorta
Hillebrand et al	2014	317 patients with BAV (30/317), Marfan syndrome, Loeys–Dietz syndrome, thoracic aortic aneurysm and dissection; 119 control subjects without genetic aortic syndrome	Not available	Circulating TGF-β1	TGF-β1 higher in BAV patients vs control subjects without genetic aortic syndrome
Harrison et al	2018	15 BAV patients categorized into 2 groups according to aortic dimensions	Stenosis/ regurgitation	Plasma proteome	Correlation with aortic diameter (among others, DNA-dependent protein kinase catalytic subunit, lumican, tetranectin, gelsolin, and cartilage acidic protein 1 showed significantly lower variability in the aneurysmal group)
Kilickesmez et al	2012	82 BAV patients categorized into 2 groups according to aortic dimensions	No or mild dysfunction	Circulating alpha 1-antitrypsin	Correlation with aortic diameter
Branchetti et al	2014	61 TAV patients, 74 BAV patients	Stenosis/ regurgitation	Circulating sRAGE	sRAGE higher in BAV patients. Correlation with tissue RAGE expression, with BAV and with

(*continued on next page*)

TABLE 1. (*continued*)

Author(s)	Year of publication	Number of patients, study design	Aortic valve dysfunction	Biomarker(s) investigated	Correlations found
					dysfunctional aortic microstructures in BAV patients
Drapisz et al	2013	20 TAV patients, 20 BAV patients	Nonstenotic	Circulating ADMA, MMP-2	ADMA and MMP-2 higher in BAV vs TAV patients; ADMA correlation in BAV patients with aortic annulus, peak aortic velocity, aortic distensibility, aortic stiffness index, and aortic strain, as well as with MMP-2 and plasma total homocysteine
Martinez-Micaelo et al	2017	18 BAV patients categorized into 2 groups according to aortic dimensions; 6 TAV healthy subjects; data validation in independent cohorts of BAV/TAV patients and healthy subjects	Stenosis/ regurgitation	Circulating miRNome→miR-718 identified as potential biomarker	Correlation with aortic diameter, independently of aortic valve morphology
Ikonomids et al	2013	21 TAV patients, 21 BAV patients with aortic aneurysm; 10 heart transplant donors (aortic tissue)	Not available	Circulating miRNAs, MMPs, TIMPs	Correlation of a combination of multiple analytes with aortic valve morphology and aneurysm
Girdauskas et al	2018	63 BAV patients, *root phenotype* categorized into 2 groups according to aortic dimensions (blood samples taken at postsurgery follow-up visits)	Regurgitation	A subset of 11 circulating miRNAs→miR-17 and miR-106a identified as potential biomarkers	Correlation with severity of aortopathy (diameter) and previous adverse aortic events

(*continued on next page*)

TABLE 1. (*continued*)

Author(s)	Year of publication	Number of patients, study design	Aortic valve dysfunction	Biomarker(s) investigated	Correlations found
Balistreri et al	2018	70 TAV patients, 70 BAV patients, categorized into different groups according to aortic dimensions	Stenosis/ regurgitation	Circulating EPCs and Notch1	Quantitative reduction of Notch1 level, EPC number/EPC impaired function in BAV patients
Balistreri et al	2018	35 TAV patients, 25 BAV patients, categorized into different groups according to aortic dimensions	Stenosis/ regurgitation	Circulating T and B lymphocyte subsets	Quantitative reduction of T and B lymphocyte cell subsets in BAV patients

Abbreviations: ADMA, asymmetric dimethylarginine; CTGF, connective tissue growth factor; EPCs, endothelial progenitor cells; MMP, matrix metalloproteinase; sENG, soluble endoglin; SOD3, superoxide dismutase 3; sRAGE, soluble receptor for advanced glycation end product; TGF-β1, transforming growth factor β1.

the only study that has documented relevance of the candidate biomarker to the course of aortic disease (namely the growth rate of the aorta over time). Differences in serum TGF-β1 levels between BAV patients and patients with no defined genetic aortic syndrome have been also found by others.[56]

Regarding RNA molecules, current data support the expression and the role of miRNAs in endothelial and smooth muscle cell homeostasis and phenotype changes associated with aortopathy progression,[57] both at local and plasmatic level. Conversely, studies focusing on lncRNA in aortic disease are still in their infancy[58] and, to the best of our knowledge, no data are currently available about their potentiality as biomarkers of BAV aortopathy.[59,60] MiRNAs and lncRNAs are generally considered as ideal disease biomarkers since their levels in plasma are reproducible, stable, and consistent among subjects, as they are protected from endogenous ribonuclease-induced degradation.[61] Among miRNAs, the expression profile of plasmatic *miR-718* is strongly influenced by dilation of the ascending aorta, inversely correlates with the aortic diameter and independently predicts aortic dilation, both in BAV and TAV patients.[62] In a study by Ikonomidis et al,[63] unique combinations of plasmatic matrix metalloproteinases, tissue inhibitors of matrix metalloproteinases, and miRNA species were associated with aortopathy in BAV vs TAV patients. A study by Girdauskas et al[64] included a selected cohort of patients with BAV insufficiency and root dilation phenotype. They identified a correlation among the levels of circulating *miR-17* and *miR-106a*, the severity of aortopathy and the occurrence of adverse aortic events, although this was analyzed in retrospect, since circulating miRNAs were tested during follow-up visits, not perioperatively. Finally, a recent analysis revealed several differences between BAV patients with less-dilated and severely dilated aorta, with particular reference to *miR-34a*, defined as a potential independent predictor of aortic dilation in BAV patients when confounding factors like age and hypertension are controlled for.[65]

Interestingly, in addition to the biomolecules described above, growing attention is also focusing on circulating cells as potential early biomarkers of BAV aortopathy. Recently, among BAV patients with valve dysfunction, a lower number of circulating endothelial progenitor cells was found in those with nondilated compared to those with dilated aortas.[66] Similar profiles have been described by the same authors for specific T lymphocyte cell subsets (namely NKT and MAIT T cells) in BAV patients with nondilated vs dilated aortas.[67] Since no longitudinal follow-up has been performed in these studies, they need to be extended in future investigations to assess the potential role of endothelial progenitor cells

or other circulating cells as early biomarkers of aortopathy, as suggested by the authors.

Conclusions: Moving Toward Individualized Risk Startification

The search for robust risk markers of BAV aortopathy is particularly lively and of critical importance given the limitations of aortic diameter. On the basis of the evidence summarized above, we suggest that beyond aortic diameter, an immediate subsequent level of risk stratification in BAV patients should be performed on the basis of the aortic phenotype: closer clinical follow-up and a more aggressive elective surgical timing appears necessary for the root phenotype form, as compared to the larger (and still prognostically heterogeneous) subpopulation of BAV patients with ascending phenotype aortic dilatation. Other important clinical risk factors to take into account in all BAV patients with aortopathy have been clearly outlined in current guidelines.[24] Thus, especially in the setting of the ascending phenotype, a combination of imaging-derived metrics of flow-related wall stress and circulating biomolecule-based risk markers have the potential of taking BAV aortopathy risk definition to a highly individualized level.

The current body of literature suggests the possibility of developing software tools to stratify the risk of aortopathy based on the noninvasive measurement of blood flow through 4D-flow sequences. Yet, some issues still represent a bottleneck in this process: a consensus on the WSS-related indices to be quantified and on the algorithms to be utilized is missing. Overcoming this limitation will be necessary to eventually provide robust criteria for risk stratification based on flow features.

Current research efforts into circulating biomarkers of BAV aortopathy is opening a multitude of opportunities for individualized risk factor discovery in BAV aortopathy. However, the large majority of studies were so far only exploratory, with limited-size patient cohorts, proposed biomarker molecules not having a pathogenetic justification, and lacked clinical validation in adequately long-term follow-up periods. Some studies suggest that a multimarker strategy could represent a valuable approach.

Well designed (eg in well-defined phenotypes) studies, including adequate control groups, with large patient numbers and long follow-up, of likely multicenter nature, are required to confirm the suitability of a precision medicine approach to stratification of the aortopathy-related risks in BAV patients.

REFERENCES

1. Michelena HI, Della Corte A, Prakash SK, et al. Bicuspid aortic valve aortopathy in adults: incidence, etiology, and clinical significance. *Int J Cardiol* 2015;201:400–7.
2. Michelena HI, Khanna AD, Mahoney D, et al. Incidence of aortic complications in patients with bicuspid aortic valves. *JAMA* 2011;306:1104–12.
3. Hardikar AA, Marwick TH. Surgical thresholds for bicuspid aortic valve associated aortopathy. *JACC Cardiovasc Imaging* 2013;6:1311–20.
4. Pape LA, Tsai TT, Isselbacher EM, et al. Aortic diameter >or = 5.5 cm is not a good predictor of type A aortic dissection: observations from the International Registry of Acute Aortic Dissection (IRAD). *Circulation* 2007;116:1120–7.
5. Rylski B, Blanke P, Beyersdorf F, et al. How does the ascending aorta geometry change when it dissects? *J Am Coll Cardiol* 2014;63:1311–9.
6. Erbel R, Aboyans V, Boileau C, et al. ESC Guidelines on the diagnosis and treatment of aortic diseases: document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). *Eur Heart J* 2014;35: 2873–926.
7. Fatehi Hassanabad A, Barker AJ, Guzzardi D, et al. Evolution of precision medicine and surgical strategies for bicuspid aortic valve-associated aortopathy. *Front Physiol* 2017;8:475.
8. Maredia AK, Greenway SC, Verma S, et al. Bicuspid aortic valve-associated aortopathy: update on biomarkers. *Curr Opin Cardiol* 2018;33:134–9.
9. Della Corte A, Body SC, Booher AM, et al. Surgical treatment of bicuspid aortic valve disease: knowledge gaps and research perspectives. *J Thorac Cardiovasc Surg* 2014;147:1749–57.
10. Della Corte A, Bancone C, Quarto C, et al. Predictors of ascending aortic dilatation with bicuspid aortic valve: a wide spectrum of disease expression. *Eur J Cardiothorac Surg* 2007;31:397–404.
11. Schaefer BM, Lewin MB, Stout KK, et al. The bicuspid aortic valve: an integrated phenotypic classification of leaflet morphology and aortic root shape. *Heart* 2008;94:1634–8.
12. Fazel SS, Mallidi HR, Lee RS, et al. The aortopathy of bicuspid aortic valve disease has distinctive patterns and usually involves the transverse aortic arch. *J Thorac Cardiovasc Surg* 2008;135:901–7.
13. Hardikar AA, Marwick TH. The natural history of guidelines: the case of aortopathy related to bicuspid aortic valves. *Int J Cardiol* 2015;199:150–3.
14. Verma S, Yanagawa B, Kalra S, et al. Knowledge, attitudes, and practice patterns in surgical management of bicuspid aortopathy: a survey of 100 cardiac surgeons. *J Thorac Cardiovasc Surg* 2013;146:1033–40.
15. Detaint D, Michelena HI, Nkomo VT, et al. Aortic dilatation patterns and rates in adults with bicuspid aortic valves: a comparative study with Marfan syndrome and degenerative aortopathy. *Heart* 2014;100:126–34.
16. Sievers HH, Sievers HL. Aortopathy in bicuspid aortic valve disease - genes or hemodynamics? or Scylla and Charybdis. *Eur J Cardiothorac Surg* 2011;39:803–4.

17. Della Corte A. Phenotypic heterogeneity of bicuspid aortopathy: a potential key to decode the prognosis? *Heart* 2011;100:96–7.
18. Kang JW, Song HG, Yang DH, et al. Association between bicuspid aortic valve phenotype and patterns of valvular dysfunction and bicuspid aortopathy: comprehensive evaluation using MDCT and echocardiography. *JACC Cardiovasc Imaging* 2013;6:150–61.
19. Della Corte A, Bancone C, Dialetto G, et al. The ascending aorta with bicuspid aortic valve: a phenotypic classification with potential prognostic significance. *Eur J Cardiothorac Surg* 2014;46:240–7.
20. Della Corte A, Bancone C, Buonocore M, et al. Pattern of ascending aortic dimensions predicts the growth rate of the aorta in patients with bicuspid aortic valve. *JACC Cardiovasc Imaging* 2013;6:1301–10.
21. Girdauskas E, Disha K, Raisin HH, et al. Risk of late aortic events after an isolated aortic valve replacement for bicuspid aortic valve stenosis with concomitant ascending aortic dilation. *Eur J Cardiothorac Surg* 2012;42:832–7.
22. Pepe G, Nistri S, Giusti B, et al. Identification of fibrillin 1 gene mutations in patients with bicuspid aortic valve (BAV) without Marfan syndrome. *BMC Med Genet* 2014;15:23.
23. Girdauskas E, Geist L, Disha K, et al. Genetic abnormalities in bicuspid aortic valve root phenotype: preliminary results. *Eur J Cardiothorac Surg* 2017;52:156–62.
24. Borger MA, Fedak PWM, Stephens EH, et al. The American Association for Thoracic Surgery consensus guidelines on bicuspid aortic valve-related aortopathy: full online-only version. *J Thorac Cardiovasc Surg* 2018;156:e41–74.
25. Burris NS, Hope MD. Bicuspid valve-related aortic disease: flow assessment with conventional phase-contrast MRI. *Acad Radiol* 2015;22:690–6.
26. Rodríguez-Palomares JF, Dux-Santoy L, Guala A, et al. Aortic flow patterns and wall shear stress maps by 4D-flow cardiovascular magnetic resonance in the assessment of aortic dilatation in bicuspid aortic valve disease. *J Cardiovasc Magn Reson* 2018;20:28.
27. Mahadevia R, Barker AJ, Schnell S, et al. Bicuspid aortic cusp fusion morphology alters aortic three-dimensional outflow patterns, wall shear stress, and expression of aortopathy. *Circulation* 2014;129:673–82.
28. Hope MD, Hope TA, Crook SE, et al. 4D flow CMR in assessment of valve-related ascending aortic disease. *JACC Cardiovasc Imaging* 2011;4:781–7.
29. Hope MD, Hope TA, Meadows AK, et al. Bicuspid aortic valve: four-dimensional MR evaluation of ascending aortic systolic flow patterns. *Radiology* 2010;255:53–61.
30. Bürk J, Blanke P, Stankovic Z, et al. Evaluation of 3D blood flow patterns and wall shear stress in the normal and dilated thoracic aorta using flow-sensitive 4D CMR. *J Cardiovasc Magn Reson* 2012;14:84.
31. Barker AJ, Markl M, Bürk J, et al. Bicuspid aortic valve is associated with altered wall shear stress in the ascending aorta. *Circ Cardiovasc Imaging* 2012;5:457–66.
32. Bissell MM, Hess AT, Biasiolli L, et al. Aortic dilation in bicuspid aortic valve disease: flow pattern is a major contributor and differs with valve fusion type. *Circ Cardiovasc Imaging* 2013;6:499–507.

33. Bissell MM, Loudon M, Hess AT, et al. Differential flow improvements after valve replacements in bicuspid aortic valve disease: a cardiovascular magnetic resonance assessment. *J Cardiovasc Magn Reson* 2018;20:10.
34. Bissell MM, Hess AT, Biasiolli L, et al. Response to letter regarding article, "Aortic dilation in bicuspid aortic valve disease: flow pattern is a major contributor and differs with valve fusion type". *Circ Cardiovasc Imaging* 2014;7:214.
35. Guzzardi DG, Barker AJ, van Ooij P, et al. Valve-related hemodynamics mediate human bicuspid aortopathy: insights from wall shear stress mapping. *J Am Coll Cardiol* 2015;25:892–900. 66.
36. Della Corte A, Quarto C, Bancone C, et al. Spatiotemporal patterns of smooth muscle cell changes in ascending aortic dilatation with bicuspid and tricuspid aortic valve stenosis: focus on cell-matrix signaling. *J Thorac Cardiovasc Surg* 2008;135:8–18.
37. Forte A, Della Corte A, Grossi M, et al. Early cell changes and TGFβ pathway alterations in the aortopathy associated with bicuspid aortic valve stenosis. *Clin Sci (Lond)* 2013;124:97–108.
38. Bollache E, Guzzardi DG, Sattari S, et al. Aortic valve-mediated wall shear stress is heterogeneous and predicts regional aortic elastic fiber thinning in bicuspid aortic valve-associated aortopathy. *J Thorac Cardiovasc Surg* 2018;156:2112–20.
39. Atkins SK, Cao K, Rajamannan NM, et al. Bicuspid aortic valve hemodynamics induces abnormal medial remodeling in the convexity of porcine ascending aortas. *Biomech Model Mechanobiol* 2014;13:1209–25.
40. Atkins SK, Moore AN, Sucosky P. Bicuspid aortic valve hemodynamics does not promote remodeling in porcine aortic wall concavity. *World J Cardiol* 2016;26:89–97. 8.
41. Allen BD, van Ooij P, Barker AJ, et al. Thoracic aorta 3D hemodynamics in pediatric and young adult patients with bicuspid aortic valve. *J Magn Reson Imaging* 2015;42:954–63.
42. Hope MD, Sigovan M, Wrenn SJ, et al. MRI hemodynamic markers of progressive bicuspid aortic valve-related aortic disease. *J Magn Reson Imaging* 2014;40:140–5.
43. van Ooij P, Potters WV, Collins J, et al. Characterization of abnormal wall shear stress using 4D flow MRI in human bicuspid aortopathy. *Ann Biomed Eng* 2015;43:1385–97.
44. Liu J, Shar JA, Sucosky P. Wall shear stress directional abnormalities in BAV aortas: toward a new hemodynamic predictor of aortopathy? *Front Physiol* 2018;9:993.
45. Piatti F, Sturla F, Bissell MM, et al. 4D flow analysis of BAV-related fluid-dynamic alterations: evidences of wall shear stress alterations in absence of clinically-relevant aortic anatomical remodeling. *Front Physiol* 2017;8:441.
46. Meierhofer C, Schneider EP, Lyko C, et al. Wall shear stress and flow patterns in the ascending aorta in patients with bicuspid aortic valves differ significantly from tricuspid aortic valves: a prospective study. *Eur Heart J Cardiovasc Imaging* 2013;14:797–804.
47. Rocchiccioli S, Cecchettini A, Panesi P, et al. Hypothesis-free secretome analysis of thoracic aortic aneurysm reinforces the central role of TGF-β cascade in patients with bicuspid aortic valve. *J Cardiol* 2017;69:570–6.
48. Kilickesmez KO, Abaci O, Kocas C, et al. Dilatation of the ascending aorta and serum alpha 1-antitrypsin level in patients with bicuspid aortic valve. *Heart Vessels* 2012;27:391–7.

49. Sarkar A, Prasad K, Ziganshin BA, et al. Reasons to investigate the soluble receptor for advanced glycation end-product (sRAGE) pathway in aortic disease. *Aorta (Stamford)* 2013;1:210–7.
50. Branchetti E, Bavaria JE, Grau JB, et al. Circulating soluble receptor for advanced glycation end product identifies patients with bicuspid aortic valve and associated aortopathies. *Arterioscler Thromb Vasc Biol* 2014;34:2349–57.
51. Drapisz S, Góralczyk T, Jamka-Miszalski T, et al. Nonstenotic bicuspid aortic valve is associated with elevated plasma asymmetric dimethylarginine. *J Cardiovasc Med (Hagerstown)* 2013;14:446–52.
52. Wheeler JB, Ikonomidis JS, Jones JA. Connective tissue disorders and cardiovascular complications: the indomitable role of transforming growth factor-beta signaling. *Adv Exp Med Biol* 2014;802:107–27.
53. Bertoli-Avella AM, Gillis E, Morisaki H, et al. Mutations in a TGF-β ligand, TGFB3, cause syndromic aortic aneurysms and dissections. *J Am Coll Cardiol* 2015;65:1324–36.
54. Takeda N, Hara H, Fujiwara T, et al. TGF-β signaling-related genes and thoracic aortic aneurysms and dissections. *Int J Mol Sci* 2018;19:E2125. pii.
55. Forte A, Bancone C, Cobellis G, et al. A possible early biomarker for bicuspid aortopathy: circulating transforming growth factor β-1 to soluble endoglin ratio. *Circ Res* 2017;120:1800–11.
56. Hillebrand M, Millot N, Sheikhzadeh S, et al. Total serum transforming growth factor-β1 is elevated in the entire spectrum of genetic aortic syndromes. *Clin Cardiol* 2014;37:672–9.
57. Albinsson S, Della Corte A, Alajbegovic A, et al. Patients with bicuspid and tricuspid aortic valve exhibit distinct regional microrna signatures in mildly dilated ascending aorta. *Heart Vessels* 2017;32:750–67.
58. Li Y, Maegdefessel L. Non-coding RNA contribution to thoracic and abdominal aortic aneurysm disease development and progression. *Front Physiol* 2017;8:429.
59. Wang S, Zhang X, Yuan Y, et al. BRG1 expression is increased in thoracic aortic aneurysms and regulates proliferation and apoptosis of vascular smooth muscle cells through the long non-coding RNA HIF1A-AS1 in vitro. *Eur J Cardiothorac Surg* 2015;47:439–46.
60. Guo X, Chang Q, Pei H, et al. Long non-coding RNA-mRNA correlation analysis reveals the potential role of HOTAIR in pathogenesis of sporadic thoracic aortic aneurysm. *Eur J Vasc Endovasc Surg* 2017;54:303–14.
61. Boon RA, Vickers KC. Intercellular transport of microRNAs. *Arterioscler Thromb Vasc Biol* 2013;33:186–92.
62. Martínez-Micaelo N, Beltrán-Debón R, Baiges I, et al. Specific circulating microRNA signature of bicuspid aortic valve disease. *J Transl Med* 2017;15:76.
63. Ikonomidis JS, Ivey CR, Wheeler JB, et al. Plasma biomarkers for distinguishing etiologic subtypes of thoracic aortic aneurysm disease. *J Thorac Cardiovasc Surg* 2013;145:1326–33.
64. Girdauskas E, Petersen J, Neumann N, et al. Evaluation of microribonucleic acids as potential biomarkers in the bicuspid aortic valve-associated aortopathy. *Interact Cardiovasc Thorac Surg* 2018;27:60–6.

65. Gallo A, Agnese V, Coronnello C, et al. On the prospect of serum exosomal miRNA profiling and protein biomarkers for the diagnosis of ascending aortic dilatation in patients with bicuspid and tricuspid aortic valve. *Int J Cardiol* 2018;273:230–6.
66. Balistreri CR, Crapanzano F, Schirone L, et al. Deregulation of Notch1 pathway and circulating endothelial progenitor cell (EPC) number in patients with bicuspid aortic valve with and without ascending aorta aneurysm. *Sci Rep* 2018;8:13834.
67. Balistreri CR, Buffa S, Allegra A, et al. A typical immune T/B subset profile characterizes bicuspid aortic valve: in an old status? *Oxid Med Cell Longev* 2018:5879281. 2018.