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Multiple biomarker panel to screen for severe aortic stenosis: results from the CASABLANCA study

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ABSTRACT

Objective Severe aortic valve stenosis (AS) develops via insidious processes and can be challenging to correctly diagnose. We sought to develop a circulating biomarker panel to identify patients with severe AS.

Methods We enrolled study participants undergoing coronary or peripheral angiography for a variety of cardiovascular diseases at a single academic medical centre. A panel of 109 proteins were measured in blood obtained at the time of the procedure. Statistical learning methods were used to identify biomarkers and clinical parameters that associate with severe AS. A diagnostic model incorporating clinical and biomarker results was developed and evaluated using Monte Carlo cross-validation.

Results Of 1244 subjects (age 66.4±11.5 years, 28.7% female), 80 (6.4%) had severe AS (defined as aortic valve area (AVA) <1.0 cm^2). A final model included age, N-terminal pro-B-type natriuretic peptide, von Willebrand factor and fetuin-A. The model had good discrimination for severe AS (OR=5.9, 95% CI 3.5 to 10.1, p<0.001) with an area under the curve of 0.76 insample and 0.74 with cross-validation. A diagnostic score was generated. Higher prevalence of severe AS was noted in those with higher scores, such that 1.6% of those with a score of 1 had severe AS compared with 15.3% with a score of 5 (p<0.001), and score values were inversely correlated with AVA (r=−0.35; p<0.001). At optimal model cut-off, we found 76% sensitivity, 65% specificity, 13% positive predictive value and 98% negative predictive value.

Conclusions We describe a novel, multiple biomarker approach for diagnostic evaluation of severe AS.

Trial registration number NCT00842868.

Key questions

What is already known about this subject?

► Severe aortic stenosis develops insidiously and is associated with markedly increased morbidity and mortality when not recognised and treated in a timely manner.

What does this study add?

► We developed and evaluated a model including age and three proteins, N-terminal pro-B-type natriuretic peptide, von Willebrand factor and fetuin-A, which identified patients with severe aortic stenosis.

How might this impact on clinical practice?

► A model composed of age and three circulating biomarkers may supplement currently available diagnostic methods to facilitate the diagnosis of severe aortic valve stenosis and may serve as the basis for population screening algorithms.

INTRODUCTION

Calcific aortic stenosis (AS) is the most common cause of valvular heart disease in the Western world, present in >20% of older adults.[1] As the proportion of elderly Americans grows, so too will the prevalence and socioeconomic burden of AS. AS progresses via an indolent process with symptoms developing at a late stage of the disease. Once symptoms occur, AS portends a dismal prognosis unless the aortic valve is replaced; without aortic valve replacement (AVR), only half of patients will survive 1 year.[2]

Current clinical practice guidelines recommend deferring AVR until AS severity reaches the severe stage and until onset of clinical symptoms or the occurrence of overt left ventricular (LV) systolic dysfunction.[3] Classic teaching suggests that survival normalises after AVR; however, evidence is mounting that survival, symptom improvement and quality of life are diminished when valve replacement is performed late. With progressive valve narrowing, compensatory LV remodelling mechanisms fail and become maladaptive, in turn resulting in irreversible myocardial injury and fibrosis. For example, we have demonstrated that the absence of LV ejection fraction (LVEF) improvement within 1 month after transcatheter AVR is associated with a tripling of the risk of 1-year all-cause mortality and fivefold increase in 1-year cardiac death,[4] and persistent LV hypertrophy is similarly associated with increased mortality.[5] Myocardial fibrosis, a late sequela

of AS, has also been associated with markedly increased risk of mortality, regardless of whether the stenotic aortic valve is replaced.[6]

Early recognition of severe AS is important for maximising health and survival after valve replacement. While clinical history is important, symptoms caused by severe AS are often incorrectly attributed to comorbid conditions or simply to advanced age, delaying appropriate diagnosis and therapy. Physical examination may be useful to identify AS and grade its severity, yet skills in such evaluation may be limited in clinical practice.[7] Echocardiography is the gold-standard diagnostic test for the diagnosis and grading of AS severity, yet several inconsistencies with the echocardiographic assessment of AS exist that may hinder appropriate clinical management decisions. Echocardiography is also time-consuming, potentially costly and requires specialised interpretation.

Together, the aforementioned observations highlight the detrimental impact of delayed intervention for severe AS and highlight the need for clinical tools to assist in the rapid identification of severe AS. We therefore sought to develop a biomarker panel to identify patients with severe AS within the Catheter Sampled Blood Archive in Cardiovascular Diseases (CASABLANCA) study. Such a blood-based biomarker panel may serve as a convenient and low-cost method of screening for severe AS.

METHODS

The design of the CASABLANCA study has been described previously (ClinicalTrials.gov NCT00842868).[8 9] Briefly, the CASABLANCA study was a prospective, single-centre, investigator-initiated, observational cohort study performed at the Massachusetts General Hospital to investigate novel biomarkers in subjects who underwent coronary and peripheral angiography with or without intervention between 2008 and 2011. Patients were referred for these procedures for several reasons, including angiography for acute coronary syndromes, stable angina, heart failure, abnormal cardiac functional test, peripheral arterial disease, and preoperatively before heart valve surgery or transcatheter intervention.

All subjects provided informed consent.

Data acquisition

Detailed clinical and historical variables and reason for referral for angiography were recorded from medical record review and subject interviews at the time of the procedure. Echocardiographic data from studies performed within 6 months of enrolment were collected retrospectively. Transthoracic echocardiographic images were obtained using standard views for routine clinical care. Recorded echocardiographic parameters include measures of AS severity (valve area, transvalvular gradients), LV chamber dimensions, hypertrophic remodelling (absolute and relative wall thickness and LV mass), systolic and diastolic function, and other valvular abnormalities. Measures of AS severity were available in patients with at least moderate AS. All measurements were performed as outlined by the American Society of Echocardiography.[10]

Biomarker testing

Blood (15 mL) was collected immediately before angiography and after the completion of angiographic procedure(s) through a centrally placed vascular access sheath. Samples were immediately centrifuged for 15 min, and plasma samples were then aliquoted on ice and frozen in a −80°C refrigerator until analysis. After a single freeze–thaw cycle, 200 µL of plasma was analysed using the Luminex 100/200 xMAP technology platform (Luminex, Austin, Texas), which uses multiplexed, microsphere-based assays in a single reaction vessel. Multiplexing was accomplished by assigning each protein-specific assay a microsphere set labelled with a unique fluorescence signature. An assay-specific capture antibody was conjugated covalently to each unique set of microspheres and bound to the protein of interest. Assay-specific, biotinylated detecting antibodies were added, followed by a streptavidin-labelled fluorescent 'reporter' molecule. We specifically assayed 109 proteins (online supplementary table 1) using a commercially available kit, known as the Myriad RBM MAP. The panel incorporates biomarkers that reflect a wide variety of pathways associated with plaque rupture/erosion and includes acute phase reactants, inflammatory markers and biomarkers of atherosclerosis.

Statistical analyses

For the purposes of this analysis, subjects were considered to have severe AS if a transthoracic echocardiogram within 6 months of enrolment demonstrated severe AS, defined by an aortic valve area (AVA) <1.0 cm^2. Since AVA is consistently measured in patients with AS, patients missing AVA measurements were assumed to be negative. Baseline characteristics in those with and without severe AS were compared using Fisher's exact test for categorical variables, two-sided two-sample t-test for normally distributed continuous variables, and Wilcoxon rank-sum test for continuous variables that were not normally distributed. Protein concentrations were log-transformed to achieve a normal distribution, and to facilitate the analysis they were rescaled to a zero-mean, unit-variance distribution, and outliers (ie, values beyond 3× median absolute deviation) were Winsorised. The case was conducted as a complete case analysis, and one patient was excluded from the analysis for missing data.

Candidate panels of proteins and clinical features were generated via least-angle regression (LARS).[8] In this method, factors were included in the model one at a time, with their coefficients determined by their correlation with the outcome. This was repeated until all factors were included in the model, and the step at which the performance plateaued resulted in our initial panel of interest. With this panel of interest, predictive analyses were run on the training set using least absolute

Table 1 Baseline patient characteristics

Patient characteristics	With severe AS (n=80)	Without severe AS (n=1164)	P values
Age, years	73.9±10.6	65.9±11.4	<0.001
Male gender	55/80 (68.8%)	831/1164 (71.4%)	0.61
Caucasian	79/80 (98.8%)	1083/1164 (93.0%)	0.06
Heart rate, beats/min	73±14	69±13	0.01
Systolic BP, mm Hg	137±21	138±23	0.75
Diastolic BP, mm Hg	71±10	73±12	0.16
Smoker	6/80 (7.5%)	172/1151 (14.9%)	0.07
Atrial fibrillation/flutter	23/80 (28.8%)	210/1164 (18.0%)	0.03
Hypertension	63/80 (78.8%)	877/1164 (75.3%)	0.59
Coronary artery disease	35/80 (43.8%)	621/1164 (53.4%)	0.11
Prior MI	12/80 (15.0%)	280/1164 (24.1%)	0.08
Heart failure	22/80 (27.5%)	233/1164 (20.0%)	0.12
Peripheral artery disease	21/80 (26.3%)	306/1164 (26.3%)	>0.99
COPD	16/80 (20.0%)	203/1163 (17.5%)	0.55
Diabetes mellitus	25/80 (31.3%)	322/1164 (27.7%)	0.52
CVA/TIA	8/80 (10.0%)	129/1164 (11.1%)	>0.99
CKD	16/80 (20.0%)	151/1164 (13.0%)	0.09
Renal replacement therapy	2/80 (2.5%)	34/1161 (2.9%)	>0.99
Prior angioplasty	6/80 (7.5%)	157/1164 (13.5%)	0.17
Prior stent	15/80 (18.8%)	373/1164 (32.0%)	0.01
Prior CABG	19/80 (23.8%)	251/1164 (21.6%)	0.67
Medication use			
ACE-I/ARB	41/78 (52.6%)	641/1161 (55.2%)	0.72
Beta-blocker	41/79 (51.9%)	836/1161 (71.0%)	<0.001
Aldosterone antagonist	5/79 (6.3%)	50/1161 (4.3%)	0.39
Loop diuretics	25/79 (31.7%)	239/1161 (20.6%)	0.03
Nitrates	9/79 (11.4%)	230/1160 (19.8%)	0.08
CCB	27/79 (34.2%)	288/1161 (24.8%)	0.08
Statin	59/79 (74.7%)	852/1160 (73. 5%)	0.90
Aspirin	52/79 (65.8%)	911/1160 (78.5%)	0.01
Warfarin	15/79 (19.0%)	175/1160 (15.1%)	0.34
Clopidogrel	10/79 (12.7%)	291/1160 (25.1%)	0.01
Echocardiographic parameters			
LVEF, %	57.1±15.7	56.4±15.2	0.71
RSVP, mm Hg	42.1±9.6	41.4±11.9	0.66
Biochemical parameters			
Creatinine, mg/dL	1.0 (0.9–1.4)	1.09 (0.90–1.33)	0.97
eGFR*, mL/min/1.73 m^2	91.7 (67. 3–107.0)	98.8 (72.7–110.2)	0.06
Total cholesterol, mg/dL	147.5±50.3	149.9±41.8	0.76
LDL cholesterol, mg/dL	83.0±40.0	81.4±33.4	0.8
Glycosylated haemoglobin, %	5.7 (5.4–6.5)	6.2 (5.7–7.1)	0.005
Glucose, mg/dL	101 (93–109)	102 (92–122)	0.48
Haemoglobin, g/dL	13.2±1.9	13.2±1.7	0.88
Biomarkers			

Continued

Table 1 Continued

Patient characteristics	With severe AS (n=80)	Without severe AS (n=1164)	P values
NT-proBNP, pg/mL	4095.0 (1692.5–15 975.0)	1385.0 (523.8–3732.5)	<0.001
von Willebrand factor, µg/mL	124.0 (91.3–175.0)	130.0 (95.0–179.0)	0.40
Fetuin-A, µg/mL	620.5 (512.3–787.3)	700.0 (585.0–832.3)	0.002

Values are mean±SD, median (IQR) or n/N (%).
*Calculated using the Chronic Kidney Disease-Epidemiology Collaboration formula.
ACE-I, ACE inhibitor; ARB, angiotensin receptor blocker; AS, aortic stenosis; BP, blood pressure; CABG, coronary artery bypass graft surgery; CCB, calcium channel blocker; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CVA/TIA, cerebrovascular accident/transient ischaemic attack; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RVSP, right ventricular systolic pressure.

shrinkage and selection operator (LASSO) with logistic regression, predicting the outcome of severe AS using only the variables in the panel of interest. This model development process was done via Monte Carlo cross-validation, using 400 iterations with an 80:20 (training:test) split. The final panel was used to create a final model with the entire sample, and this model was then evaluated to predict severe AS. Discrimination of the final model was assessed by calculating the area under the receiver operating curve (AUC), and calibration was determined using Akaike or Bayesian information criteria (AIC and BIC, respectively) and the Hosmer-Lemeshow goodness of fit test. The score of the final model was grouped into quintiles to create a five-level clinical risk score. All statistics were performed using R V.3.3 software (R Foundation for Statistical Computing, Vienna, Austria); p values are two-sided, with a value <0.05 considered significant.

RESULTS

Patient population

We enrolled 1251 subjects who underwent coronary and peripheral angiography with and without intervention between 2008 and 2011. Overall, 981 subjects underwent coronary angiography, 155 peripheral angiography, and 115 both coronary and peripheral angiography. Of these, 1244 had available blood samples and clinical data for analysis. Subjects were on average 66±11 years old and 28.8% (358 of 1244) were female.

Table 1 details the characteristics of those subjects with (n=80; 6.4%) and without severe AS; of those without severe AS, moderate stenosis was present in 2.0% (26 of 1244). Sex and many comorbid conditions were similar between those with and without severe AS. However, compared with those without, subjects with severe AS were older (74±11 vs 66±11 years) and more often afflicted with atrial fibrillation/flutter (28.8 vs 18.0%; p=0.03). Patients with severe AS were less likely than those without severe AS to have undergone a prior stent (18.8 vs 32.0%; p=0.01) and were less often receiving a beta-blocker (51.9 vs 72.0%; p<0.001) or antiplatelet agent (aspirin: 65.8 vs 78.5%; p=0.01; clopidogrel: 12.7 vs 25.1%; p=0.01). Loop diuretics were more often prescribed with severe AS (31.7 vs 20.6%; p=0.03).

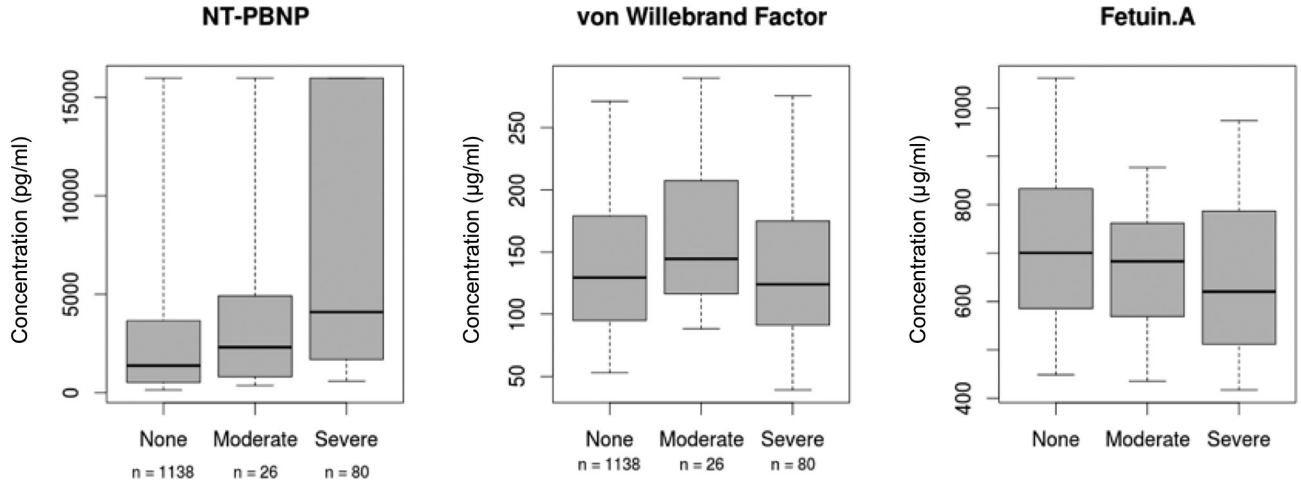

Figure 1 Distribution of NT-proBNP, von Willebrand factor and fetuin-A in patients with and without aortic stenosis. Levels of circulating biomarkers are shown in patients without significant AS, moderate AS and severe AS. Dark lines indicate median values and grey bar the IQR. Error bars indicate the range of the rest of the distribution to 1.5 times the IQR. AS, aortic stenosis; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Table 2 Model fit characteristics

Model	AUC	AIC	BIC	Hosmer-Lemeshow p values
Model 1: age	0.70	558.4	568.6	0.18
Model 2: age+NT-proBNP	0.74	540.5	555.9	0.94
Model 3: age+NT-proBNP+vWF	0.75	530.0	550.5	0.06
Model 4: age+NT-proBNP+vWF+fetuin-A	0.76	530.4	556.0	0.44

AIC, Akaike information criterion; AUC, area under the curve; BIC, Bayesian information criterion; NT-proBNP, N-terminal pro-B-type natriuretic peptide; vWF, von Willebrand factor.

Model for identification of severe AS

LARS was used to identify independent predictors of severe AS. These factors included age and concentrations of three biomarkers: N-terminal pro-B-type natriuretic peptide (NT-proBNP), von Willebrand factor (vWF) and fetuin-A. Concentrations of NT-proBNP demonstrated direct correlation with AS, with a progressive increase in circulating NT-proBNP concentrations with worsening AS severity (figure 1; p<0.001, Kruskal-Wallis rank-sum test), while concentrations of circulating fetuin-A progressively decreased with worse AS (p=0.003). vWF concentrations did not clearly correlate with AS severity (p=0.19), although the direction of vWF's effect in the final model was negative, with lower concentrations associated with severe AS. Using Monte Carlo cross-validation, this panel achieved a cross-validated AUC of 0.74.

A final combined model of age, NT-proBNP, vWF and fetuin-A was created using the entire population with LASSO with logistic regression. This model was strongly predictive of severe AS (OR=5.9, 95% CI 3.5 to 10.1, p<0.001). To examine the model's performance and the relative contribution of each parameter, we assessed discrimination, calibration and Hosmer-Lemeshow goodness of fit for identification of severe AS. Addition of biomarkers to age resulted in improved AUC of the receiver operating characteristic curve, minimisation of AIC and BIC, and with non-significant Hosmer-Lemeshow p values (table 2). This final model demonstrated discrimination with an insample AUC of 0.76 (p<0.001).

We examined the distribution of the final model's diagnostic score for each patient with their status for severe AS. A clinical risk score was derived from the score generated by the model, with the five quintiles of the score corresponding to a five-level risk score. Higher prevalence of severe AS was noted in those with higher scores (figure 2), such that 1.6% of those with a risk score of 1 (lowest risk) had severe AS compared with 15.3% with a risk score of 5 (highest risk) (p<0.001). When modelled as a continuous measure, higher AS scores were directly correlated with the proportion of subjects with severe AS (p<0.001) and inversely correlated with AVA (r=−0.35; p<0.001).

Receiver operator characteristic analyses were performed and operating characteristics calculated (figure 3). Using a score cut-off determined by the optimal Youden's Index, we found 76% sensitivity, 65% specificity, 13% positive predictive value and 98% negative predictive value. Likelihood ratio for positive results (LR+) was 2.17 and 0.37 for negative results (LR−).

We evaluated the levels of circulating biomarkers and the AS score within subtypes of severe AS (table 3). We found marked differences in the levels of NT-proBNP across AS subtype, with the highest levels noted in patients with low LVEF, low aortic valve gradient (AVG) AS, and the lowest levels in those patients with AS with preserved LVEF and low AVG (p<0.001). A similar although non-significant pattern of abundance was noted for circulating vWF (p=0.055). Fetuin-A was highest in the preserved LVEF and low AVG group and lowest in those with preserved LVEF and high AVG (p=0.01). The AS risk score did not differ between AS subtypes.

DISCUSSION

The identification of AS may be challenging to recognise and grade. Furthermore, broad application of tools to assess presence or severity of AS such as echocardiography—although useful—may not be practical in some populations. As a consequence, a substantial proportion of valvular heart disease is not diagnosed within the

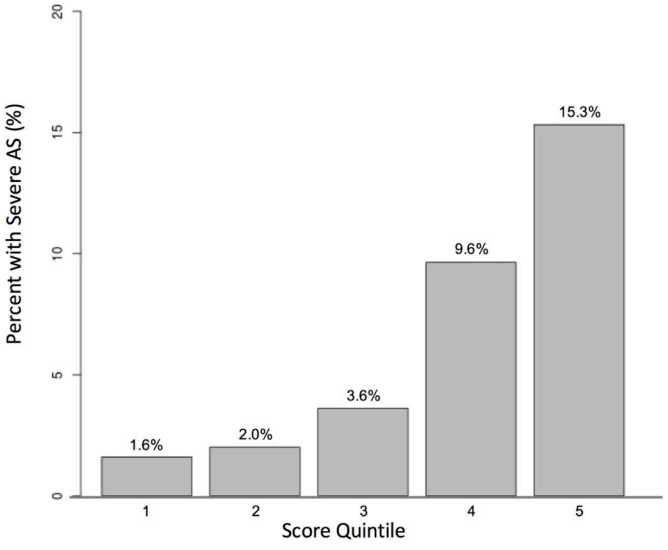

Figure 2 Prevalence of severe aortic stenosis (AS) within a quintile-based five-level diagnostic score. Increasing prevalence of severe AS is noted at higher scores, such that 1.6% of those with a score of 1 (lowest risk) had severe AS compared with 15.3% with a score of 5 (highest risk) (p<0.001).

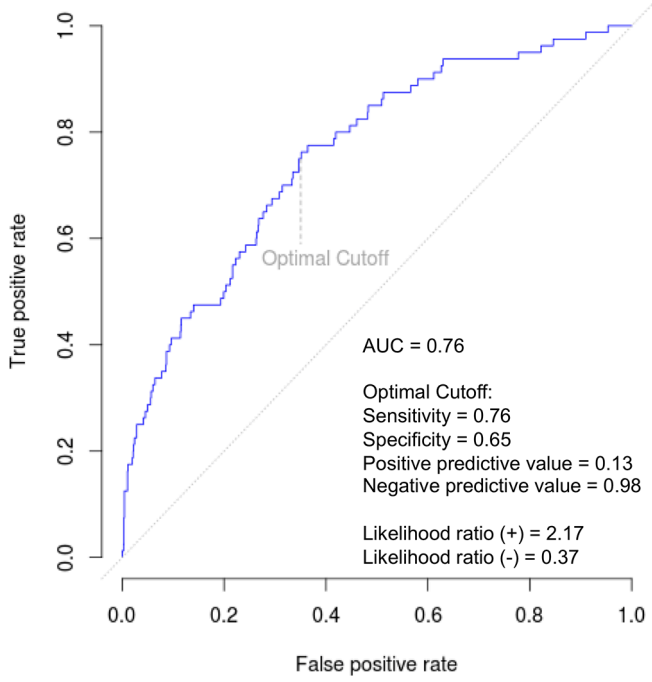

Figure 3 Receiver operating characteristic curve for aortic stenosis (AS) score. The AS score demonstrated good discrimination with an insample area under the curve (AUC) of 0.76. The sensitivity, specificity, positive predictive value and negative predictive value at the optimal cut-off are depicted.

general population.[11] Availability of a non-invasive, easily interpreted and cost-effective tool to screen for the presence of AS is therefore of clinical value. Using patients undergoing coronary and peripheral angiography within the CASABLANCA study, we developed and evaluated a robust, non-invasive, clinical plus multimarker approach for the identification of severe AS. This model, which includes age and concentrations of NT-proBNP, vWF and fetuin-A, was adapted into a clinically applicable score, which performed well within the CASABLANCA study cohort with strong sensitivity, specificity and negative predictive value. The score may therefore add a novel tool to our clinical armamentarium for the identification of patients with severe AS, in particular for excluding its presence in a patient who might otherwise be suspected as suffering from the diagnosis.

AS is an indolent disease that culminates in clinical decompensation and necessitates valve replacement, without which 1-year mortality is exceedingly high.[2] In fact, mortality due to untreated symptomatic severe AS exceeds that associated with several advanced cancer diagnoses. While survival is thought to improve on valve replacement, recent evidence suggests persistently elevated morbidity and mortality risk in patients treated at a late disease state. Adverse outcomes are in part driven by irreversible maladaptive LV remodelling and fibrosis.[4–6] In addition, frailty, sarcopaenia and poor functional status worsen with time and contribute to diminished survival and recovery after AVR.[12 13] Timely identification of severe AS is therefore of critical importance to the maintenance of health in the vulnerable elderly population.

A detailed physical examination with cardiac auscultation is often relied on to screen for valve lesions, including severe AS. When cardiac auscultation reveals an abnormal heart murmur, an echocardiogram is often performed to confirm or exclude a pathological cardiac lesion.[3] Unfortunately, limited proficiency with cardiac auscultation among practitioners contributes to the underdiagnosis of AS.[14 15] Furthermore, even with comprehensive echocardiography, the gold-standard diagnostic tool, accurate identification and grading of AS severity often prove challenging due to poor imaging windows, inaccurate quantification of LV outflow tract dimensions and discordant metrics of AS severity (ie, AVA, mean gradient and peak velocity).[16 17] Inconsistencies regarding the evaluation of AS severity occur due to measurement error and due to the disruption of the physiological relationship between AV area and gradient that occurs as a consequence of diminished transvalvular flow.[3 18 19] Together, these factors contribute to the frequent late recognition of severe AS and highlight the need for novel objective metrics that will supplement current diagnostic strategies and aid in the timely recognition of at-risk patients.

While biomarkers may be of prognostic value in those with AS, our data add significant understanding regarding the role of biomarkers for the diagnosis of AS. Concentrations of circulating natriuretic peptides rise with AS disease severity due to cardiomyocyte stretch and pressure overload and have been shown to predict survival

Table 3 Biomarkers in subtypes of aortic stenosis

Biomarker	Mean AVG ≥40 mm Hg (n=46)	LVEF <50% and mean AVG <40 mm Hg (n=10)	LVEF ≥50% and mean AVG <40 mm Hg (n=17)	P values
NT-proBNP	4920 (1677.5–15 975)	15 9755 (11 493.75–15 975)	2080 (898–3410)	<0.001
vWF	127 (92.5–171.75)	185.5 (145.5–262)	117 (80–172)	0.055
Fetuin-A	594.5 (499.25–667)	603 (501.75–855.25)	781 (656–872)	0.01
AS risk score	6.24±1.58	6.46±1.21	5.36±1.29	0.08

Values are mean±SD, median (IQR).
AS, aortic stenosis; AVG, aortic valve gradient; LVEF, ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; vWF, von Willebrand factor.

and clinical outcomes in patients with AS and after transcatheter and surgical AVR.[20–22] Clinical practice guidelines articulate a possible role for elevated natriuretic peptide concentrations to inform surgical intervention in asymptomatic patients[23]; reliance on NT-proBNP has not been universally endorsed, however.[3] For example, while NT-proBNP may be elevated in those with AS, LV hypertrophy due to AS may normalise wall stress and therefore attenuate the anticipated rise in the biomarker. Because there is also considerable overlap in natriuretic peptide concentrations across elderly patients with and without AS,[24] a strategy of integrating NT-proBNP with orthogonal biomarkers may inform clinical practice better than NT-proBNP alone.

Several studies have demonstrated abnormalities in vWF high-molecular-weight multimers in subjects with severe AS.[25 26] As blood flows through a severely stenotic valve, shear forces unfold these multimers, resulting in increased susceptibility to proteolytic cleavage.[25] The resultant haematological deficiencies form the clinical basis for the acquired type 2A von Willebrand syndrome and gastrointestinal angiodysplasia (Heyde's syndrome), often noted with severe AS,[25] which may resolve with alleviation of AVG.[26] Association between circulating vWF concentrations in those with and without severe AS were less direct than NT-proBNP; however, vWF concentrations were selected by LARS during the model development process, and their presence improved calibration of the AS model, supporting the adoption of this biomarker into a multimarker approach for AS discrimination.

Fetuin-A is a liver-derived protein that binds and solubilises calcium and phosphate and in turn regulates soft tissue mineralisation. Reduced circulating fetuin-A concentrations have been implicated as a mediator of calcific cardiovascular disease seen in patients with end-stage renal disease and valve disease.[27] As shown here, a recent analysis suggested that patients with AS have lower circulating fetuin-A than control subjects.[28] Much as with NT-proBNP and vWF, fetuin-A concentrations were selected by LARS to remain in the final aggregate multiple biomarker approach for AS diagnosis, and the presence of this protein in the model improved model discrimination while maintaining equivalent calibration.

While the size of the CASABLANCA cohort, the large number of proteins assayed, and the unbiased and methodical statistical approach are strengths of the current study, several limitations warrant attention. First, the CASABLANCA study enrolled subjects undergoing invasive cardiovascular angiography, irrespective of the clinical indication. The cohort is therefore diverse with low prevalence of severe AS. We anticipate that the clinical application of the AS score in select patients with higher pretest probability of having severe AS may result in improved model performance. Second, the AS score possessed low positive predictive value, a consequence of the low prevalence of severe AS within our population. Such performance characteristics are commonly noted for screening tests applied to the general population.[29] Third, subjects with severe AS undergoing angiography were largely symptomatic patients being evaluated for AVR. We were consequently not able to examine the interplay between the AS score and clinical symptomatology. Fourth, the relatively small number of AS cases limited the statistical power of our attempts to evaluate the value of the AS score in discriminating the degree of AS severity or AS subtype (preserved LVEF high gradient vs paradoxical preserved LVEF low gradient vs low LVEF low gradient AS). Future efforts will seek to externally validate the AS score with larger patient cohorts and to determine the value of the AS score in aiding the grading of AS severity.

In conclusion, we have developed a non-invasive, biomarker-supported strategy for screening for severe AS. This approach includes age and three protein biomarkers indicative of distinct biological pathways previously implicated in the pathophysiology of AS. The model we describe could theoretically be a cost-effective addition to the clinical armamentarium for evaluating those with suspected AS, facilitating the identification of this high-risk patient cohort and fostering timely valve replacement. Further efforts are needed to examine the utility of incorporating the predictive model into clinical practice.

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