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Impact of trans-cutaneous aortic valve implantation on the speed of Acquired von Willebrand Syndrome correction in patients suffering from Aortic Stenosis.

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Introduction

Trans-Aortic Valve Implantation

Aortic Stenosis is the most common valvular disease in developed countries, affecting 2 to 7% of patients over 65 and up to 3% of patients over 75 [1]. Most recent european guidelines define it as an aortic orifice area under 1 cm^2 or under 0,6 cm^2/m^2. The increased myocardial load most often leads to congestive heart failure. It is also associated with a higher incidence of cardio-vascular morbidity and mortality [2].

If left untreated, symptomatic AS often implied a high short term mortality rate [3]. Up until recently, only aortic valve replacement offered a definite solution to this disease [4]. However, observational studies have helped identify sub-groups of patients at increased risk and sometimes even unacceptably high risk of surgery related morbidity and mortality.

Although aortic balloon valvuloplasty had initially wielded high hopes for an alternative to surgery, disappointing short term results had relegated it to a second hand solution, offered only as a bridge to surgery or to inoperable patients [4].

The emergence of a functional percutaneously implantable valvular prothesis in the late 1990's, thought-up by a French cardiologist, Alain Cribier, at first wrought skepticism from the cardiology community [5].

However, after some initial studies yielding promising results [6], recent years have seen a rapid development of the technique dubbed Trans-Aortic Valve Implantation (TAVI), crowned by the end of its first decade of life, with the encouraging success of the PARTNER A and B studies [7, 8].

HEYDE'S SYNDROME

In addition to its cardio-vascular consequences, aortic stenosis has also been linked to an increased number of gastro-intestinal bleeding cases. This association was first noted by Edward Heyde, specialized in internal medicine, who called attention to it through a letter to the New England Journal of Medicine in 1958 and has since been named Heyde's syndrome [9]. The bleeding was later linked to colic angiodysplasia in patients with AS [10].

Also some controversy at first surrounded the very existence of Heyde's syndrome, it essentially ended in 1987 when King et al showed that aortic valve replacement successfully cured the GI bleeding in over 93% of cases whereas direct gastro-intestinal intervention only succeeded in 5% of cases [11].

Further large scale retrospective studies confirmed the association, concomitantly, however, pointing out the infrequent nature of symptomatic angiodysplasia-linked GI bleeding as an AS complication [12].

ACQUIRED VON WILLEBRAND SYNDROME

Heyde's syndrome is at least in part explained by hemostatic abnormalities associated with aortic stenosis. The most clearly demonstrated culprit is Acquired von Willebrand Syndrome (AVWS) type 2A-like, due to loss of high molecular weight multimers (HMWM) of von Willebrand Factor (vWF) [13].

VFW is a string-shaped multimeric plasma glycoprotein composed of 500 kDa dimer "beads" and weighing between 500 and 20 000 kDa. Synthesized in endothelial cells and megakaryocytes, it plays an essential role in platelet-subendothelium adhesion as well as in platelet-to-platelet interaction. Bundled up into a spherical shape in laminar blood flow, high shear forces secondary to vessel wall injury causes it to uncoil, revealing the fixation sites to exposed sub-endothelial collagen

and to the platelet receptor glycoprotein (GP) Ibα/IX and also to it's specific protease the ADAMTS-13 (A Disintegrin And Metalloproteinase with ThromboSpondin).

AVWS is characterized by loss of these HMWM. Although an increased clearance of unknown mechanism of these multimers was first hypothesized as the cause, one study demonstrated the problem lay in augmented proteolysis [14].

HMWM are downgraded into lower weight multimers through cleavage of a metalloprotease sensitive amino acid bond (between Tyr842 and Met843) exposed only in the multimers' elongated and therefore active conformation. This metalloprotease, known as ADAMTS-13 therefore serves to regulate vWF activity by reducing the number of active forms [15]. Thus mechanical forces play in the regulation of primary hemostasis in blood clotting and this mechanoenzymatic regulated cleavage is a newly described regulation of an enzymatic activity [16].

Hence, situations of increased shear stress, revealing the ADAMTS 13 sensitive bond, can lead to the loss of these straightened out HMWM and to the development of AVWS type 2A-like. Shear stress and its hematological consequence on HMWM loss make up the missing link of Heyde's Syndrome.

This also explains the moderate bleeding risk-increase and its tendency to manifest in angiodysplasias. These microvascular malformations, themselves sources of increased shear stress due to fast circulating blood flow and small vessel diameters, favor vWF multimer consumption. Therefore only the largest forms of HMWM can offer a sufficient source of local hemostasis and protection from GI bleeding, the very forms that we now know are lacking in AS [10].

Pareti's work also showed that reduced platelet count accompanied the reduction of the HMW multimers in AS, inferring that the increased shear stress-induced conformation changes of the vWF, in addition to the aforementioned enhanced proteolysis, may promote vWF A1-domain binding to platelet glycoprotein Ibα/IX, transiently responsible for platelet aggregation related con-

sumption [14]. Recently another group challenged this hypothesis of proteolysis suggesting that multimeric profile of cardiovascular associated AVWS is different than those from 2A patients and is not in favor of proteolysis [17].

In 2003, we confirmed this association in AS and went as far as mapping the correlation between HMWM deficiency and the echocardiagraphic translation of shear stress : trans-aortic gradient [18]. Patients with severe AS had significantly lower HMWM than control patients. This resulted in altered vWF-mediated primary hemostasis due to lowered sub-endothelial collagen and platelet interacting functions as shown from a lower ratio of collagen-binding activity to antigen (vWF:CB/Ag) and a longer closure time on the platelet-function analyzer (PFA 100). Plotting mean aortic transvalvular gradient and HMWM percentage, they also demonstrated an almost linear impact of the former on the later.

We later quantified a similar relation between left ventricular outflow tract (LVOT) velocity and AVWS severity, showing the same deficiencies in HMWM percentage, vWF:CB/Ag, and PFA 100 (ADP and epinephrine) than in AS and even identifying cutoff points in gradients in the specific setting of obstructive hypertrophic cardiomyopathy (OHCM) [19].

These same studies also showed that even partial rectification of the abnormal shear stress translated into correction of the AVWS. Surgical aortic valve replacement led to a normalization of the patients' hematological profile, except in cases of mismatch in which the trans-aortic gradient remained increased. Reduction in LVOT velocity through optimal management of OHCM patients also led to increased HMW multimers and vWF:CB/Ag.

Reduced metalloprotease cleavage once the abnormal increase in shear stress has subsided therefore translates into higher levels of HMWM and normalized vWF activity.

WHERE WE STAND

However, the speed in which the biological profile reverts to a normal state remains unknown.

Insofar as we know, no one has of yet performed focused studies on the speed of AVWS correction after treatment of the source of increase shear stress, be it AS or another cause.

When demonstrating reversal of the HMWM deficiency, our own previous work (demonstrating reversal in all but one patient) [18] and that of others such as Panzer et al [20] made use of an arbitrary six months waiting period before comparative hemostasis assessments.

It seemed logical to respect a safety "washout" period during which post-operative hemostatic imbalances would be expected especially due to cardiopulmonary bypass consequences.. Surgery is a commonly known source of inflammation and therefore hemostasis activation. More specifically, AS correction through aortic valve replacement requires the use of extra-corporeal life support (ECLS). Not only does the circulation of blood through the artificial circuit promote inflammation and hemostatic activation despite the existence of modern day biomaterials, but ECLS itself has been proven a source of increased AVWS-inducing shear stress [21].

We recently developed an experimental model using banding of rabbit aortas in a beating-heart setting, however, inferring that AVWS may be rapidly reversed. In this model, aortic stenosis using an inflatable device led to HMWM deficiency within minutes of balloon inflation through the afore-mentioned shear stress mechanism. Interestingly, a return to unimpeded blood flow conditions (i.e. lower shear stress) measurably improved the biological profile of the animals within minutes of balloon deflation and total reversal of the induced VWF anomalies after 30 minutes [22].

However, recent years have seen the development of trans-aortic valve replacement (TAVI) and this fast evolving, previously described (see introduction) technique allows for a percutaneous treatment of AS relieving us from the ECLS "black box" effect. Deployment of the prothesis

within the diseased valve almost instantaneously lifts the outflow obstruction, leading to immediate reduction of trans-aortic gradient and subsequently the abnormal shear stress.

The purpose of this study was to ascertain that TAVI would correct the loss of HMWM of vWF and therefore AVWS and to determine the speed at which this correction took place.

Methods

PATIENTS

Between December 2011 and April 2012, we included 32 and 13 consecutive patients suffering from aortic stenosis having respectively been selected for TAVI and surgical aortic valvular replacement (AVR).

Inclusion criteria for all patients were:

- trans-aortic valvular gradient ≥ 40 mmHg or valvular area ≤ 1 cm^2 or ≤ 0,6 cm^2/m^2.

Inclusion criteria for TAVI patients were as follows:

- a high surgical risk as determined by the Euroscore or a major comorbidity,
- unanimous consensus among members of the local heart team either that surgical aortic valve replacement was not suitable or that TAVI was safer.

Patient preference did not represent sufficient grounds for choice of a percutaneous method over conventional surgical aortic valve replacement.

Exclusion criteria:

- Patient refusal,
- patients under 18 years of age,
- patients not competent to give consent.

TAVI [23-25]

Aortic annulus sizing was performed using data gathered from trans-thoracic echocardiography (TTE), tans-esophageal echocardiography (TEE) and angioscan, allowing us to determine the optimal diameter of the prosthetic valve.

Depending on the afore-mentioned sizing, we used one of two the SAPIEN heart valve (Edwards Lifesciences) available with diameters of 23 mm and 26 mm and respective stent-heights of 14.5 and 16 mm.

Injected scans of the femoral access routes and aortic calcifications as well as possible aneurysms helped determine the use of a trans-femoral (TF-TAVI) or trans-apical (TA-TAVI) approach.

The procedure was performed in the catheterization laboratory of the CHRU de Lille cardiology hospital. Patients systematically underwent general anesthesia and were pretreated with aspirin and antibiotics. Per procedural heparin was injected at UI/kg and antagonized immediately before femoral access closure using half dose protamine sulfate.

A temporary electrode was introduced into the right ventricle through the femoral vein contra lateral to the TAVI main access side.

A TEE was systematically performed during and after implantation of the Edwards-SAPIEN prothesis to control pre-deployment valve positioning and verify the absence of significant aortic regurgitation (AR).

The SAPIEN aortic stented valve was set on a balloon catheter immediately before implantation:

- For TF-AVR a 14F sheath was placed using the Seldinger method after an angiographically guided puncture of the main femoral artery using a crossover technique. The Retroflex system was used for valve delivery, supported by an extra stiff guide-wire introduced retrogradely through the diseased valve into the left ventricle.
- For TA-AVR, deployment of the prothesis was performed using an anterograde approach. This method entails a left anterolateral minithoracotomy, opening of the pericardium and an incision of the left ventricular apex lateral to the left anterior descending coronary artery, allowing sheath incision and the introduction of a dedicated delivery catheter [26].

In both cases, implantation implied two consecutive steps, both performed under rapid ventricular pacing (180 beats/mn) to insure partial cardiac standstill:

- Aortic valvuloplasty with a 20- or 23-mm balloon,
- Followed by valve deployment after optimal positioning with the annulus using fluoroscopy, angiography and TEE. Deployment was achieved through expansion of the valve stent by inflating of the delivery balloon, thereby compressing the native diseased valve.

In case of post-deployment TEE showing AR ≥ 2/4 valve impaction was optimized through a second balloon inflation. Significant regurgitations were also sought out through a final control angiography.

The sheath was removed and the access site closed. In the TD-AVR, the femoral puncture site was mended using a PROGLIDE percutaneous closure system (Abbott Vascular, Illinois, USA). In the TA-AVR, surgical closure was performed.

Procedural success was defined as the implantation of a functional EWARDS SAPIEN valve and patient survival.

ECHOCARDIOGRAPHIC EVALUATION

Using a Vivid 7 or a Vivid 9 (General Electrics) or a iE33 echocardiographic system (Phillips), we assessed hemodynamic characteristics of the aortic valve by transthoracic echocardiography at base line and seven days post TAVI.

The mean and peak trans-valvular pressure gradients were calculated using the modified Bernoulli equation, and the effective orifice area (EOA) using the continuity equation.

According to per-procedural TEE, patients were classified into three groups:

- Those presenting with no significant post-TAVI aortic regurgitation (AR),

- Those presenting with AR ≥ 2/4 requiring optimization of valve deployment through a second balloon inflation,
- Those with respected post-TAVI AR.

BLOOD COLLECTION AND LABORATORY ASSAYS

Blood samples were collected for the assessment of the various measured parameters in the entire patient population.

Patients underwent blood sampling:

- immediately before primary balloon inflation (baseline),
- consecutively 5, 30 and 180 minutes after valve implantation,

There was no scheduled follow-up appointment in the design of the study.

PRIMARY HEMOSTASIS ASSESSMENT [18, 19]

Platelet-related hemostasis was tested with a platelet-function analyzer (PFA-100; Dade Behring, Deerfield, Ill) by determining closure time with adenine diphosphate cartridges (normal value, less than 114 seconds). The platelet-function analyzer is a high-shear system for in vitro testing of platelet function that simulates primary hemostasis after injury to a small vessel. It is a highly sensitive way to screen patients for von Willebrand factor defect.

The multimeric structure of plasma vWF was analyzed by electrophoresis with 0.1% sodium dodecyl sulfate and 1.5% agarose gel. The percentage of the HMWM (%HMWM, >15 mers) was determined after densitometric scanning, as described previously [27]. The same pool of normal platelet-poor plasma was used as reference in each gel electrophoresis. The lower limit of the normal range for the %HMWM is 10.5%, which is defined as 2 SD below the mean value for normal plasma samples, as reported previously. The ratio of HMWM percentages of patients over that of the control (HMWM > 15m/c) was calculated each time so as to standardize results.

The result of a primary hemostasis assay was considered impaired when the value of the assay was beyond the predefined normal value. Interseries variability assessed in plasma controls for vWF: HMWM 15 mers (10.4%) was judged satisfactory. The accuracy of the closure time with collagen-epinephrine or collagen-ADP cartridges is evaluated to 12.4% and 12.7%, respectively, by the manufacturer.

STATISTICAL ANALYSIS

Continuous variables with Gaussian distribution are given as mean ± SD. Continuous variables with no Gaussian distribution are given as median [25th–75th] percentiles. Categorical variables are given as percentage (numbers) of patients with the respective attribute. Normality was tested using the Shapiro-Wilk test. Bivariate comparisons were conducted using the t-test for normally distributed continuous variables or the Mann-Whitney test for not normally distributed variables. Bivariate comparisons for categorical variables were performed using the Fisher test. Multivariate analysis for continuous variables were conducted using the oridnary one-way ANOVA test.

All analyses were conducted with Prism (version 6.0; GraphPad Software, San Diego, CA).

Results

PATIENTS CHARACTERISTICS

Clinical characteristics

The baseline clinical characteristics of the study population are shown in Table 1.

TABLE 1. Clinical characteristics of the 32 TAVI patients

Age (year)	81.7 ± 5.5
Male, n (%)	17 (53)
BMI (kg/m^2)	27.3 ± 4.6
Hypertension, n (%)	24 (75)
Atrial fibrillation, n (%)	18 (56)
Coronary artery disease, n (%)	16 (50)
Percutaneous coronary intervention < 6 months, n (%)	3 (9)
Cerebral vascular events, n (%)	5 (16)
Peripheral vascular disease, n (%)	5 (16)
Renal failure (CG GFR < 30 mL/mn), n (%)	2 (6)
COPD, n (%)	11 (34)
NYHA class, n (%)	
I	0 (0)
II	13 (40)
III	12 (38)
IV	7 (22)
Logistic euroSCORE	16.3 [13.3-21.5]
	19.3 ± 13.9

BMI, Body mass index; COPD, chronic obstructive pulmonary disorder; NYHA, New York Heart Association; euroSCORE, European System for Cardiac Operative Risk Evaluation; CG GFR: Cockroft & Gault glomerular filtration rate.

Data are mean ± standard deviation unless otherwise stated. In case of non normal distribution: median [1st quartile-3rd quartile].
Logistical Euroscore did not pass normality test but is also represented as mean ± SD for readability's sake.

Morphological characteristics

The echocardiographic and scanographic findings before TAVI and surgery are shown in Table 2.

TABLE 2. Transthoracic echocardiography characteristics of the 32 patients

	TAVI patients (n = 32)	Surgical patients (n = 13)	p =
LVEF (%)	53 ± 12	45 ± 19	0.07
Patients with PEF, n (%)	23 (72)	6 (46)	0.17
LV end-diastolic diameter (mm)	47.8 ± 12.4	51.8 ± 10.4	0.34
Aortic valve area (cm^2)	0.71 ± 0.22	0.75 ± 0.17	0.65
Indexed effective orifice area (cm2/m2 of body-surface area)	0.39 ± 0.13	0.41 ± 0.11	0.75
Mean aortic gradient (mmHg)	50 ± 14	40 ± 15	0.03
Maximum aortic velocity (m/s)	4.5 ± 0.5	4.0 ± 0.8	0.08

LVEF, left ventricular ejection fraction.
Data are mean ± standard deviation unless otherwise stated.

The tranthoracic echocardiographic findings before and after TAVI are shown in Table 3.

TABLE 3. Annulus diameter of the 32 TAVI patients

By TEE (mm)	22.8 ± 2.1
By TDM (mm)	25.2 ± 5.2

Data are mean ± standard deviation.
Diameter by TDM is obtained through calculation of the mean between long and short diameters of the oval shaped aortic annulus.

The tranthoracic echocardiographic findings before and after TAVI are shown in Table 4.

TABLE 4. Evolution of the echocardiographic characteristics of the 32 TAVI patients

	Before TAVI	After TAVI	p =
LVEF (%)	53 ± 12	57 ± 16	0.0165
Patients with PEF, n (%)	23 (72)	23 (72)	1
LV end-diastolic diameter (mm)	47.8 ± 12.4	49.6 ± 5.9	0.33
Aortic valve area (cm^2)	0.71 ± 0.22	1.83 ± 0.53	< 0.0001
Indexed effective orifice area (cm2/m2 of body-surface area)	0.39 ± 0.13	1.02 ± 0.34	< 0.0001
Mean aortic gradient (mmHg)	47 [41-58]	9 [7-13]	< 0.0001
Maximum aortic velocity (m/s)	4.5 ± 0.5	2.1 ± 0.5	< 0.0001

LVEF, left ventricular ejection fraction.
Data are mean ± standard deviation unless otherwise stated. In case of non normal distribution: median [1st quartile-3rd quartile].

CONFIRMATION OF CORRELATION

The ratios of HMWM percentages from the 32 patients, who underwent TAVI over that of the control (HMWM>15m/c) were negatively correlated with the mean transvalvular gradient (r^2=0.203; p<0.0001) (Fig. 1).

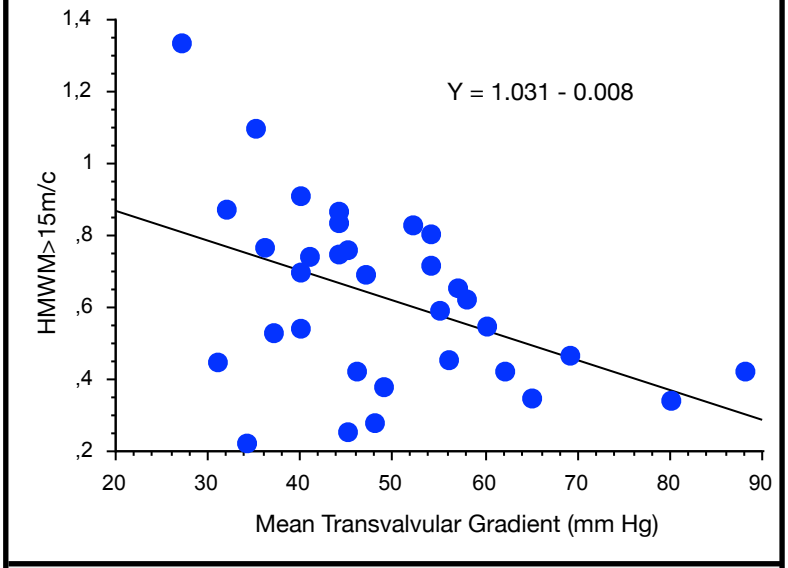


Figure 1. Regression line plotting mean transvalvular gradient against the ratio of highest molecular weight Von Willebrand factor multimers over control (r^2=0.203, p<0.0001).
HMWM>15m/c : von Willebrand factor highest molecular weight multimer percentage ratios (patients/control)

CORRECTION OF HWMW DEFICIENCY AFTER SURGERY

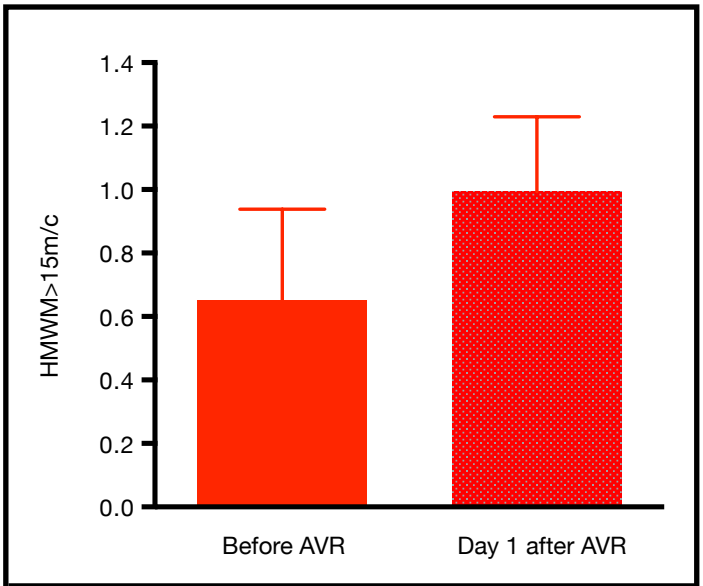
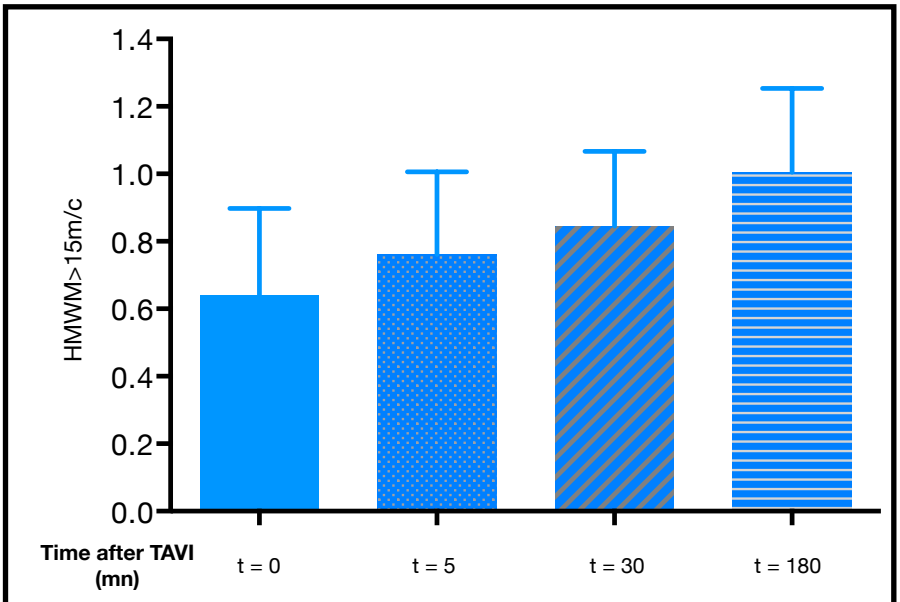


Figure 2. Increase in means of the ratios of highest molecular weight von Willebrand factor multimers over control before and one day after AVR (p=0.0051).
AVR: Surgical aortic valvular replacement
HMWM>15m/c : von Willebrand factor highest molecular weight multimer percentage ratios (patients/control)

The ratios of HMWM percentages from the 13 patients, who underwent surgical aortic valvular replacement over that of the control (HMWM > 15m/c) were significantly higher after than before surgery (p=0.0051) (Fig. 2).

SPEED OF VWF HMWM CORRECTION



Mean	0.639	0.761	0.846	1.005
Std Dev	0.259	0.246	0.221	0.249

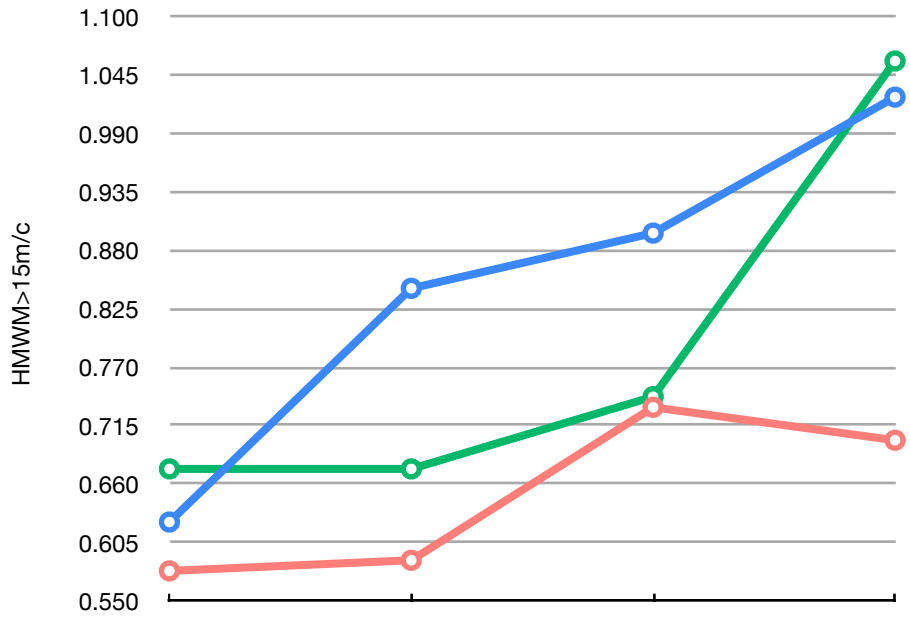
Figure 3. Increase in means of the ratios of highest molecular weight von Willebrand factor multimers over control before and 5, 30 and 180 minutes after TAVI (p<0.0001).
HMWM>15m/c : von Willebrand factor highest molecular weight multimer percentage ratios (patients/control)

There was a statistically significant increase in ratios of HMWM percentages from patients over that of the control between the blood samples taken at progressively increased intervals after TAVI (p<0.0001) (Fig. 3).

SUBGROUPS ACCORDING TO AORTIC REGURGITATION

When classified into sub-groups according to post-TAVI aortic regurgitation (no post-TAVI AR, post-TAVI AR corrected through a second balloon inflation and respected post-TAVI AR), we observed different profiles of von Willebrand factor highest molecular weight multimer percentage ratios corrections.

Patients without post-TAVI AR presented with early increase in HMWM whereas patients with post-TAVI AR showed little to no correction of the deficit. However, in the subgroup that underwent a second balloon inflation to optimize valve deployment, HMWM began to increase after correction of the AR.



Time after TAVI (mn)	t = 0	t = 5	t = 30	t = 180
No post TAVI AR (n=22)	0.624 ± 0.302	0.844 ± 0.244	0.896 ± 0.217	1.024 ± 0.237
Corrected post TAVI AR (n=7)	0.674 ± 0.161	0.674 ± 0.076	0.742 ± 0.126	1.058 ± 0.191
Respected post TAVI AR (n=3)	0.578 ± 0.169	0.588 ± 0.118	0.732 ± 0.235	0.701 ± 0.133

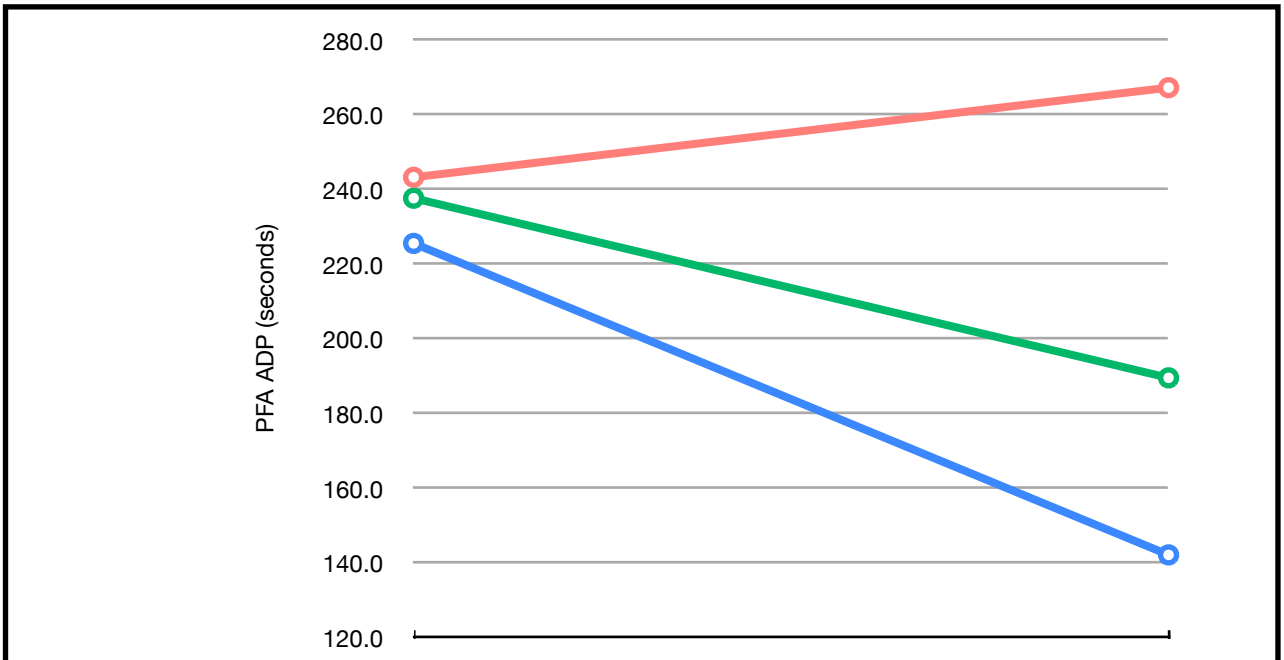
Figure 3. Progressive correction of HMWM deficit according to the existence of post TAVI aortic regurgitation and a possible second balloon inflation within the valve to correct it if present (values are expressed as mean ± SD) ($p<0.0001$ for effect of time on HMWM ratio percentages variation; $p=0.03$ for interaction between time and aortic regurgitation status).

HMWM>15m/c: von Willebrand factor highest molecular weight multimer percentage ratios (patients/control)
TAVI: trans-aortic valvular replacement
AR: aortic regurgitation

The effect of time on HMWM percentage ratios showed a significant difference between baseline and samples at 5, 15 and 180 minutes ($p<0.0001$). Interaction between time and AR status was also significant ($p=0.03$).

CORRECTION OF PFA

We observed a similar distinction in PFA-100 clotting time profiles between the three same sub-groups.



Time after TAVI (mn)	t = 0	t = 180
No post TAVI AR (s), (n=22)	226 ± 81	142 ± 73
Corrected post TAVI AR (s), (n=7)	243 ± 54	267 ± 57
Respected post TAVI AR (s), (n=3)	238 ± 62	190 ± 93

Figure 4. Progressive correction PFA-100 closure time according to the existence of post TAVI aortic regurgitation and a possible second balloon inflation within the valve to correct it if present (values are expressed as mean ± SD) ($p=0.0009$ for effect of time on HMWM ratio percentages variation; $p=0.18$ for interaction between time and aortic regurgitation status).

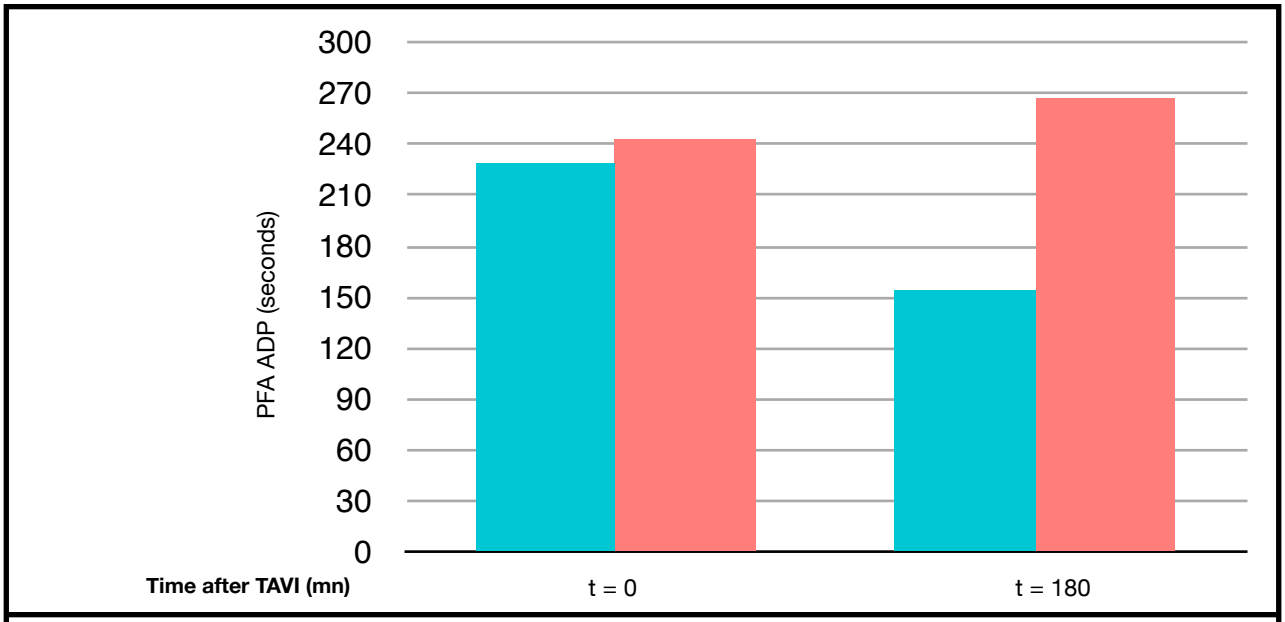
PFA-ADP: Platelet function analyser clotting time with adenine diphosphate cartridges
TAVI: trans-aortic valvular replacement
AR: aortic regurgitation

Patients without post-TAVI AR presented with a decrease to near normal values after TAVI. In a similar manner, patients who underwent a second balloon inflation and therefore a secondary correction of the initial post-TAVI AR, also showed improvement in PFA clotting time also less pro-

nounced. Finally, PFA clotting times of patients with uncorrected post-TAVI AR did not decrease but rather increased.

The effect of time on PFA showed a significant difference between baseline and samples at 5, 15 and 180 minutes (p=0.0009), however interaction between time and AR status did not reach significance (p=0.18).

When considering only the end result and dividing the patients into two groups according to the presence or no of post-TAVI AR, although still not reaching significance, we found a trend towards interaction between time and AR (p=0.096).



AR present at end of procedure (s), (n=3)	243 ± 54	267 ± 57
No AR present at end of procedure (s), (n=29)	229 ± 76	154 ± 80

Figure 5. Progressive correction PFA-100 closure time according to the existence of post TAVI aortic regurgitation at the end of the procedure (values are expressed as mean ± SD) (p=0.096 for interaction).

PFA-ADP: Platelet function analyser clotting time with adenine diphosphate cartridges
AR: aortic regurgitation

Discussion

Although HMWM deficiency due to increased shear stress in AS is now generally accepted, the immediate effects of aortic gradient rapid decrease on VWF were up until now still unclear. The present study is the first to describe the speed of AVWS correction after aortic stenosis treatment.

The key message is the rapid correction of AVWS after TAVI, which could not be studying after corrective surgery of aortic stenosis due to the "black-box" effect brought on by the use of ECLS.

CONFIRMATION OF CORRELATION

We confirmed what our previous work had shown: that HMWM deficiency increased with mean aortic pressure gradient and therefore that type 2A AVWS was related to stenosis-induced shear stress [18].

RESULTS COMPARABLE TO SURGICAL TREATMENT

The study of AVR patients allowed us to demonstrate that, even in patients exposed to the confounding effects of ECLS, HMWM percentages ascended to the levels of that of the control patients as early as day one after after corrective surgery.

The total reversal of AVWS after correction of AS similar to the one observed in TAVI patients (see below) emancipates us from the risk of a procedural-specific effect.

A RAPID CORRECTION OF BIOLOGICAL PARAMETERS

Intermediate blood samples at 5 and 30 minutes showed that the von Willebrand factor highest molecular weight multimer percentages obtained in TAVI patients increased immediately after prothesis expansion with progressive and continuous improvement, finally equalling those of the control patients as early as 180 minutes after procedure, demonstrating total reversal of the AVWS.

The possibility of reliable analyzes of early samples immediately after correction of AS, without ECLS interference, therefore allowed us to establish that shear stress-related HMWM deficiency

reversal is rapid and linear, leading to full recovery of von Willebrand factor anomalies within 3 hours of shear stress reduction.

This confirms data from our experimental model, specifically that lifting the causal shear stress leads to the reversal of the ensuing HMWM deficiency within hours [22].

The mechanisms leading to this speedy recovery, however, are still unclear.

Increased release of VWF?

AS favors shear stress-induced proteolysis and cleavage of HMWM of VWF, insufficiently counter-balanced by physiological release.

While working with patients with moderately obstructive hypertrophic cardiomyopathy (OHCM), we had observed that punctual rises in cardiac output through exercise testing did not modify the overall concentration of HMWM, seemingly indicating that transient aggravation of the obstruction has no immediate worsening effect on the AVWS and that the cumulative effects of a heightened gradient mattered more than rapid increments in shear stress [19].

The effect of VWF clearance through platelet interaction essentially concerning lower weight multimers [28], HMWM decrease is primarily due to proteolysis. Therefore Heyde's Syndrome could be described as a new-found balance between maximized yet insufficient HMWM release and cumulative ADAMTS 13-dependent proteolysis.

This would explain that a rapid correction of the obstruction due to AS through TAVI and the ensuing interruption of ADAMTS 13-dependent cleavage, through persistence of the enhanced physiological release of VWF, would lead to the rapid increase of HMWM and the reversal of the type 2A AVWS.

Lateral association

However, another possible explanation might be the recuperation of lateral associations between plasma VWF HMWM. The VWF present in plasma, have been shown to form thiol-disulfide re-

versible covalent bonds in their string-shaped shear stress-induced conformation, resulting in larger forms of HMWM and therefore heightened platelet adhesion capability [29].

This oxydo-reduction dependent association is also sensitive to ADAMTS 13 cleavage, though in this case, independently from the proteolytic activity of the protease. The enzyme reduces Cys2431-Cys2453 disulfide bond in the C2 domain, lowering the number of larger multimers [30].

In high shear stress situations, such as AS, these ropes of associated VWF strings would therefore form but also be increasingly exposed to ADAMTS 13 activity.

Correction of AS might help to reduce this phenomenon and favor unimpeded lateral self-association, resulting in an increased number of highest molecular forms of VWF.

THE INFLUENCE OF PERSISTENT AORTIC REGURGITATION

Peri-prothetic aortic regurgitation (AR) is a frequent observed phenomenon after TAVI. Often due to prosthesis/annulus discongruence [31], recent studies have also identified moderate to severe AR (≥ 2/4) as the most grievous complication of TAVI with dismal impact on patient survival [32]. Follow-up at two years show it to be the main risk factor for all cause mortality and cardio-vascular death for TAVI patients (with odds ratios of 4.89 and 7.9) [33].

Balloon post-dilatation (BPD) is a widely used method for optimizing prothesis expansion and re-ducing post-TAVI moderate to severe AR without inducing prothesis dysfunction [34].

Using TEE, we identified those patients with post-TAVI significant AR and observed its impact on AVWS.

Patients without AR ≥ 2/4 after TAVI showed immediate (5 mn) and continuous (30 and 180 mn) improvement of the original HMWM VWF deficiency. However, those with significant AR, pre-sented with no such significant increase in multimers.

After BPD however, an efficient correction of AR led to a similar increase of HWMW as those with no post-TAVI significant AR, with comparable levels of HWMW ratios at 180 mn.

Consequently, persistence of moderate to severe AR after BPD resulted into steady HMWM deficiency.

We explain this unremitting AVWS as the consequence of sustained heightened shear stress, no longer due to AS but AR, yet with similar effect on VWF transition from coiled to string conformation and susceptibility to ADAMTS 13 cleavage.

THE CONSEQUENCES ON PFA AND ITS USE

Since lack in VWF HMWM leads to platelet aggregation deficiency, it seemed logical to assume that clotting time would vary the same way as highest molecular weight multimer percentage ratios.

Patients without post-TAVI AR presented with decreased PFA clotting time. A comparable yet slightly inferior improvement took place among the group of patients with a post-TAVI treated with BPD whereas those with significant post-TAVI AR maintained a stable platelet aggregation dysfunction.

Although the interaction between time and AR did not reach significance, we found a definite trend towards interaction between time and presence of AR after procedure, inferring that AVWS reversal seemed to lead to a decrease of PFA-100 measured clotting time in patients free of post-TAVI AR. The lack of significance is, in our opinion, mainly due to the small number of patients (3/32) who ended up with a significant AR at the end of the TAVI.

Although a higher number of included patients is required to definitely confirm this trend, PFA clotting time variation mirrored therefore seem to mirror those of HMWM ratios and that AVWS rectification translated into that of PFA determined aggregation function.

STUDY LIMITATIONS

This study concentrated on immediate biological repercussions of AS correction without recording bleeding events. Therefore clinical consequences of HMWM deficiency before and after TAVI were not analyzed and more specifically the impact of AVWS on procedural bleeding.

The limited number of patients and therefore the even lower number of post-TAVI persistent moderate to severe AR only allowed us to infer a relationship between AR and uncorrected AVWS but this association merits further and larger scale studies to confirm it.

Summary

Correction of type 2A von Willebrand syndrome after treatment of aortic stenosis through TAVI begins within the first minutes after the drop in shear-stress and complete rectification of hemostasis is obtained three hours after valve implantation. This translates into reduced clotting times with platelet function analysis.

Persistent high shear-stress due to post-procedural aortic regurgitation impedes the reversal of the hemostatic abnormalities. This observation may offer insight on one of various possible causes of increase mortality rates in patients presenting with significant post-TAVI aortic regurgitation.

Clinical perspectives

Although it did not reach significance, we found a definite trend towards the interaction between time and aortic regurgitation for the correction of PFA clotting time in the absence of post-TAVI AR. Further studies are warranted to determine the possible use of PFA as an on-site test of TAVI procedural success and more specifically the absence of significant AR after valve implantation. This method could complement morphological appraisals of peri-prothetic regurgitation severity, mainly TEE which is ever more often being described as an insufficiently discriminative tool especially when in the presence of moderate regurgitation.

Multimodal quantification to guide balloon post-dilatation would appear all the more desirable that peri-prothetic regurgitation is now recognized as the complication with most impact on TAVI patient mid-term prognosis [32].

Also of great prognostic value are post TAVI bleeding complications especially in case of transapical approach. If it necessary to take into account the increased risk of bleeding in patients presenting with AVWS in the pre-procedural setting, we also believe it essential to reassess this risk post-procedurally all the more so that the hemostatic abnormalities regress within hours of AS correction.

If the link between PFA clotting time and HMWM should be confirmed through further investigation, this technique, due to its ability to generate on-site results, might also help identify patients at highest bleeding risk and guide adequate follow-up procedures.

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Abstract

Background

Acquired von Willebrand syndrome is a hematological complication of aortic stenosis, identified mainly through deficiency of the highest molecular weight multimers of von Willebrand factor. Surgical aortic valvular replacement leads to a correction of this disease but the “black box effect” of extra-corporeal life support has up until know rendered the measurement of the speed of this correction unfeasible. Recent years have seen the development of trans-aortic valve implantation as an alternative to aortic valvular correction of aortic stenosis in patients unsuitable for surgery or at high-risk patients who may still be suitable for surgery,

Methods

We enrolled 32 consecutive patients selected for trans-aortic valve implantation for the treatment of aortic stenosis. The multimeric structure of von Willebrand factor and platelet function under conditions of high shear stress were assessed at baseline and five and thirty minutes as well as three hours after procedure. We divided the population into three sub-groups: those presenting with respected post-procedural peri-prothetic aortic regurgitation, those with aortic regurgitation corrected by a second balloon inflation, and those without aortic regurgitation.

Results

Correction of the acquired hemostatic abnormalities began as early as five minutes after valve implantation and increased progressively to reach control group values three hours after procedure: mean von Willebrand factor highest molecular weight multimer percentage ratios of patients over control respectively 0.639, 0.761, 0.846 and 1.005 at five, fifteen and one-hundred and eighty minutes ($p<0.0001$). The sub-group of patients with post-procedural aortic regurgitation showed no significant hematological improvement. Patients undergoing second balloon inflation presented

with a rise of the multimer ratios only after correction of the regurgitation. Platelet function was significantly improved three hours after procedure than at baseline.

CONCLUSION

Correction of type 2A von Willebrand syndrome after treatment of aortic stenosis through TAVI begins within the first minutes after the drop in shear-stress and complete rectification of hemostasis is obtained three hours after valve implantation. This translates into reduced clotting times with platelet function analysis. Persistent high shear-stress due to post-procedural aortic regurgitation impedes the reversal of the hemostatic abnormalities.

Key words: aortic stenosis, aortic regurgitation, von Willebrand syndrome, TAVI, multimers

Résumé en Français

Contexte

Le syndrome de Willebrand acquis est une complication hématologique du rétrécissement aortique, caractérisé par un déficit en multimères de haut poids moléculaire du facteur von Willebrand (VWF). Ce déficit est corrigé par le remplacement valvulaire aortique chirurgical mais l'effet "boîte noire" lié à la circulation extra-corporelle a jusque maintenant rendu impossible l'étude précise de la cinétique de cette amélioration. Une alternative à la chirurgie a vu le jour ces dernières années: le remplacement valvulaire aortique par voie percutanée, nommé TAVI, chez les patients contre-indiqués à la chirurgie ou bien à haut risque chirurgical.

Méthodes

Nous avons consécutivement inclus 32 patients sélectionnés pour une procédure TAVI et avons étudié la répartition de formes de haut poids moléculaire du VWF ainsi que l'agrégabilité plaquettaire par PFA avant, puis à cinq minutes, quinze minutes et trois heures après la procédure. Trois sous-groupes ont été déterminés en fonction de l'apparition post-procédurale d'une fuite aortique péri-prothétique respectée ou corrigée par post-dilatation au ballon ou bien de l'absence de fuite.

Résultats

Un début de correction du déficit en formes de VWF de haut poids moléculaire est observable dès la cinquième minute après TAVI, et progresse jusqu'au retour à un profil hémostatique normal à la troisième heure: la moyenne des ratios de formes de VWF de haut poids moléculaires des patients sur celui d'un contrôle normal était respectivement de 0.639 avant la procédure et de 0.761, 0.846 and 1.005 à cinq, trente et cent quatre-vingt minutes ($p<0.0001$). Le sous-groupe des patients chez qui persistait une fuite aortique significative non corrigée après TAVI ne montrait pas d'amélioration hématologique, alors que les patients bénéficiant d'une post-dilatation présentaient une cor-

rection seulement après disparition de la fuite. L'étude de l'agrégabilité par PFA était significativement améliorée trois heures après le TAVI.

CONCLUSION

La réversion du syndrome de Willebrand type 2A associé au rétrécissement aortique débute donc dès les première minutes après la diminution des forces de cisaillement et la normalisation complète du profil hémostatique est obtenue trois heures après implantation de la valve percutanée. Ceci mène à une amélioration mesurable de l'agrégabilité plaquettaire par PFA. Une persistance de l'élévation des forces de cisaillement par la survenue d'une fuite aortique péri-prothétique s'oppose à la correction du syndrome de Willebrand.

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Date de Soutenance : Mercredi 12 Septembre 2012

Titre de la Thèse : Impact de l'implantation d'une valve aortique percutanée sur la cinétique de correction du syndrome de Willebrand acquis chez le sujet porteur d'une sténose aortique.

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Cadre de classement : *DES Medecine Cardio-vasculaire*

Mots-clés : Syndrome de Willebrand, Sténose aortique, Insuffisance aortique, multimères, TAVI

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Composition du Jury :

Président : Monsieur le Professeur Christophe Bauters

Assesseurs : Monsieur le Professeur André Vincentelli, Madame le Professeur Sophie Susen

Directeur de Thèse: Monsieur le Professeur Eric Van Belle

[redacted]