QUANTIFICATION OF AORTIC VALVE REGURGITATION

MASTER THESIS
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Measurement of aortic valve regurgitation after valve-sparing root replacement - a quantitative and intraoperative method

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Preface

Here it is, my thesis on the quantification of aortic valve regurgitation. After 10 months of work at the department of Cardiothoracic surgery in the LUMC, this project is finished. I managed to stretch my time as a Clinical Technology student at the TU Delft long enough, but with this thesis, it finally comes to an end.

During one of my clinical internships in the LUMC, while sitting in the Intensive Care Unit, a doctor walked in and asked for a student to help with a project on aortic valve regurgitation. And so it began, the collaboration between Bardia and me, which finally resulted in this master thesis. This project combined my clinical and technical knowledge gained during my studies at the TU Delft. I learned more about the clinical background of aortic root diseases and spent time observing them in the operating theatre. On the other hand, my technical knowledge of fluid dynamics and the world of medical technology was enhanced. I was fortunate to receive great help from my supervisors during this project.

Firstly, I would like to thank Bardia for the special collaboration we had over the past year. I enjoyed the (sometimes really long) coffee breaks where we discussed the project and many other things. Secondly, Susanne and Hubald, thank you for all your input during our regular meetings. You pushed me to think further and helped make this project a success. Lastly, I would like to thank the last member of my committee, Jenny. Although we haven't had many meetings, I felt that I could always reach out to you when I was stuck on the project. Thank you!

I would also like to express my gratitude to everyone in the departments of Cardiothoracic Surgery and Medical Technology for making this project happen and making my time at the LUMC more enjoyable. A special thanks to everyone who helped me with the experiments. Without their helping hands, I would not have been able to obtain any results. Finally, I would like to thank my friends and family for their mental support and for entertaining distractions when I needed them.

I am proud of the final result and grateful for everything I have learnt along the way. I am curious about what the future holds for me as a *Klinisch Technoloog*.

Laura Koopman
Rotterdam, February 2023

Contents

List of abbreviations

3D	Three dimensional
AR	Aortic regurgitation
AVP	Aortic valve visualisation and pressurisation
AVR	Aortic valve replacement
CPB	Cardiopulmonary bypass
EACTS	European Association for Cardio-Thoracic Surgery
EROA	Effective regurgitant orifice area
ESC	European Society of Cardiology
ISO	International Organisation for Standardisation
LV	Left ventricle
mL/min	Millilitres per minute
MR	Magnetic resonance
PHT	Pressure half-time
PVC	Polyvinyl chloride
PISA	Proximal isovelocity surface area
RMSE	Root mean square error
Rvol	Regurgitant volume
TEE	Transesophageal echocardiography
UV	Ultraviolet
VC	Vena contracta
VSRR	Valve-sparing root replacement

Summary

Introduction - Aortic valve regurgitation (AR) is a common type of aortic valve disease that affects the surrounding tissues of the heart. Surgery may be required in patients with acute or chronic AR, aortic root disease or ascending aortic dissection. A viable alternative to traditional aortic valve replacement that offers encouraging clinical outcomes is aortic valve-sparing root replacement (VSRR). However, reoperation due to recurrent or residual AR has a high incidence. Transesophageal echocardiography (TEE) is the gold standard for intraoperatively evaluating residual AR. Evaluation is performed after aortic declamping; therefore, a longer bypass time is inevitable when additional repair is required. Furthermore, quantification of AR with TEE can be challenging. Therefore, the need for an early and quantitative assessment method rises. The main objective of this study was to identify the relationship between the intraoperative and quantitative assessment of the repaired aortic valve and the post-declamping echocardiographic assessment after VSRR. Echocardiographic parameters were studied to find the optimal parameter to identify this relationship. Furthermore, variables that influence the amount of leakage in the quantitative and intraoperative assessment of the repaired valve were identified. Two *in vitro* experiments were performed to investigate the contribution of these variables to the leakage of sufficient and insufficient aortic valves.

Experiment 1 - The effective regurgitant orifice area was the optimal parameter to obtain the relationship between echocardiographic and quantitative assessment. It was implemented in an experimental setup as the orifice area. In addition to this parameter, the contribution of the orifice shape, pressure and fluid viscosity to the leakage was examined using a custom representation of the ascending aorta and the aortic valve. The orifice area was shown to have the most significant contribution to the amount of leakage, followed by the pressure and shape of the orifice. These three variables will be assessed in a second experiment. The viscosity of the fluid did not influence the leakage.

Experiment 2 - In a second experiment, the contribution of different variables to sufficient and insufficient aortic valves was evaluated at different pressures. The experiment was carried out in three cardiac tissues, in which first the contributions to the baseline leakage of a sufficient valve were evaluated. Two methods were used to create insufficiency. The first involved a punch and the second applied a more physiological approach of leaflet manipulation. The graft water permeability and the anastomosis leakage determined the leakage in a sufficient aortic valve state. The leakage of an insufficient aortic valve was almost completely determined by the size of the insufficiency. Graft permeability and anastomosis leakage could be neglected in the insufficient valve state. The leakage of the punched valve was comparable to the expected regurgitant volume based on the echocardiographic guidelines for that area. Leaflet manipulation resulted in less leakage than expected.

Conclusion - The contribution of several variables to the leakage of sufficient and insufficient aortic valves was investigated in two *in vitro* experiments. The results of this study show that the contributions of each of these variables could be quantified. As a result, the leakage of an insufficient aortic valve could be quantified in a static, pressurised *in vitro* setting. This is the first step in obtaining the relationship between the quantitative amount of leakage and echocardiography, to improve intraoperative assessment of the repaired aortic valve after VSRR.

1|Introduction

1.1 Background

Valve-sparing aortic root replacement

Aortic regurgitation (AR) is a common type of aortic valve disease and its severity increases with age [1]. Echocardiography is the primary imaging modality used to diagnose and follow-up on AR [2, 3]. In a study in which echocardiograms of 3589 unselected men and women were analysed, at least a trace of AR was observed in 3.3% of men and 1.1% of women in the age range of 26 to 39 years. This number increases to 10.0% for men and 10.1% for women, in the age group of 70 to 83 years [4]. AR is mainly caused by abnormalities of the aortic leaflets or root. It can affect the surrounding structures of the aortic valve, root and, in the case of chronic AR, even the left ventricle (LV) [1, 2].

Urgent surgery may be required in patients with acute AR, which is caused mainly by infective endocarditis and aortic dissection. The indications for surgery in patients with chronic AR or aortic root disease (aortic root or ascending aortic aneurysm with or without AR) are less urgent and depend on the symptoms of the patient, the status of the LV and the dilation of the aorta [3]. Depending on the characteristics of the patient, surgical treatment options are conventional aortic valve replacement (AVR, with or without replacement of the proximal ascending aorta) or aortic valve-sparing root replacement (VSRR) [3, 5]. AVR, whether it is performed with a mechanical or a biological valve, has some limitations. Mechanical valves require the lifelong use of anticoagulants and biological valves fail over time due to degeneration [6]. Moreover, serious complications occur after AVR with both types of valves, such as thromboembolic events (0.6-2.3% per patient-year), endocarditis (0.5% per patient-year) and anticoagulant-related bleeding (1% per patient-year) [7–9].

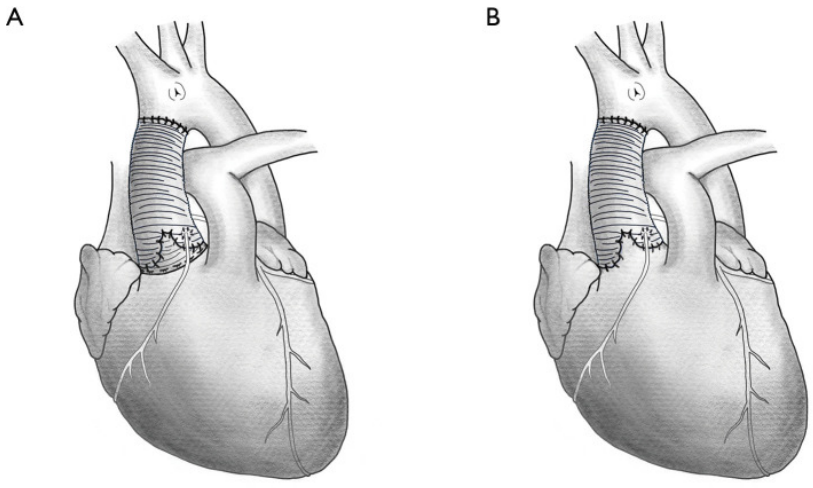


Figure 1: *The reimplantation (A) and remodelling (B) techniques of aortic valve-sparing root replacement, visualised by Zhou and colleagues [10].*

Because of the complications after AVR, the preferred procedure in selected patients is VSRR, according to the most recent European Society of Cardiology and European Association for Cardio-Thoracic Surgery (ESC/EACTS) guidelines on valvular heart disease [2]. Patient characteristics, feasibility of valve preservation and expected durability of the repaired valve are key factors in determining the suitability for VSRR [11]. Studies comparing AVR and VSRR demonstrate that the clinical outcomes of VSRR are superior to AVR, in terms of overall survival and valve-related events [12, 13].

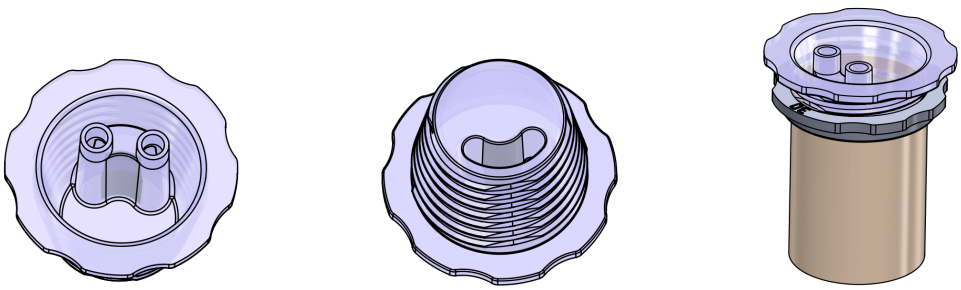
In VSRR, the aortic root, including the Valsalva sinuses and a part of the ascending aorta, is replaced by an aortic prosthesis. Numerous techniques to perform VSRR have been described, but the most widely used are the David reimplantation and the Yacoub remodelling techniques (Figure 1). With the first technique, the valves and their commissures are reimplanted in the tubular prosthesis to replace the sinuses of Valsalva and the proximal part of the ascending aorta [14]. The Yacoub remodelling technique uses a custom prosthesis fitted between the commissures to recreate the Valsalva sinuses [15].

Despite the encouraging clinical outcomes of VSRR, the possibility of reoperation due to recurrent or residual AR is one of the most common complications. A study amongst 764 patients after VSRR reported that after 8 years, 10% of the patients required AVR to treat recurrent AR [16]. The likelihood of requiring reoperation to re-repair or replace the aortic valve has increased to 17.1% after 15 years [17, 18]. In 82% of the patients, severe symptomatic AR is the main indication of reoperation, however, the underlying pathophysiology of recurrent AR remains unclear [18].

Intraoperative assessment

In the intraoperative setting, the gold standard to evaluate the repaired aortic valve and to diagnose residual AR is transesophageal echocardiography (TEE) after the aorta is declamped and weaning from cardiopulmonary bypass (CPB) is initiated [3, 19]. The valve is assessed based on morphology and functioning, using qualitative, semi-quantitative and quantitative measures [19]. The severity of AR is classified based on a combination of parameters and the clinical judgment of the cardiologist [20].

Post-aortic declamping evaluation of the repaired valve using TEE has some disadvantages. When residual AR is identified, additional repair of the aortic valve is required. This means the aorta must be reclamped and reopened, resulting in an inevitably longer CPB time, which potentially increases the patient's perioperative risk of, amongst others, neurological deficits [21, 22]. Another limitation of this evaluation method is that echocardiographic quantification of residual AR can be challenging, as it is time-consuming and requires a high level of expertise [23]. As a result, a method is needed to quantitatively assess the result of aortic valve repair at an earlier stage of the procedure. Multiple methods have been described, but no approach is currently widely used in the VSRR workflow [24].



(a) *AVP device, top view.*

(b) *AVP device, bottom view.*

(c) *AVP device attached to graft.*

Figure 2: *Technical drawings of the aortic valve visualisation and pressurisation (AVP) device in different views. Drawings from Nimbler Medical.*

The aortic valve visualisation and pressurisation (AVP) device is an example of a device that is used to perform early quantitative and visual assessment of the repaired valve (Figure 2). The device is attached to the aortic graft with a ring to close off the aortic root, while the heart is still arrested. A transparent solution is used to pressurise the root using one of the device connectors. Simultaneously, an endoscope is introduced into the device to perform visual inspection of the valve. The flow of the transparent solution is measured to quantify the leakage of the repaired valve in millilitres per minute (mL/min) [25]. The total amount of leakage is measured, including, for example, leakage resulting from the permeability of the prosthesis or anastomosis. However, it is unclear how much leakage is physiologically normal and how much indicates residual AR.

1.2 Problem statement and report outline

It is desirable to incorporate quantitative and early assessment of the repaired aortic valve into the current VSRR workflow. Therefore, the quantitative assessment method using the AVP device and the current gold standard of TEE after aortic declamping must be related. Understanding this relationship makes it possible to determine the acceptable amount of leakage after VSRR, leading to a more standardised procedure and improved intraoperative decision making. The following research question can be derived:

What is the relationship between the intraoperative and quantitative assessment of the leakage of an insufficient aortic valve and the post-declamping echocardiographic assessment after valve-sparing root replacement surgery?

This research question leads to the following sub-questions:

1. What are the echocardiographic parameters used to grade the severity of AR and which parameter would be optimal to relate to the quantitative measurement of the leakage with the AVP device after VSRR?
2. Which variables influence the amount of leakage after VSRR as measured with the AVP device and which should be included in an *in vitro* experiment?
3. What is the contribution of the variables from sub-question 2 independently, when tested in a non-physiological *in vitro* experiment, and which variables should be studied in a more physiological experiment?
4. What is the contribution of the variables from sub-question 3 and other variables, when tested in sufficient and insufficient aortic valves?

First, the echocardiographic parameters used to grade the severity of AR will be examined to choose the optimal parameter to identify the relationship with the quantitative amount of leakage. Secondly, the quantitative assessment of the repaired valve after VSRR will be studied, to identify variables that influence the measured leakage by the AVP device. Both topics will be discussed in Chapter 2.

Next, in an *in vitro* experiment with a non-physiological setup, the contribution of some of these variables is investigated. The methods performed in this experiment as well as the results will be covered in Chapter 3. This knowledge was applied in a more physiological *in vitro* experiment, where the variables with the most significant contribution from the first experiment were studied in cardiac tissue. Furthermore, in this setup, the contribution of variables that can only be assessed in cardiac tissue, such as anastomosis leakage, was evaluated. The second experiment is discussed in Chapter 4. Because this experiment is referred to as a scientific study, some information in this thesis is repeated.

To summarise, this research includes a first step in determining the relationship between the echocardiographic parameters used to grade the severity of AR and the quantitative amount of leakage of an insufficient aortic valve after VSRR in mL/min. This is done by studying the contributions to this leakage in two *in vitro* experiments. The contribution of the variables and their translation into an intraoperative setting are topics for future research, which is discussed in Chapter 5.

2|Assessing aortic valve regurgitation: echocardiographic parameters and intraoperative contributions

2.1 Echocardiographic parameters to grade the severity of aortic regurgitation

Using quantitative, semi-quantitative and qualitative echocardiographic parameters, the severity of aortic regurgitation (AR) can be classified as mild, moderate or severe (Figure 3) [19]. The qualitative parameters are based on valve morphology, the colour flow and the continuous Doppler wave of the regurgitant jet. The semi-quantitative measurements include pressure half-time (PHT) and vena contracta (VC) width. Lastly, quantitative parameters are represented by the effective regurgitant orifice area (EROA) and the regurgitant volume (Rvol). In clinical practice, the evaluation of the severity of AR is often based on semi-quantitative parameters. However, the prognostic value of quantitative parameters has been shown to supersede that of the semi-quantitative parameters [26].

Parameters	Mild	Moderate	Severe
Qualitative			
Aortic valve morphology	Normal/abnormal	Normal/abnormal	Abnormal/flail/large coaptation defect
Colour flow AR jet width[a]	Small in central jets	Intermediate	Large in central jet, variable in eccentric jets
CW signal of AR jet	Incomplete/faint	Dense	Dense
Diastolic flow reversal in the descending aorta	Brief, protodiastolic flow reversal	Intermediate	Holodiastolic flow reversal (end-diastolic velocity >20 cm/s)
Diastolic flow reversal in the abdominal aorta	Absent	Absent	Present
Semi-quantitative			
VC width (mm)	<3	Intermediate	≥6
Pressure half-time (ms)[b]	>500	Intermediate	<200
Quantitative			
EROA (mm^2)	<10	10–19; 20–29[d]	≥30
R Vol (mL)	<30	30–44; 45–59[d]	≥60
+ LV size[c]			

AR, aortic regurgitation; CW, continuous wave; LA, left atrium; EROA, effective regurgitant orifice area; LV, left ventricle; R Vol, regurgitant volume; VC, vena contracta.
[a]At a Nyquist limit of 50–60 cm/s.
[b]Pressure half-time is shortened with increasing LV diastolic pressure, vasodilator therapy, and in patients with a dilated compliant aorta or lengthened in chronic AR.
[c]Unless for other reasons, the LV size is usually normal in patients with mild AR. In acute severe AR, the LV size is often normal. *Accepted cut-off values for non-significant LV enlargement:* LV end-diastolic diameter <56 mm, LV end-diastolic volume <82 mL/m^2, LV end-systolic diameter <40 mm, LV end-systolic volume <30 mL/m^2.
[d]Grading of the severity of AR classifies regurgitation as mild, moderate, or severe, and subclassifies the moderate regurgitation group into 'mild-to-moderate' (EROA of 10–19 mm or an R Vol of 20–44 mL) and 'moderate-to-severe' (EROA of 20–29 mm^2 or an R Vol of 45–59 mL).

Figure 3: *Grading the severity of aortic regurgitation with qualitative, semi-quantitative and quantitative parameters. Table from of the European Society of Cardiology [19].*

The semi-quantitative and quantitative parameters shown in Figure 3 are discussed in order to find the optimal echocardiographic parameter to identify the relationship with the quantitative amount of leakage of a regurgitant aortic valve. This parameter will be included in an *in vitro* experimental setup to study the influence on the measured leakage in millilitres per minute (mL/min). The selected parameter should meet some requirements. It should be easy to measure in an experimental setup

and adjustment should be possible.

To obtain sufficient information on valvular morphology, the mechanism and severity of AR, different echocardiographic modalities are used in two- and three-dimensional modes [27]. Doppler echocardiography, the most important modality, plots blood flow velocities versus time, based on the Doppler shift. The two forms of this modality are continuous and pulsed wave Doppler. Additionally, colour Doppler imaging can be performed in the pulsed wave Doppler mode to provide a visual representation of the AR jet [28]. The (semi-)quantitative echocardiographic parameters are derived from the Doppler signals using the Bernoulli and the continuity principle. The principles are based on the law of conservation of energy and state that the total energy of an isolated system remains constant over time [29].

- **Pressure half-time** - The PHT represents the rate of equalisation of the aortic and left ventricular (LV) pressure. Rapid PHT indicates a large pressure difference and therefore a higher AR grade [27]. A PHT longer than 500 ms is compatible with mild AR, whereas values less than 200 ms indicate severe AR. It is important to keep in mind that the relaxation characteristics of the LV and the aorta influence the PHT. In a more compliant ventricle, as happens when the LV has adapted to chronic AR, the PHT will be overestimated and the AR grade will therefore be underestimated [30]. Furthermore, the orientation of the Doppler beam is of great importance, as it must be aligned with the AR jet [31].

- **Vena contracta width** - The VC width represents the smallest area of the regurgitant jet, which is formed immediately downstream from the orifice [32]. The parameter is analysed perpendicular to the jet, in early to mid diastole [31, 33]. A larger VC width represents a higher AR grade. Mild AR is indicated when the VC width is under 3 mm, and severe AR is indicated when the width is over 6 mm [19]. The parameter might over- or underestimate the severity of AR when the shape of the jet is irregular or when multiple jets are present [34].

- **Effective regurgitant orifice area** - The EROA represents the three-dimensional VC width and is generally obtained using the proximal isovelocity surface area (PISA) method. This method is based on the assumption that the velocity of blood increases as it gets closer to the regurgitant orifice, creating concentric, roughly cylindrical circles of increasing velocity and decreasing area [35]. The zone where this occurs is the flow convergence zone. The orifice area is calculated based on the size of this zone and the peak velocity of the AR jet [27, 36]. The PISA method is, however, subjective to some limitations, since it assumes a hemispheric flow convergence zone, a circular orifice and a central regurgitant jet [27, 36, 37]. An EROA less than 10 mm^2 corresponds to a mild AR grade and an EROA greater than 30 mm^2 is assessed as severe AR. A mild-moderate AR corresponds to an EROA of 10-19 mm^2 and a moderate-severe AR corresponds to an EROA of 20-29 mm^2 [19].

- **Regurgitant volume** - The Rvol represents the difference in the volumes of aortic outflow and mitral inflow per beat [38]. It can be derived from the EROA by dividing it with the velocity-time integral of the regurgitant flow [27]. Mild AR is concluded when the Rvol is less than 30 mL/beat, mild-moderate AR corresponds to a Rvol of 30-44 mL/beat, moderate-severe AR to a volume of 45-59 mL/beat and severe AR is concluded in the case of a Rvol of more than 60 mL/beat [19].

Based on the requirements, the EROA is the most suitable parameter to include in the experimental setup. This parameter is easy to measure and adjust according to the sizes as given in the guidelines. Furthermore, the parameter is more accurate than the semi-quantitative parameters because it comprises more sub-classifications. The EROA is represented in the experimental setup by several orifice areas corresponding to mild, moderate and severe AR, as classified by the European Society of Cardiology [19].

2.2 Variables influencing the leakage of a repaired aortic valve

The echocardiographic assessment of the repaired aortic valve after aortic declamping in valve-sparing root replacement (VSRR) only offers indirect information on the size of the residual AR and the morphology of the valve. With the use of the aortic valve visualisation and pressurisation (AVP) device, the valve can be assessed by direct visual confirmation and quantitative measurement of leakage. Additional details about the repaired valve can be acquired, such as the leakage caused by the permeability of the prosthesis. The variables influencing the measured leakage will be identified using a systematic impact analysis method. The method should include estimating the probability and impact of a variable on the leakage measured by the AVP device. An example of such a method is the Healthcare Failure Mode and Effects Analysis [39, 40]. This method is used to prospectively evaluate possible process failures in healthcare. The steps of this analysis will be applied to identify the variables with the highest contribution to the measured leakage of a repaired aortic valve using the AVP device. These variables will be included in an experimental setup to study this contribution.

The impact analysis of the variables starts with a brainstorming session to determine the steps in the assessment of the repaired aortic valve after VSRR and to identify the potential variables of influence. Second, based on the author's observations and experience, an estimation is made of the probability that the variable will impact the outcome, as well as the expected size of this impact. The probability and expected size of the impact are divided into five categories (Table 1). To obtain a final impact score, the probability and the size of the impact are multiplied. Variables with a final impact score higher than the cut-off of 70 are taken into consideration to be included in the experimental setup.

Table 1: *Probability and impact scores for the variables of influence.*

Probability	**Score**	**Impact**	**Score**
Frequent	10	Severe	10
Probable	9	Significant	9
Occasional	8	Medium	8
Remote	7	Measurable	7
Improbable	6	Insignificant	6

There are various steps in the evaluation of the aortic valve after VSRR using the AVP device:

0. **Baseline situation** - The VSRR procedure is completed, the patient is still on complete cardiopulmonary bypass and the heart is arrested. This is the baseline situation from where the assessment is performed.
1. **Attachment of the AVP device** - A ring corresponding to the size of the graft is used to secure the AVP device after it is inserted into the graft. The perfusion tube is connected to the device and the graft with the device is held by the surgeon or assistant.
2. **Fluid pressurisation in the aortic root** - A perfusion pump with clear cardioplegia is started and the aortic root is filled and de-aired. Once the root pressure reaches 80 mmHg, the flow is reduced to maintain this pressure.
3. **Leakage measurement** - The pressure in the aortic root is constant at 80 mmHg. The flow measured by the perfusion pump represents the leakage of the repaired valve. This situation is maintained for at least one minute. Simultaneously, a scope is inserted in the AVP device to visually assess the repaired valve based on the morphology, coaptation height and competency.
4. **Uncoupling of the device** - The flow is stopped and the AVP device is decoupled from the graft. If necessary, additional valve repair can be performed and the assessment can be repeated.

All variables of influence in each step in this process and their expected probability and impact scores can be found in Appendix A. The variables of interest with a final impact score greater than 70 can

be brought back to six subjects, as presented in Table 2. In the first experiment, the competency of the repaired valve, the pressure and the fluid viscosity will be studied. In the second experiment, the influence of the graft permeability, the anastomosis and the AVP device connection is tested, along with the variables with the highest influence in the first experiment. The competence of the repaired aortic valve will be represented by different orifice areas, corresponding to the EROA sizes from the echocardiographic guidelines, as discussed in the previous section. The pressures that will be tested are those of normal diastolic pressure (80 mmHg) and up to 20 mmHg lower to study the influence of increasing pressure. Instead of blood, milk will be used to study the influence of viscosity, since milk has a viscosity comparable to that of blood [41, 42]. Besides the identified variables, the influence of the orifice shape will be evaluated, since the echocardiographic guidelines assume a circular orifice. Besides a circular orifice, the leakage through a triangular and two non-central orifices with both half the desired area is evaluated.

Table 2: *Impact analysis results. Variables with a high contribution (impact score greater than 70) to the measured leakage of a repaired aortic valve after valve-sparing root replacement, measured by the aortic valve visualisation and pressurisation device.*

Variable	**Corresponds to**	**Method**	**Values**	**Experiment**
Repair	Incompetent valve	Orifice area	0, 10, 19.5, 30 mm^2	1 and 2
Pressure	Filling status LV, Aortic root pressure	Root pressure	60, 70, 80 mmHg	1 and 2
Fluid	Fluid viscosity	Water or milk	0.9, 2.1 mPas	1
Graft	Graft permeability	Graft permeability	-	2
Anastomosis	Anastomosis commissure, Anastomosis annulus	Baseline leakage	-	2
AVP device	Device incorrect, Incorrect ring size	Incorrect ring, ring not tight	Correct, incorrect	2

AVP = aortic valve visualisation and pressurisation; LV = left ventricle.

3|Evaluating the contribution of different variables to aortic valve regurgitation in a representative model

This experiment investigates the contribution of different orifice areas, pressures, fluid viscosity levels, and orifice shapes to the leakage through an orifice, representing an insufficient aortic valve. The orifice areas correspond to the effective regurgitant orifice area (EROA) sizes from the echocardiographic guidelines to grade aortic regurgitation (AR) [19]. The goal of this experiment is to identify the variables with the highest contribution to the leakage, and to include them in a follow-up experiment.

3.1 Materials and methods

3.1.1 Preparation and setup

Representation of the aorta and the aortic valve

The experiment was conducted in a customised representation of the ascending aorta and the regurgitant aortic valve with different levels of insufficiency. The design requirements for this representation are summarised in Table 3.

Description	Value
Must haves	
Waterproof	-
Diameter	29 mm
Length	81 mm
Pressure resistant	120 mmHg
Diameter connection	6.35 mm
Nice to haves	
Transparent	-

(a) *Requirements for the aorta.*

Description	Value
Must haves	
Waterproof	-
Orifice areas	10, 19.5, 30 mm^2
Orifice shapes	Circular, triangular, double
Pressure resistant	120 mmHg
Nice to haves	
Transparent	-

(b) *Requirements for the aortic valve.*

Table 3: *Design requirements for the custom representation of the aorta and aortic valve.*

- **Materials** - The material of the complete representation should be waterproof. Furthermore, according to the International Organisation for Standardisation guidelines for vascular prostheses (ISO 7198), prostheses should be tested at a maximum pressure of 120 mmHg [43]. Therefore, the material of the representation should be pressure resistant up to 120 mmHg. Lastly, to be able to check if the representation is de-aired, transparency would be nice to have.
- **Dimensions** - The inner diameter of the aortic representation should match the inner diameter of the proximal ascending aorta, which has a mean value of 29 mm in healthy subjects [44]. The length of the aortic representation should correspond to the mean length of the ascending aorta in healthy subjects, which is 81 mm [45]. The wall thickness must be sufficient to resist a pressure of 120 mmHg without expanding. The valve representation should have orifice areas of 10, 19.5 and 30 mm^2, corresponding to the EROA sizes in the echocardiographic guidelines [19]. The shapes of the orifices should be circular, triangular and double non-central circular.

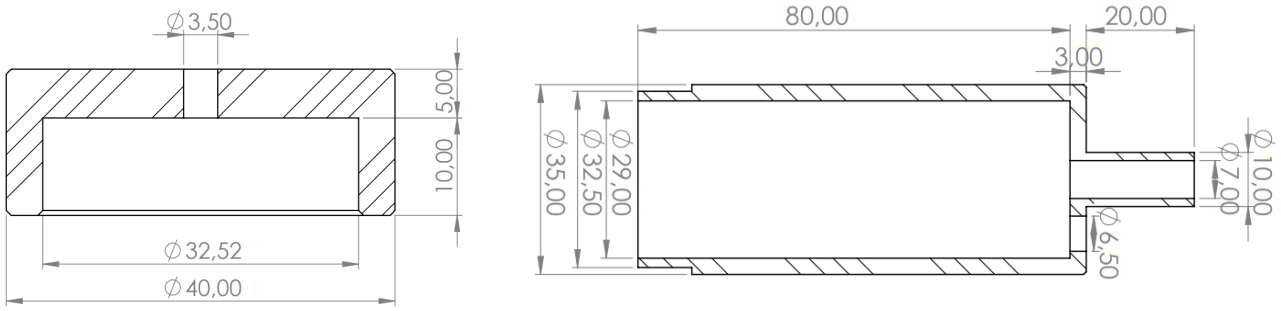
- **Connections** - The representation of the aorta and aortic valve should be able to connect to a standard 6.35 mm diameter perfusion tube.

Following the requirements, ten tubes (representing the ascending aorta) and lids with different orifice shapes and areas (representing insufficient aortic valves) were designed and three-dimensional (3D) printed. The lids had an inner diameter of 32.52 mm, an outer diameter of 40 mm and a height of 15 mm. Each lid was designed with a different orifice in three sizes and three shapes, resulting in nine combinations. One closed lid was designed for baseline measurements. In the double circular orifice, the area of each orifice was calculated by dividing the desired area by two. The orifices were located halfway between the centre of the lid, at a distance of 60 degrees apart. The dimensions of the triangular shape were calculated using:

$$Triangular\ area\ [mm^2] = \frac{\sqrt{3} \cdot (2 \cdot r)^2}{4} - 3 \cdot (\frac{60}{360} \cdot \pi \cdot r^2), \tag{3.1}$$

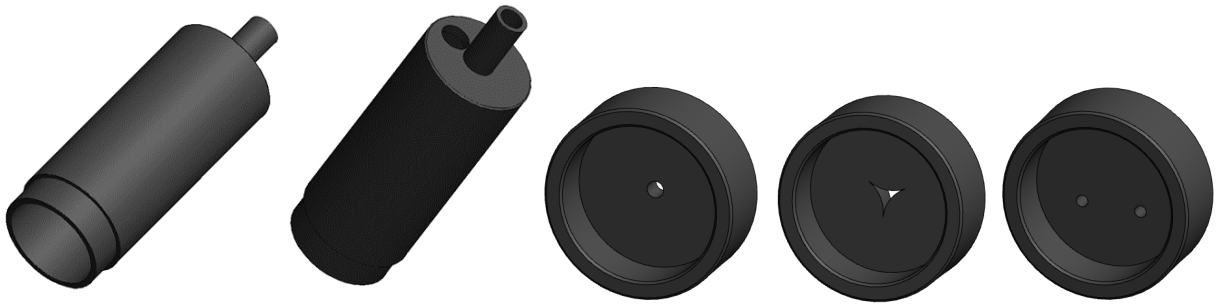
where: r = orifice radius corresponding to the desired orifice area, mm.

Corresponding the number of lids, ten tubes were designed with one open end and one end with an extruded pipe (height 20 mm, inner diameter 7 mm, wall thickness 1.5 mm) to connect to a perfusion tube. Next to this small tube, a 6.5 mm diameter hole was created for a second perfusion connector. The tube had a length of 80 mm, an inner diameter of 29 mm and a wall thickness of 3 mm. The lid could be attached to the narrowed bottom part of the tube, which had a wall thickness of 1.25 mm. The technical drawings of the tube and lids can be found in Appendix B and partly in Figure 4.



(a) *Lid with a 10 mm^2 circular orifice.* (b) *Tube.*

Figure 4: *Technical drawings of one of the lids (a) and a tube (b). Dimensions are given in millimetres.*



(a) *Tube.* (b) *Tube.* (c) *Circular.* (d) *Triangular.* (e) *Double.*

Figure 5: *Image of the custom tube (a and b), a lid with a 10 mm^2 circular orifice (c), a lid with a 10 mm^2 triangular orifice (d) and a lid with two non-central orifices, both with an area of 5 mm^2 (e).*

The tube and lids were created using a 3D printer with the stereolithography printing process with clear resin as material (Form 3, Formlabs GmbH, Berlin, Germany). The tube with support beams was post-processed in isopropyl alcohol and dried for 15 minutes with ultraviolet (UV) light. The support beams were removed and the edges were scrubbed. The tube and lid were glued together using a drop of clear resin and dried out using 15 minutes of UV light. Images of the tube and three of the lids are shown in Figure 5.

Experimental setup

Figure 6 shows a schematic overview of the experimental setup. The representation of the aorta and aortic valve with the orifice area of interest was placed on a laboratory stand and connected to a centrifugal pump (iLA activve, Novalung GmbH, Heilbronn, Germany) and a four-litre reservoir through standard sized perfusion tubes and connectors. Using a perfusion connector, an analogue pressure gauge was connected to the tube, to measure the pressure in the tube obtained using the pump. The pressure gauge was mounted on a laboratory stand at the same height as the tube. A servo motor with bent polyvinyl chloride (PVC) pipes was placed under the tube to lead the fluid to bins. The amount of fluid leaking through the orifice was measured to obtain the primary outcome of leakage in millilitres per minute (mL/min). When no measurement was performed, the fluid leaked through the pipes in the bin on the ground. When the motor was activated, it was rotated 90 degrees and the fluid was collected in the bin on the scale for 10 seconds.

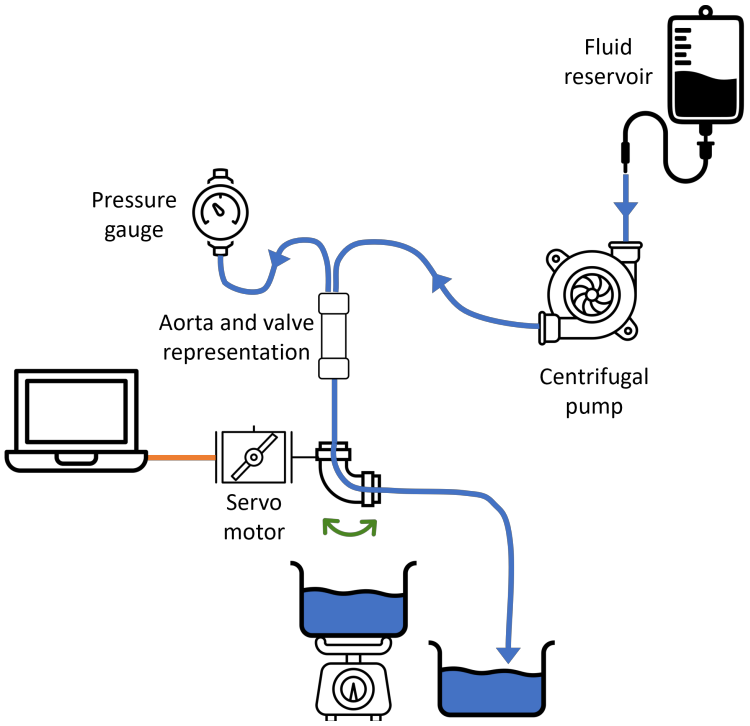


Figure 6: *Schematic overview of the setup for experiment 1. The fluid flows from the reservoir into the representation of the aorta and insufficient aortic valve with the use of the centrifugal pump. The pressure is measured using an analogue pressure meter. The leakage through the orifice in the representation is guided to bins through a polyvinyl chloride pipe, which can be rotated using the servo motor to lead the fluid in the bin on the scale.*

3.1.2 Measurement protocol

Prior to the experiments, a test run was performed for the tubes with all three sizes of circular orifices, to check if pressure could be obtained. It was found that no pressure was obtained in the larger orifice areas of 19.5 and 30 mm^2. Therefore, the values of the variables stated in Table 2 (Chapter 2) were

lowered. The orifice areas were adjusted to 5, 10 and 14.5 mm^2. Pressures of 40 and 50 mmHg were added since in the tube with the 14.5 mm^2 orifice area, maximum pressure of 60 mmHg was obtained.

The contribution of the different parameters was evaluated in a full factorial design of the experiment. With nine different orifices, two fluids and three to five pressures, this led to 78 different combinations excluding the baseline measurements. The full study protocol can be found in Appendix C.

The baseline tube with the closed lid was placed in the setup. The pressure in the tube was increased to 40 mmHg by increasing the pump flow. The tube was inspected by eye on sites of leakage for one minute. This was repeated for pressures of 50, 60, 70, 80 and 120 mmHg.

The baseline tube was replaced with the tube with a 5 mm^2 circular orifice. The tube was filled with water, leaving as little air as possible. The pressure was increased to 40 mmHg by increasing the pump flow. When the leakage was constant, the servo motor was rotated to measure the leakage for 10 seconds. The measurement was performed three times and repeated at 50, 60, 70 and 80 mmHg of pressure. This was repeated in all nine tubes, first with water and then with milk. The leakage from the tube with the 14.5 mm^2 orifice area could only be measured at 40, 50 and 60 mmHg.

3.1.3 Data analysis

To convert the measured leakage in grams per 10 seconds to the leakage in mL/min, the average of three measurements was multiplied by 6 and divided by the density of the fluid (water: 0.997 g/mL, milk: 1.035 g/mL) [46]. The data were presented as mean±standard error. The standard error was calculated with the formula:

$$SE = 6 \cdot (\sigma/\sqrt{n}), \tag{3.2}$$

where: SE = standard error, mL/min;
σ = standard deviation, mL/min;
n = number of repetitions, 3.

The influence of pressure, fluid viscosity, orifice size and orifice shape were visualised by plotting the relationship between the variable and the leakage in mL/min. To evaluate the leakage of the orifice areas corresponding to the echocardiographic guidelines (10, 19.5 and 30 mm^2), polynomial curve fitting was performed on the data for each pressure.

To determine the influence of each variable on the leakage, a multiple linear regression analysis was performed. The data pre-processing started with splitting the data into dependent and independent variables. The leakage was selected as the dependent variable. Pressure, fluid type, orifice area and shape were chosen as independent variables. Next, numerical data of the pressure and orifice area were normalised. To include categorical data, the data were encoded using a dummy data frame. The fluid viscosity level was presented as 0 for water and 1 for milk. For the orifice shape, the circular shape was presented as 0 and a different shape (triangular or double circular) was presented as 1. The data were merged again and split into a test and train set in a 20:80 ratio, with a random state of 3. After pre-processing, a multiple linear regression analysis was performed. The analysis resulted in a value for the intercept and a coefficient for each independent variable, representing the contribution to the leakage. P-values were calculated for each coefficient. A p-value lower than 0.05 was considered to be significant. To quantify the performance of the model, the adjusted R-squared and the root mean squared error (RMSE) were calculated.

3.2 Results

3.2.1 Baseline

No leakage was observed at all pressures for the baseline measurements.

3.2.2 Contribution of variables

Influence of orifice area and pressure

Figure 7 shows the effect of an increasing orifice area on the water leakage through a circular orifice for different pressures. The figure shows leakage varying from 862.21±1.30 mL/min (40 mmHg) to 1151.03±0.60 mL/min (80 mmHg) for the 5 mm^2 orifice. The results increased from 1821.89±2.07 mL/min to 2334.46±2.03 mL/min (40 to 80 mmHg, respectively) for an orifice area of 10 mm^2. Lastly, for the orifice area of 14.5 mm^2 the leakage differed from 2777.99±3.16 mL/min (40 mmHg) to 3148.83±3.66 mL/min (60 mmHg).

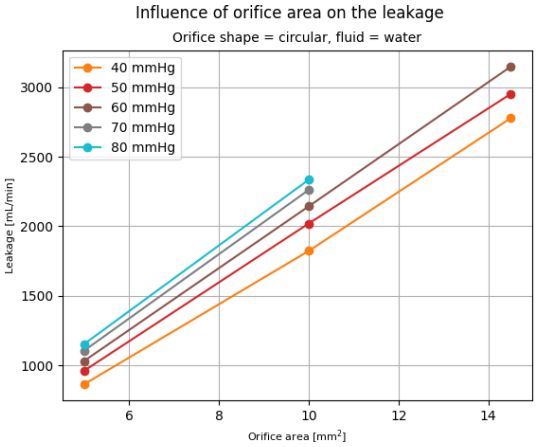
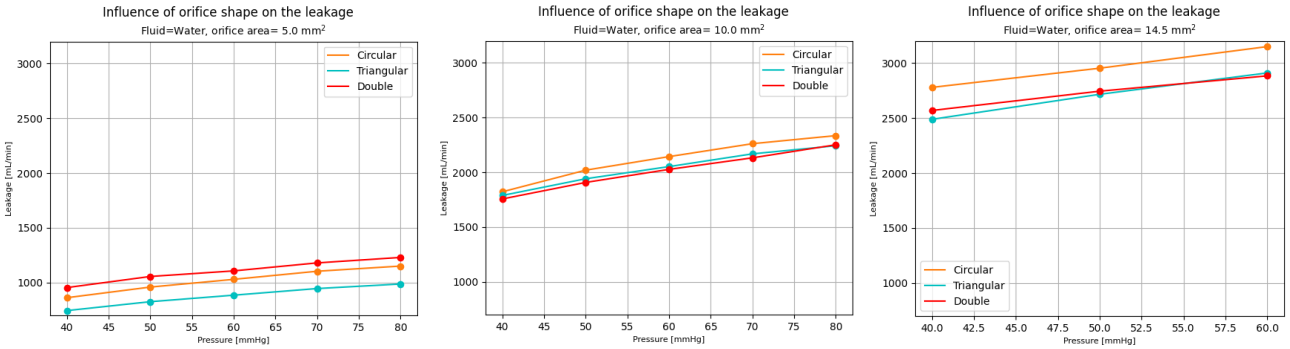


Figure 7: *The influence of an increasing orifice area on the leakage.*

Influence of orifice shape

In Figure 8, the influence of the orifice shape on water leakage is plotted for the three different shapes. The area of 5 mm^2 (Figure 8a) showed the largest leakage for the double orifice shape (maximum 1229.89±1.24 mL/min at 80 mmHg). The circular orifice shows less leakage (1151.03±0.60 mL/min at 80 mmHg), followed by the triangular shape (987.42±0.48 mL/min at 80 mmHg). For the orifice areas of 10 mm^2 (Figure 8b) and 14.5 mm^2 (Figure 8c), the circular orifice showed the highest leakage (respectively 2334.46±2.03 mL/min at 80 mmHg and 3148.83±3.66 mL/min at 60 mmHg). Depending on the pressure, the triangular or double circular shape followed with the second largest leakage.

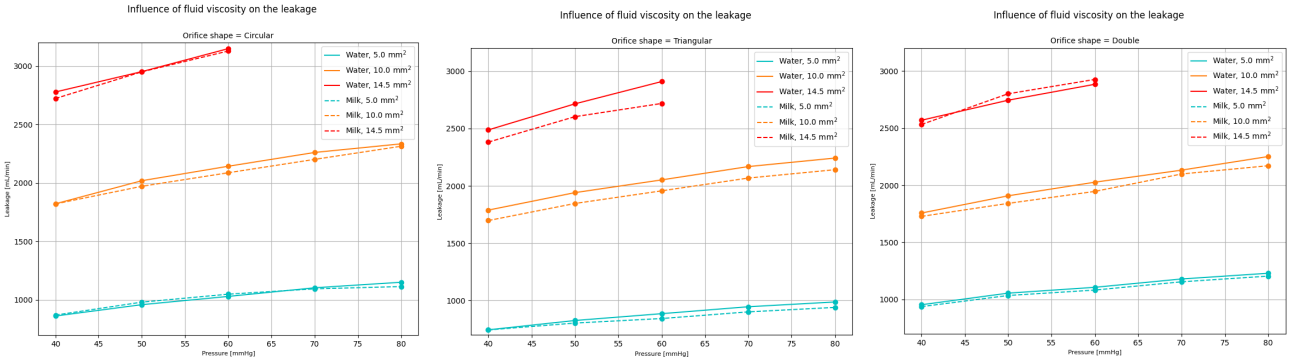


(a) *Orifice area 5 mm^2, water* (b) *Orifice area 10 mm^2, water* (c) *Orifice area 14.5 mm^2, water*

Figure 8: *The influence of different orifice shapes on the leakage in various orifice areas.*

Influence of fluid viscosity

The influence of the final variable, fluid viscosity, is shown in Figures 9 for all areas and shapes. The difference between leakage with water and milk varied with pressure and orifice shape. The maximum difference was observed for the triangular shape and was 42.33 mL/min (5 mm^2, 60 mmHg), 91.02 mL/min (10 mm^2, 40 mmHg) and 190.92 mL/min (14.5 mm^2, 60 mmHg).



(a) *Orifice shape: circular* (b) *Orifice shape: triangular* (c) *Orifice shape: double*

Figure 9: *The influence of fluid viscosity from water and milk on the leakage in different orifice shapes.*

3.2.3 Estimated leakage corresponding to the echocardiographic guidelines

When we applied a polynomial fit to the data from the water leakage through a circular orifice at 60, 70 and 80 mmHg of pressure, we obtained the following polynomials to estimate the leakage in mL/min based on the orifice area in mm^2:

$$Leakage = 219.07 * area \tag{3.3}$$

$$Leakage = 226.09 * area \tag{3.4}$$

$$Leakage = 233.45 * area \tag{3.5}$$

The polynomials were used to estimate the expected leakage for the orifice areas of 10, 19.5 and 30 mm^2 for pressures of 60 (Equation 3.3), 70 (Equation 3.4) and 80 (Equation 3.5) mmHg, as discussed in Chapter 2. The results are shown in Table 4.

Table 4: *Expected water leakage for circular orifice areas at different pressures. The orifice areas correspond to the effective regurgitant orifice areas from the echocardiographic guidelines.*

Orifice area [mm^2]	**Pressure [mmHg]**	**Leakage [mL/min]**
10	60	2190.70
	70	2260.90
	80	2334.50
19.5	60	4271.87
	70	4408.76
	80	4552.28
30	60	6572.10
	70	6782.70
	80	7003.50

3.2.4 Multiple linear regression analysis

After data pre-processing, data were split into one dependent variable (1x78 values) and four independent variables (4x78 values). Splitting the data into a test and train set led to sizes of 62 for the training set and 16 for the test set. The analysis was carried out, and the intercept values and coefficients were combined in the following equation.

$$Leakage = 1883.36 - 92.74 \cdot shape - 51.85 * viscosity + 141.25 \cdot pressure + 719.74 \cdot area, \quad (3.6)$$

where: $Leakage$ = leakage, mL/min;
$Shape$ = orifice shape, for circular, 1 for triangular and double circular;
$Viscosity$ = fluid viscosity, 0 for water, 1 for milk;
$Pressure$ = tube pressure, mmHg;
$Area$ = orifice area, mm^2.

All coefficients except the one for fluid viscosity showed to have a p-value less than 0.05. The p-value of the fluid viscosity coefficient was 0.062. The model was shown to have a R^2 of 0.984 and a RMSE of 87.85 mL/min.

3.3 Discussion

This experiment evaluated the contribution of different variables in a representative model of the aorta and aortic valve. Tubes, representing the ascending aorta, and lids, representing the aortic valve with nine different orifice areas, were designed and 3D printed. The contribution of the size of the orifice area, the orifice shape, pressure and fluid viscosity to the leakage was independently examined. The aim of this experiment was to identify the variables that should be studied in a second, more physiological *in vitro* experiment.

According to the results, the orifice area had the greatest contribution to the measured leakage. The contribution of the orifice shape and pressure was smaller, but still significant. Regarding the last variable of fluid viscosity, changing the fluid from water to milk did not affect the measured leakage. Based on the findings, the follow-up experiment in cardiac tissue will examine the influence of the orifice area, orifice shape and pressure.

The results of the measured leakage showed to be lower than expected in advance. Based on calculations using fluid dynamics principles, the expected leakage for the circular orifice could be calculated. The expected fluid velocity through the orifice could be calculated using the Bernoulli equation [47]:

$$P + 0.5 \cdot \rho \cdot v^2 + \rho \cdot g \cdot y = constant, \quad (3.7)$$

where: P = pressure, Pa;
ρ = fluid density, kg/m^3;
v = velocity of the leakage, m/s;
g = gravitational force, m/s^2;
y = tube height, m.

The calculated velocity could be converted to the expected flow of the leakage through the orifice, using the area of the orifice:

$$Q = v \cdot A, \quad (3.8)$$

where: Q = leakage flow, m^3/s;
v = leakage velocity, m/s;
A = orifice area, m^2.

The calculations resulted in an expected leakage, which was up to 22% higher than measured in the experiment. The fluid dynamics principles used to calculate the expected leakage, however, assume steady flow and negligible energy losses due to friction, which causes an overestimation of the measured leakage. The behaviour of flow and pressure loss caused by friction forces can be accounted for with the discharge coefficient [48, 49]. This dimensionless coefficient can be determined using:

$$C = Q/(2 \cdot dP/d) \cdot A, \tag{3.9}$$

where: C = coefficient of discharge, -;
Q = leakage, m^3/s;
dP = pressure difference, mmHg;
d = tube diameter, m;
A = orifice area, m^2.

The calculated discharge coefficient varied between 0.85 and 0.95, leading to a decreased discrepancy between the measured and expected leakage of 9%. However, the discharge coefficient has some limitations as well. For example, in turbulent flows, the discharge coefficient may be affected by the turbulence intensity and pressure fluctuations that result from it [50]. Whether flow is turbulent or laminar can be indicated by the Reynolds number. For the settings in this experiment, the Reynolds number was calculated and it was shown to be consistently higher than the cut-off value of 4000 for fully turbulent flow [51]. The remaining discrepancy between the expected and measured flow can partly be explained by this effect and the limitations of this study.

A first limitation of this study concerns the experimental setup. In a test run of the experiment, no pressure was obtained in the tubes with orifice areas of 19.5 and 30 mm^2. As a result, the leakage at these orifice areas had to be approximated based on the fit of the measured leakage of smaller areas. A discrepancy is expected between the calculated leakage based on this fit and the actual leakage. Since the friction contribution increases as the flow increases, the actual leakage for the orifice areas 19.5 and 30 mm^2 will be lower than expected based on the polynomial fit. Second, a relatively large difference was observed between the different orifice shapes of the 5 mm^2 orifice area. This can be explained by the inaccuracy in the orifice shapes resulting from the 3D printing process. For the small orifice areas, resin residue was found in the triangular and double orifices. The double orifice had to be manually reopened, leading to larger orifice areas than originally intended. Therefore, the results of the leakage from the small orifice area should be considered with caution.

To estimate the quantitative amount of leakage of regurgitant aortic valves, this experiment was conducted in a representative model. Another experimental method of estimating the leakage was described by DeGroff and colleagues, who made use of finite element modelling to investigate the isovelocity surface area flow convergence method in AR [52]. Different flow rates and orifice areas were modelled and the regurgitant flow rate was simulated. An overestimation of the regurgitant flow was seen using this method, when compared to echocardiography. For future research, finite element modelling might allow for the quantification of AR while accounting for tissue characteristics and fluid viscosity and other influences.

Following the results, it can be determined which variables should be included in the follow-up experiment. The contribution of the variables is summarised in the results from the linear regression analysis. When all other variables are kept constant, an increase in the variable of interest by one point results in an increase in the outcome as indicated by the coefficient. This means that the influence of the orifice area is nearly seven times higher than the influence of pressure. The analysis also revealed that despite of the higher velocity of milk, no difference was seen between the measurement with water and milk. This can be explained by the fact that fluids with low viscosity, like water and milk, do not have an effect on turbulent flow, which is the case for all settings in this experiment [53]. Therefore, in this experiment, the viscosity does not affect the leakage. It is expected that, based on

viscosity, water and blood show similar results in the quantitative measurement of the repaired aortic valve in a clinical setting.

In conclusion, the measured amount of leakage through an orifice depends on the contribution of the orifice area, orifice shape and pressure. This was evaluated in a 3D printed representation of the aorta and the aortic valve in an *in vitro* experimental setup. In a follow-up experiment, the effects of these parameters will be studied in a more physiological setup with cardiac tissues.

4|Quantifying aortic valve regurgitation in vitro: the analysis of sufficient and insufficient valves

Abstract

Background - Valve-sparing aortic root replacement (VSRR) is rising in popularity for young patients requiring surgery because of chronic or acute aortic regurgitation (AR), aortic dissection or aortic root disease. Quantitative and early intraoperative assessment of the repaired aortic valve after VSRR can identify residual AR. The relationship with the gold standard, transesophageal echocardiography (TEE) after aortic declamping, needs to be made to implement the quantitative method in the VSRR workflow.

Methods - In the present study, we obtained to quantify the leakage of sufficient and insufficient aortic valves under influence of different pressures in an *in vitro* experiment. Insufficiency was induced by a 4 mm diameter punch and by a more physiological approach of leaflet manipulation. The leakage was compared to the parameters from the echocardiographic guidelines.

Results - Leakage of sufficient valves varied from 183.71±0.43 (40 mmHg) to 521.85±1.72 mL/min (80 mmHg) and was mainly caused by graft water permeability and the anastomosis. The maximum water permeability of the 24 and 30 mm diameter grafts were 1.72±0.02 and 3.34±0.03 mL/cm^2/min, respectively. Leakage of an insufficient valve with a punch (area 12.6 mm^2) showed similar results as stated in the echocardiographic guidelines. Leakage resulting from physiological insufficiency was evaluated for five different orifice areas and showed less leakage than expected based on the guidelines.

Conclusion - This study showed the quantification of AR in a static, pressurised *in vitro* experiment. This research contains the first step towards identifying a relationship between the quantitative measurement and echocardiographic assessment to grade the severity of AR after VSRR.

4.1 Background

Aortic valve-sparing root replacement (VSRR) is an appealing alternative to more conventional aortic valve replacement (AVR) in young patients with ascending aortic aneurysm with aortic regurgitation (AR), ascending aortic dissection, or aortic root dilatation with or without AR. Recent studies comparing AVR and VSRR demonstrate that the clinical outcomes of VSRR are superior to AVR in terms of overall survival and valve-related events (thromboembolic events, bleeding, and endocarditis) [12, 13]. Despite the encouraging results, the possibility of reoperation due to recurrent or residual AR is one of the most common complications. A study amongst 764 patients after VSRR reported that after 8 years, 10% of the patients required AVR to treat recurrent AR [16]. Even higher percentages of AVR incidence at 5, 10, and 15 years follow-up of 8.4%, 12.8%, and 17.1%, respectively, are shown by a more recent study [18]. The primary indication for reoperation was severe symptomatic AR in 82% of the patients. However, the underlying pathophysiology of recurrent AR remained unclear.

Intraoperative transesophageal echocardiography (TEE) after aortic declamping is the current gold

standard for assessing the repaired aortic valve after VSRR [19]. However, longer cardiopulmonary bypass times result from the need to repair or replace the valve when the repair is unsatisfactory [21, 22]. A second limitation is that echocardiographic quantification of residual AR can be challenging, as it is time-consuming and requires a high level of expertise [23]. Since the prognostic value of quantitative measurements has been demonstrated to exceed that of semi-quantitative parameters, quantification is essential [26].

As a result of the disadvantages of the current intraoperative assessment method using TEE, the need for quantitative and intraoperative assessment in an earlier stage of the procedure rises. Although several solutions have been proposed, no approach is widely used in the VSRR workflow [54–57]. The aortic valve visualisation and pressurisation (AVP) device is an example of such a device, offering visual and quantitative assessment of the repaired valve in an earlier stage of the procedure [25]. The AVP device allows for additional and direct information on the repaired aortic valve. However, to include the early quantitative assessment of the repaired aortic valve in the VSRR workflow, its diagnostic value needs to be proven. Therefore, the relationship between the current gold standard, TEE after aortic declamping, and the quantitative assessment using the AVP device must be established.

By inducing AR with various effective regurgitant orifice area (EROA) sizes according to the echocardiographic guidelines (Table 5) and measuring the leakage, the relationship between the echocardiographic and quantitative assessment methods can be identified. It is important to consider the contribution of all variables that influence the measured leakage, such as pressure or graft permeability, to the detected leakage. A pilot experiment was performed in which the influence of the orifice area, pressure, orifice shape and fluid viscosity was tested in a three-dimensional (3D) printed representation of the aorta and aortic valve. According to the results of this experiment, the orifice area has the greatest impact on leakage, followed by pressure and orifice shape. In this second experiment, we attempt to quantify the leakage of sufficient and insufficient aortic valves in a static and pressurised situation. First, we identify the contribution of different variables (AVP device connection leakage, graft water permeability and anastomosis leakage) in the sufficient aortic valve state. The influence of induced insufficiency is then studied using a punch hole and a more physiological approach of leaflet manipulation.

Table 5: *Quantitative parameters to grade the severity of aortic regurgitation. Retrieved from the European Society of Cardiology [19].*

AR grade	EROA [mm^2]	Rvol [mL/beat]
Mild	<10	<30
Mild - moderate	10 - 19	30 - 44
Moderate - severe	20 - 29	45 - 59
Severe	>30	>60

AR = aortic regurgitation; EROA = effective regurgitant orifice area; Rvol = regurgitant volume.

4.2 Methods

4.2.1 Preparation and setup

Two porcine hearts (including a part of the ascending aorta) and one aortic homograft were prepared for the experiments. The first porcine heart (subject 1) was prepared by performing VSRR using the reimplantation technique with a 3.0 cm piece 30 mm diameter aortic prosthesis (Hemashield Platinum Double Woven Velour, Getinge AB, Göteborg, Sweden) and 4-0 sutures (Surgipro^{TM} II Monofilament Sutures, Covidien/Medtronic, Minneapolis, USA) [14]. The coronary arteries were closed to prevent washout from the aortic root. In the second porcine heart (subject 2), only the aortic valve and a

part of the ascending aorta (approximately 1 cm supracommissural) were preserved. The coronary arteries were closed again. Using 4-0 sutures, an anastomosis was created with a 2.1 cm piece of 24 mm diameter graft (Hemashield Platinum Double Woven Velour, Getinge AB, Göteborg, Sweden). Lastly, the aortic homograft was prepared by creating an anastomosis between a 1.5 cm piece of 24 mm diameter graft and the aorta (approximately 1 cm supracommissural), again with 4-0 sutures. The homograft was subjected to a two-day experiment and the tissue will be referred to as subject 3a on day 1 and subject 3b on day 2.

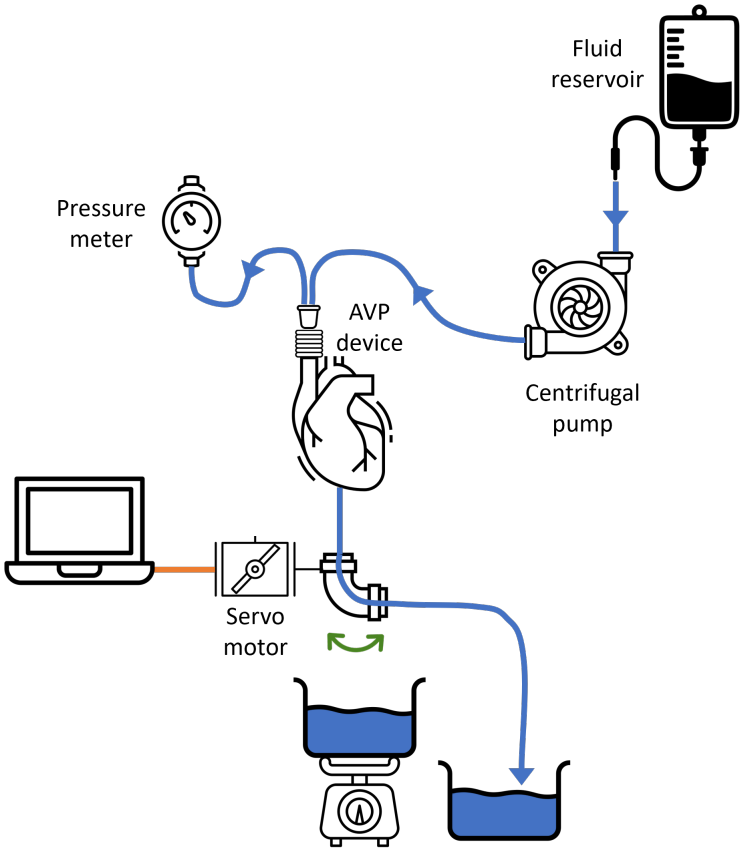


Figure 10: *Schematic visualisation of the experimental setup. Water flows from the reservoir through the centrifugal pump into the aortic root through the aortic valve visualisation and pressurisation (AVP) device. The pressure inside the root is measured using an analogue pressure gauge. The leakage is guided to one of the bins through polyvinyl chloride pipes. The pipes can be rotated using the servo motor to lead the water in the bin on the scale.*

Figure 10 shows the schematic visualisation of the experimental setup. The subject of interest was placed in an open system with a centrifugal pump (iLA Activve, Novalung GmbH, Heilbronn, Germany). The AVP device was attached to the graft using a ring corresponding to the diameter of the graft. The analogue pressure gauge, which was positioned at the same height as the subject of interest, and the pump were both connected to the device. Leakage was collected in a bin on a scale for a predetermined time of 10 seconds using rotating pipes. To visualise the valve and record videos, a 30-degree scope (Visera Elite, Olympus, Tokyo, Japan) was inserted into the AVP device.

4.2.2 Measurement protocol

For each setting, the measurement was carried out in the same approach to obtain the primary outcome of the measured leakage in millilitres per minute (mL/min). When the leakage was constant, the servo

motor was activated to rotate the pipes, which guide the water to the bin on the scale for 10 seconds. This was carried out three times per setting. The full study protocol can be found in Appendix D.

Baseline

AVP device connection leakage - Two thin pieces of rubber were inserted into two parts of aortic graft, one measuring 7.5 cm in length and 30 mm in inner diameter and the other 11 cm in length and 24 mm in inner diameter. Each graft was connected to two AVP devices to create a closed environment. The connectors of one of the AVP devices were sealed off. The pump flow was increased to a root pressure of 40 mmHg and the leakage was evaluated. The measurement was repeated for 50, 60, 70, 80 and 120 mmHg of pressure.

Graft water permeability - In order to evaluate the graft water permeability, the rubber was removed from the grafts and measurements were repeated at the same pressures as described to test leakage from the AVP device connection.

Sufficient state - The aortic root was filled with water and de-aired prior to the measurement. Next, the flow was increased such that a root pressure of 40 mmHg was obtained. The leakage of the three prepared subjects with a sufficient aortic valve was evaluated at pressures of 40 to 80 mmHg, in steps of 10 mmHg. For subject 1, this measurement was only carried out at 60, 70 and 80 mmHg of pressure. The sufficient state measurement for subject 3 was performed on both test days.

Insufficient aortic valve

Punch - Following the baseline measurements, a 4 mm diameter (12.6 mm^2 area) aortic punch (4 mm Aortic Punch, Medtronic, Minneapolis, USA) was used to create an orifice in the centre of one of the aortic cusps in subject 2. The subject was positioned in the setup, root pressure was increased to 40 mmHg and the leakage was measured. The measurement was repeated at pressures of 50, 60 and 70 mmHg. A pressure higher than 70 mmHg could not be obtained. At each pressure, the scope was inserted and videos were recorded.

Leaflet manipulation - Two 7-0 sutures (Surgipro II Monofilament Sutures, Covidien/Medtronic, Minneapolis, USA) were introduced through the nodule of one of the cusps of subject 3. Insufficiency resulted from one of the sutures being pulled and sufficiency was restored by pulling the other suture. The subject was positioned in the setup, and the pressure was increased while pulling the suture to obtain a small insufficiency. The other suture was kept under tension to preserve the orifice area of the insufficiency. Measurements were performed and videos were taken at pressures of 40 to 80 mmHg, in steps of 10 mmHg. Insufficiency was increased and measurements were performed at the same pressures. On the second test day, the experiment was repeated at three levels of insufficiency.

4.2.3 Data analysis

For all measurements, the initial steps of the data analysis were similar. To convert the measured leakage in grams per 10 seconds to the leakage in mL/min, the average of the three measurements was multiplied by 6 and divided by the density of water (0.997 g/mL). The data were presented as mean±standard error. The standard error was calculated using the following formula:

$$SE = 6 \cdot (\sigma/\sqrt{n}), \tag{4.1}$$

where: SE = standard error, mL/min;
σ = standard deviation, mL/min;
n = number of repetitions, 3.

Baseline

AVP device connection leakage - To obtain the leakage of one AVP device connection in mL/min, the measured leakage was divided by two.

Graft water permeability - The leakage of the AVP device connection is subtracted from the measured leakage. The graft water permeability in mL/cm^2/min is calculated by dividing the leakage by the graft area. The following formula is used to determine the area of the graft in cm^2:

$$Graft\ surface = \pi * diameter * height \tag{4.2}$$

Sufficient state - The leakage resulting from the connection of the AVP device and the permeability of the graft is subtracted from the total measured leakage to determine the leakage of the sufficient aortic valve state for each subject.

Insufficient aortic valve

Punch - The measured leakage was subtracted with the sufficient valve state leakage. The estimated regurgitant volume (Rvol) in mL per beat was calculated according to the formula:

$$Rvol = leakage_{80} * diastole/frequency, \tag{4.3}$$

where: $Rvol$ = regurgitant volume, mL/beat;
$Leakage_{80}$ = leakage at 80 mmHg, mL/min;
$Diastole$ = diastolic fraction, -;
$Frequency$ = cardiac frequency, bpm.

Using data from measurements at lower pressures, a polynomial fit was used to estimate the leakage at 80 mmHg. Normal values for the fraction of diastolic duration and the frequency are 0.6 and 60 beats per minute, respectively [58]. The estimated Rvol was compared with the echocardiographic Rvol for the same orifice area.

Leaflet manipulation - Again, the leakage from the sufficient state is subtracted from the measured leakage at each pressure. Using ImageJ (ImageJ, U.S. National Institutes of Health, Maryland, USA), the minimum and maximum areas of insufficiency were measured from the scope images. The diameter of the graft was used as a reference size. The average of the minimum and maximum areas were used for further analysis. In order to estimate the Rvol for the EROA sizes as specified for mild to severe AR in the echocardiographic guidelines, a polynomial fit was performed on the data from the smaller orifice sizes in subject 3b at 80 mmHg of pressure. The estimated Rvol was calculated using Equation 4.3.

4.3 Results

4.3.1 Baseline

The leakage of the sufficient valve state increased with pressure and varied from subject to subject. Subject 1 revealed the least total amount of leakage, up to 183.71±0.43 mL/min at 80 mmHg (Figure 11). The highest total leakage was observed in subject 2, measuring 521.85±1.72 mL/min at 80 mmHg. The leakage resulting from the connection of the AVP device and the graft's water permeability was subtracted from the measured leakage at each pressure. At 120 mmHg, the leakage of the AVP device connection reached a maximum of 4.90±0.58 mL/min. The maximum water permeability values for the 24 and 30 mm diameter grafts were 1.72±0.02 and 3.34±0.03 mL/cm^2/min, respectively.

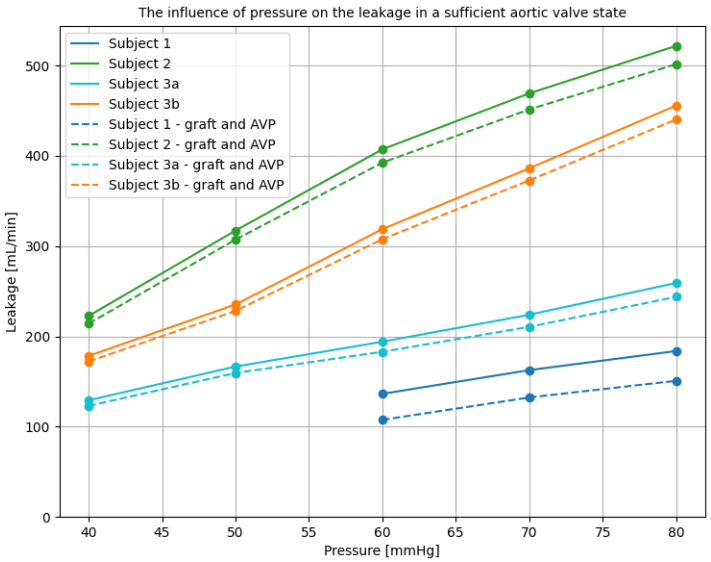


Figure 11: *The leakage of different subjects in the sufficient aortic valve state. The dotted line represents the baseline leakage, where the leakage resulting from the graft permeability and the AVP device is subtracted from the total measured leakage.*
AVP = aortic valve visualisation and pressurisation device.

An example can be given for subject 3a at 60 mmHg, where the total measured leakage was 194.04 mL/min. 2.12 mL/min could be assigned to the leakage resulting from the AVP device connection. The water permeability of the 2.4 cm diameter graft was 0.80 mL/cm^2/min at a pressure of 60 mmHg. The graft had a length of 1.5 cm, and together with a diameter of 2.4 cm, this resulted in an area of 11.3 cm^2. Therefore, a total leakage of 9.04 mL/min could be attributed to the water permeability of the graft. The baseline leakage for subject 3a at 60 mmHg resulted in 182.88 mL/min.

4.3.2 Insufficient aortic valve

Punch insufficiency

The measured leakage of subject 2 with a punched orifice ranged from 2353.06±20.62 at 40 mmHg to 2999.82±8.27 mL/min at 70 mmHg (Figure 12), after subtracting the leakage from the sufficient valve state. The expected leakage at 80 mmHg was calculated using the polynomial:

$$y = 21.44 \cdot x + 1482.57, \tag{4.4}$$

where: y = leakage, mL/min;
x = pressure, mmHg.

This resulted in an expected leakage of 3197.77 mL/min at 80 mmHg, with a root mean square error (RMSE) of 13.78 mL/min. The leakage was converted to an expected Rvol using Equation 4.3 and revealed to be 36.77 mL/beat.

According the echocardiographic guidelines, the EROA and Rvol for mild-moderate AR are 10-19 mm^2 and 30-44 mL/beat, respectively. Following this linear relationship, the Rvol for an EROA of 12.6 mm^2 results in 34 mL/beat, which was about 2 to 3 mL/beat less than expected based on the measurements in subject 2 (Table 6).

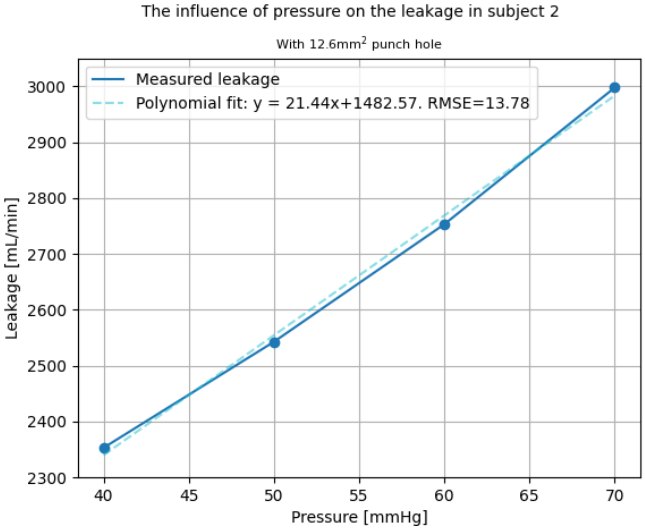


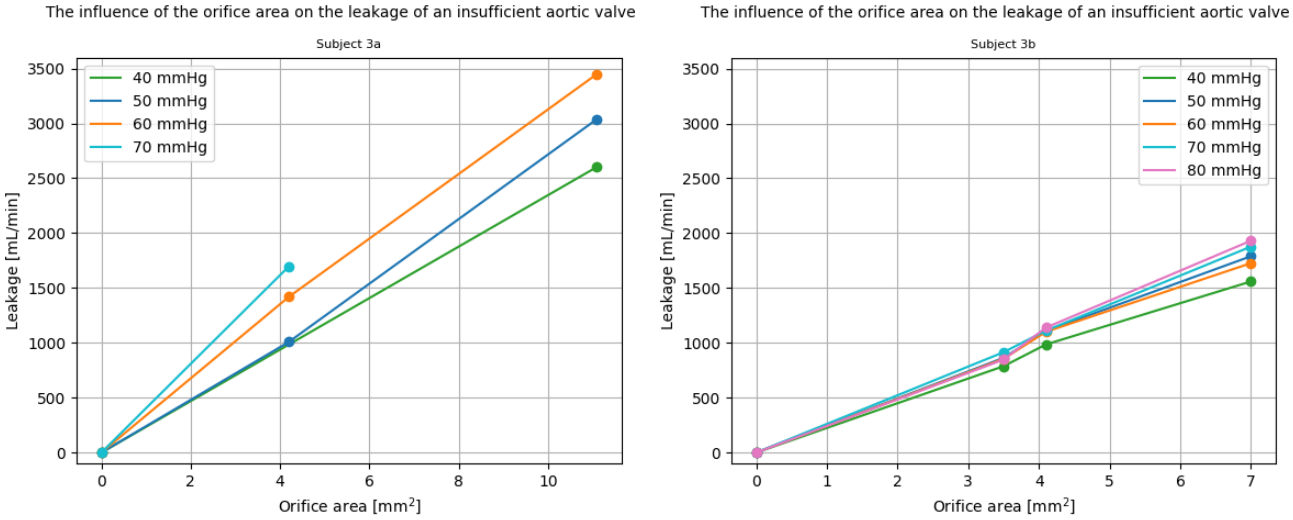
Figure 12: *The influence of pressure on the leakage of an insufficient aortic valve using a 4 mm diameter punch, including the polynomial fit.*
RMSE = root mean square error.

Table 6: *Expected leakage for a 12.6 mm² punch area based on the echocardiographic guidelines and the polynomial fit on the measured leakage [19].*

Orifice area [mm²]	**Expected leakage guidelines [mL/beat]**	**Expected leakage polynomial fit [mL/beat]**
12.6	34	36.77

Physiological insufficiency

Subject 3a was evaluated with a small and large regurgitant area of 4.2±1.2 mm² and 11.1±1.7 mm² (Figure 13a). The leakage for the small orifice area (minus baseline) ranged from 1008.65±15.60 mL/min (50 mmHg) to 1696.96±28.59 mL/min (70 mmHg). For the larger area, the measured leakage ranged from 2603.19±62.21 mL/min (40 mmHg) to 3449.89±64.09 mL/min (60 mmHg).



(a) *Subject 3a* (b) *Subject 3b*

Figure 13: *The influence of orifice area on the leakage for various pressures, in an aortic homograft.*

In subject 3b, three different orifice areas were evaluated: 3.5±0.8, 4.1±0.9 and 7.0±0.9 mm^2 (Figure 13b). The measured leakage varied from 787.83±1.29 mL/min (40 mmHg) to 848.09 ±1.82 mL/min (80 mmHg) for the smallest orifice area. For the medium insufficiency, the leakage ranged from 986.08±7.16 mL/min (40 mmHg) to 1139.48±2.19 mL/min (80 mmHg). Lastly, the largest orifice area revealed a leakage from 1560.02±2.78 mL/min (40 mmHg) to 1931.70±0.67 mL/min (80 mmHg).

The data of subject 3 (Figure 13) were fitted to calculate the expected leakage based on the orifice area for each pressure. The polynomials had an RMSE ranging from 0 to 87.43 mL/min. The expected leakage in mL/min for the EROA sizes as mentioned in the echocardiographic guidelines was calculated using the polynomial fit from subject 3b at 80 mmHg:

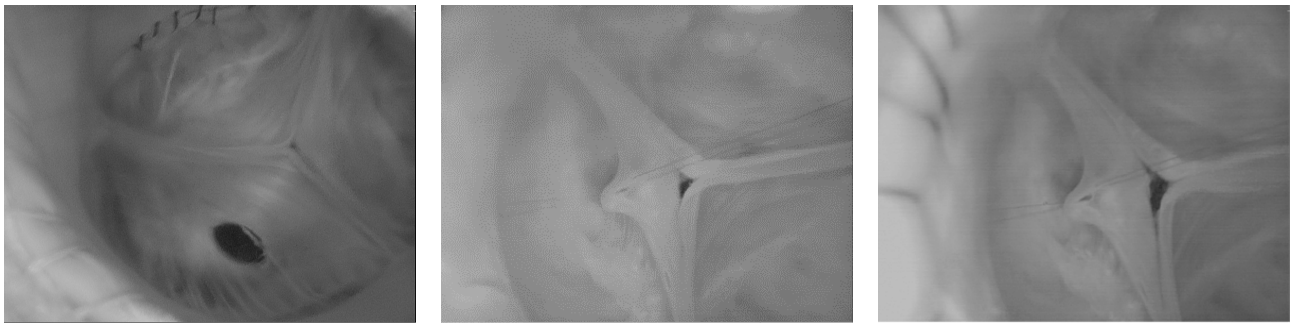
$$Leakage = 276.82 * area, \tag{4.5}$$

where: $Leakage$ = leakage, mL/min;
$Area$ = orifice area, mm^2.

For the EROA's of 10, 19.5 and 30 mm^2 the estimated leakage was revealed to be 2768.20, 4013.89 and 8304.60 mL/min, respectively. This leakage in mL/min was converted to a Rvol of 32, 60 and 95 mL/beat. The results are summarised in Table 7.

Table 7: *Aortic regurgitation classification based on the guidelines and the measured leakage. AR = aortic regurgitation; EROA = effective regurgitant orifice area; Rvol = regurgitant volume.*

AR grade	EROA [mm^2]	Rvol [mL/beat]	Estimated Rvol [mL/beat]
Mild	<10	<30	<32
Mild - moderate	10 - 19	30 - 44	32 - 60
Moderate - severe	20 - 29	45 - 59	61 - 95
Severe	>30	>60	>95



(a) *Subject 2 with a punched valve (50 mmHg)*

(b) *Subject 3b with medium insufficiency (40 mmHg)*

(c) *Subject 3b with large insufficiency (50 mmHg)*

Figure 14: *Scope images of insufficient aortic valves induced by different methods and with different root pressures.*

4.4 Discussion

The present study demonstrated the contribution of several variables to the leakage of sufficient and insufficient aortic valves, to quantify residual AR of a repaired valve after VSRR. First, in a sufficient aortic valve state, the contribution of the AVP device leakage, the leakage resulting from the graft permeability and the sufficient valve state was studied. Next, insufficiency was induced by creating orifices with the use of a punch and by a more physiological method, where the leaflets were manipulated. The leakage of insufficient valves was measured and compared with the echocardiographic

guidelines for the same orifice area.

According to the results, the graft water permeability and the sufficient valve state leakage showed to contribute to the baseline leakage. The graft water permeability was shown to be consistent with the manufacturer's data, which claim a permeability of less than 5 mL/cm^2/min. The resulting leakage in the sufficient valve state can be contributed to the anastomosis leakage. In insufficient aortic valves, the leakage is nearly entirely determined by the size of the insufficiency. The punched aortic valve leakage follows the echocardiographic parameters for that area. The leakage resulting from a more physiological insufficiency correspond to the guidelines for only the EROA of mild AR.

To include the early and quantitative assessment in the current VSRR workflow, the relationship with echocardiography needs to be identified. Regarding the echocardiographic assessment, it is shown that the diagnostic value of the quantitative parameters is higher than that of semi-quantitative parameters [26]. However, the application of the proximal isovelocity surface area (PISA) method used to calculate the quantitative parameters has some limitations [20, 59]. The fluid dynamics principles used in this method assume, amongst others, a hemispheric flow convergence zone, a circular orifice, a central regurgitant jet and laminar flow [27, 36, 37]. Regarding this last assumption, even in a healthy patient, the aorta can be subject to turbulent blood flow [60]. Patients with AR exhibit a higher incidence of complex and swirling flows, which are indicative of turbulent blood flow, compared to healthy individuals [61].

Second, in an insufficient aortic valve, it is probable that the orifice shape is non-circular. When the orifice is non-circular, the impact of frictional forces becomes more significant. In this experiment, the leakage of an insufficient aortic valve was assessed in two different orifice shapes. It was observed that the circular orifice conformed to the echocardiographic guidelines, whereas the non-circular orifice produced different outcomes. Because of the non-circular orifice and the likelihood of turbulent flow, it can be stated that there is a high probability that the quantitative evaluation of AR using echocardiography overestimates the severity of AR. The establishment of a relationship between echocardiography and the quantitative amount of leakage may lead to a more accurate grading of the severity of AR.

The experimental findings showed that the estimated Rvol for moderate and severe AR grades is significantly higher than what is expected based on echocardiographic guidelines. This difference can be attributed to two factors. First, in large insufficiency areas, it was not possible to obtain pressure in the aortic root. Consequently, the leakage in the orifice areas that correspond to the echocardiography guidelines needed to be estimated based on data from smaller areas. Since friction contribution increases as flow increases, the actual leakage for the orifice areas 19.5 and 30 mm^2 will be lower than expected based on this estimation. Consequently, there is likely to be a discrepancy between the calculated leakage based on the polynomial fit and the actual leakage, particularly for more severe AR grades.

In addition, to convert the leakage in mL/min from the polynomial fit to the Rvol in mL/beat, assumptions had to be made regarding the diastolic duration and cardiac frequency. It was assumed that the regurgitant jet remains constant in size throughout the entire diastolic duration. However, as the heart is in a dynamic state, there is a build-up of pressure which results in an overestimation of the expected Rvol based on the polynomial fit. Nevertheless, this assumption is also made in echocardiographic measurements, where the grade of AR is determined based on a static view during the maximum diastolic opening of the valve [19].

The present experiment was conducted under static and pressurised conditions, which were found to be difficult to maintain and proved to be unstable, particularly at high flow rates. This raises the question of whether this static condition is representative of the physiologically dynamic aortic valve. In a previous study by Steindl and colleagues, AR was induced in porcine hearts and echocardiographic

assessment was performed under static and dynamic conditions [56]. The results showed similar regurgitant jet existence and morphology in both situations. Therefore, it is believed that the static measurements obtained after VSRR using the AVP device can be related to the dynamic situation in which echocardiographic assessment is performed.

Another limitation of this study is that not all variables influencing the leakage of the sufficient and insufficient aortic valve were assessed, such as the influence of suture holes. Previous studies have shown that the leakage from a single 5-0 mono-filament suture hole is 1.93 mL/min (at 120/80 mmHg pulsatile flow) [62]. The contribution of the suture hole leakage to the leakage of an insufficient valve is expected to be negligible.

In this experiment, the AVP device was used to quantify the leakage of an insufficient aortic valve. Other methods and devices to perform intraoperative assessment after VSRR have been described [54–57, 63]. Okita and colleagues described a method using a flexible scope to visualise the aortic valve from the left ventricle, enabling visualisation of the AR jet if it is present [55]. In a study by Ikeno and colleagues, the competence of the repaired valve can be estimated by assessing the rate of pressurisation of the aortic root, the so-called mean pressure build-up [63]. A low pressure buildup suggests a poorly repaired aortic valve. In contrast, the AVP device used in this study provides both quantitative and visual information regarding the pressurised repaired valve.

By incorporating early quantitative assessment of the repaired valve in the VSRR workflow, the understanding of residual AR after VSRR might be improved. The use of a device such as the AVP can provide direct and additional quantitative information about the repaired valve in an early stage of the procedure. In contrast, echocardiography assesses the repaired valve using an indirect projection. If the relationship between the actual amount of leakage and the echocardiographic grading is established, the interpretation of the quantitative amount of leakage would be improved. This could result in a more accurate classification of the grade of AR into more sub-categories since additional information is provided with direct measurement. This enables improved intraoperative decision making and therefore increased clinical outcomes of VSRR procedures.

In conclusion, this research investigated the contribution of several variables to the leakage of sufficient and insufficient aortic valves in a static, pressurised *in vitro* experimental setup. This is the first step towards identifying the relationship between the intraoperative and quantitative assessment of the leakage of an insufficient aortic valve and the post-declamping echocardiographic assessment after VSRR.

5|Summary and concluding discussion

This thesis sought to make the first step in determining the relationship between quantitative and intraoperative assessment of repaired aortic valve leakage in millilitres per minute and the grading of aortic regurgitation (AR) assessed by transesophageal echocardiography (TEE) after valve-sparing root replacement (VSRR). The effective regurgitant orifice area (EROA) was found to be the optimal echocardiographic parameter to implement in the experimental setup, where it was represented as the orifice area. In addition to the orifice area, the contribution of three other variables to the measured leakage was studied in a custom representation of the aorta and the aortic valve. The other parameters included the aortic root pressure, orifice shape and fluid viscosity. In a second experiment, the contribution of the orifice area, orifice shape and pressure was evaluated in sufficient and insufficient aortic valves in cardiac tissues, with the use of the aortic valve visualisation and pressurisation (AVP) device. Furthermore, the contribution of graft water permeability, AVP device connection leakage and anastomosis leakage was assessed in the second experiment.

The first experiment in a custom representation of the aorta and the insufficient aortic valve made it clear that the orifice area has the greatest influence on the measured leakage. Additionally, the orifice's shape and pressure both contribute to the outcome. The findings of the second experiment demonstrated how anastomosis leakage and graft water permeability influence the leakage in a sufficient aortic valve state. The influence of variables could be disregarded when compared to the leakage of an insufficient aortic valve, where the leakage was almost entirely determined by the area of the regurgitant orifice. The punched aortic valve leakage follows the echocardiographic parameters of that area. The leakage resulting from a more physiological insufficiency correspond to the guidelines for only the EROA of mild AR.

According to the findings of the study, it is possible to quantify the contribution of each of the variables to the total amount of leakage in sufficient and insufficient aortic valves. As a result, the grade of AR can be roughly identified for small orifice areas in a static, pressurised environment. This is the first step in obtaining the relationship between the quantitative amount of leakage in millilitres per minute and echocardiography, to improve intraoperative assessment of the repaired aortic valve after VSRR.

When we compare the results of the two experiments in more detail, a discrepancy can be seen. In general, a higher leakage is observed in the first experiment for the same measured orifice area as in the second experiment. The measurement of the orifice area in the second experiment is, however, subjective to a significant level of uncertainty. The orifice area was measured based on the two-dimensional scope image of the insufficient aortic valve. Taking into account the anatomy of the valve, a larger orifice area is expected. In echocardiography, the assessment of the EROA is also considerably more valuable with three-dimensional imaging, since it allows for measurement of the exact shape and size by an enhanced plane orientation of the aortic valve [64]. As a result, it is anticipated that the measured area from the scope images will be smaller than the actual orifice area.

To identify the relationship between echocardiography and the quantitative assessment using the AVP device, the experimental setup has to be enhanced. The setup should enable quantitative and echocardiographic assessment of the orifice area of insufficient aortic valves. A stable insufficiency with

a known orifice area should be induced and quantitative assessment should be performed. Additionally, the EROA should be assessed using echocardiography. The echocardiographic assessment of the EROA demands a pulsatile flow with an echo-enabling fluid. An example of such a setup is the Cardiac Biosimulator (LifeTec Group, Inc, Eindhoven, The Netherlands), which is an *ex vivo* beating heart simulator that can be used to perform valve surgery with real-time echocardiographic imaging and videoscopic vision [65]. With the use of a similar setup, it is possible to quantify AR using the AVP device in a static state and evaluate it using real-time echocardiography in a dynamic state, in order to establish the relationship between the two assessment methods.

Second, it is important to collect intraoperative and quantitative data on the outcomes of VSRR. The visual and quantitative evaluation obtained using the AVP device should be compared with post-aortic declamping TEE data. This approach enhances the understanding of the quality of the repaired aortic valve after VSRR and provides valuable information about the clinical significance of the measured leakage, as the grade of AR can be monitored during patient follow-up.

Incorporating early quantitative measurement of the repaired valve into the VSRR workflow will improve intraoperative assessment and decision making. Measurements with a device such as the AVP device offer additional information regarding the repaired valve when compared to echocardiographic assessment. It allows for direct visualisation of the valve and quantifies the total leakage, thereby providing information about variables such as graft permeability. In contrast, in echocardiography, only the valve morphology and the regurgitation itself can be assessed using an indirect projection of the insufficient valve. When all contributions to the measured leakage and the relationship with echocardiography are known, it will be possible to accurately differentiate between the grades of AR, while additional information about the nature of the regurgitation is provided. This results in improved decision-making during VSRR procedures and subsequently enhances clinical outcomes.

In conclusion, the contribution of different variables to the leakage of sufficient and insufficient aortic valves was investigated in two *in vitro* experiments. This research includes a first step to determine the relationship between the intraoperative and quantitative assessment of the leakage of an insufficient aortic valve and the post-declamping echocardiographic assessment after VSRR.

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A|Prospective impact analysis

Impact analysis variables of influence	
Author: Laura Koopman	
The goal of this analysis is to find the variables of influence on valve assessment using the AVP device.	
The exact influence of these variables can be tested in an experimental environment based on the results.	
Procedure	Valve-sparing aortic root replacement
Location	CVIC LUMC
Disciplines	Perfusionist
	(thoracic)anesthesist
	Thoracic surgeon
Devices	Anesthesia: ventilator, transesophageal echo probe, medication pumps
	Surgical: small tools, AVP device, aspirator, etc.
	Perfusionist: ECMO device
Present	Surgery table: 3 surgeons and 1 assistent
	Perfusionist
	Surgery assistent
	Thoracic anesthesist
	Nurse anesthesist
Explanation contents	
Steps	0: baseline situation
	1: connecting the AVP device
	2: running the fluid
	3: measure the pressurized root
	4: disconnect the AVP device
Situation	Detailed description of the step
Variables	The variable with possible influence on the amount of leakage
Influence	The way in which this variable can influence the procedure.
Probability	Improbable/remote/occasional/probable/frequent -> 6/7/8/9/10
Impact	Insignificant/measurable/medium/significant/severe -> 6/7/8/9/10
Risk score	Probability times severity
Consequence	Will the variable be tested in an experimental environment?
Translation	How will this variable be tested?
Validation	How is measured if this variable is taken into account and if the influence can be measured?

Figure 1: *General introduction and explanation impact analysis of the variables of influence.*

Step 0: baseline situation before the usage of the AVP device

Author	Laura Koopman
Date	25-2-2023

Description of situation

The valve-sparing aortic root replacement procedure is performed.
The patient is on complete cardiopulmonary bypass and the heart is not beating.
The quality of the repair of the valve can be assessed from this situation
Note: the variables of influence in this baseline situation, affect all the other steps as well.

Variable of influence	Method of influence	Probability	Impact	Impact score
Temperature OR	Lower than body temperature	Frequent	Insignificant	Low
Temperature patient	Lower than physiological state	Frequent	Insignificant	Low
Workspace	Small	Probable	Measurable	Low
Hemodynamic stability patient	Lower than physiological state	Frequent	Insignificant	Low
Breath movement patient	Absent	Probable	Insignificant	Low
Dynamics heart	Absent	Frequent	Insignificant	Low
Filling status heart	Lower	Frequent	Medium	Very high
Angle aortic root vs valve	Different angle than physiological	Frequent	Insignificant	Low
Position patient	Horizontally	Frequent	Insignificant	Low
Pressure by esophagus	Higher because of TEE	Frequent	Insignificant	Low
Blood clotting	Disrupted because of medication and temperature	Frequent	Insignificant	Low
Alarms	Distracting for surgeon	Probable	Insignificant	Low

(a) *Step 0.*

Step 1: Connection of the AVP device to the graft

Author	Laura Koopman
Date	25/02/2023

Description of situation

The bottom of the AVP device is inserted in the graft as far as possible.
The device is attached to the graft using a ring corresponding the graft size.
The perfusion tube is connected to the AVP device.
The graft and the device are held by the surgeon/assistent.

Variable of influence	Method of influence	Probability	Impact	Impact score
Diameter of graft	Bigger than physiological	Probable	Insignificant	Low
Diameter of graft	Smaller than physiolocal	Probable	Insignificant	Low
Permeability of the graft	Higher	Frequent	Measurable	High
Wall thickness of graft	Harder to attach to device	Remote	Insignificant	Very low
Large ring	Harder to attach device	Occasional	Significant	High
Small ring	Harder to attach device	Occasional	Significant	High
Attachment of ring device	Not firm enough	Occasional	Severe	Very high
Position of graft and device	Unsteady	Probable	Measurable	Low
Device too shallow in graft	Incorrect attachment	Occasional	Significant	High
Device too deep in graft	Graft breaks	Improbable	Severe	Low
No firm attachment tube to device	Tube releases	Remote	Significant	Low
Too firm attachtment tube to device	Hard to release	Improbable	Insignificant	Very low
Material of AVP device	Porous/contains a hole	Improbable	Significant	Low

(b) *Step 1.*

Figure 2: *Steps 0 and 1 in the impact analysis of the variables of influence.*

Step 2: Fluid pressurization in the aortic root

Author	Laura Koopman
Date	25/02/2023

Description of situation

A pump with clear cardioplegia is started and runs through the tube to the patient.
The fluid is flowing into the aortic root through an opening in the AVP device.
The pressure in the aortic root builds up to a pressure of 80 mmHg
The flow is turned down so a constant pressure is obtained.

Variable of influence	Method of influence	Probability	Severity	Risk score
Low fluid viscosity	More leakage than compared to blood	Occasional	Significant	High
High fluid flow	Pressure rises uncontrollable	Occasional	Medium	Low
Low fluid flow	Takes too much time	Probable	Measurable	Low
Large diameter perfusion tube	High flow	Remote	Measurable	Very low
High length tube	Takes too much time	Remote	Insignificant	Very low
High resistance tube	High pressure	Improbable	Significant	Low
Position of graft compared to device	Not 0 degrees	Remote	Medium	Low
Flow of cardioplegia	Continuous instead of pulsatile	Improbable	Significant	Low
A lot of fluid in thorax	Limits flow	Remote	Medium	Low
Breathing movement patient	Off	Frequent	Insignificant	Low
Bad repair quality of the valve	80 mmHg not obtained	Occasional	Severe	Very high
Suture holes at valve level	Leakage when big	Occasional	Significant	High
Suture holes at annulus level	Leakage when big	Occasional	Significant	High
Ring of AVP device	Leakage when too lose	Remote	Severe	High
Traction on device	too much	Remote	Medium	Low
Error in pressure measurement	Inaccurate	Improbable	Severe	Low

(a) *Step 2.*

Step 3: Assessment of the valve in the pressurized root

Author	Laura Koopman
Date	25/02/2023

Description of situation

The pressure created by the cardioplegia in the aortic root is constant at a level of 80 mmHg.
The leaking fluid is measured based on the flow from the perfusion pump and the properties of the tube.
This situation is maintained for at least one minute and the leakage is measured.
At the same time, a scope is inserted in the AVP device.
The scope is used to assess the repair quality of the valve based on coaptation height, symmetry and insufficiency.

NB: The tube properties, fluid properties, device properties, pump properties are included in step 2 and their influence is not included in step 3.

Variable of influence	Method of influence	Probability	Severity	Risk score
Too high fluid flow	Inconsistent pressure	Remote	Significant	Low
Repair quality of the valve	Coaptation height, symmetry, insufficiency by eye	Remote	Severe	High
Too high pressure in the root	More leakage	Occasional	Severe	Very high
Too low pressure in the root	Less leakage	Occasional	Severe	Very high
Pressure applied by the scope on the device	Unstable measurement	Occasional	Medium	Low
Movement because of the scope	Unstable measurement	Occasional	Medium	Low

(b) *Step 3.*

Figure 3: *Steps 2 and 3 in the impact analysis of the variables of influence.*

Step 4: Disconnection of the AVP device

Author	Laura Koopman
Date	25/02/2023

Description of situation

This step does not influence the amount of leakage, but is described to give the full image of the procedure.
The fluid flow is stopped and the tube is uncoupled from the AVP device.
The suture is cut from the graft and the AVP device.
The device is taken out of the graft.
Potential additional valve repair is performed and the assessment is repeated.

Variable of influence	Method of influence	Probability	Severity	Risk score
Suture is cut too hard	Graft is damaged	Improbable	Severe	Low
The device is pulled out too hard	Any of the sutures is damages	Improbable	Severe	Low

(a) *Step 4.*

Results of the impact analysis of the influence of variables on the measured leakage

Variable	Method	Score	Category
	Step 0		
Filling status left ventricle	If the left ventricle is filled, pressure difference is lower, leading to lower measured leakage	80	Pressure
	Step 1		
Graft permeability	Higher graft permeability results in higher leakage	70	Graft
Device ring too large	Device not correct attached, higher leakage	72	AVP device
Device ring too small	Device not correct attached, higher leakage	72	AVP device
Device not firm attached	Device not correct attached, higher leakage	80	AVP device
Device too shallow	Device not correct attached, higher leakage	72	AVP device
	Step 2		
Low fluid viscosity	Viscosity of water is lower than blood, leading to higher leakage	72	Fluid
Incompetent valve	The valve is incompetent, so no pressure can be build. Leakage is very high.	80	Repair
Sutures commissure level	More suture holes result in more leakage	72	Anastomosis
Sutures annulus level	More suture holes result in more leakage	72	Anastomosis
AVP device ring loose	Device not correct attached, higher leakage	70	AVP device
	Step 3		
Incompetent valve	Higher leakage in an incompetent repaired valve	70	Repair
Too high root pressure	More leakage when the pressure is too high	80	Pressure
Too low root pressure	Less leakage when the pressure is too low.	80	Pressure

Variable	Method to test	Values	Experiment
Repair	Orifice area	0, 10, 19, 30 mm2	1 and 2
Pressure	Root pressure	60, 70, 80 mmHg	1 and 2
Fluid	Water or milk	0.9, 2.1 mPas	1
Graft	Graft permeability	-	2
Anastomosis	Baseline leakage	-	2
Device	Incorrect ring, not tight	Correct, incorrect	2

(b) *Results.*

Figure 4: *Step 4 and the results of the impact analysis of the variables of influence.*

B Technical drawings representation experiment 1

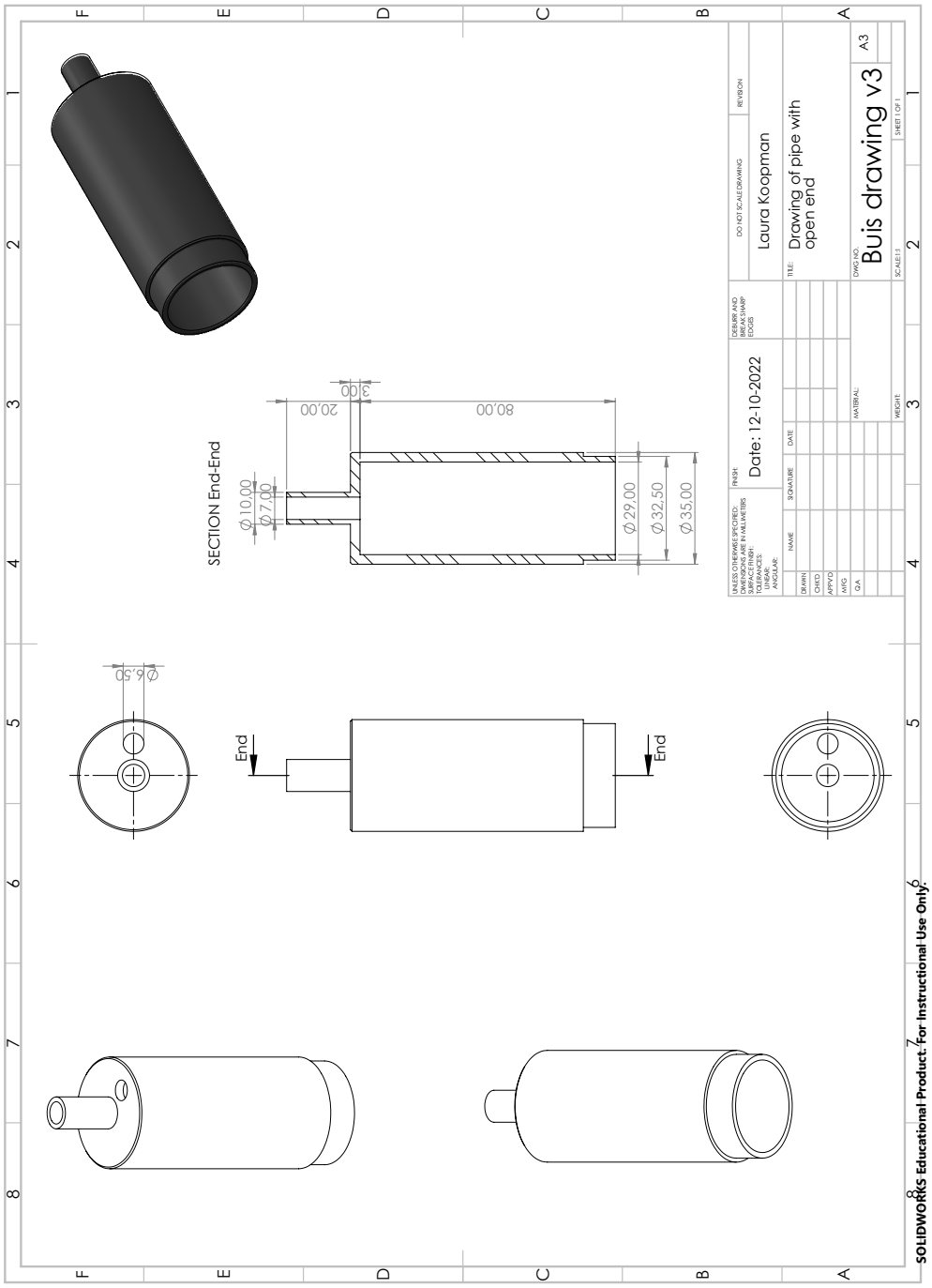


Figure 1: *Technical drawing of the tube.*

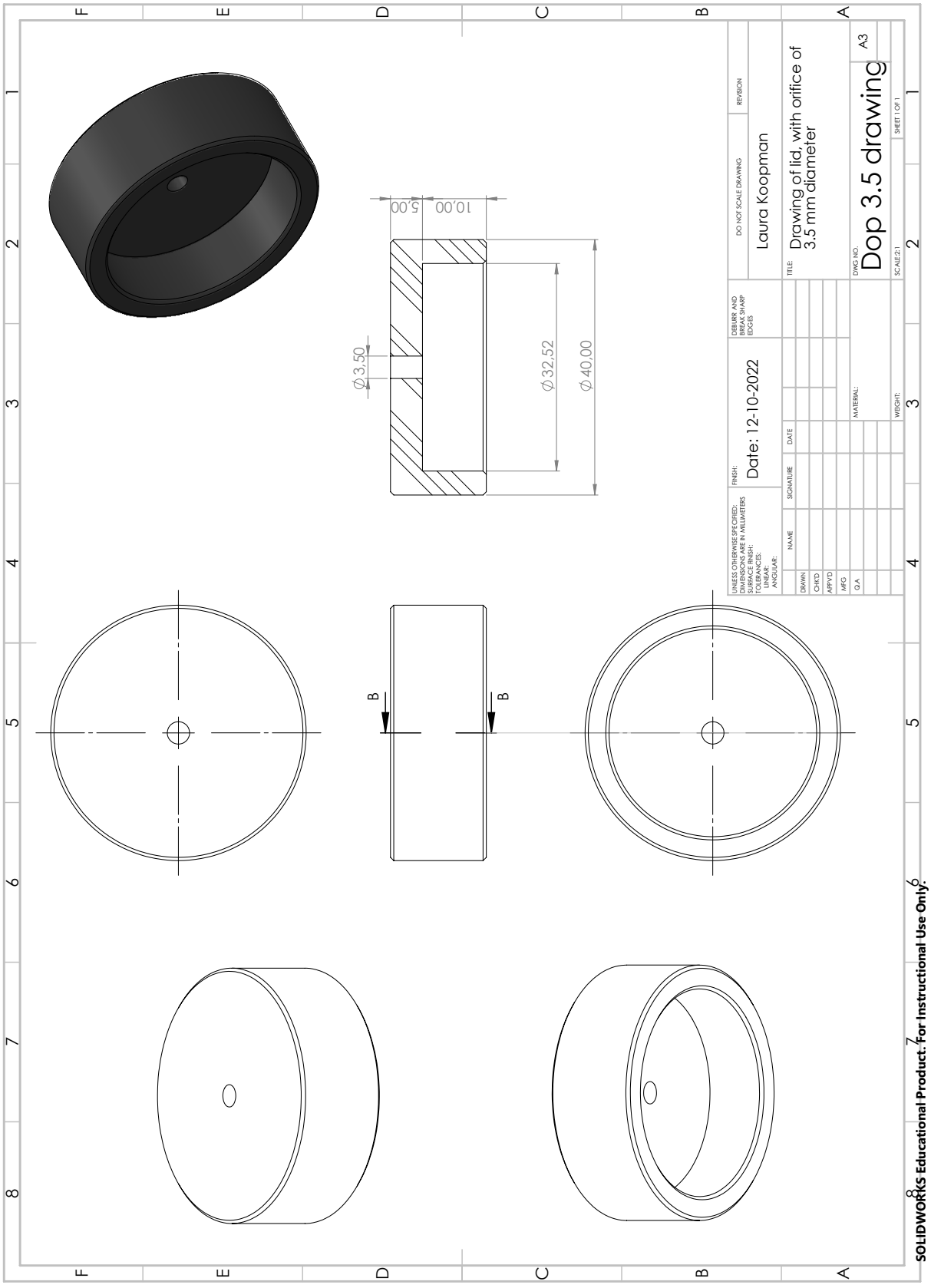


Figure 2: *Technical drawing of the circular orifice with 10 mm^2 area.*

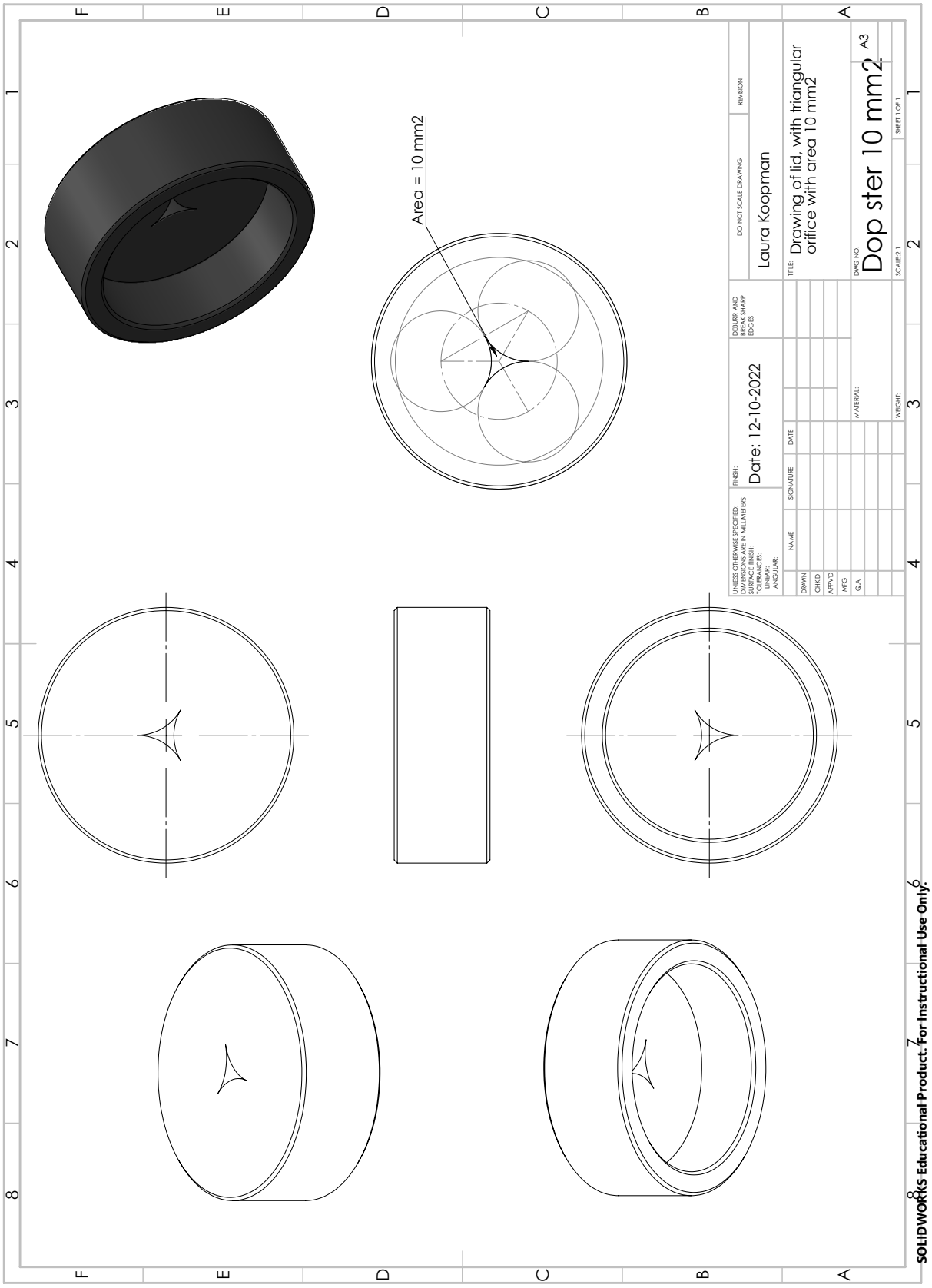


Figure 3: *Technical drawing of the triangular orifice with 10 mm^2 area.*

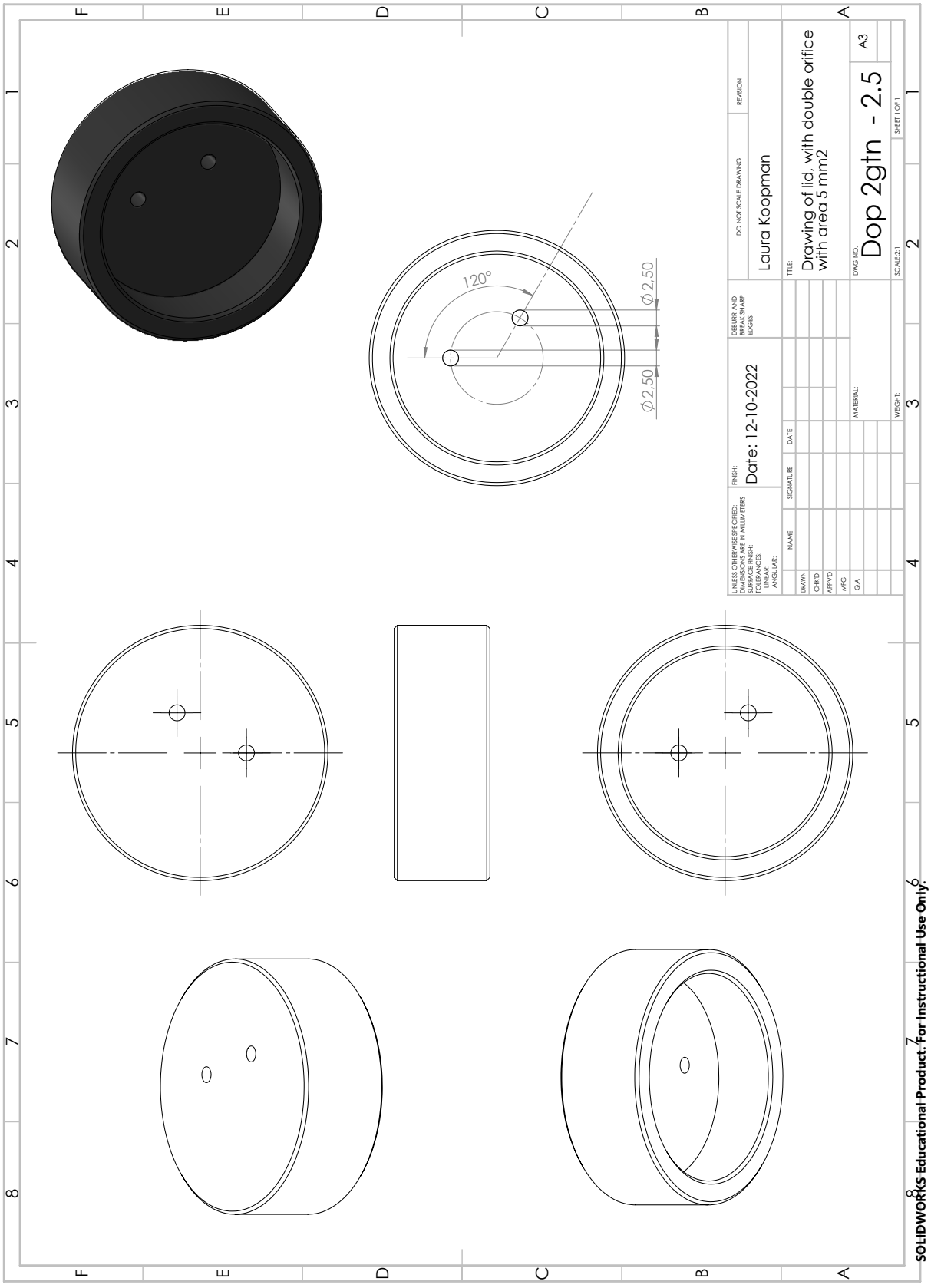


Figure 4: *Technical drawing of the non-central double circular orifice with 5 mm^2 area.*

C|Protocol experiment 1

Experiment setup leakage part 1

Location: LUMC Medical Technology, C0
Date: October 2022

Background

To quantify the amount of leakage of an insufficient aortic valve, a relation between the leakage in [mL/min] and the EROA (effective regurgitant orifice area) measured with echocardiography is made. The EROA represents the size of the orifice in the insufficient aortic valve in mm^2. Two experiments are set up to study this relation and how various factors, like the pressure or the orifice's shape, affect it. This is carried out in the first experiment using a simplified model with a pipe with an orifice representing the aorta with the insufficient valve. In the second experiment, a porcine heart will be used to test this relation.

The goal of this first experiment is to study the influence of different variables on the total amount of leakage through an orifice in millilitres per minute. The tested variables are:

- Orifice area → 5, 10 and 14.5 mm^2
- Orifice shape → round, triangular or double
- Pressure → 40 to 80 mmHg
- Fluid viscosity level → water and milk*

The influence of the variables will be tested in a full factorial design of experiment.

Hypothesis

Hypotheses are described for the influence of the four variables mentioned above:

The amount of leakage will increase when the orifice size and pressure are increased.

The amount of leakage will not significantly change when the orifice shape is changed.

The amount of leakage will decrease when the fluid viscosity is increased.

* Milk and blood have a similar value for dynamic viscosity

Required hardware

Table 1: Required hardware

Present?	What	Notes
X	NovaLung iLA active infusion pump with console and trolley	Centrifugal pump with flow up to 10000 rpm
X	Pressure meter	Analogue, with membrane
X	10 3D printed pipes with different orifices	See SolidWorks drawings 1 closed, 9 different holes See Table 3
X	Fluid reservoir	4 L
X	Tubes and connectors	3/8 inch tubes 3x 15 cm ¼ inch tubes: 1x 150 cm, 1x 20 cm, 1x 5cm 3/8 to ¼ inch adapter 2 female-female adapters 3 perfusion adapters
X	Funnel	
X	Stainless steel clamp	ECMO
X	Syringe	
X	Scale	Measures in mg, accuracy 0.01 g
X	2 bins for leaking water	At least 4 L
X	Plastic shield	
X	Towels	
X	Tap water	
X	Full fat UHT Milk	At least 4 L
X	Laptop, pen and paper	
X	Camera	Mobile phone
X	Tube holder	Borrow from Medical Technology
X	Servo motor 180 degrees	Order online[1]
X	2 Drain-pipes	Gamma[2]
X	Hook, bolt and nut	To connect pipe and motor[3]
X	Arduino, Grove shield and wires	Borrow from Medical Technology
X	Tape	Gamma

[1] https://www.otronic.nl/a-64574091/servos/servo-mg996r-180-graden/

[2] https://www.gamma.nl/assortiment/martens-bocht-45-pvc-grijs-2x-lijmverbinding-32x32-mm/p/B302014

[3] https://www.gamma.nl/assortiment/versterkingshoek-verzinkt-30x30-mm-4-stuks/p/B105165

Figures 1-4: Female-female adapter [4], male-male adapter [4], perfusion adapter [5] , 3/8 to ¼ inch adapter[6]

Figure 4: 4 L fluid reservoir [7]

Figure 5:Pressure dome [8]

[4] https://www.argonmedical.com/products/monitoring-accessories
[5] https://iremedy.com/medline-DPL10007
[6] https://europe.medtronic.com/xd-en/healthcare-professionals/products/cardiovascular/cardiopulmonary/custom-packs.html
[7] https://www.terumocv.com/products/ProductDetail.aspx?groupId=1103&familyID=6&country=1
[8] Personal photo

Figures 5 and 6: The NovaLung iLa activve pump and monitor [9] [10]

Table 2: 3D printed lids

Orifice area(mm^2)	Orifice shape	Lid number
0	-	0
5	Round	1
5	Triangular	2
5 (2*2,5)	Double	3
10	Round	4
10	Triangular	5
10 (2*5)	Double	6
14.5	Round	7
14.5	Triangular	8
14.5 (2*7,25)	Double	9

Required software

- Excel file "*Results experiment 1"*. This file also shows the order of the experiments.
- Arduino file *"Servo motor experiment 1"* to control servo motor

[9] https://www.inspiration-healthcare.com/downloads/brochure-85.pdf

Experiment setup

A. Before setup

1. Create setup with motor (see Figure 10 for total setup)
   To improve the accuracy and lower the random error, a setup is created to automatically transfer the amount of leakage in 10 seconds to the bin placed on the scale. The rest of the time, the leakage is collected in the other bin.
   a. Connect the plastic part (delivered with the motor) to the servo motor

Figure 7: Servo motor with plastic parts [11]

   b. Using a small bolt and nut, connect the metal hook to the plastic part and make sure the connection is solid.
   c. Create a hole in the pipe, at the outer side, a couple of cm from the edge

Figure 8: Drain pipe. Red circle indicates location of the hole for connection to the metal hook.[2]

   d. Using a bolt and nut, connect the pipe to the metal hook.
   e. Connect another pipe to the first pipe to create a greater distance for the flow.
   f. Place the Arduino in the Grove shield and connect it to the computer
   g. Connect the servo motor to the Grove shield with the Arduino at pin D6.
   h. Place start/stop button on pin D4.
   i. Connect the Arduino to the laptop and open the file “Servo motor experiment 1”

[11] https://www.az-delivery.de/nl/products/az-delivery-servo-mg995

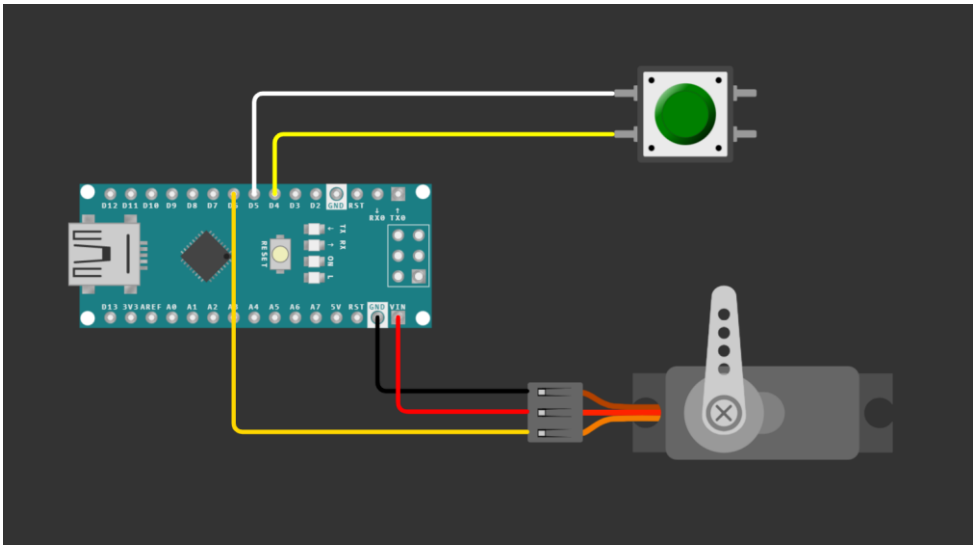


Figure 9: Arduino setup without grove shield

Figure 10a and b: Drain pipes connected to the metal hook and to the servo motor

B. Create setup

1. Pipe and pressure system
    a. Place the pipe in line with the holder at the edge of the table, as low as possible.
    b. Connect the perfusion adapter to the hole of the pipe
    c. Connect 20 cm of ¼ inch tube to the adapter
    d. Connect the other side of the tube to the pressure dome
        i. Make sure the membrane in the pressure dome is pulled to the side of the pipe
    e. Connect the pressure dome to the analogue pressure meter using a male-male adapter
    f. Place the pressure dome at the same height as the orifice of the pipe, in the second laboratory holder

    g. At the protruding part of the pipe, connect the 5 cm ¼ inch part of a perfusion tube
    h. Connect a perfusion adapter to the tube

i. Connect the adapter to the 150 cm long perfusion tube using a female-female adapter. Make sure the tube does not touch the bin on the scale to prevent incorrect measurements.
j. Using a 3/8 to ¼ inch adapter, connect the tube to the tube on the Novalung centrifugal pump
k. Using a 20 cm long 3/8 inch tube, connect the pump to the fluid reservoir

l. Fill the reservoir with **4L tap water**
m. Put the pump on power supply and turn it on
n. Start the pump by turning off the priming mode and clicking the button 'pump'
    i. Click on the arrows to skip through the menu
o. Fill the whole system with water increasing the pump. Release the air from the tubes, lower the pump to 0 rpm and place a clamp on the 150 cm long tube.
    i. Hold the pipe with the orifice upside down while filling it, to release the air.
p. Tare the scale

2. Bins
    a. Place the bin on the scale, right under the orifice of the pipe
    b. Place the other bin on the left next to the first bin
    c. Place the plastic shield between the bins and cover it with a towel (to catch leaking water drops)
    d. Put the scale on power supply, turn it on and tare it

3. Servo motor system
    a. Attach the servo motor with the pipe to the bottom of the table
    b. Place the laptop and the Arduino on the table, as far away from the rest of the setup as possible to prevent water damage.
    c. Open the Arduino file *"Servo motor experiment 1"* and run it to test if the pipe is able to make the turn
        i. Make sure the right Arduino is selected on the right port
        ii. Select for board: *Arduino nano every*
4. Open the excel file *"Results experiment 1"* on the laptop
5. Make photos of setup

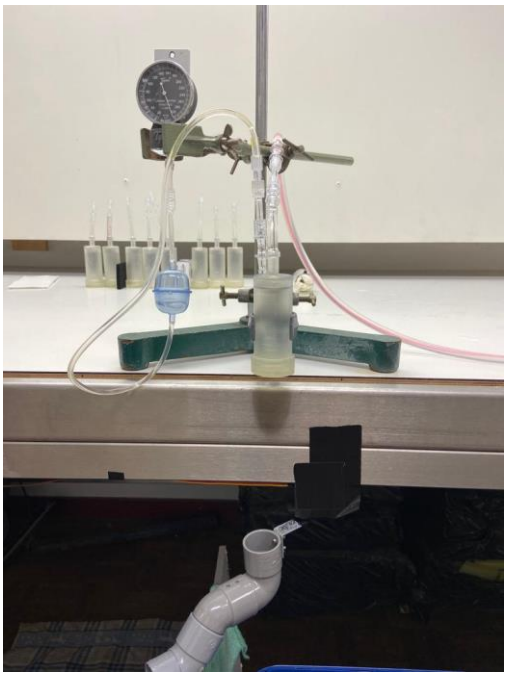


Figure 11 and 12: Photos of setup

C. Perform experiment

The experiment with different settings will be performed 78 times (repetitions not included):

$$\#\ repetitions = \#\ fluid\ viscosity\ levels * \#\ orifice\ parameters * \#\ pressure\ levels$$

The pipes with orifice areas of 5 and 10 mm^2 will be tested at 5 different pressures (40, 50, 60, 70, 80). The pipes with orifice area 14.5 mm^2 will be tested at the lowest 3 pressures (40, 50, 60). See Table 3 for an indication of possible experimental settings. The full table of settings can be found in the Excel file "*Results experiment 1*".

First, the experiments will be conducted with water. The leakage is measured at increasing pressures and is replaced with the next pipe, ascending in size and changing of shape.

The experiments are repeated 3 times to reduce random error.

Table 3: Example of three of the experimental settings

Run	Fluid	Area	Shape	Pressure	Rep 1	Rep 2	Rep 3	Mean 10	Mean 60
1	Water	5	Round	40	x	x	x	x	6*x
57	Milk	10	Round	60	x	x	x	x	6*x
77	Milk	14.5	Double	50	x	x	x	x	6*x

Part 1 – variable influence

Water

Pipe 0 (baseline)

1. Make sure the pipe is in the 0 degree position and the scale is tared.
2. Open the clamp and increase the pressure to 60 mmHg
   a. Wait and inspect the possible sites of leakage
3. Increase the pressure to 70 mmHg and inspect again
4. Increase the pressure to 80 mmHg and inspect again
5. Lastly, increase the pressure to 120 mmHg and inspect
6. Drop the pump level to 0 rpm
7. Write down if any leakage was observed.

Pipe 1

1. Replace the baseline pipe with pipe 1 (10 mm^2, round)
2. Fill the system with water by increasing the pump and opening the stopcock.
   a. Again, to release the air from the system by turning the pipe upside down during filling
3. Put the clamp on the tube when the system is filled with water
4. Place the pipe in the holder and tare the scale
5. Open the clamp
   a. Note: when the clamp is opened, the pump will detect backflow and raises an alarm. To prevent this, turn up the pump to 100 rpm before opening the clamp.
6. Increase the pump so the pressure is increased to 40 mmHg and wait until the flow is stable
   a. The water is now leaking in the left bin through the drain pipes
7. Hit the start button to run the Arduino script and wait 10 seconds
8. When the drain pipes are turned back to 0 degrees, close the tube with the clamp

    a. The pump does not need to be lowered
9. Read the scale and note the weight and the rpm level
    a. Excel file *"Results experiment 1"*
10. Tare the scale and empty the bins in the reservoir
    a. Note: the centrifugal pump is preload dependent, so the level in the fluid reservoir should be restored after each repetition
11. Repeat steps 5-10 3 times
12. Open the clamp and increase the motor speed so the pressure is increased to 50 mmHg and perform steps 5-10 3 times.
13. Perform step 12 for pressures 60, 70 and 80 mmHg.
14. Lower the pump to 0 rpm
15. Empty the bins in the reservoir and tare the scale
16. Clean the workspace by cleaning the water
17. Remove the pipe from the holder

<u>Pipes 2 – 6</u>

Repeat the steps as described for pipe 1 above for the pipes with the orifice sizes and shapes of pipe 2 to 6.

<u>Pipes 7 to 9</u>

Repeat the steps as described for pipe 1 for the first three pressures (40, 50 and 60 mmHg).

<u>Milk</u>

1. Empty the bins and reservoir in the drain
2. Fill the reservoir with 4L full fat UHT milk
3. Repeat the experiment described above for water, but now with milk

D. End experiment

1. Disconnect everything and clean workspace
    a. Flush the system with water after the repetitions with milk are performed to clean the inside of the system
2. Save files

D|Protocol experiment 2

Experiment setup leakage part 2

Location: LUMC Medical Technology D0
Date: 15th, 16th and 29th of December 2022; 3rd and 10th of January 2023

Background

The goal of this experiment is to study the influence of pressure and orifice size on the leakage through an insufficient valve. In October 2022, the first experiment is performed, to study the influence of different parameters on the exit flow through an orifice. This was performed in a 3D printed tube and lid, representing the aorta and the aortic valve. Different orifice shapes and sizes were printed on the lids. The following parameters were tested: pressure level, fluid viscosity level, orifice area and orifice shape. It was found that the pressure level, orifice shape and area influence the amount of leakage.

In this second experiment, some of these parameters will be tested in a pig heart. The final goal of this study is to obtain a relation between the leakage of an (insufficient) aortic valve in millilitres per minute and the EROA (effective regurgitant orifice area) as measured with echocardiography.

The parameters that will be tested in this second experiment are:

- Pressure level → 40 to 80 mmHg
- Orifice area → different sizes
- Orifice shape → circular punch and physiological insufficiency

The leakage will be evaluated by quantitively measuring the amount using a scale. Furthermore, echocardiographic images will be tried to obtain.

To have a complete overview of the variables of influence the influence of the anastomosis, graft water permeability and AVP device connection will be tested as well.

Hypotheses

- *The relation between the amount of leakage in ml/min can be related to the size of the EROA in mm^2.*
- *The size of the orifice area had the largest influence on the amount of leakage of an insufficient aortic valve.*
- *The pressure level in the aortic root influences the amount of leakage of an insufficient aortic valve.*
- *The influence of the leakage from the anastomosis, graft permeability and AVP device connection is negligible when compared to the influence of the orifice size.*

Required hardware

Present?	What?	Notes
X	NovaLung iLa activve pump	With console and trolley
X	Pressure meter	Analogue, with pressure dome
X	2 Fluid reservoirs	4L capacity
X	2 funnels	1 for reservoir and 1 for holding the heart
X	Tube	To transport water to bins
X	Perfusion tubes	40 cm 3/8 tube 50 cm ¼ tube 2 cm 1/8 tube
X	Connectors	2 male-male connectors 3/8 to ¼ connector 1 perfusion adapter 3/8 y-connector
X	Stainless steel clamp	ECMO
X	2 syringes	20 and 60 mL
X	Water	Tap
X	Milk	For echocardiography
X	Towels	
X	Porcine hearts	Pick up on 14th of December in cooling box
X	Aortic homograft	
X	2 bins for leaking water	
X	Scale	Accuracy 0.01g
X	Philips echo probe and monitor	Xavier and Camille Borrow from J9 room 17
X	Scope and monitor	30 degrees scope Olympus
X	2 AVP devices	With rings for connection to graft
X	2 Hemashield woven aortic grafts	Diameter 30 mm, length 30 cm Diameter 24 mm, length 30 cm
X	Piece of rubber	Ultra thin, to insert in graft
X	Laptop	
X	USB 8GB	For echo images For probe images
	Gloves	
X	Sutures (4-0, 2-0), forceps, scissors	
X	2 laboratory stands with 6 holders in total	From exp part 1 and another from D1
X	Servo motor 180 degrees	From exp part 1
X	2 Drain-pipes	From exp part 1
X	Hook, bolt and nut	From exp part 1 Also to create insufficiency
X	Arduino, Grove shield and wires	From exp part 1
X	Tape	From exp part 1

Required software

- Excel file "*Results experiment 2"*. This file also shows the order of the experiments.
- Arduino file *"Servo motor experiment"* to control servo motor

Experiment setup

1. Before setup

1. Pick up the heart
    - Five pig hearts will be picked up on the 14th of December in a cooling box.
2. Prepare impermeable graft
    - Insert a rubber piece in the graft and cut off the end
    - Attach the AVP devices to both ends of the grafts and make sure the rubber covers the full graft
3. Prepare setup with servo motor. See protocol part 1 for the extensive explanation. In short:
    - The servo motor is connected to the drain pipes. The cable is connected to an Arduino and a laptop. The motor will turn the pipes 90 degrees, stays in this position for 10 seconds and turns back.

2. Create setup

1. Perform "David procedure". [1] on subject 1.
    - Materials: 1 porcine heart, 30 mm Hemashield graft (length 3 cm), gloves, sutures (size 4-0 and 2-0 with pledges), forceps, scissors
    - Dissect the aortic root along the level of the sinuses and the commissures
        1. Note: the coronary arteries will not be reimplanted to prevent washout from the aortic root
    - Fixate the graft at the level of the ventriculo-aortic junction using the 2.0 sutures with pledged
    - Reimplant the valve in the graft. First, fixate the three commissures at the right height in the graft. Perform the running suture line from commissure to commissure.

2. Create anastomosis:
    - Materials: 1 porcine heart (subject 2) and 1 aortic homograft (subject 3), 24 mm Hemashield graft, gloves, sutures (size 4-0), forceps, scissors
    - Subject 2: remove the heart and a part of the aorta such that only the aortic valve and a small piece (2 cm) of ascending aorta is possible. Close the coronary arteries to prevent washout from the root. Create an anastomosis between the graft and the ascending aorta using 4-0 sutures.
    - Subject 3: Create the anastomosis between the ascending aorta and the homograft using 4-0 sutures.

3. Prepare the AVP device connection measurements.
   To test the leakage caused by the device connection, the device is connected to a graft that is made impermeable to water using a rubber.
    - Attach the AVP device in the correct size to both ends of the graft.
    - Make sure the connection is tight by pulling the graft apart.
    - Close one end of the AVP device with perfusion stops

1 de Kerchove L, Mosala Nezhad Z, Boodhwani M, El Khoury G. How to perform valve sparing reimplantation in a tricuspid aortic valve. Ann Cardiothorac Surg 2013;2(1):105-112. doi: 10.3978/j.issn.2225-319X.2013.01.16

4. Prepare the graft permeability measurements.
    - Remove the rubber from part 2 and reconnect the AVP devices to both ends of the graft. Note the length of the graft.
    - Make sure the connection is tight by pulling the graft in the length.
    - Close one end of the AVP device with 2 perfusion stops

5. Create total setup.
    - <u>Pump connections:</u>
    - Connect the reservoir to the centrifugal pump using 15 cm 3/8 inch tube
    - Connect the pump to a 15 cm 3/8 inch tube.
    - Using a connector (3/8 to ¼ inch), connect the tube to a 50 cm long piece of ¼ inch tube.
    - Attach a perfusion adapter to the ¼ inch tube. The other end will be connected to the AVP device.
    - Connect the pressure dome to the pressure meter using a 1/8 inch tube piece of 2 cm and a male-male adapter.
    - Connect the pressure dome to a male-male adapter. This side will be connected to the AVP device in a later stage.
    - Make sure the two open ends (on the pump side and on the pressure meter side) contain Luer connections to be connected to the AVP device in a later stage.
    - Turn on the pump and disable the priming mode by clicking the arrows on the screen.
    - Fill the reservoir with 4L water
    - Fill the system with the water and make sure there are as little air bubbles as possible.
    - Put a clamp on the tube to stop the flow
    - Turn the pump down to 0 rpm.

    - <u>Laboratory stands.</u>
    - Place the two stands next to each other at the edge of the table. Connect one holder to the left stand and 5 holders to the right stand
    - Place a funnel in the lowest position on the right stand. Make sure the lower opening of the funnel is above the ground.
        1. Connect a tube to the funnel such that the water will leak directly in the pipes on the servo motor.
    - Place three holders about 15 centimetres above the holder with the funnel. These holders will be used to create the insufficiency. Therefore, put a plastic nut in each holder. Place a plastic bolt in the nut and turn it a couple of times to make sure it is connected. Do not fully turn it down.
        1. Make sure the bolts are in the same line such that a symmetric insufficiency can be created.
    - Place a fourth holder on top of the other three, approximately 8 cm higher. The AVP device will be held to this holder.
    - Place a pressure meter in the left holder and adjust the height such that the dome is at the level of the aortic root.
    - See images below

- <u>Echocardiography device and scope.</u>
- Put the echocardiography machine and the scope screen on power supply.
- Put a cover over the ultrasound probe and set aside.
- Connect the scope to the screen
- Perform recordings and save them on a USB. Open the images/movies on the laptop
- Open the ImageJ program.
- Test if the images can be imported and a surface area can be measured.

- <u>Servo motor system.</u>
- Using tape, attach the servo motor under the table, directly under the funnel
- Place the bin on a scale directly under the table.
- Turn on the scale and click *Tare*.
- Place the other bin next to the first bin, on the left side.
- Place a screen between the bins.
- Connect the Arduino to the laptop and test if the script runs correctly by pressing the button.

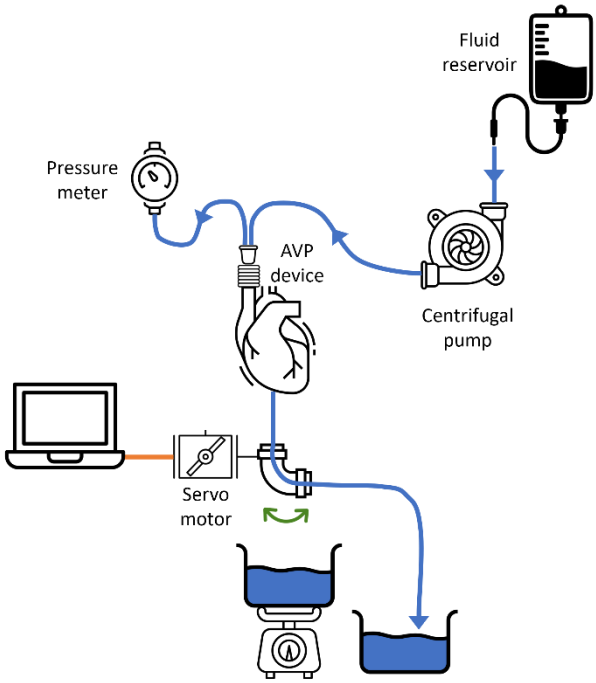
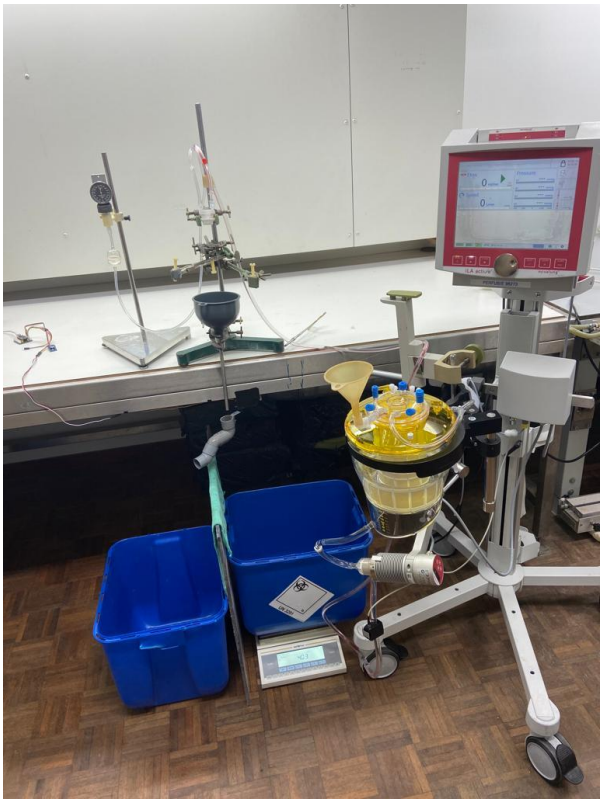
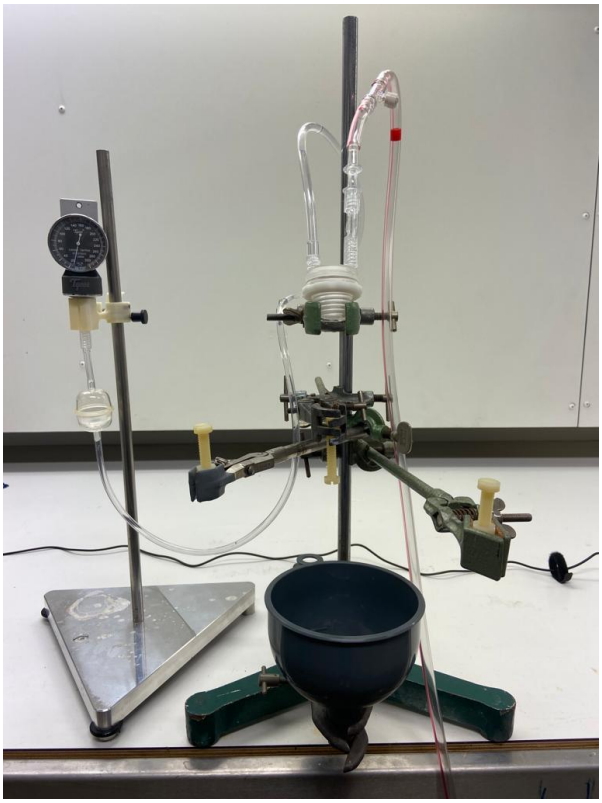
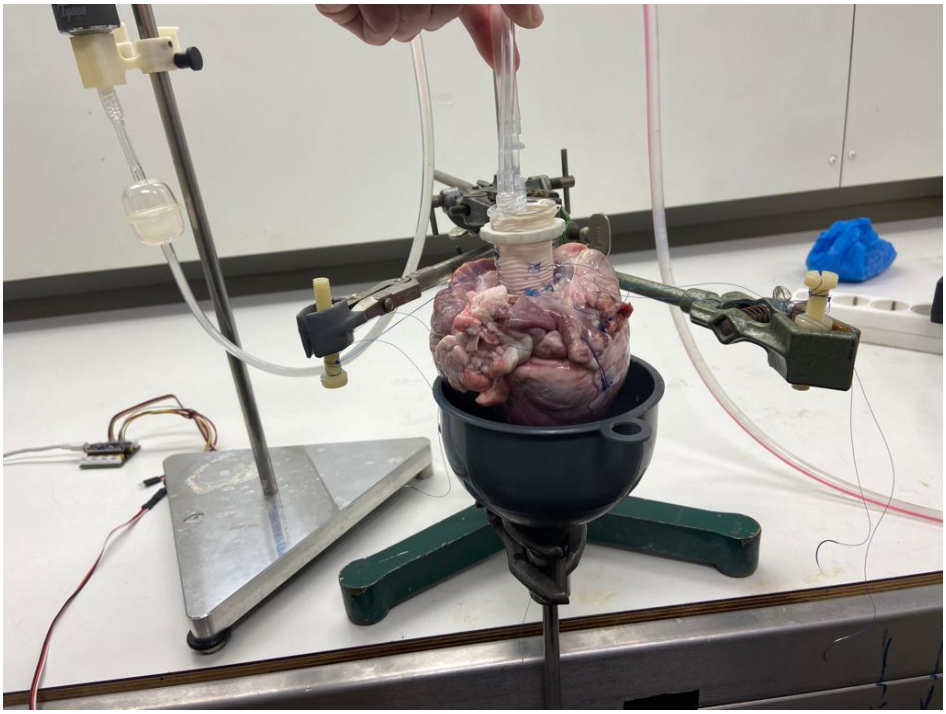


Figure 1: Schematic of setup







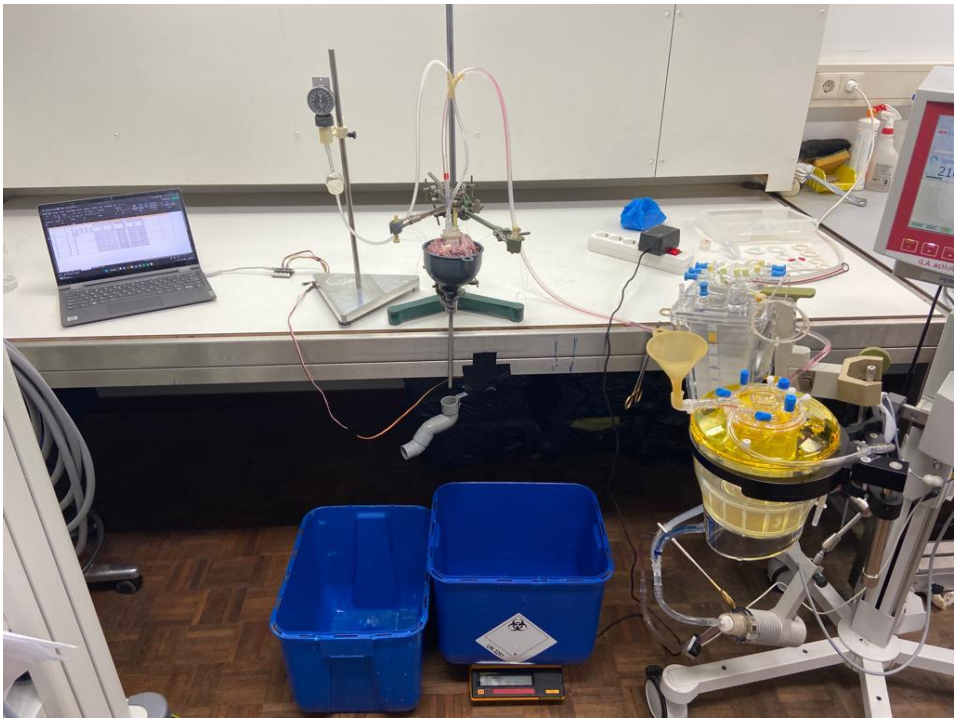


Figure 2a-f: Photo's of total setup

3. Perform experiment

Step 1 → The influence of the AVP device connection.

This step will be performed before the 15th of December.

1. Take the piece of the graft with rubber and two AVP devices
    a. Note the exact length of the graft
2. Connect one connector to the perfusion adapter at the pump side
3. Connect the other connector to the pressure meter
4. Close the openings of the AVP device on the other end with perfusion stops
5. Fill the system and make sure no air is in the tubes
    a. Keep the graft upside down and release the air by opening the stop
6. Increase the pressure to **60 mmHg** and measure the amount of leakage.
    a. If the leakage shows a constant flow: tare the scale and click the button on the Arduino. Repeat the measurement 3 times
    b. If the leakage is too small to measure: observe and wait 10 minutes. Catch the leaking fluid in these 10 minutes in the bin on the scale.
    c. Take photos of the location of leakage (i.e. Luer lock connection, ring or other location)
    d. Note the amount of leakage in Excel in grams
7. Increase the pressure to **70 mmHg** and repeat step 6
    a. Note leakage in excel in grams
8. Increase the pressure to **80 mmHg** and repeat step 6
    a. Note leakage in Excel in grams
9. Increase the pressure to **150 mmHg** and repeat step 6.
    a. Needed for document Marc
    b. Note leakage in Excel in grams
10. Turn down the pump and remove the graft with the AVP devices

Step 2 → The influence of the graft permeability

1. Take a new piece of 30 mm Hemashield graft of 8 cm (not impregnated)
    a. Measure and note the exact length of the graft in Excel
2. Attach the two AVP devices firmly on both ends
3. Connect one AVP device to the adapter on the pump side and to the pressure meter on the other side
4. Close both ends of the other AVP device with a stop
5. Fill the system and make sure there are as few air bubbles as possible
    a. Attach the pressure meter after the graft is filled with water if a lot of air is present
6. Increase the pressure to **60 mmHg**
    a. If the leakage is showing a constant flow: measure it three times using the Arduino setup
    b. If the leakage is too small to measure: observe and wait 10 minutes. Catch the leaking fluid in these 10 minutes in the bin on the scale.
    c. Take photos of the leakage location
    d. Note the amount of leakage in Excel. The amount of leakage caused by the AVP device connection is subtracted from this.
7. Increase the pressure to **70 mmHg** and repeat step 6
    a. Note the amount of leakage in grams
8. Increase the pressure to **80 mmHg** and repeat step 6

    a. Note the amount of leakage in grams
9. Increase the pressure to **120 mmHg** and repeat step 6
    a. Note the amount of leakage
10. Turn down the pump and disconnect the AVP devices
11. Repeat the measurement for the 24 mm diameter graft

Step 3 → Leakage of a sufficient valve

First, the amount of leakage will be measured using the scale. At the same time, the valve will be inspected using the scope.

1. Place the heart (subject 1, after David procedure) in the funnel on the laboratory stand
    a. Make sure fluid can leak through the funnel by creating space between the heart and the funnel with, for example, a stick
2. Connect the AVP device to the graft with the correct ring and attach it firmly
3. Connect the AVP device to the pump and pressure meter using the Luer connections
4. Fill the system with fluid, including the heart. Release the air from the root, if present.
5. Increase the pump up to a pressure of **40 mmHg** and insert the scope in the AVP device.
    a. Make screenshots from the valve and take photos of the locations of the leakage
        i. The screenshots will be processed after the experiment to study the orifice size (if present)
    b. Measure the amount of leakage when the flow is stable
        i. Repeat this three times and note the amount in the Excel file
        ii. The influence of the graft permeability and the AVP device connection will be subtracted from this number
    c. Put a clamp on a tube to stop the flow
    d. Empty the bins in the reservoir
6. Increase the pump up to pressures of **50, 60, 70 and 80 mmHg** and repeat step 5 for each pressure.

7. Attach the fluid reservoir with the milk to the pump and fill the system.
8. Increase the pump such that a pressure of **60 mmHg** is obtained.
    a. Observe the valve with the ultrasound probe with the short and long-axis views
        i. Xavier and Camille will try to obtain images regarding the valve morphology and coaptation height
        ii. Try to make Doppler images to obtain views from the regurgitant jet (if present) and try to calculate the vena contracta
        iii. Store the images on a USB
    b. Observe the valve with the ultrasound probe with the apical view
        i. Try to obtain jet visibility and EROA measurements (if the insufficiency is present)
        ii. Store the images on a USB
    c. Put a clamp on a tube to stop the flow
    d. Empty the bin in the reservoir
9. Reset the setup by cleaning the workspace and filling the reservoirs again

Repeat this process for the other two prepared hearts to measure the baseline leakage.

Step 4 → Leakage of an insufficient valve (small insufficiency, ~5 mm²) in subjects 2 and 3

1. Place subject 2 in the experimental setup.
2. Attach a suture on each commissure and tighten it on the bolts in the nuts
3. Turn the bolts such that a small insufficiency is created in the aortic valve
    a. Note: put a weight on it if it is not stable
4. Increase the pressure to **80 mmHg** and take a screenshot with the probe. Take the picture such that the full aortic valve is seen, but no graft.
5. Stop the flow using the clamp.
6. Load the screenshot on the computer using a USB or a DVI-HDMI connection.
7. Using ImageJ, calculate the area of the insufficiency
    a. Set the scale in ImageJ by inserting the size of the graft as a reference using: *Analyze → Set scale.* Crop the image if necessary.
    b. Draw the insufficiency using the *freehand selections* button
    c. Measure the area using: *Analyse → Measure.* The area should be approximately **5 mm²**. Write down the exact size of the insufficiency.
    d. If the area is much smaller or larger → adjust the bolts to change. If necessary, check the surface area again

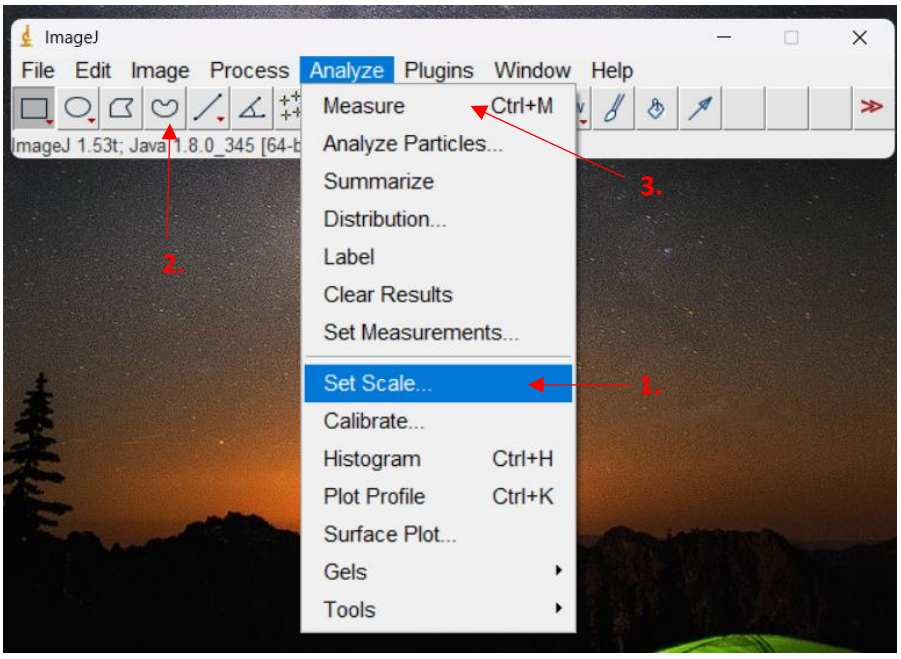


Figure 3: Steps to calculate the orifice area using ImageJ.

8. Increase the pressure to **40 mmHg** and insert the scope in the AVP device.
    a. Make screenshots from the valve and take photos of the locations of the leakage
        i. The screenshots will be processed after the experiment to study the actual orifice area, with this lower pressure level.
    b. Measure the amount of leakage in ml/min when the flow is stable
        i. Repeat this three times and note the amount in the Excel file
    c. Stop the flow using a clamp
    d. Empty the bins in the reservoir
9. Increase the pressure to **50, 60, 70 and 80 mmHg** and repeat step 8 for each pressure.

10. Attach the fluid reservoir with the milk to the pump and fill the system.
11. Increase the pump such that a pressure of **60 mmHg** is obtained.
    a. Observe the valve with the ultrasound probe with the short and long-axis views
        i. Xavier and Camille will try to obtain images regarding the valve morphology and coaptation height
        ii. Try to make Doppler images to obtain views from the regurgitant jet and try to measure the vena contracta
        iii. Store the images on a USB
    b. Observe the valve with the ultrasound probe with the apical view
        i. Try to obtain jet visibility and EROA measurements
        ii. Store the images on a USB
    c. Stop the flow using a clamp
    d. Empty the bin in the reservoir
12. Reset the setup by cleaning the workspace and filling the reservoirs again

13. Repeat the measurements with subject 3.

Step 5 → Leakage of an insufficient valve (other areas)

Repeat step 4 (leakage of an insufficient valve (small insufficiency, ~5 mm^2)) but turn the bolts such that the insufficiency increases to a larger orifice area.

- Use lower pressure levels if the pressure can't be obtained when the pump is at maximal rpm.
- Repeat this at multiple areas in both subjects to obtain sufficient information about the leakage of an insufficient aortic valve.

4. End experiment

a. Clean up and return everything
b. Leave the setup such that the experiments on the other days can be performed. See figure 2 for the total setup.