Optimizing Myocardial Oxygen Delivery
Clinical Impact of Coronary Retroperfusion Strategies

Frank Harig
Department of Cardiac Surgery
University Hospital Erlangen, Friedrich Alexander University, Erlangen, Germany

Joachim Schmidt
Department of Anaesthesiology
University Hospital Erlangen, Friedrich Alexander University, Erlangen, Germany

Michael Weyand
Department of Cardiac Surgery
University Hospital Erlangen, Friedrich Alexander University, Erlangen, Germany
1 Treatment Strategies of Coronary Artery Disease

1.1 Historical Aspects

In healthy hearts, the coronary arteries deliver nourishing metabolites and oxygen to the myocardial tissue, so that ATP is generated in the mitochondria and the contractile apparatus built of actin and myosin filaments is enabled to perform the “power stroke”, i.e. contractions called systole. In the case of coronary artery disease (CAD), oxygen supply does not fulfil the demands of the cardiomyocytes (coronary insufficiency) and the heart’s contractility diminishes (hibernating myocardium) and the oxygen demands of the whole organism are no longer fulfilled (myocardial insufficiency).

Patients suffer from angina pectoris as a symptom of CAD, which was initially described by William Heberden in 1772. The underlying pathophysiology of obstructions of coronary arteries has been described by Herrick (1912) who published a landmark article in JAMA 1912 called “Modern concept of coronary thrombosis and myocardial infarction”. Despite the knowledge of causality, treatment options were limited for a long time. As pharmacological treatment since 1840, nitroglycerin, a potent vasodilator, was applied [C. Hering], and inhalated as amyl nitrate since 1867 (Sir T. Lauder Brunton).

Surgical options were not yet established in this time. Some milestones had to be passed. One of them has been the first open cardiac operation in 1896 by Thomas Rehn in Frankfurt a.M., Germany. But because of obstacles of contemporary surgeons, surgical procedures had to wait until the 1960’s. First, Alexis Carrel, a French surgeon working at Rockefeller Institute in New York since 1906, had to establish anastomotic techniques and perfusion devices (this was awarded with the Nobel Prize 1912). Further, Werner Forssmann had to invent the catheterization of the heart in Berlin, Germany, 1929 (he received the Nobel Prize together with Cournand & Richards in 1956). The first coronary angiogram was performed by F. Mason Sones at the Cleveland Clinic, Ohio in 1958, when he spontaneously pushed the catheter forward into the right coronary artery (RCA). The invention and clinical refinement of the heart-lung-machine by John Gibbon (first use in 1953 in Boston, MA) made it possible to work on the heart without placing the patients at the risk of circulatory collapse. With this knowledge and technical support, the preconditions for a solution of the underlying problem of CAD, the stenotic coronary vessels were given and surgeons began to patch the stenoses to place interpositions and finally to bypass the stenotic coronary artery. It is noteworthy that those pioneers had to work against the massive criticism from the medical colleagues.

Coronary artery bypass grafting was experimentally and clinically evaluated in the 1960’s (first clinical CABG was performed by the team of Robert Goetz in 1960 (New York, RITA- Bypass), David Savistin in 1962, then Vasili Kolessov in Russia (Feb 1964, St. Petersburg, LITA- Bypass). Garret, Dennis, DeBakey in the US in Nov 1964 (reported 1973). The first series were published by Rene Favaloro 1967 during his time at Cleveland Clinic, Ohio, preferring the vena saphena graft. The left internal thoracic artery (LITA, also called Mammamian artery, LIMA) with better long-term patency rates was used and promoted by George Green, New York. In 1967, CABG procedures were performed by Rene Favaloro in Cleveland, Dudley Johnson in Milwaki, Michael DeBakey in Houston and David Sabiston in Duke University. In Germany, the first CABG was performed in 1969 by Prof. Gerd Hegemann in Erlangen.

In 1977, the catheter-based techniques were clinically inaugurated by Andreas Gruntzig who performed in Zurich, Swiss, the first percutaneous transluminal catheter angioplasty (PTCA). Since then, the direct revascularization techniques like the non- invasive percutaneous catheter interventions (PCI)
and the invasive coronary surgery (CABG) are in direct concurrence offering the best method for the treatment of CAD.

Nowadays, on the basis of several clinical trials (e.g. SYNTAX-study (Mohr et al., 2013)) guidelines attempt to provide clinicians with some information on decision-making in CAD, i.e. when and how to apply the best method for any coronary morphology (i.e. left main stem stenosis, 1-, 2-, or 3 vessel disease) (Stephan, et al., 2012). Nevertheless, large knowledge gaps and grey zones remain.

1.1 The Need for Alternatives to Established Techniques

Although being in the focus of investigators since 1898 (Pratt, 1898), this anatomical “back door” of the heart’s perfusion, the coronary veins, have lost investigators interest.

This concept is based on the observations of Pratt more than 100 years ago (Pratt, 1898). In 1898, he could show in experiments with cats, that the blood supply via the coronary sinus could prevent myocardial infarction and stabilize the pumping heart. Since then, in the first half of the 19th century, observations and hypotheses were evaluated in animal models and technically refined. Basic aspects of pathophysiology and anatomy in retroperfusion techniques were investigated, so that the feasibility and protective effect of retroperfusion in experimental ischemia could be demonstrated (Pratt, 1898; Roberts et al., 1943).

Nowadays, with better technical support and refined understanding of pathophysiology, a revival of this technique seems to be useful in order to have an additional treatment option for sick heart suffering from oxygen shortage. In today’s daily surgical practice, coronary surgery often has to face coronary arteries with difficult conditions to perform bypass grafting. Patients become older (in 2009, more than 50% are older than 70 years and 12% of CABG are performed in pts. older than 80 years) and the number of redo operations is growing (2009: 8.7%) (Gummert et al., 2009).

Thus technical considerations like obliterated vessels, intramural course and small vessel disease in a growing patient population suffering from diabetes demonstrates limitations to cardiac surgeons efforts. In this context, arterialization of of cardiac venous system may lead to an optimization of myocardial blood flow to ischemic regions.

1.2. Interventions on the Coronary Sinus in Animal Models

1.2.1. Global Permanent Retroperfusion

Investigators have used different animal models dealing with arterialization of the coronary sinus (CS). Roberts placed in an experiment with dogs an arterio-venous shunt between the Truncus brachiocephalicus and the CS using a canula made of glass. Later on, the carotid artery was used as an autologous bypass graft placed between aorta descendens and the CS (Roberts et al., 1943).

On the basis of these experiments, Beck started a series of 350 operations in dogs anastomosing the carotid artery directly to the CS. The first use in humans was started in 1948. A segment of the saphenous vein (SVG, saphenous vein graft) was placed between the aorta and the CS. Two weeks later, in a second step called Beck II procedure, the CS was partially occluded in order to reduce the amount of blood flow shunting directly to the venous part instead of flowing to the myocardial capillary bed (Beck et al., 1948a; 1948b; 1954). The wording “global retroperfusion” was used because of the fact that the arterial flow reached the complete venous system and not only the ischemic region. Some surgeons like Bakst could achieve relief from angina (Bakst et al., 1956), and some pts. with severe CAD were offered
This procedure. But due to an unacceptable high mortality rate, this procedure had to be abandoned. Histopathologically, the wall of the CS showed severe damage resulting in myocardial edema and hemorrhagia, signs of a reduced drainage of venous blood.

Thus, the technique of global retroperfusion (RP) had been given up. But the selective use of retroperfusion techniques was something special. Anatomical considerations support the idea of selective RP. Ludinghausen analyzed in 1987 the anatomical variance of the human coronary venous system in 350 heart specimens (Ludinghausen, 1987). He could show that in 13% the venous drainage of vast regions of the left ventricle is not directed into the coronary sinus directly. In the case of global retroperfusion these regions would not have been perfused and thus did not have had any benefit regarding additional oxygen supply. This anatomical characteristic could also be shown in animal studies of dogs by Hahn and Kim (1952). They found the Vena cordis magna (Anterior cardiac vein), draining directly into the Right Atrium in dogs. In the case of global RP, these veins did not show intimal proliferation, whereas veins that have been exposed to arterial pressure developed intimal proliferation.

Retroperfusion studies in dogs have to take into account the peculiarity of the canine coronary system of having a good collateral coronary supply, in contrast to the human and porcine coronary system having coronary end arteries.

1.2.2. Direct Selective Retroperfusion

In the 1970’s the technique of retroperfusion was refined on the basis of a better pathophysiological understanding. The arterio-venous connections/ anastomoses were established more selectively, by creating connections between the veins of the ischemic myocardial regions and the IMA (internal mammary artery) or by interposing a segment of the SVG (saphenous vein graft). The technical feasibility could be proved by some working groups (Arealis et al., 1973; Bhayana et al., 1974; Park et al., 1975; Kay & Suzuki, 1975). Further pathophysiological investigations were undertaken. Hochberg (Hochberg, 1977) used radioactive microspheres (isotope $^{114}$Cer) in order to quantify the flow quotient of subendocardial to epicardial flow. Longterm observation for 3 to 5 months were made in a canine model using 18 dogs. These encouraging results showed a late bypass flow that was nearly as high as it was initially, histological analyses showed that coronary venous bypasses did not show signs of sclerosis or thrombosis, nor signs of interstitial edema or myocardial hemorrhagia (Hammond et al., 1967; Hochberg et al., 1979; Rhodes et al., 1978).

Despite these positive results of venous retroperfusion, the fast propagation and spreading of arterial revascularization techniques in the 1960’s (CABG) and 1970’s (PTCA) prevented retroperfusion techniques from achieving clinical relevance. The capacity for interventional cardiology rapidly increased (so that Germany today is international leader in PCI treatment of CAD). This led to the nowadays usual but often not guideline- conformed treatment of CAD by placement of stents, whereas the surgical treatment is reduced since some years (Bundesgeschäftsstelle Qualitätssicherung (BQS) Bundesauswertung, 2006; Gummert et al., 2009).

Discussions about the right choice of treatment is reactivated since the SYNTAX-Trial showed positive long term survival and less collateral damage for patients choosing the initially more invasive but on the long way successful treatment. Both techniques CABG and PCI have their limits in treating patients with end stage coronary artery disease (CAD). Some studies quantify this cohort of patients by 15 -20% (Bundesgeschäftsstelle Qualitätssicherung (BQS) Bundesauswertung, 2006; Gummert et al., 2009), so that alternative methods will be of greater importance in the future.
2 Current Studies for Selective Retroperfusion of Cardiac Veins: Retrobypass in an Experimental Porcine Model

In ischemic hearts, venous retroperfusion is a potential myocardial revascularization strategy. The goal underlying retrograde coronary sinus (CS) perfusion is perfusion of the ischemic myocardium proximal to the occlusion or stenosis. The lack of suitable target vessels remains a challenge for aortocoronary bypass grafting in end stage coronary heart disease. This study aimed to investigate the arterialization of cardiac veins as an alternative myocardial revascularization strategy in an experimental long term model in pigs.

In 2.1, the technical and functional aspects of a pig model of acute myocardial infarction and retroperfusion was refined with respect to the azygos connection. Previous animal studies on interspecies anatomic differences in mammals have concentrated on the venous connections of the vessels draining the myocardium and have demonstrated a need for further feasibility studies of the pig model that focus on hemodynamic performance (Harig et al., 2010). Global retroperfusion after ligation of the ramus interventricularis paraconalis (equivalent to the left anterior descending artery in humans) was performed. Hemodynamic performance was significantly better in pigs that underwent coronary sinus perfusion. For the first time, we showed that effective retrograde flow and thus hemodynamic stability was achieved by ligation of the azygos vein. Therefore, experiments focusing on global retroperfusion will benefit from effective inhibition of the blood flow through the azygos vein.

In 2.2 and following, selective retrograde perfusion of a coronary vein (aorta to coronary vein bypass, retrobypass) was studied in a pig model of myocardial ischemia. Retroperfusion (RP) of the concomitant vein of the LAD was performed after ligation of the ramus interventricularis paraconalis (equivalent to the left anterior descending artery (LAD) in humans).

Hemodynamic was significantly better in pigs that underwent selective retroperfusion with proximal ligation of vena cordis magna compared with all other animals. Long term survival was significant better in those pigs than in all other groups. Histological follow-up studies showed significant lower area of necrosis in all animals of the retroperfusion group. Venous retroperfusion can be an effective technique to achieve long term survival after acute LAD occlusion in a pig model. In this setting, proximal ligation of V. cordis magna is mandatory.

2.1 Methods: Anatomical preconditions of an adequate animal model

2.1.1 Anatomical studies of the Azygos system in pigs

Background: Retroperfusion using the venous system is of interest as a potential myocardial revascularization strategy in ischemic hearts. This study aimed to refine the technical and functional aspects of an acute pig model of myocardial infarction and retroperfusion with respect to the azygos connection.

Methods: Under anaesthesia and with hemodynamic monitoring of 16 male Landrace pigs (Sus scrofa domestica), median sternotomy and aorta-to-coronary sinus catheterization were performed. The animals were divided into 4 groups. For all groups, an acute infarction was simulated by ligation of the ramus paraconalis (equivalent to the left anterior descending artery, LAD), in groups 1 and 2 (n=8) without coronary sinus (CS) perfusion, in groups 3 and 4 (n=8) with CS perfusion for 1h. The azygos vein was left open in groups 1 and 3, and ligated in groups 2 and 4. Hemodynamic performance and
intraoperative angiograms were analyzed.

**Results:** The presence of an azygos connection and great cardiac vein (vena cordis magna) was verified in all animals via intraoperative angiography (see Figure 1 and 2). Hemodynamic performance in group 4 (CO: 4.9 L/min) was significantly better than in groups 1, 2 or 3 (CO, 2.9, 3.2, 2.8 L/min respectively) (see Table 1).

**Conclusions:** Global retroperfusion was efficient in preventing hemodynamic deterioration after LAD occlusion, but ligation of an azygos connection was essential to achieving effective retrograde flow. Therefore, a basic requirement for long-term successful experiments focusing on global retroperfusion is to ensure effective inhibition of the blood flow via the azygos vein.

![Retrograde angiogram of the heart with labeled vessels](image)

**Figure 1:** Anatomical studies of the cardiac venous system in pigs: Venogram after application of contrast medium into the V. azygos. Retrograde blood flow is indicated.
Figure 2: Anatomical study of the cardiac venous system in pigs. Venogram. After ligation of the V. azygos, contrast medium indicates the retrograde blood flow.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LAD ligation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>preop. 0</td>
</tr>
<tr>
<td></td>
<td>azYGos vein</td>
</tr>
<tr>
<td></td>
<td>n = 16</td>
</tr>
<tr>
<td>EF [%]</td>
<td>60.5 ± 5</td>
</tr>
<tr>
<td>CO [L/min]</td>
<td>5.6 ± 1.4</td>
</tr>
<tr>
<td>HR [1/min]</td>
<td>78.7 ± 12</td>
</tr>
<tr>
<td>ST [mm]</td>
<td>0.2 ± 1</td>
</tr>
<tr>
<td>MAP [mmHg]</td>
<td>65.6 ± 5</td>
</tr>
</tbody>
</table>

Table 1: Hemodynamic values after LAD ligation. In groups 1 and 2, without coronary sinus (CS) perfusion, in groups 3 and 4, with CS perfusion, in groups 1 and 3, the azygos vein was left open, in groups 2 and 4 it was ligated. Abbreviations: LAD, left anterior descending artery; CS, coronary sinus; EF, ejection fraction; CO, cardiac output; HR, heart rate; ST, ST-segment elevation; MAP, mean arterial pressure; n.s. not significant. Methods used: 1: picco, 2: TEE, transesophageal echocardiography; 3: LAP, left atrial pressure; 4: ECG, electrocardiogram.
2.2 Histological Studies: Selective Coronary Venous Retroperfusion (Scvrp) Preserves Myocardium Effectively in Experimental Ischemia

Objectives: Cardiac veins are in the focus for myocardial revascularization strategies as alternatives for patients without suitable coronary target vessels. We studied retroperfusion techniques in a long term pig model and focused on histological examinations.

Methods: In 25 landrace pigs different retroperfusion models were performed. Acute infarction was simulated by LAD ligation. In group A (n=10), the left internal thoracic artery (LITA) was anastomosed to the Vena interventricularis anterior (selective RP) (Figure 3).

Figure 3: Intraoperative situs of a porcine heart after retrobypass using IMA graft. (1) The upper white tourniquet (at 12:00) ligates this vein and prevents central shunt via coronary sinus. (2) The downer (at 6:00) ligates the LAD. (3) Between the tourniquets, left internal thoracic artery (LITA) is anastomosed to the Vena interventricularis anterior, establishing retroperfusion.

In group B (n=5) coronary sinus (CS) perfusion (global RP) was established by an aorta-to-CS shunt with ligation of the V. azygos sinistra (P+L+). In group C (n=5) global RP was performed without ligation of the azygos vein (P+L-). In group D (n=5, control) LAD was ligated without RP and without ligation of the azygos connection (P-L-). After termination, all hearts were excised, cut into slices and histologically examined (Figure 4 and 5).
Figure 4: Histologic examination of the IMA bypass graft. Histologic slices (Sirius Red): LITA after 90d: magnification, A:40x, B:100x; C:200x, D:400x.

Figure 5: Histologic examination of the LV myocardium. Sirius Red staining. Histologic slices of left ventricular myocardium 90d after retroperfusion- above: basis of LV; down: apex of LV; magnification, A: 40x, B: 100x, D:100x, E: 400x, H.E. staining- magnification, C:100x, F:200x, Sirius Red staining.
**Results:** In group D (control), the hearts showed large infarcted areas (32.4% of the LV-Area) after LAD-ligation. With global RP in group C (P+L-), the infarcted area was reduced (19.8% of the LV) as was in group B (P+L+) (infarcted area, 10.9% of the LV). In group A (selective RP), the infarcted area was significantly reduced (1.1% of the LV). Subanalysis showed apical regions as most vulnerable (Figure 6).

![Figure 6: Histogram of the area of necrosis of LV area in different groups.](image)

In all groups, ischemia was induced by ligation of the left anterior descending artery (LAD); group A: selective retroperfusion (RP), with perfusion (P+) and ligation (L+) of the anterior descending vein (ADV); group B, global RP, with both, perfusion and ligation of the azygos vein; group C, global RP, only with perfusion of the ADV, without ligation of the azygos vein; group D, control, no perfusion, no ligation, as in all groups, LAD was ligated.

**Conclusions:** In global RP studies in pigs, ligation of the azygos connection is mandatory to save myocardium. Selective retroperfusion protects myocardium from infarction after experimental LAD occlusion. Apical regions of examined hearts seem to be at highest risk.

### 2.3 Pathophysiological Coronary Perfusion Studies: Influence of Flow & Pressure on Myocardial Contractility in Selective Coronary Venous Retroperfusion (Scvrp)

**Background:** Selective coronary venous retroperfusion is considered as an option for end stage coronary artery disease. As hyperperfusion of veins may cause damage to the endothelium and myocardial edema, the evaluation of flow and pressure conditions is important for further clinical application of selective retrograde perfusion therapy in humans.

**Methods:** After approval from the local veterinary office, retrobypass surgery was performed in 16 animals and left internal thoracic artery was anastomosed to the anterior cardiac vein (Figure 7). Complete hemodynamic monitoring was installed and myocardial contractility (dP/dt) was evaluated. For intracoronary flow measurements, Combo Map system (Volcano) was used. A correlation analysis (contractility - blood pressure; and contractility - blood flow) was performed.
Figure 7: angiogram of retrobypass. Intraoperative angiogram of retroperfusion. Arrows indicate LITA graft (solid line) and blood flow in the anterior cardiac vein distal to the anastomosis (dotted line).

**Results:** Blood flow measurements showed values between 45 and 65ml/min in the anterior cardiac vein (VIVA). Intravasal blood pressure in the VIVA showed a mean venous pressure between 35mmHg (baseline) and maximum systolic value of 72mmHg. Correlation analysis ($R^2=0.911$) showed a maximum increase in pressure over time (1100mmHg/s) for blood pressure values in the VIVA between 65 and 75 mmHg. Below 60mmHg and above 80mmHg, contractility decreased below 900mmHg/s (Figure 8). Blood flow correlation analysis ($R^2=0.602$) showed an equivalent optimal range between 50ml/min and 70ml/min. Below 50ml/min and above 70ml/min, contractility dropped below 900mmHg/s (Figure 9).
**Figure 8:** Analysis of contractility and blood flow. Myocardial contractility depends on blood flow: High values for contractility are observed in a range of blood flow between 50 and 70ml/min. Circle means baseline measurement, triangle: after 1h without vasodilation (no nitrotriglycerid), parallelogram: measurement after 1h with vasodilatating by application of nitrotriglyceride.

**Figure 9:** Perfusion studies. Correlation analysis of contractility and blood pressure. Myocardial contractility depends on blood pressure: High values for contractility are observed in a range of blood pressure around 70mmHg. Small white parallelogram means: mean value.
Conclusions: Myocardial contractility showed optimal performance within blood pressure ranges between 65 and 75mmHg and blood flow ranges between 50 and 70ml/min. These observations may serve as an early indirect sign of impaired myocardial integrity. Optimal pressure and flow patterns are preconditions for selective retroperfusion therapy.

2.4 Studies of Hemodynamics: Selective Arterialization of Vena Cordis Magna (Retrobypass) Prevents Hemodynamic Instability after LAD Occlusion

Objectives: The lack of eligible target vessels remains a challenge for aortocoronary bypass grafting in end stage coronary heart disease. Since the coronary sinus is a standardized access for retrograde cardioplegia, the arterialization of venous vessels is in the focus of research for alternative myocardial revascularisation strategies. Therefore the technical feasibility of this technique was investigated in a porcine long term model.

Methods: After anaesthesia, hemodynamic monitoring and median sternotomy of 16 landrace pigs, an aorta- to- coronary- vein (V. cordis magna, VCM) bypass (Retrobypass) was performed. Acute ischemia was simulated by LAD ligation. In group A.1 (in Figure 10 called RB+L+), LAD was ligated, a venous bypass performed and the VCM was proximally ligated. In group B.1, same procedure was performed without VCM ligation (in Figure 10 called RB+L-). Two control groups were added to the study design: group A.2 (in Figure 10 called RB-L+), in order to show effects of only ligating VCM, and group B.2 (in Figure 10 called RB-L-), in order to show effects of only performing LAD- ligation with no therapeutic intervention. Intraoperative angiograms, hemodynamic performance (Cardiac output, CO and stroke volume, SV) and survival time were analyzed.

Results: Hemodynamic performance in group A.1(RB+L+) was significantly better than in group B.1 (CO, 7.0ml/min vs. 3.2, p<.05; SV, 48ml vs. 29, p<.05). An open bypass could be verified angiographically in all animals of group A.1. Distal flow could only be established by proximal ligation of VCM. In all other groups, there was no long term survival after 4h. In group A.1, long term survival was 83% (10/12 animals) with a cumulative survival of 891 days, mean 99 d.

Conclusions: Retrobypass is an effective technique to achieve long term survival after acute LAD occlusion in a pig model. Proximal ligation of V. cordis magna is mandatory. Additional experiments will focus on structural and functional investigations.

2.5 Immunological Studies in Selective Retroperfusion: Cytokine Release after Retrobypass Surgery in Pigs

Background: This study aimed to analyze the effectiveness of selective retrograde perfusion of cardiac veins in pigs. Therefore we analyzed the cytokine release and typical parameters of cardiac ischemia during an acute infarction model. As the myocardium is a source of cytokines in ischemia/ reperfusion, it is interesting to get information about the cytokine release in retroperfusion.

Methods: In phase I of the study, 15 German landrace pigs (sus scrofa domestica) received cardiac bypass grafting under general anesthesia. After median sternotomy a retrobypass was established in off-pump cardiac surgery. A thirty minute period of ischemia by ligating the LAD was followed by retrograde reperfusion. Serial blood samples were taken to determine the concentration of lactate, troponin I, TNF-α, IL-6, IL-8 and IL-10. To assess the long-term effects of selective retroperfusion, troponin I was repeatedly measured three months after implanting the retrobypass. Furthermore, blood
Figure 10: Hemodynamic effect of retroperfusion in ischemia. Studies on cardiac output were performed in different groups, all with myocardial ischemia induced by LAD ligation: The above thin lines represent cardiac output (CO) of animals with retrobypass (RB+) and ligation of the anterior cardiac vein, ACV (L+). The triangle indicates the mean value of this group. The dotted lines indicate the CO of animals without retrobypass and with ligation of the ACV (RB-L+); or with retrobypass but without ligation of the ACV (RB+L-); or without any intervention (RB-L-). Those animals did not survive myocardial ischemia longer than one hour.

Results: In the implantation period, the increase of troponin I (Figure 11a) suggests a myocardial ischemia, and therefore demonstrates this model of ischemia to be effective after LAD occlusion. The serum concentration of troponin I, the most specific blood parameter for myocardial damage, showed normal levels one month (data not shown) and three months (Figure 11b) after surgery. So were the lactate levels three month after the bypass grafting (data not shown). These findings were underlined by the analyzed cytokine concentrations, which were quantified by a porcine specific ELISA. An inter-individual variance in the determined cytokine release patterns was observed.
**Figure 11a:** Effect of selective retroperfusion on perioperative troponin I release.

**Figure 11b:** Effect of selective retroperfusion on myocardial troponin I release after 90d.
The proinflammatory cytokines TNF-α (Figure 11c), IL-6 (Figure 11d), IL-8 (Figure 11e) showed a peak after the surgical trauma of median sternotomy. After the next trauma and possible trigger of cytokine release, the myocardial ischemia, a further cytokine peak could not be observed. During the observation period, plasma levels of the antiinflammatory cytokine IL-10 could not be detected (data not shown).

**Conclusions:** In this pig model of venous retroperfusion we could observe courses of myocardial metabolite (lactate) and enzyme parameters (troponin I). The analyzed cytokine trends showed a typical peak as an adequate answer to the surgical trauma. Ischemia and subsequent selective retroperfusion did not further increase cytokine levels. These findings encourage the hypothesis that selective retroperfusion may be an adequate and effective alternative method for myocardial revascularization. Cytokines underlie several potential influence factors, which limit their appropriateness as specific markers for cardiac ischemia.

![Figure 11c: Effect of selective retroperfusion on TNF-α release.](image-url)
2.6 Discussion of Experimental Findings

In this study, pigs are used for experimental scientific research because they have a comparable cardiac anatomy and physiology to humans. The study was conducted in strict compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals. All procedures were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and the German Animal Protection Law of 1998, and IACUC approval was obtained from the local Veterinary Office. In the following chapters the authors try to focus on special aspects of retroperfusion, the refinement of the methods and for the first time, it is shown that it is possible to perform a regional venous retrobypass (selective arterialization of the VIVA) in a long term pig model.

The importance of methods- Anatomical preconditions in an adequate animal model

In the methodical part of study, the clinical importance of the azygos connection in a pig model of retroperfusion was analyzed. During acute ischemia causing by LAD ligation, the impact of simultaneous...
global retrograde perfusion of the CS by means of an aorta-to-coronary sinus (ACS) shunt was studied by analyzing hemodynamic parameters. As expected, it was found that mid-LAD occlusion reduced cardiac output and worsened cardiac and circulatory parameters. Previous studies using animal retroperfusion models in dogs, pigs and sheep did not focus on the potential obstacle created by anatomical variation. Global retroperfusion prevented hemodynamic deterioration only if it was used in combination with azygos ligation (Harig et al., 2010).

**Hemodynamical and histological studies- Selective coronary venous retroperfusion preserves myocardium effectively in experimental ischemia**

In the clinical part of the study, we studied the impact of selective retrograde perfusion of the vena interventricularis anterior (VIVA) by means of a retrobypass (LITA to VIVA) by analyzing hemodynamic, histologic and other parameters.

As expected, we found that mid-LAD occlusion reduced cardiac output and worsened cardiac and circulatory parameters that were consistent with myocardial ischemia. Selective retroperfusion of the
VIVA prevented **hemodynamic** deterioration only when it was combined with proximal ligation of the LAD vein (vena cordis magna). When selective retroperfusion was performed in addition to proximal ligation of the VCM, arterialized blood was prevented from flowing into the CS (bypassing the heart) and was redirected to the ischemic myocardium (flow reversal). In this acute infarction model, selective retroperfusion led to improved hemodynamic stability, an indirect sign of sufficient myocardial oxygen supply.

These clinical parameters are consistent with the **histological** studies. The use of the retrobypass in combination with the ligation of the vena cordis magna reduced the area of necrosis significantly. In the histologic analysis of heart slices, the apical region showed a tendency towards higher percentage of histologically necrotic myocardium (5-8%) in contrast to mid ventricular and basal parts (1-3%). After ligation of the LAD the potential of retroperfusion seems to be a little bit limited. This is interpreted as a limited retrograde perfusion capability of the graft, but on the other side endothelial dysfunction due to high pressure and consecutive edema, swelling and capillary occlusion is also a possible explanation.

In global RP studies in pigs, ligation of the azygos connection is mandatory to save myocardium. Selective retroperfusion protects myocardium from infarction after experimental LAD occlusion.

**Pathophysiological Coronary Perfusion Studies: Influence of Flow & Pressure on Myocardial Contractility in Selective Coronary Venous Retroperfusion**

Optimal pressure and flow patterns are preconditions for selective retroperfusion therapy. In former experimental studies concerning retroperfusion, the modification of blood flow and blood pressure has been essential: The negative effect of high pressure on vascular endothelium was shown by Hammond *et al.* (1967). He could find histomorphological changes in the coronary sinuses of dogs beyond a pressure of 60mmHg. An interesting observation in studies on flow-dependent myocardial contractility in pigs could be made by Verdow, Serrhuys *et al.* (1988) They found an optimal myocardial contractility in a range of flow rates between 60ml and 80ml/min.

In our studies myocardial contractility showed optimal performance within blood pressure ranges between 65 and 75mmHg and blood flow ranges between 50 and 70ml/min. These observations may serve as an early indirect sign of impaired myocardial integrity. In the coronary sinuses of humans, flow rates of 120ml/min (with high interindividual ranges) could be measured at rest. In the V. interventricularis anterior, flow rates between 50 and 80ml/min could be measured (Swan *et al.* 1971).

**Immunological Studies in Selective Retroperfusion: Retrobypass Does Not Further Increase Surgical Proinflammatory Cytokine Release in Pigs**

In former studies on the cytokine response of patients after coronary artery bypass grafting, we could find an immediate peak of IL-10 and IL-8. The proinflammatory IL-6 followed some hours later as an indicator of SIRS (systemic inflammatory response syndrome) (Harig *et al.*, 1999a; 1999b; 2001).

In this pig model of venous retroperfusion the analyzed proinflammatory cytokine trends of TNF-α, IL-6 and IL-8 showed a typical peak as an adequate answer to the surgical trauma. Ischemia and subsequent selective retroperfusion did not further increase cytokine levels.

These findings encourage the hypothesis that selective retroperfusion may be an adequate and effective alternative method for myocardial revascularization. Cytokines underlie several potential influence factors, which limit their appropriateness as specific markers for cardiac ischemia.
2.7 Additional Aspects and Summary

Angiogenesis

Some investigators (Weigel et al., 2007) analyzed the effect of pressure-controlled coronary sinus occlusion (PICSO) on the activation of proangiogenetic genes like HO-1 (Häm-Oxygenase 1) and VEGF (Vascular endothelial growth factor) by exerting pulsatile pressure on coronary venous endothelium. Mohl and coworkers hypothesized that neoangiogenesis may be the trigger for the positive effects of retroperfusion.

Staged procedure

Choy et al. (2006a; 2006b) introduced a novel strategy of ligating the vein of the target region in a first step, leading to an increasing wall thickness of coronary venules and then, in a second step, after remodeling of the coronary veins, creating the retrobypass as an alternative method of myocardial oxygen delivery. Resuming the broad spectrum of investigations concerning retroperfusion one can state that the impact of this method may lead to a broader clinical application for the treatment of end stage coronary disease.

References


Bundesgeschäftsstelle Qualitätssicherung (BQS) Bundesauswertung 2006, Koronarchirurgie, Abs. II 61, S. 2.32 (German Federal Office of Quality Assessment, Federal Data Analysis 2006, CABG, par.II61, p.2.32)


Pratt, F.H. (1898). The nutrition of the heart through the vessels of thebesius and the coronary veins. American Journal of Physiology, 1, 86-103


