

*Are Non -or Low-Contrast Agents
Alternatives to Contrast flushing in
Coronary Artery Optical Coherence
Tomography Regarding Diagnostic
Length and Image Quality?
– A pilot study*

Cecilie Halvorsen Egeland



Master of Health Sciences

UNIVERSITETET I OSLO

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**Can non -or low-contrast fluids serve as an
alternative to contrast fluids for flushing in
coronary artery optical coherence tomography,
with preserved diagnostic image length and
image quality?
– A pilot study**

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IV

Abstract

Aim: Clinical applicability with Optical Coherence Tomography (OCT) is limited by the use of contrast as flushing agent for removal of blood during image acquisition, due to the nephrotoxicity of contrast. Therefore, a contrast saving alternative is needed, however, it remains unclear whether other fluids may be used. We investigated if other contrast agents, diluted iodine-contrast or non-iodine containing fluids, might be utilized as flushing agents during OCT with preserved image quality.

Litterature framework: Promising results for non-contrast fluids as a contrast alternative for removal of blood in OCT image acquisition have been shown in previous studies.

Methods: In two anaesthetized pigs OCT were obtained from the left anterior descending and right coronary arteries (n=4). In both vessels we compared non-contrasts; Macrodex 60, Voluven, Ringer Acetat, diluted-contrast (Iomeron 150 mg I/mL) and contrast (Iomeron 350 mg I/mL and Visipaque 270 mg I/mL) to a reference contrast (Visipaque 320 mg I/mL) at identical infusion pressure and flow rates, over the same segment in each vessel. Diagnostic image length with discriminable wall layers $\geq 270^\circ$ of the circumference was measured and we assessed the overall subjectively evaluated image quality.

Results: As compared to reference, no statistical significant differences were observed for the fluids regarding diagnostic image length. However, a somewhat lower diagnostic vessel length was observed for Ringer Acetat and Iomeron 150 ($p=0.068$) compared to the reference. An overall lower image quality was observed for the non-contrast fluids compared to the reference. Blood pressure and heart rate did not vary during the experiment.

Conclusions: Even if the pilot study was underpowered, the data may indicate that both the diluted-contrast and non-contrast fluids may have a potential to replace contrast in OCT flushing, and should be investigated further.

Sammendrag (Norsk)

Formål: Optisk koherens tomografi (OCT) er begrenset grunnet bruk av jod-holdige kontrastmidler for å skylle bort blodet under billedtakning. Kontrastbesparende alternativ er ønskelig siden kontrastvæsker kan føre til kontrastindusert nefropati, det er uklart om andre væsker kan benyttes. Vi undersøkte om andre kontrastmidler, redusert-kontrastmidler eller ikke-kontrast væsker kunne være et alternativ til kontrast ved OCT billedtakning, og allikevel oppnå god diagnostikk.

Teoretisk forankring: Det er utført få studier på å finne alternativer til kontrast ved OCT, men et par studier har vist at ikke-kontrast holdige væsker har et potensiale for å erstatte bruk av kontrastmidler ved OCT.

Metode: I to intuberte griser utførte vi OCT billedtakning fra venstre fremre og høyre koronar arterie. I begge årene injiserte vi ikke-kontrastholdige væsker: Macrodex 60, Voluven, Ringer Acetat, samt kontrast med lavere jod-innhold (Iomeron 150 mg I/mL), kontrast (Visipaque 270 mg I/mL og Iomeron 350 mg I/mL) og en referanse kontrast (Visipaque 320 mg I/mL) med samme volum, injeksjonshastighet og trykk. Væskene ble sammenlignet mot referanse kontrasten på diagnostisk billedlengde med synlige karlag i $\geq 270^\circ$ av sirkumferensen, samt subjektive vurderinger av billedkvaliteten innenfor den diagnostiske bildekvaliteten.

Resultater: Ingen av væskene gav en statistisk signifikant forskjellig i diagnostisk billedlengde sammenlignet med referansen. Ringer Acetat og Iomeron 150 ($p=0.068$), viste en noe dårligere diagnostisk billedlengde sammenlignet med referansen. Den subjektive vurderingen av billedkvaliteten var noe lavere for de ikke-kontrastholdige væskene ($p=0.49$) sammenlignet med referansen. Det var lite variasjon i blodtrykk og elektrokardiogram under datainnsamlingen.

Konklusjon: Selv om studiet har lav statistisk styrke, viser dataene at kontrastvæske med lavt jod-innhold og ikke-kontrastholdige væsker har et potensiale for å kunne erstatte kontrastvæske under billedtakning ved OCT. Dette potensiale bør undersøkes ytterligere.

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Oslo, May 25, 2016

Cecilie Halvorsen Egeland

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Abbreviations

CIN	Contrast induced nephropathy
eGFR	Glomerular filtration rate
FFR	Fractional flow reserve
IVUS	Intravascular ultrasound
LAD	Left anterior descending artery
Lmw-dextran	Low molecular weight dextran
NIR-light	Near-infrared light
OCT	Optical coherence tomography
PCI	Percutaneous coronary intervention
RCA	Right coronary artery
SCA	Selective coronary angiography
sCR	Serum creatinine

1. Introduction

1.1 Coronary optical coherence tomography and contrast flushing

Fourier-Domain optical coherence tomography imaging (OCT) has recently been introduced as a supplementary useful tool for clinical use in coronary artery imaging, during selective coronary angiography (SCA) and percutaneous coronary intervention (PCI) (1). This imaging modality is safe (2, 3), with an adequate reproducibility (4). The images are created with near infrared light (NIR-light), providing images of the coronary vessel wall with a high resolution down to 10 μm (5). These detailed images allow for the study of coronary anatomy and pathology, including the vessel wall, lumen, plaque changes, dissections and stent placement (6).

Although, OCT may provide clinical important information, there are some disadvantages, especially due to the use of iodine-based contrast agents for flushing during OCT image acquisition. Modern OCT equipment used in clinical practice today requires a blood free vessel to perform image acquisition and to achieve diagnostic images. Contrast agents with high viscosity like Visipaque (iodixanol, GE Healthcare) has been recommended for clearance of blood during image acquisition, mainly because of the low arrhythmogenic potential and the high viscosity which is helpful for erasing blood through the whole image acquisition time (6). However, iodine-based contrast agents in general have some potential side effects due to their nephrotoxicity, and high volumes increase the chance for developing contrast induced nephropathy (CIN) (7). CIN is an acute kidney injury caused by the exposure of iodine-based contrast media (8). In patients with normal kidney function studies have showed that functional and structural changes induced by contrast agents are of negative significance (7, 9). However, some patient subsets have risk factors for developing CIN. The main risk factor is an existing reduced kidney function, other risk factors are: age > 75 years, heart failure (NYHA class 3-4), diabetes mellitus and female gender (7).

Although the use of iodine-based contrast agents has been recommended for OCT imaging, this recommendation does not seem to be based on broad empirical evidence. However, few studies have been performed comparing iodine-based contrast agents and non-contrast (no content of iodine) agents on image quality. Two studies (10, 11) compared an iodine-based contrast fluid (low-osmolar, Omnipaque 350 mg I/mL and iso-osmolar Visipaque 320 mg I/mL) with low molecular weight dextran (lmw-dextran), which is a colloid solution normally used for increasing the plasma volume during hypovolemic shock (12). One of these studies was published after the data collection for this pilot. No significant difference between lmw-dextran and iodine-based contrast regarding image quality was found (10, 11). Another small study compared iodine-based contrast (Visipaque 320 mg I/mL) to Voluven, also a colloid solution used for hypovolemia, containing starch (13). The result showed that a 50/50 mixture of contrast and Voluven seemed to be an efficient alternative to contrast flushing alone (14). To my knowledge, no studies have so far compared contrast with low-contrast fluids, meaning contrast agents with a much lower content of iodine.

The experience with the first generation of coronary OCT (Time-Domain OCT), where an occlusion balloon was expanded proximal for the segment of interest, image acquisition was performed using non-contrast agents such as saline, Ringer's solution, or a mixture of contrast and saline/Ringer's solution with acceptable results (15, 16).

In this pilot study we wanted to compare; contrast, low-contrast and no-contrast flushing fluids on subjective image quality by measuring a continuous length of good image quality frames using a blinded model, based on own clinical practice, experience with OCT and medical literature. If non-contrast or low-contrast fluids can provide adequate quality OCT images, it would be beneficial for patients with risk factors for achieving CIN.

1.2 Aims of the study

The overall study aim is to examine whether it was possible a possibility to reduce or replace the volume of iodine-based contrast agent administered during image acquisition with OCT, and still achieve adequate image quality in which will provide good diagnostic

information about the coronary artery wall. The primary aim of the study was to compare a standard iodine-based contrast with other contrast agents, a lower iodine concentration of contrast and non-contrast agents on diagnostic OCT image length. The secondary aim was to investigate the subjectively evaluated image quality within the diagnostic image lengths. Due to the recommendation for using Visipaque in OCT image acquisition, the contrast agent Visipaque 320 mg I/mL was chosen as reference fluid for comparison.

1.3 Hypothesis

Based on the aims of the pilot study the null hypothesis is the following:

There is no statistical significant difference between the use of the standard contrast agent Visipaque 320 mg I/ml compared with the other contrasts, low-contrast or non-contrast fluids regarding diagnostic image length by OCT with preserved image quality, and subjectively rated image quality.

2. Theory

2.1 Selective coronary angiography, percutaneous coronary intervention and coronary imaging

Selective coronary angiography (SCA) is a well-established imaging modality and examination of the coronary arteries (figure 1). The coronary arteries supply the heart muscle with oxygen-rich blood, and commence at the start of ascending aorta from the right and left sinus of Valsalva. Coronary anatomy consists of the right coronary artery and the left coronary artery (17). The coronary artery wall consists of three layers: intima, media and adventitia (figure 2). The purpose of a SCA is to investigate the presence of coronary artery disease, to access the location of the disease and extent of coronary atheroma, and to decide upon a therapeutic strategy (17). If treatment is given during a SCA, this is termed percutaneous coronary intervention (PCI), and includes stent inplacement, balloon dilatation and atherectomy (18). SCA is an invasive technique, where a catheter is inserted via the radial or femoral artery, and advanced over a wire to the coronary ostium. Visualization of the arteries is made possible by selective injection of iodine-based contrast agents through the catheter during pulsed x-ray recording (18). The presence of stenosis (narrow parts in the artery that decreases blood flow) or occlusion (obstruction/blockage of an artery) of the arteries can be detected on the images. Medical indications for performing a SCA exam are stable angina, unstable angina, cardiac arrest, ST-elevation myocardial infarction, non-ST elevation myocardial infarction (19), as well as preoperative investigations with valve diseases, cardiomyopathies, and congenital heart diseases.

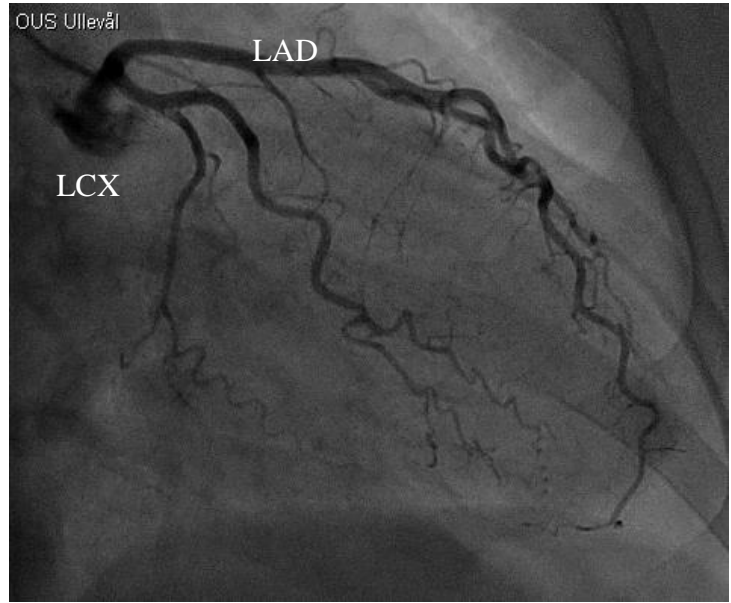


Figure 1. Selective coronary angiogram with normal anatomy of the left coronary arteries.

Normal human heart anatomy of the left coronary arteries (LCX: left circumflex, LAD: left anterior descending artery). Picture reprinted from Oslo University Hospital, unit Ullevål.

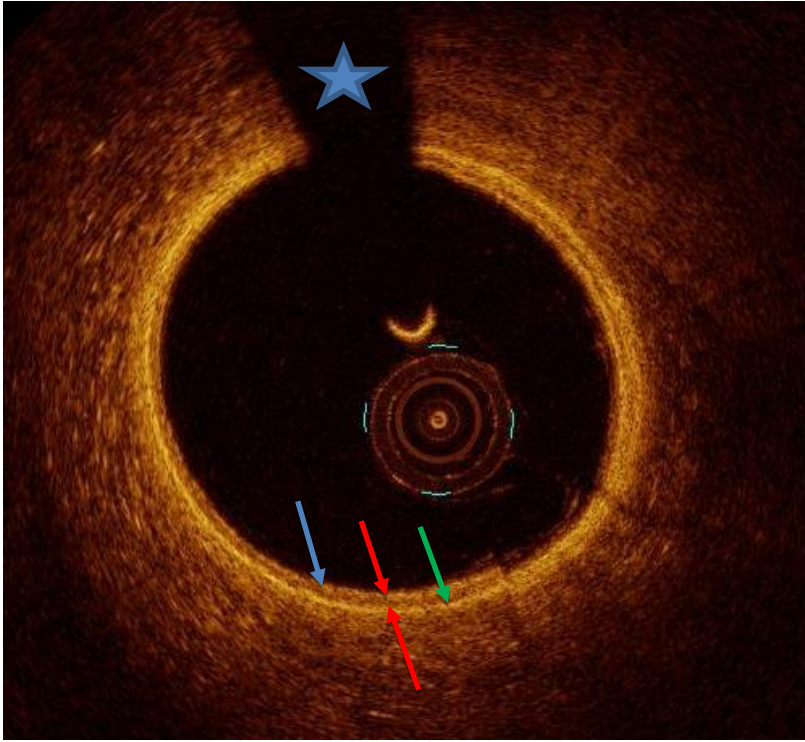


Figure 2. OCT image frame with normal vessel layers.

Optical coherence tomography (OCT) image of a normal right coronary artery in porcine #1. All the three wall layers are visualized: Intima (blue arrow) is depicted as a thin yellow line closest to the black artery lumen; Media (red arrows) is the darker area between the red arrows; and Adventitia (green arrow) is the outer yellow line. The asterisk indicates shadow from the coronary wire.

SCA is an established method for the visualization of atherosclerotic disease. However, due to the two-dimensional images and low sensitivity for identifying lesions of positive remodeling (growth of plaque without narrowing the artery) and diffuse disease (20), as well as eccentric stenosis, over-projection and bifurcations, SCA does not provide details of the vascular wall, and can therefore not identify vulnerable plaques (21).

To overcome the limitations of SCA there are several intravascular modalities for gaining supplementary information such as intravascular ultrasound (IVUS), fractional flow reserve (FFR) and OCT. There is a need for improving the characterization of the coronary pathology to better understand the factors associated with acute coronary syndromes, as well as developing better therapeutic strategies (22). FFR measures functional significance of stenosis, whereas IVUS and OCT provide a visualization of the intraluminal and transmural anatomy of the coronary arteries (23). OCT and IVUS are analogous imaging methods, but IVUS use ultrasound waves to perform image acquisition (6). IVUS gives a resolution down to 100-200 μm and a tissue penetration of 4-8 mm, whereas OCT provides a resolution down to 10 μm and a tissue penetration of 1-2 mm (24). Due to their differences in resolution OCT is better for detailed information in the near field, whereas IVUS is more suitable for a more overall recognition of the vessel wall (23).

2.2 Optical coherence tomography (OCT)

OCT is a relatively novel modality for supplementary imaging during SCA. Due to the detailed images the technique has been shown to have a great potential in intravascular imaging for both research and for clinical applications (21, 25). The image acquisition of the biological structures of the coronary artery wall is based on reflection of near infrared light (NIR-light) which is sent into the tissue. The intensity of the reflected or backscattered NIR-light is taken as a measure of depth (26). The technology is based on the optical one-dimension low-coherence reflectometry that uses a Michelson interferometer and a broadband light source. Around 25 years ago transverse scanning, called b-scan, enabled a two dimensional imaging of the eyes retina and coronary vessel wall (24, 27), and proved OCT to be a promising technique. First in the mid-1990s the OCT technology

showed a potential for use in cardiology for evaluation of atherosclerosis, and in 2000 it became available for human use (20).

2.2.1 Clinical use of optical coherence tomography (OCT)

Even though OCT has been available for clinical use for more than a decade, the clinical diagnostic value has not been well documented. Due to the limited controlled data published supporting its prognostic role, OCT does not have an established clinical indication in the European Society of Cardiology guidelines (28). However, many consider the technique as being very promising regarding its clinical value (29). The international working group for intravascular optical coherence tomography standardization and validation has presented a consensus document where among other topics the clinical values of OCT were presented (1). Evidence from multiple, well-designed, cohort studies with sufficient statistical power have showed that anatomical and pathological structures are well detected by OCT, including normal anatomy, intimal thickening, atherosclerotic plaque, atheroma, thrombus (figure 3) fibrous plaque, fibrocalcific plaque, lipid pools, fibrous cap, thin-capped fibroatheroma, and ruptured plaques (1). - All of these anatomical and pathological structures can provide valuable information about the pathological processes in the vessel wall, and can contribute to the choice of treatment. The consensus document (1) also acknowledges OCT to be beneficial for stent assessment due to the clinical evidence present. Stents are made of metal alloy, and the stent struts are well visualized with OCT since they are opaque to light. Stent prolapse (prolapse of tissue between the stent struts), malapposition (gap between the stent to and vessel wall), strut coverage (stent struts coverage of tissue) can easily be detected with OCT. Thus, OCT may provide valuable information if a patient return for a SCA due to new symptoms. If the SCA shows that there might be a stent failure, OCT images can help with different strategies of treatment depending on what kind of mechanical stent problem is present (1).

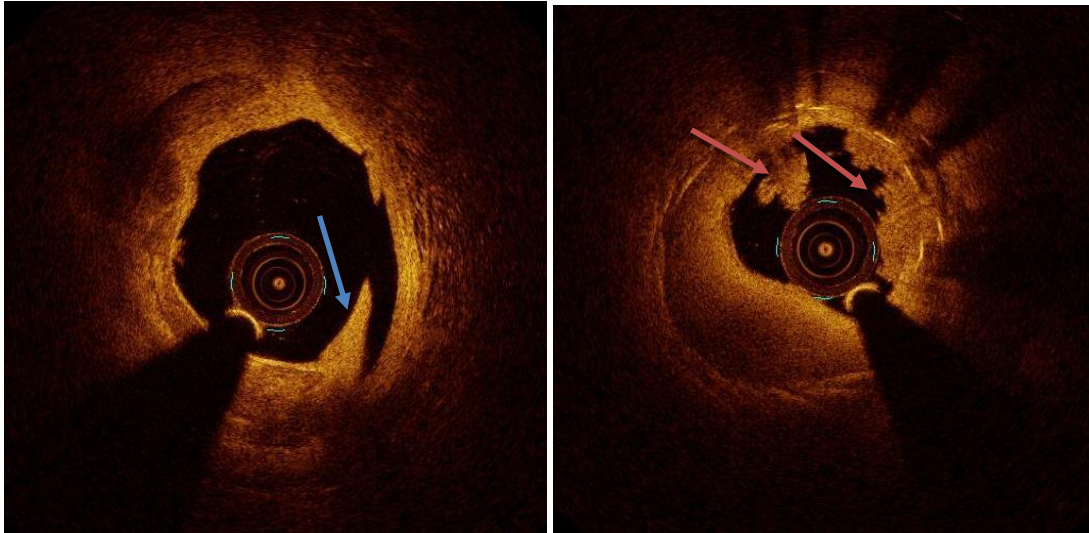


Figure 3. OCT images of pathology

Optical coherence tomography of a patient with ST-elevation myocardial infarction due to stent thrombosis who had a percutan coronary intervention with stenting done 5 days ago. Left panel: rupture of the intima wall layer (dissection) distally to a stented segment (blue arrow). Right panel: Thrombus in the stented area (red arrow). Pictures reprinted from Oslo University Hospital, unit Ullevål.

2.2.2 Physical principles of optical coherence tomography

OCT is a light-based modality based on interference between light waves, and provides high resolution cross-sectional images of tissue microstructure (1), or more specific the changes in the optical properties of the tissue/sample (30). To perform OCT image acquisition an optical fiber is inserted into the vessel lumen. This optical fiber emits light and also records the reflections simultaneously while being rotated and pulled back along the artery (15). The background for image creation is the comparison of the back-reflected intensities from the two arms (figure 4) (31). The intensities of the light is measured and digitally concerted into a grey scale creating an image (32). For further information regarding physical principles regarding OCT see Appendix 1.

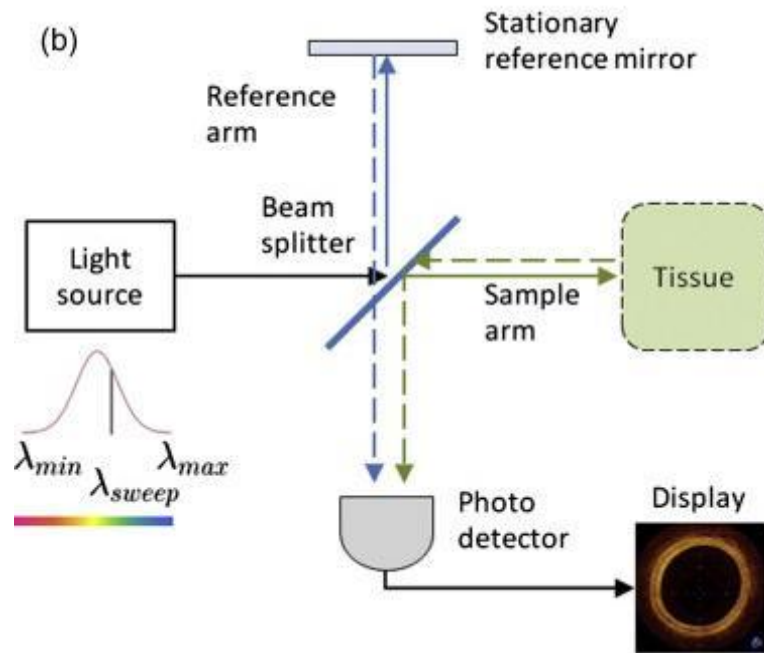


Figure 4. Illustration of the physical principles of OCT

Illustration of optical coherence tomography. The near-infrared light is sent out from the light source at different wavelengths into the beam splitter. The beam splitter divides the light into two, travelling to the reference arm and the sample arm. The returning light is then sent to the photo detector for identification of differences in intensity (33). Reprinted with permission from Elsevier/Medical Image Analysis, Vol 18, In-vivo segmentation and quantification of coronary lesions by optical coherence tomography images for a lesion type definition and stenosis grading, Simona Celi, Sergio Berti, p. 1157-1168, 2014, (See Appendix 2 for permissions).

Before the image acquisition, eventual presence of blood in the OCT catheter has to be removed to avoid attenuation of the NIR-light. A small syringe is attached to the catheter purging blood out of the catheter. An automatic image acquisition begins when the artery is cleared from blood, and clearance is made possible by using a mechanical injector or manual injection for injection of blood-replacing fluid.

As with any device introduced to a coronary artery, caution is also necessary with OCT. The coronary wires and catheters into a coronary artery always contains a small risk of complications, such as coronary artery dissections, intramural hematoma, coronary artery perforation and occlusion of side branches (34). As with any intravascular device, care should be taken while advancing the OCT catheter into the artery, since intravascular modalities include a risk in itself by being an invasive procedure (20). During clearance of blood and image acquisition chest pain and electrocardiogram changes may occur, this may be due to coronary spasms, caused by the presence of the catheter or the injection of contrast dye. This might be removed by intracoronary injection of nitroglycerine prior to

insertion of the OCT catheter. Though, OCT has proven to be safe (35, 36), and these possible discomforts have been showed to resolve immediately after the image acquisition. There has been a theoretical concern regarding local heating caused by NIR-light, but this has not been observed to be an issue in studies so far (20).

2.2.3 Optical coherence tomography image quality

A high quality of the constructed OCT images is a prerequisite for identifying the different structures and pathological processes in the coronary artery wall. Good/optimal image quality can be defined as a visualized boarder between the artery lumen and vessel wall in $\geq 270^\circ$ of the circumference (figure 5) (10). As the coronary wire over which the OCT catheter is advanced always creates a sector shaped shadow it is impossible to visualize the entire circumference (360°) in one frame. Therefore, 270° has been used as minimum accepted circumference. In a normal artery visualization of the intima, media and adventicia should be present. In this study the term diagnostic image length indicated a length of continuous cross-sectional frames of good image quality.

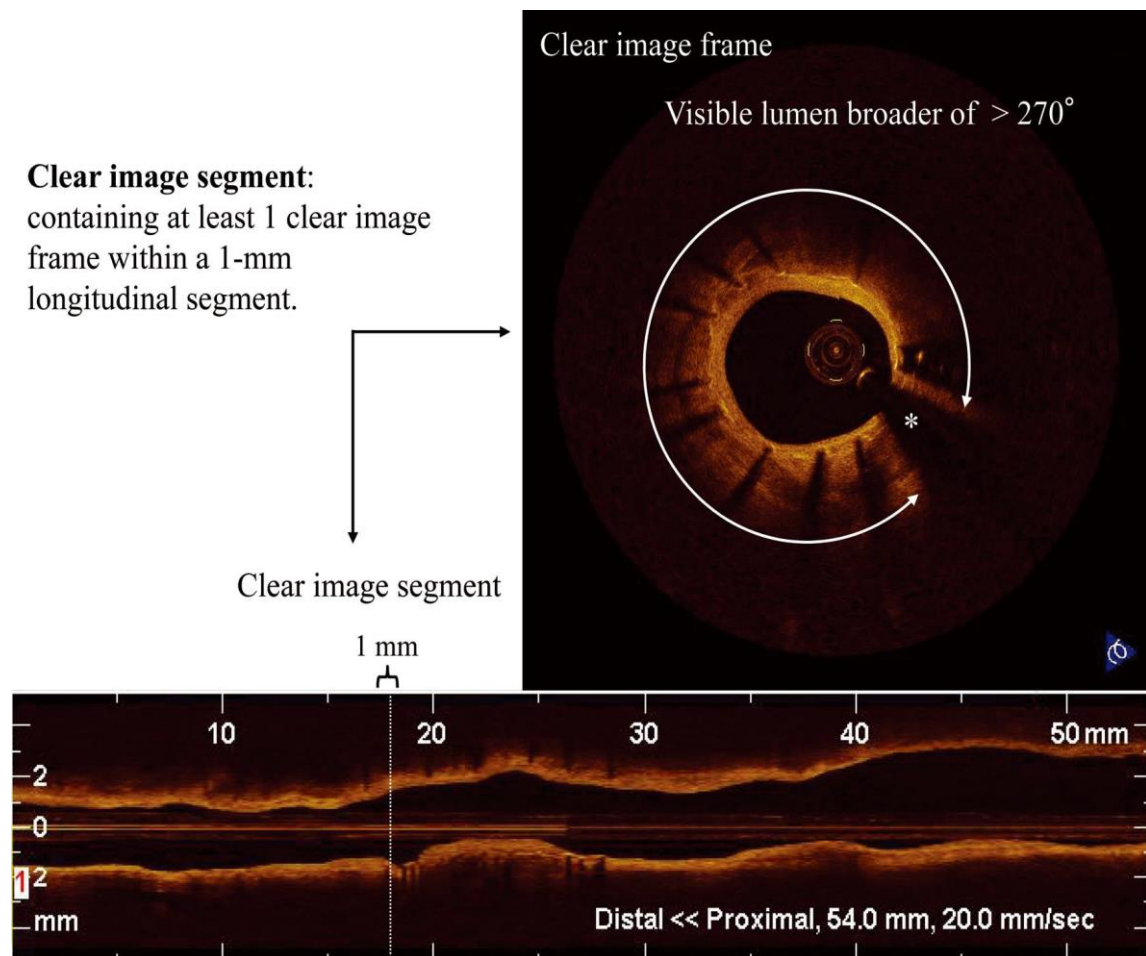


Figure 5. Definition of clear image segment/image frame

Definition of clear optical coherence tomography (OCT) image segment, with visible lumen boarder in $> 270^\circ$. A complete OCT recording is 54mm (10). Upper right panel: An axial OCT frame showing a clear boarder between the lumen and vessel wall in a stented artery. Shadow from the coronary artery is indicated by an asterisk, whereas the circular structure in the middle lumen is the OCT catheter. Lower panel: A longitudinal image of the complete image acquisition (54mm), the white dotted line shows the localization of the axial frame in the upper right panel. Reprinted from *Circulation Journal*, Vol 76, Comparison of contrast media and low- molecular-weight dextran for frequency- domain optical coherence tomography, Yuichi Ozaki, Takashi Akasaka, p. 922-927, with permission from *Circulation Journal* (Permissions in Appendix 2)

There are several aspects that may affect the quality of an OCT image such as OCT catheter position, image artifacts, but most importantly, lack of clearance of blood (figure 6) in the artery. OCT requires a blood free artery, which in practice means that the blood has to be replaced with another fluid to perform image acquisition due to the physical properties of blood (37). If optimal clearance of blood is not acquired residual blood in

the artery will reduce the OCT image quality and decrease the level of diagnostic information.

OCT image acquisition relies on the penetration and backscattering of the NIR-light to make the cross sectional images of an OCT image recording. Biological structures strongly scatter the light (38), leading to attenuation of the NIR-light (39). The mismatch in refractive index, which is how light propagates from one medium to another, between the erythrocyte cytoplasm and blood plasma also cause a severe scattering of blood, this makes it difficult to achieve high quality imaging (40).

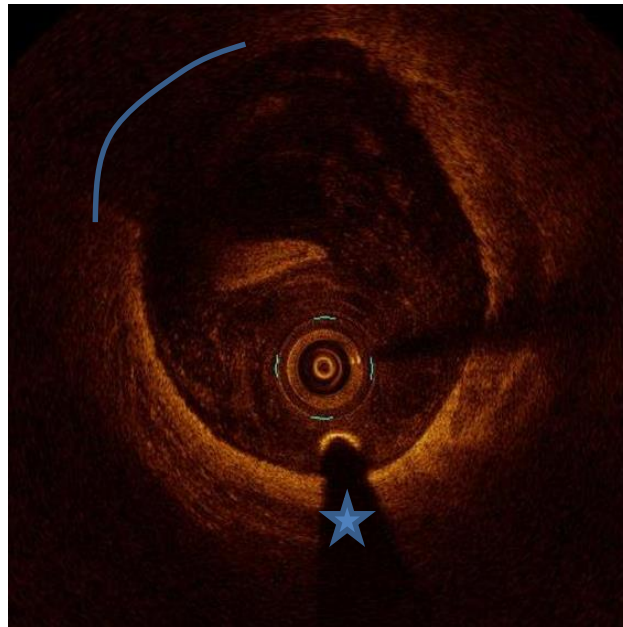


Figure 6. Poor OCT image quality

Optical coherence tomography image with residuals of blood, and blood in the catheter. In the upper left area of the artery (blue curve) the border between the vessel lumen and artery wall is not visible. This makes it impossible to determine which pathological or anatomical structures that is present in this area. Wire shadow is indicated by a blue asterisk. Image is reprinted from local database at Oslo University Hospital, Unit Ullevål.

2.2.4 Flushing/Contrast agents in optical coherence tomography

The main purpose of using viscous iodine-based contrast agents for flushing during an OCT recording is that the higher the viscosity of the fluid, the lower administrated

volume is required (41). Viscous contrast, for example Visipaque, is especially recommended due to the high viscosity and the low risk for arrhythmias (6).

Contrast is the iodine based agent in which are used to visualize the coronary arteries during SCA (42) and the use of contrast agents has recently increased in medical practice, as medical investigations using contrast has increased (43). Iodine-based contrast agents are being used ruinously in the coronary catheterization laboratory, it is a familiar fluid for the physicians and is known to be safe for intracoronary injections. Contrast agents can be divided into three main groups based in their osmolality; high- iso- and low-osmolar. The high-osmolar contrasts are more nephrotoxic than the low-osmolar and iso-osmolar contrast agents (44), and should therefore be avoided. - Two contrast agents used widely in clinical practice are the low-osmolar Iomeron (iomeprol, Bracco Imaging) and the iso-osmolar Visipaque (iodixanol, GE Healthcare). Presently, no studies have showed up to now a superiority between these (8). These two, as well as other available low-osmolar and iso-osmolar contrast agents, have similar renal safety profile (42). Given the widely use of contrast agents for intracoronary imaging a challenge is to reduce the amount contrast needed, as the volume administrated is closely related to the risk of achieving contrast induced nephropathy (CIN) (9). In addition to the risk of CIN, administration of contrast containing fluids have several other potential side effects as example nausea, urticaria, dyspnea, arrhythmias (45, 46).

2.3 Contrast induced nephropathy

CIN is a feared complication after angiography where iodine-based contrast media cause an acute kidney (8). It is associated with increased morbidity and mortality (47), as well as prolonged hospitalization (48). CIN is characterized by an increase in serum creatinine (sCR) and decrease in urine excretion. CIN is defined as an increase in sCR by $\geq 0,3$ mg/dL or 1,5 times the baseline sCr within the past 7 days, and a decrease in urine volume to $< 0,05\text{mL/kg/h}$ after exposure to contrast, or an increase of 25 % over baseline or \geq mg/dL within 3 days of contrast dye, and in the absence of other alternative cause (8).

The risk for achieving CIN is positively correlated with the amount of iodine-based contrast administered (9, 44, 49, 50). Therefore, the volume of contrast administered should be kept as low as possible (51). For the general population the chance for achieving CIN after contrast administration is considered low, but for some patient subset the risk may be very high (52). Factors such as patient characteristics, clinical setting and other modifiable factors affect the risk factors for development of CIN (43). The Contrast Media Safety Committee of the European Society of Urogenital Radiology guidelines have presented several risk factors for CIN in which are glomerular filtration rate (eGFR) < 60, especially in combination with one or several of these: diabetic nephropathy, dehydration, congestive heart failure, low left ventricular ejection fraction, recent myocardial infarction (<24 hours), intra-aortic balloon pump, peri-procedural hypotension, age > 70 years, known or suspected renal failure, concurrent administration of nephrotoxic drugs, administration of high-osmolar contrast agents, large doses of contrast agent and multiple contrast agents administration within a few days (49). Intravenous prehydration for patients with decreased kidney function should be performed before administration of contrast (49). Intraarterial administration of contrast, as being used in coronary angiography has been shown to be more nephrotoxic when compared to intravenous administration (43). Even if CIN is a well-known cause for acute renal failure, the underlying mechanisms are not completely understood, however, increasing evidence suggests that a combination of renal ischemia and a direct toxic effect on the tubular cells in the kidney has an impact (43).

Approximately 10 % of hospital acquired acute renal failures are caused by CIN, and CIN is the third ranked cause of acute renal failure after dehydration and nephrotoxic drugs (47). In addition to these risk factors the SCA procedure itself carries a risk for CIN, especially regarding volume of contrast administered during SCA and PCI. The timing of a SCA and PCI can also increase the risk for development of CIN. The risk is increased in patients with circulatory collapse, and in those who had a myocardial infarction less than 24 hours previously. The risk is also increased if contrast is administered less than 48 hours following a previous exposure to contrast. To reduce the risk of CIN prehydration with isotonic solution should be completed before and after the exposure if possible (47) on patients with decreased kidney function. A SCA in combination with PCI treatment may more often lead to a higher volume of iodine-based

contrast being used. In addition, if there is clinical indication for supplementary information with OCT, this would lead to even higher administered volume.

2.3.1 Alternatives to contrast flushing in optical coherence tomography

Because of the nephrotoxicity of contrast agents some studies have examined alternatives to contrast flushing in OCT image acquisition. Two studies comparing low molecular weight (lmw)-dextran as an alternative to contrast flushing has been performed (10, 11), as well as a study investigating if Voluven may serve as an alternative (14).

Good image quality was observed with the use of saline or Ringer Acetat as well as contrast in the earlier generation of coronary OCT (32). In this early generation of OCT an occlusive balloon was inflated proximal from the segment of interest, leading to a stop of blood flow. Isotonic saline or Ringer's solutions were solutions that might be flushed out of the distal end of the balloon during image acquisition (53), providing adequate diagnostic images. However, the inflation of the balloon could cause trauma to the vessel wall, and therefore is the flushing technique more gentle to the vessel wall.

There are several non-contrast fluids available in the coronary catheterization laboratory with a potential for replacing contrast in OCT. Crystalloids and colloids are fluids for intravenous use for hypovolemia. Ringer Acetate and saline are common crystalloids. Colloids contains larger molecules, and the greater the colloid osmotic pressure the greater expansion of plasma. Hydroxyethyl starch, such as Voluven (Fresenius Kabi), is a D-glucose polymer from durra or corn, and is primary used for volume therapy. Dextranes, as Macrodex 60 (dextran-70, Meda AB) are artificial polysaccharide, and provides a plasma expansion of 20 % (54), and are mainly used for volume therapy (55). Though, there are some potential side effects by administrating these volume therapeutic fluids, since they often are injected in great volumes. Dextran has a reported anaphylactic incidence to be 0.27% (55) and dose-negative effects on hemostasis, however this is an issue when large volumes are injected, and the doses used for OCT recording are relatively small (56). High volumes of Voluven can lead to dilution of blood components as blood clotting (13), whereas high volumes of Ringer Acetate can lead to overloading

the circulatory system when heart or kidney failure is present, all of these effects are rare (57).

Lmw-dextran has been examined for OCT flushing in a study comparing safety to the first generation coronary OCT which used an occlusive technique. There was no statistically significant difference in image quality between the occlusive technique compared to flushing with lmw-dextran (58). Lmw-dextran has also successfully been used for coronary flushing in other coronary imaging modalities such as coronary angiography (59).

Ozaki et al. (10) investigated if lmw-dextran had the potential to replace contrast flushing for OCT image acquisition, and therefore reduce the risk for renal impairment. In the study by Ozaki et al (10) a low-osmolar contrast agent (Omnipaque 350 mg I/mL, GE Healthcare) was compared to dextran-40 mixed with lactated Ringer's solution (Low Molecular Dextran L Injection). By looking at clear image segments (segments of 1 mm containing at least one clear image frame) they compared image quality for $\geq 270^\circ$ of the circumference, and there was no significant difference between the lmw-dextran/lactated Ringer mixture and contrast. They concluded that lmw-dextran had the potential to replace contrast for clearance of blood during OCT recordings, and that there would probably become an increased use of lmw-dextran instead of contrast for OCT flushing.

In 2014, by Frick et al. (11), investigated further if lmw-dextran had the potential to be an alternative to contrast flushing in OCT. They compared Visipaque 320 (GE Healthcare) to dextran-40 (Hospira, Inc.). There was no statistically significant difference observed in image quality and counted clear image segments. After correction for the refractive index, no statistically significant differences in area measures were present. The authors concluded that image acquisition in OCT with lmw-dextran is feasible, safe and provides similar results compared to contrast (11).

A study comparing another colloid solution, Voluven, to contrast has also been performed. In a pilot study with one swine Hamdan et al. (14) compared image quality after flushing with either Visipaque 320, Voluven or a mixture of 50/50 concentration of Visipaque 320 and Voluven. After the pilot Voluven alone was discarded due to statistical

significant worse image quality, but performed a comparison of the 50/50 mixture against contrast successfully on six patients with optimal image quality with no statistical significant difference between the flushing agents on image quality, as well as assessment of thrombus, intima thickness, lumen area, stent struts and detection of vasa vasorum (14).

These studies show that there may be a potential for an alternative to contrast flushing in OCT image acquisition, with preserved image quality.

3. Methods

3.1 Overview of the study

In this study, we investigated if it was possible to reduce or replace the use of contrast agents as flushing fluids during image acquisition with OCT, with a preserved image quality. Therefore, in the present study we compared six flushing fluids of different contents of iodine against a recommended contrast dye as reference (Visipaque 320 mg I/mL) on OCT image length and quality. In an animal model OCT image acquisition was quantified as diagnostic image length and subjective image quality in the left and right coronary arteries.

Each of the seven fluids was injected in the same segment in each of the four coronary arteries at equal infusion rates, and each porcine served as its own control.

The pilot study used a crossover design, a variant of repeated measures design in which the animals were exposed to all flushing conditions in sequence. This means that the comparison is within-subject instead of between subject (60).

A pilot study gives an overview over personnel and data management, as well as scientific reasons like safety and estimation of the results (61). Due to the uncertainty of the feasibility of study design with many fluids injected into an artery, we planned to start with a small sample to investigate if any of the fluids would show a potential to replacing contrast flushing, which would support previous studies. By performing a pilot, there was also a possibility test the feasibility of the design due to uncertainty to the injections, as well as look at the measurement outcomes, the overall standardization of the data collection and team work.

3.2 Animals

This pilot study was completed using two female porcine, 3 months of age and weighing respectively 26 and 31 kg. We considered it unethical to administrate many injections

(7x2) in a human subject due to exposure of iodine-based contrast, possible procedural complications and discomfort, and the risk for serious arrhythmias. Porcine were selected since they share several anatomic and physiologic characteristics with human hearts, with similar coronary artery system. The size of the heart and the cardiovascular system of a four month old porcine is equal to the growth of a human into the mid-teens (62) Therefore, porcine are one of the major animals used in medical research. Because of these similarities to the human heart, we decided upon a porcine model. The study protocol consisted of 7 injections of different flushing fluids for each of the two coronary arteries for each subject. Even though, it has been well documented that repeated injections during OCT imaging is well tolerated in porcine (63), it was unclear if as many as fourteen (7x2) repeated injections could carry the risk for serious arrhythmias that would require treatment.

3.2.1 The coronary arteries

Both the right coronary artery (RCA) and the left anterior descending coronary artery (LAD) of each swine ($n = 4$ arteries) (figure 7) were chosen in each porcine, which mean that the porcine contributed with two arteries each. For this pilot study, we chose to consider the two arteries in each individual porcine as independent from each other, thus the data are significantly treated as the recordings came from four individual porcine. Although this handling of data is in violation of the principle of independence this procedure was chosen for the pilot. Due to differences in the physical behavior of the arteries, such as the location at the heart and size differences, the research group meant that this violation of independence could be defended for the pilot study. The flow dynamics in the RCA and LAD are different, and are therefore one of the reasons why these two arteries were the chosen to perform the data collection from, with the possibility of providing more data variety. The flow profiles the LAD and RCA differ, in particular caused by different pressure in the right and left ventricles (64). The LAD is located on the left ventricle wall, where the ventricular pressure is greater. The left ventricle is from where the blood is pumped out in the aorta, and therefore a higher pressure is needed. The right ventricle delivers blood to the pulmonary artery, where a much lower pressure is needed. Therefore does the LAD and the RCA have different flow

profiles (65). Normal blood pressures in humans are for the left ventricle 90-140 mm Hg (systolic pressure), 8-15 mm Hg (diastolic pressure) and for the right ventricle 15-25 mm Hg (systolic pressure), 0-8 mm Hg (diastolic pressure) (54).

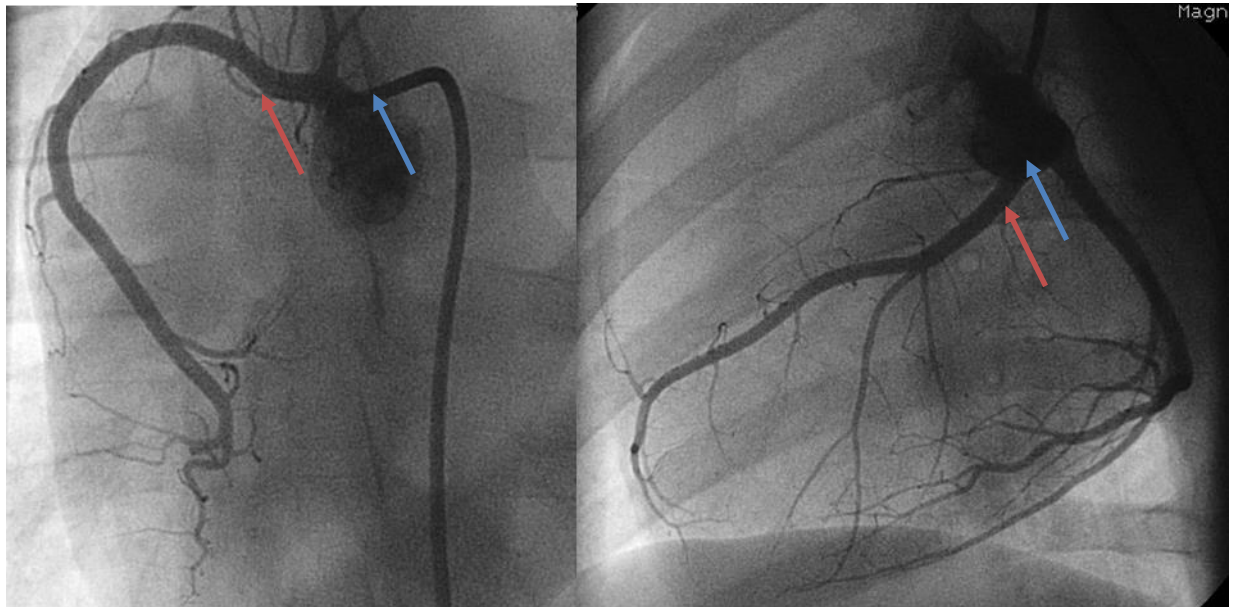


Figure 7. SCA from porcine # 2

Selective coronary angiograms (SCA) from Porcine #2. Contrast dye creates a dark silhouette of the coronary arteries. Left panel: SCA of the right coronary artery (RCA). 6 French coronary guide catheter (blue arrow) introduced to the RCA origin (red arrow). Right panel: SCA of the left coronary arteries (left anterior descending (LAD) and left circumflex (LCX)). The LAD and LCX share a common left main stem before they separate and supply different areas of the heart muscle. Compared to RCA more side branches are seen in LAD and LCX. 6 French guide catheter in left main stem (blue arrow). LAD is indicated with a red arrow.

3.3 Instruments

The OCT system used for data recordings (C7-XR FD-OCT, St.Jude/Lightlab Imaging Inc., Westford, MA, USA) consists of an imaging catheter, a motorized pullback device and an imaging console. The image console contains the display, the light source, data storage and signal processing units (29). (For images of the C7-XR FD-OCT, see Appendix 1).

To achieve OCT images with the C7-XR system, the OCT Dragonfly™ has to be positioned over a regular coronary wire (0,014 inches) distally to the region of interest in

the coronary artery (25). The catheter has a diameter of 2,7 French and has a single mode optical fiber which is enclosed in a hollow metal torque wire. The torque wire rotates with a speed of 100 rounds per second, and has a marker 10 millimeters distally to the optical lens (20). This means that the catheter imaging tip is pulled backwards while it is rotating in order to scan the artery along the vessel lumen (66).

3.4 Procedures

The procedures were done in an equal fashion, examining one porcine on two consecutive days, with the same clinicians and radiographers involved in the same coronary catheterization laboratory. The anaesthetized porcine were placed in a supine position on the coronary catheterization laboratory bench. Intravenous infusions of sedatives were started before the porcine entered the laboratory, and these infusions were ongoing through the whole procedure (Propolipid, Fresenius Kabi) introduction dose 15 mg/kg/h, maintenance dose 20 mg/kg/h, and Fentanyl (Actavis) introduction dose 20 mg/kg/h, maintenance dose 50 mg/kg/h). The porcine were also intubated and artificially ventilated, and were given intramuscular Ketamine (Ketalar, Pfizer) 33 mg/kg before ventilation and intravenous access were established. A 6 French (6Fr) femoral artery access was established by an experienced clinician. A j-shaped wire was introduced to the ascending aorta, and a 6Fr guide catheter was positioned into the coronary ostium. Since the RCA and LAD origins from opposite sides of the ascending aorta, different shaped coronary guide catheters had to be used for localization and for optimal fitting into the coronary ostium. A 6 Fr FR4 catheter were used for intubating the RCA and a 6Fr FL3.5 catheter were used for the LAD (Runway, Boston Scientific). Both the catheters were placed in a steady position in the artery ostium. When the placement of the catheters was completed selective coronary angiograms were performed. After recording the first angiograms and identifying reference marks a thin coronary wire was advanced into the distal end of the coronary artery (PT graphix intermediate 0.014", Boston Scientific). Side branches on the coronary artery were observed, to serve as anatomical references to maintain OCT pullbacks of the same segment in the artery for each fluid. The OCT catheter was then inserted over the coronary wire to an area in the coronary artery with side branches as landmarks. Immediately before each image acquisition, contrast was

inserted into the artery while the live-mode imaging was on the OCT, to ensure that the distal end of the catheter was in the exact same position.

The OCT recordings were performed with an automatic image acquisition, as the OCT modality has a sensor that detects clearance of blood and subsequently starts recording along the artery automatically. Using a mechanical injector (Medrad injector, Bayer Healthcare), an equal flush protocol was used on all the recordings: flushing volume 16 mL, flow rate 4 mL/s, pressure 300 PSI (pounds per square inch)/2068 kPa.

The flushing fluids were numbered and their content were blinded for the operator and at the laboratory staff, and injected in the same order. Due to the changing of containers for the mechanical injector, care was taken to flush out remaining fluid from previous recording before a new flushing fluid injection took place. Automatic calibration was also performed before and after each recording. Before performing measurements of the created OCT images, calibration is necessary by adjusting the Z-offset around the catheter, which is the zero-point setting for the system (25), since the catheter has a standardized size used as reference (67).

In both the RCA and LAD OCT image acquisition were performed using seven different contrast and non-contrast flushing agents (table 1).

Table 1: Flushing fluids used for coronary flushing in the experiment. The rows present the name and pharmaceutical companies of the fluids. The columns describe what kind of fluid, iodine content, and name in which the fluids will be called further in the thesis.

Name	Type	Iodine content	Renamed
Visipaque (GE Healthcare)	Contrast	320 mg I/mL	Visipaque 320
Visipaque (GE Healthcare)	Contrast	270 mg I/mL	Visipaque 270
Iomeron (Bracco Imaging)	Contrast	350 mg I/mL	Iomeron 350
Iomeron (Bracco Imaging)	Diluted-contrast	150 mg I/mL	Iomeron 150
Macrodex 60 Dextran-70 (Meda AB)	Non-contrast Colloid solution	None	Macrodex 60
Voluven (Fresenius Kabi)	Non- Contrast Colloid solution	None	Voluven
Ringer Acetat (Baxter)	Non-contrast Crystalloid solution	None	Ringer Acetat

The non-contrast agents were a room temperature (23°C), whereas the contrast agents were stored in a heating cabinet before injection (37°C) before flushing. An experienced SCA and OCT operator/clinician performed the cardiac catheterization and OCT procedure.

Mean arterial blood pressure (MAP) and electrocardiogram (ECG) was monitored continuously during the experiment to ensure similar hemodynamics for each recording, and to detect serious arrhythmias. MAP was measured invasive in the ascending aorta

through the distal tip of the coronary guide catheter, whereas heart rhythm was measured by a 5-lead ECG showing the electrical activity. This observation of ECG and hemodynamics was of importance to know that the porcine was not under stress, as well as ensuring that in each porcine there was no changes that could affect the recordings. ECG and MAP were registered for each injection both days.

3.5 Image treatment and analysis

After collecting the data, the OCT images were stored and analyzed on an offline OCT-workstation. With the intention to perform interobserver reliability analysis we originally planned that three observers, with long OCT experience, blinded to the identity of the porcine and flushing agent, performed the OCT image analysis. However, due to the small sample size, leading to difficulty regarding repeated measurement analysis, the decision fell on completing the analysis with only one observer with large OCT -and research experience and without involvement in designing the study.

The main outcome in this pilot is the diagnostic image length, which is measured in millimeters, and represents the continuous diagnostic length out of 54 millimeters that consisted of frames with optimal or good subjective image quality. Subjective image quality is a secondary outcome, measuring the overall image quality within the diagnostic length

Diagnostic image length

Diagnostic image length describes the length of continuous images along the artery of at least good subjective image quality, which are image frames that will give diagnostic information about the coronary artery wall, detecting anatomy and potential pathology. Each of the 28 recordings from the OCT image acquisition was evaluated in a frame-by-frame fashion. A single OCT recording is 54 mm and consists of 270 axial frames (image plane perpendicular to the axis along the vessel). Each frame assessed in one of four categories of subjective image quality (0 = discarded, 1 = limited, 2 = good/slightly limited, 3 = optimal).

To achieve optimal image quality (category 3) there would not be visible blood in the vessel lumen, and the coronary wall should be visible in at least 270° of the circumference, and the three vessel layers of the vessel should be visible. Good/slightly limited image quality (category 2) represents good image quality, where the vessel wall and its structures are well visualized in $\geq 270^\circ$ of the circumference, but there might be small appearances of blood in the vessel lumen. With limited image quality (category 1) there are moderate appearances of blood leading to loss of details of the vessel wall, leading to lack of diagnostic information. Discarded image quality (category 0) represents no diagnostic value at all, due to lack of a successful blood removal.

Categorizing a subjective image quality score has been done in previous study (14). Measuring length of diagnostic quality (discernable border between lumen and vessel wall $> 270^\circ$) as a total of clear image segments have also been performed previously (10, 11). We chose not to measure diagnostic length within clear image segments, where just one frame in the segment (of 1 mm) had to have diagnostic image quality. Instead we measured a continuous diagnostic image length in millimeters (containing subjective image quality 2 or 3) with a cut-off at the first frame with a subjective image quality score of 1 or 0. In the clinic, the clinician or coronary laboratory staff is used to measure lengths of OCT recordings in millimeters, and is a method that our observers were used to perform, so it did not require further training. Measuring diagnostic length in millimeters containing good or optimal image quality frames has as far as we know not been performed earlier, but it does share similarities to clear image segments, but we believed that this would be an adequate method for measuring especially by including a continuous length of good image quality frames.

The continuous OCT image length consisting of an overall subjective image quality scores (2 and 3) were measured and defined as “diagnostic image length”, with a maximal obtainable value of 54 mm. Since diagnostic image length is measured in millimeters, the variable is a ratio scale, due to the threshold of 54 mm for image acquisition. This data type has a known and equal distance between the units of measurement, and they are categorized by the measurement of ranges of variables (68), and all the arithmetic functions can be applied to ratio scales. Ratio scales typically includes time, weight, and as in this study length (69).

Subjective image quality

As a secondary outcome we assessed the overall subjective image quality. The image quality is measured by subjective rating of visual quality of the images on a 4-point scale (0-3), as described under diagnostic image length. These two variables are connected, since the overall subjective image quality score of a diagnostic image length had to be 2 or 3. In the overall score in the diagnostic length there might be scores of either 2 or 3, but the overall image quality score registered is represented by the score who had a major representation. -We wanted to measure the overall image quality within the diagnostic length, to see how the image quality of the different fluids was compared to the reference. These measures were performed only with integer numbers, and represent the overall image quality of each recording. The subjective image quality variable is categorical ordinal, since the data is ordered on the basis of an operational defined property of characteristics (68).

The measurement form for both diagnostic length and image quality is included as Appendix 3.

3.5 Flushing fluids

The independent variables are variables that are chosen, cannot be controlled, and they do not rely on the other variables (70). Seven different fluids with different content of iodine and chemical composition were chosen; contrast, low-contrast and non-contrast.

We decided to use the recommended viscous contrast fluid Visipaque 320 (iodine content 320 mg I/mL) (6) as a reference fluid which all other potential contrast and no-contrast flushing fluids were compared to.

In our cardiac catheterization laboratory practice, a low-osmolar contrast agent, Iomeron 350, is used both for SCA and OCT image acquisition. This contrast fluid has a slightly higher iodine content (350 mg I/mL) than the reference. In addition to chemical composition is Iomeron 350 less viscous than the reference contrast fluid (Figure 8).

For examination of a flushing agent with a moderately lower content of iodine than the reference, Visipaque 270 was used (270 mg I/mL). A flushing fluid, Iomeron 150, with less than half of the iodine content (150 mg I/mL) was also chosen to be compared to the reference, and was used as a diluted contrast fluid.

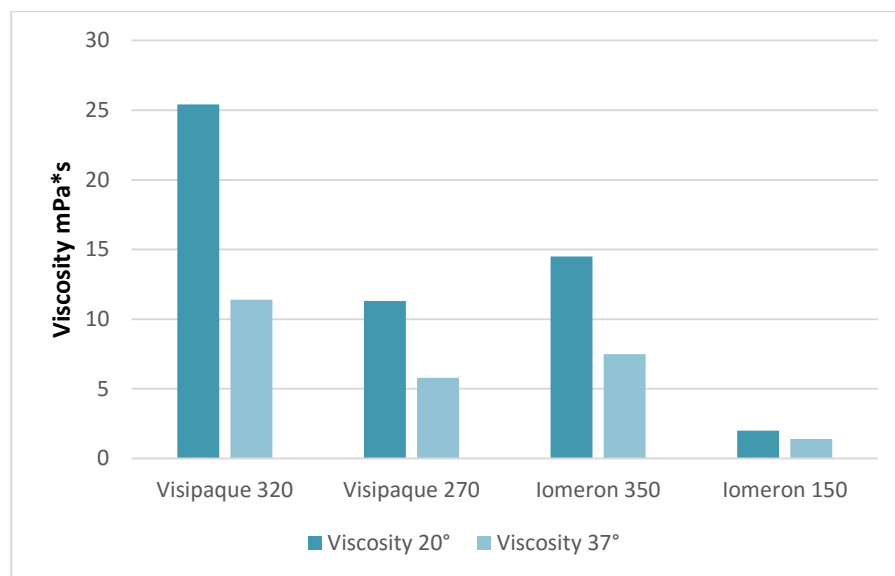


Figure 8. Diagram over the viscosity of the contrast fluids

The figure shows the viscosity in contrast agents at various temperatures. Visipaque 320 displays the greater -and Iomeron the lesser viscosity (45, 46).

Dextrans, are colloid solutions, and are available in two different concentrations, 40-dextran and 70-dextran. In this pilot study we used 70-dextran (Macrodex 60, 6g/100mL(55)), as it is easy available in our hospital. Dextrans are mainly used for volume therapy in critical ill patient, and have a maximum 24 hour dosage for 1.5g/kg (55). Voluven is another available colloid solution, and is one of the most frequent used blood plasma substitutes (71). Ringer Acetate and Voluven are also non-contrast fluids that are available on the coronary catheterization laboratory at all times and these two non-ionic containing fluids were examined.

All in all, six different fluids were chosen to be compared to the reference (Visipaque 320): Three iodine-containing contrast fluids: Visipaque 270, Iomeron 350, Iomeron 150 and three non-iodine containing fluids: Macrodex 60, Voluven and Ringer Acetat.

Unfortunately, there was not possible to get viscosity (dynamic viscosity (Pa*s)) values from the pharmaceutical companies of the non-contrast fluids. It was decided that viscosity measurements would be useful, however due to a lack of resources a viscometer could not be located.

3.5 Statistical Analysis

All the analysis in the master thesis was performed with data generated from one observer. Originally we wanted to perform an interobserver protocol using kappa for categorical data and intraclass correlation 3,1 for the continuous data. This was not possible to carry, due to the small sample.

This pilot study had a small selection ($n=4$). From the data generated we wanted to perform a post-hoc power analysis, using the reference and one of the non-contrast containing fluids. This post-hoc analysis was calculated at a statistical web site (statisticalsolutions.net), and the mean from the reference fluid was set as $m(0)$, whereas the mean from Macrodex 60 was set as $m(1)$. We decided upon Macrodex 60 since previous studies have shown that lmw-dextran has the potential to replacing contrast in OCT flushing (10, 11). The sample size was set to 4, as the data would be handled, even if this is in conflict with the principle of independence. The α -level was set to 0.05, making the probability for a type I error to occur at 5 %. Type-I errors refers the risk of believing there is an effect, when there actually is not. Power is calculated by $1-\beta$, the β -level represents type II-error, which occurs when we do not believe that there is an effect in the population when there actually is one (72). The minimum acceptable chance for a type II-error to occur is 0.20 (20%), therefore a power at 0.80 (80%) would be acceptable. We also chose to see from our generated data which sample size that would be required to achieve a power at 0,80 and a α -level at 0,05, and performed a post-hoc sample size analysis.

The result from the post-hoc power analysis gave a power = 0.61. Post-hoc sample size from the same generated data (chosen power 80%, α -level 0.05) gave a sample = 7.

If the null hypothesis is kept from our analysis of the generated data this will support results from previous studies, showing that there might be a potential to replace contrast for OCT flushing. The alternative hypothesis for this pilot study was that there is a statistical significant worse difference between a fluid or fluids when compared to the reference. If a fluid differs statistical significantly reduced on diagnostic image length, it might be seen as a less potential fluid for replacement of the reference. But, there might be a possibility for a fluid to perform statistically significantly better on diagnostic image length than the reference, this would as a non-significant result contribute to keep the null-hypothesis and reject the alternative hypothesis. However, because of to the low power, there is a risk for keeping the null hypothesis when there actually is a statistical significant worse difference (73).

The statistical analysis for diagnostic image length and subjective image quality were performed in SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22. Armonk, NY). Due to the small selection it was not possible to rely on the assumption of normal distribution, and inspection of normality histograms did not support a normal distribution. Therefore; a non-parametric test was chosen, to compare the different fluids on diagnostic image length against Visipaque 320. In the testing of the diagnostic image length, we wanted to test if the alternative fluids did not do statistically significant worse than the reference to be considered a potential alternative fluid for OCT flushing. To perform the analysis of the diagnostic image length the Wilcoxon signed-rank test was chosen, since the all the fluids were tested in the same subjects (porcine). Since this is a non-parametric test the data is ranked, and for the Wilcoxon signed rank test the sign of difference (negative or positive) is also assigned to the rank (74).

The subjective image quality analyses, which are categorical ordinal data, were performed using a contingency table (2 x 2), using a chi-square test. There are two assumptions to recognize: the frequencies represent individual counts and the categories are mutually exclusive (75). In a chi-square test the expected counts should be greater than 1 and 80 % of the expected counts should be ≥ 5 (76). A sample size of 4 did not meet the assumptions of counts in the cells of the 2 x 2 contingency table. Therefore, a Fisher's exact test was used. This test is an alternative to the chi-square for small samples, and is also based on the observed columns and rows of a table (ibid). Like the

Wilcoxon signed rank test six analyses were completed, where the reference fluid was compared to one after one of the other fluids.

3.6 Ethics

Performing research involving animals, requires justification, since animals cannot give a consent to participate (77). Even though, it is generally accepted that animal research is justified, it does not mean that every project involving animals are. Therefore, as with research involving humans, there is an official regulation organ for approving which studies on animals that are allowed (ibid). The Norwegian Animal Research Authority (NARA) is the Norwegian regulation organ for this matter.

Before the data collection, NARA approved this study (Appendix 4). In all studies that involve animals there are three main concerns: replacement, reduction and refinement. These three Rs are also constituted in the Norwegian law: The regulation about animal studies (Forskrift om forsøk på dyr) in §9 (78). Before planning the study, a literature search was done in medical databases like MEDLINE, PubMed. At the databases online search words as OCT, OCT and coronary arteries, OCT and contrast, OCT and coronary and contrast were found. At that time only one study was relevant regarding the issue of alternatives to contrast flushing. The study was performed by Ozaki et al. where contrast and lmw-dextran were compared, and lmw-dextran showed a potential for replacing contrast (10). Other studies regarding OCT were to test the safety of the non-occlusive OCT generation. When planning the study protocol studies regarding other colloids (Voluven) was performed and taken under consideration. All in all, we believed that this was an issue for further investigation. At the greater databases as Up-To-Date we did not find any information regarding this issue. The literature review confirmed that there was yet still little research done regarding this matter, and therefore we wanted to carry out the project, starting with a pilot study.

Replacement implies whether it is possible to conduct the study without the use of animals. While considering how we wanted to collect the data, contact was established with physicists at Oslo University Hospital, Ullevål and Rikshospitalet, as well as with a physicist at the Norwegian Defense Research Establishment who works with infrared

light. Primarily, we wanted to investigate if there was a possibility to build a special cylinder, working as a vessel, to perform image acquisition with OCT and different fluids. This was not possible. Since it would not be ethical to perform as many injections in a human coronary artery due to the risk of arrhythmias, other complications, and a great volume of contrast, an animal model were suggested.

Reduction implies to use as few animals as possible. This model, with as many fluids as we wanted to use, had not been done before. The decision fell on that a couple of porcine were enough for the data collection for the pilot. This should be enough to provide data to perform the sample size analysis, but also the analysis of the main variables to see if there was a difference present compared to the reference. We also believed that it would be possible to see if there was a trend that one or several of the fluids might would have the potential to replace today's recommendation/reference. The staff involved in the data collection also works at the coronary catheterization laboratory, working daily with catheterization of the coronary arteries, as well as being well experienced with OCT. This also plays a part in reduction, since the environment and procedure were well known.

Refinement implies to as little harm as possible towards the animals. The porcine used roam freely in an approved place, and before our data collection they were fasting, but with free access to water. Before entering the coronary catheterization laboratory, the porcine were anesthetized by injection with intramuscular Ketamine (Ketalar, Pfizer). This injection may cause discomfort for approximately 30 seconds. The porcine where also intubated and placed in general anesthesia before entering the coronary catheterization laboratory. During the procedure the porcine had ongoing infusions of analgesic and anesthetic medication, and ECG and MAP were continuously monitored, to make sure that there was no sign of distress. The use of OCT on porcine models has been done before, and is well tolerated by the animals (63). After the experiment the porcine were euthanized by giving 20 mL of potassium chloride.

4. Results

In the result both mean and median for diagnostic image length for the fluids will be presented in millimeters. The subjective image quality, which is based on subjective measures, categories between 0-3, will also be presented as both mean and median.

Electrocardiogram (ECG) and mean arterial blood pressure (MAP)

The porcine had continuous registration with ECG and MAP:

For porcine # 1 the MAP and ECG were at baseline: MAP = 85, ECG = 105. The mean (SD) values for MAP and ECG during the data collection for porcine #1 were: MAP = 76 (10), ECG = 109 (5). For porcine # 2 the MAP and ECG were at baseline: MAP = 103, ECG = 96. During the data collection for porcine # 2 the mean values (SD) were: MAP = 114 (7), ECG = 94 (3). No arrhythmias or changes in hemodynamics were observed during the experiments.

4.1 Diagnostic image length

Diagnostic image length represents a continuous length in millimeters of OCT image frames with subjective image quality of 2 or 3. In the table and figures the reference contrast fluid will be presented first, followed by the contrast fluids, diluted-contrast fluid and non-contrast fluids.

Table 2: Diagnostic image length (mm) of the seven flushing agents used in the study (n = 4). Data are given as median (interquartile range (IQR)). For descriptive purposes the mean (\pm SD) is also reported. The p-values from the Wilcoxon signed rank test denote the statistical value associated with comparisons to the reference fluid – the Visipaque 320.

Diagnostic image length			
Flushing agent	Median (IQR)	Mean (\pmSD)	P - value
Visipaque 320 *	54.0 (6.0)	52.0 (4.0)	-
Iomeron 350	46.0 (9.8)	46.8 (5.4)	0.10
Visipaque 270	54.0 (3.0)	53.0 (3.0)	0.66
Iomeron 150	40.0 (18.3)	40.8 (9.6)	0.068
Macrodex 60	49.5 (13.5)	47.5 (7.4)	0.47
Voluven	42.5 (15.8)	41.8 (8.3)	0.14
Ringer Acetat	32.5 (32.5)	28.5 (17.4)	0.068

*Reference fluid

As shown in table 2, compared to reference there were no statistical significant differences in diagnostic image length for the various fluids. However, both the iso-osmolar contrast agents, Visipaque 320 and Visipaque 270, were associated with the greatest numerically diagnostic image length. Iomeron 350, was associated with a somewhat shorter numerically diagnostic image length with more variation (SD 5.4) when compared to reference. By flushing with diluted-contrast, Iomeron 150, we observed a numeric diagnostic image length of approximately 40 mm, with a somewhat variation in the measures. One of the colloids, Macrodex 60 gave a numeric diagnostic image length close to 50 mm. The other colloid fluid, Voluven, provided a diagnostic image length > 40 mm. Ringer Acetat had the numerically lowest diagnostic length of approximately 30mm, with a marked variation (SD 17.4) compared to the reference fluid and the other fluids.

As indicated by the p-values in table 2, none of the different fluids tested differed statistically significant compared to reference (significance level 0.05). However, Iomeron 150 and Ringer Acetat provided a somewhat lower diagnostic image length compared to the reference ($p = 0.068$).

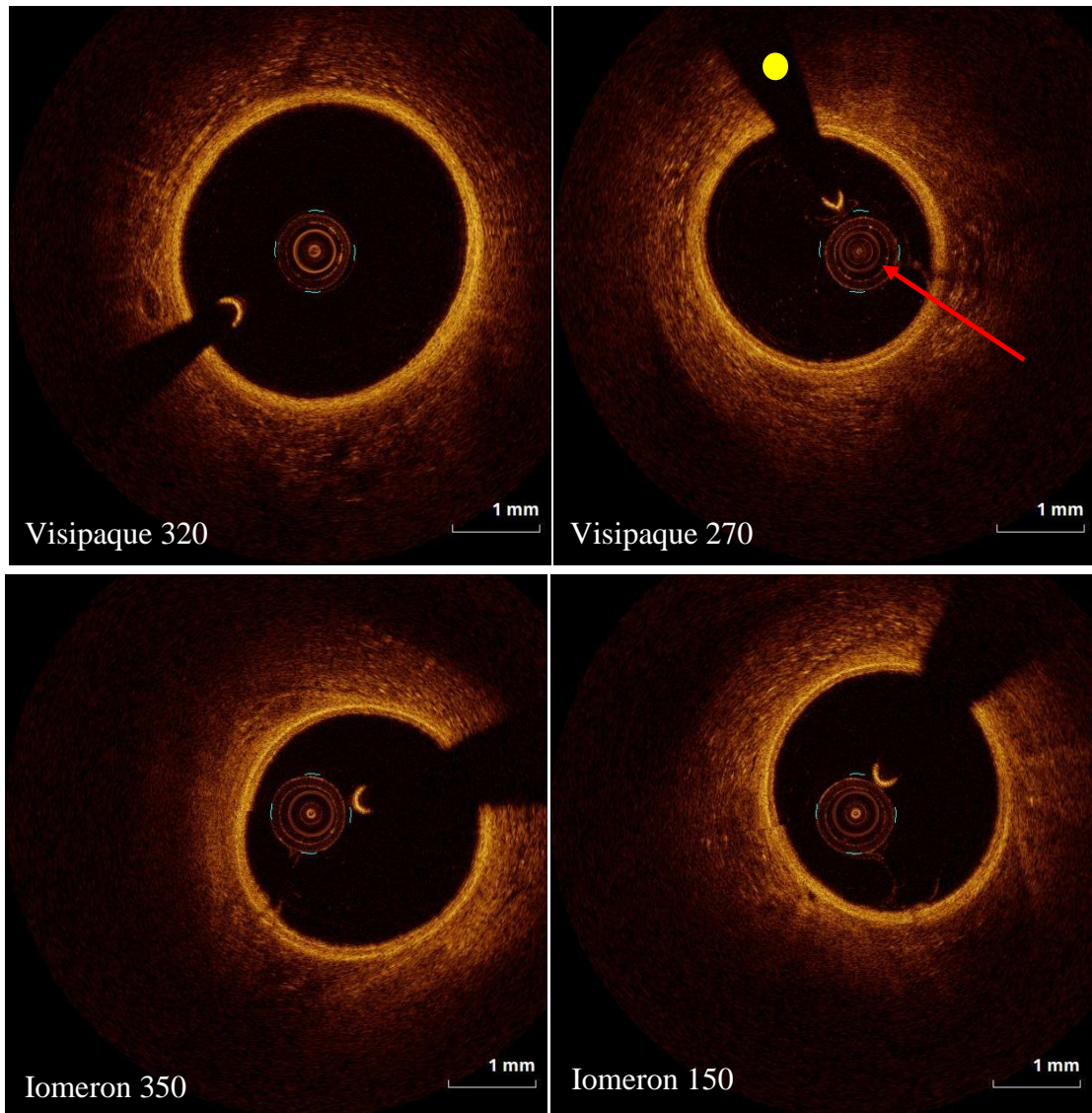


Figure 9. OCT images from the data collection, contrast fluids

Optical coherence tomography (OCT) recordings with various contrast containing flushing fluids. All images are from the distal RCA in porcine # 1. Please note that all three layers of the vessel wall are visualized in all the four frames. The yellow dot illustrates the shadow from the coronary guide wire which are present in all the frames. The red arrow points at the Dragonfly OCT-catheter which are placed in a blood free lumen (black)

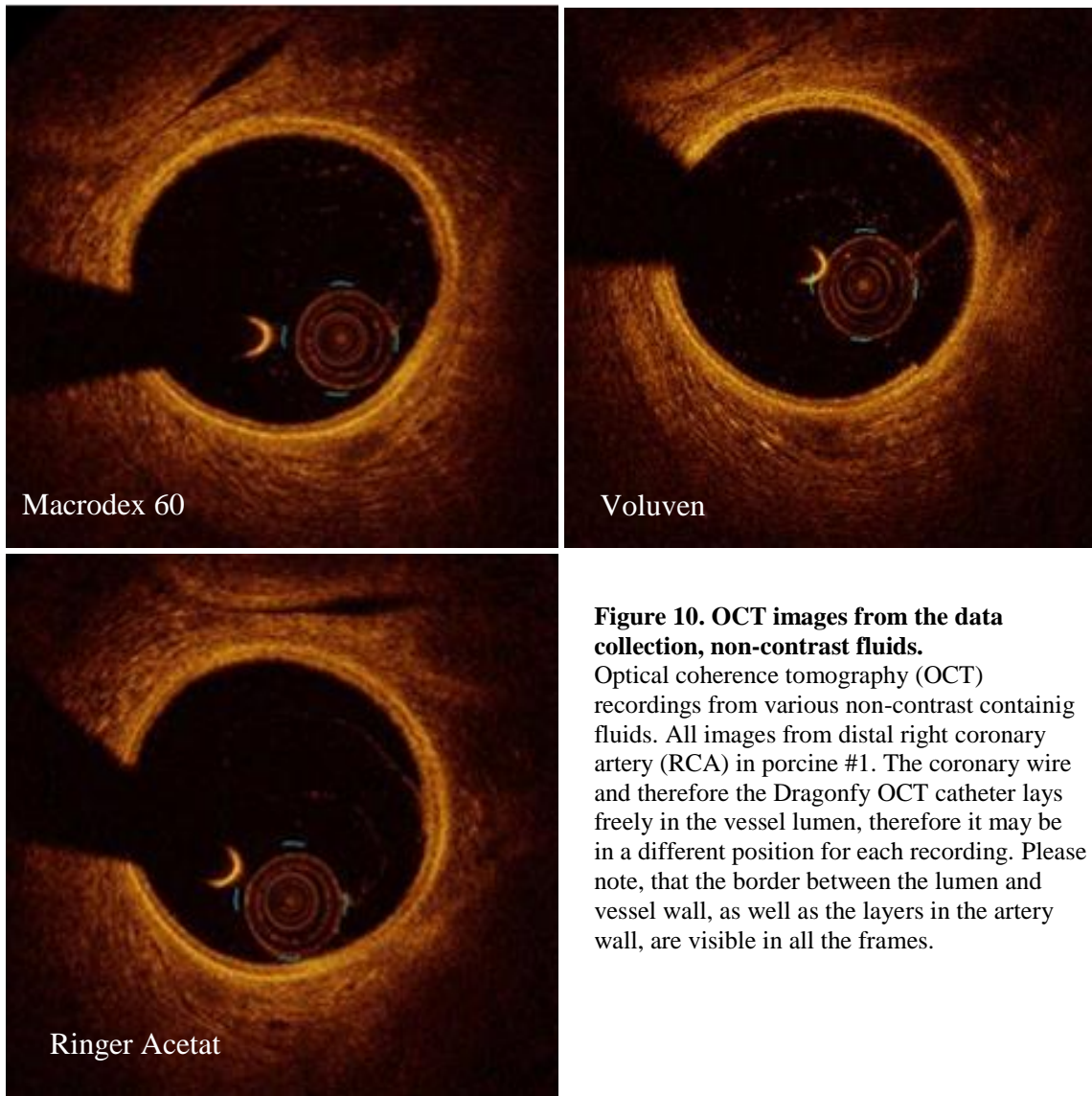


Figure 10. OCT images from the data collection, non-contrast fluids. Optical coherence tomography (OCT) recordings from various non-contrast containing fluids. All images from distal right coronary artery (RCA) in porcine #1. The coronary wire and therefore the Dragonfy OCT catheter lays freely in the vessel lumen, therefore it may be in a different position for each recording. Please note, that the border between the lumen and vessel wall, as well as the layers in the artery wall, are visible in all the frames.

4.2 Subjective image quality

Subjective image quality presented and analyzed represents the scores of 2 (good) or 3 (optimal) within the diagnostic image length. In the table the reference contrast fluid will be presented first, followed by the contrast fluids, diluted-contrast fluid and non-contrast fluids.

Table 3: The overall subjective image quality within the diagnostic image length of the seven flushing agents used in the study (n = 4). Data are given as median (interquartile range (IQR)) and mean (\pm SD). The p-values denote the statistical value from the Fisher’s exact test associated with comparisons to the reference fluid – the Visipaque 320.

Subjective image quality			
Flushing agent	Median (IQR)	Mean (\pmSD)	P - value
Visipaque 320 *	3.0 (0.8)	2.8 (0.5)	-
Iomeron 350	3.0 (0.8)	2.8 (0.5)	1.00
Visipaque 270	3.0 (0.0)	3.0 (0.0)	1.00
Iomeron 150	3.0 (0.8)	2.8 (0.5)	1.00
Macrodex 60	2.0 (0.8)	2.3 (0.5)	0.49
Voluven	2.0 (0.8)	2.3 (0.5)	0.49
Ringer Acetat	2.0 (0.8)	2.3 (0.5)	0.49

*Reference fluid

The fisher’s exact test tested the overall subjective image quality within the diagnostic image length compared to the reference fluid. The two subjective image quality categories were good (category 2) and optimal (category 3 (figure 11)). As the p-values in table 3 shows, was the subjective image quality slightly better ($p=1.00$) by flushing by contrast containing fluids than for flushing performed by the non-contrast containing fluids ($p = 0.49$) when compared to the reference.

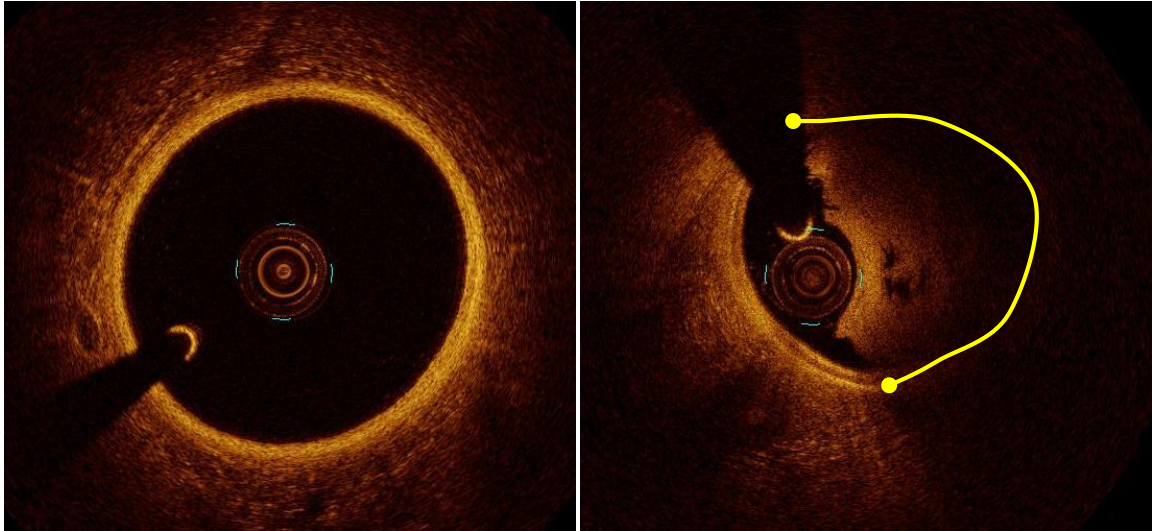


Figure 11. OCT images from the data collection with optimal and discarded image quality

Images from the pilot study, as an example of optimal versus discarded image quality. Left panel: Optimal optical coherence tomography (OCT) recording frame (subjective score 3), where the three different layers in the artery wall are visible. Right panel: Non-diagnostic frame from OCT recording (score 0-discarded), showing lack of visible lumen/vessel border due to blood (yellow curve). Just a small area of the vessel wall is visible.

5. Discussion

The overall aim of the current study was to investigate if diluted- or non-contrast flushing fluids could reduce the need for iodine-containing contrast fluids during OCT image acquisition, -with preserved image quality.

We observed no statistical significant differences in diagnostic image length or image quality for the flushing fluids when compared to the reference fluid, leading to rejection of the alternative-hypothesis. Therefore, the fluids tested should be investigated further to assess the potential to replace contrast flushing in OCT.

5.1 Quality of the OCT recordings

The observed diagnostic image length of the two contrast agents investigated (Visipaque 270 and Iomeron 350), were not statistically significant different compared to the reference fluid. Compared to the reference fluid, we did not observe statistically significant difference in diagnostic image length for the diluted-contrast ($p=0.068$). However, we did observe a trend suggesting that Iomeron 150 was associated with shorter diagnostic image length than the reference fluid. In contrast to the findings in another small study (14) where the authors reported lower image quality for OCT flushing with Voluven when compared to contrast, we did not observe a statistically significant difference in diagnostic image length between Voluven and the reference contrast. Voluven flushing provided clinically acceptable OCT recordings and should be considered for future investigations. In the clinical practice Voluven is also a fluid which is common for the coronary catheterization staff, and is easily available. For the other colloid fluid, Macrodex 60, the observed numerically image length was approximately 50 mm, which is comparable to the reference. OCT flushing by colloids, such as Macrodex 60, have also been investigated by others (10, 11) who observed preserved image quality in total of clear image segments when compared to contrast reference. In the current study similar diagnostic image length was observed for OCT flushing by Macrodex 60 as for reference. Compared to contrast reference, we observed lower diagnostic image length for Ringer Acetat, with a large variation in diagnostic image length. However, in

our small study, diagnostic image length for Ringer Acetat did not differ statistically significant on compared to reference. The hemodynamic conditions were stable in each flushing fluid and can probably not explain the large variation in image length observed for Ringer Acetate. Even if no differences in OCT quality by Ringer was observed, the large variation suggests that Ringer Acetat may not be an optimal alternative to contrast in OCT flushing.

The findings in the current study suggests that colloids might be used for flushing during OCT image acquisition, and should be further explored as flushing fluids in future larger studies. Our findings for OCT flushing by Macrodex 60 confirms others findings in lmw-dextran, and supports their conclusion that dextrans might have a potential for replacing contrast in OCT flushing.

Viscosity has been presented to be a physical property of importance for OCT flushing, and is one of the main reasons Visipaque is recommended as flushing fluid (6). Macrodex 60 is a glucose polymer and Voluven is based on starch (54), and these fluids might be considered to be more viscos than crystalloid fluids and diluted-contrasts. We were unable to measure viscosity (dynamic viscosity, Pa*s) for the current study, due to failed attempts to locate a viscometer. We made contact with the pharmacological manufacturers for viscosity properties of the different non-contrast fluids, however, in the replies they were unable to define the viscosity unit (Pa*s or mm²/sec). In previous studies (10, 11) kinematic viscosity of lmw-dextran and Visipaque 320 have been reported as 3.7 mm²/sec and 8.7 mm²/sec, indicating that Visipaque 320 has a greater viscosity than lmw-dextran. However, both of the fluids has greater viscosity than water (kinematic viscosity 1 mm²/sec). Frick et al. (11) suggests that the differences in viscosity might be the reason why a larger volume for lmw-dextran was injected than for contrast in their study where these two fluids were compared for OCT. However, due to different injection volumes, is it difficult to ascertain the degree of impact the viscosity had for total good image cross-sectional frames. Macrodex 60 is more viscous than water. Viscosity might indicate why an observed longer numerically diagnostic length was seen compared to the crystalloid, Ringer Acetate. Even though the dynamic viscosity for the colloids and crystalloid fluid is unknown in the current study, might this physical property affect the flushing length. However, other physical properties of the fluids do probably also affect the ability to clear the artery from blood, and more information about

the physical properties is needed to understand which factors that affect the OCT flushing. For the contrast-containing fluids does a decrease in temperature increase the viscosity. The contrast fluids were stored in containers fitted for the mechanical injector in a heating cabinet at 37°C before injection as in clinical practice. However, changing the containers takes a couple of minutes, this may lead to a decrease in temperature due to differences of temperature between the heating cabinet and laboratory (room temperature). The changes of containers took fairly the same time for all of the contrast fluids. The temperature should have been measured to ensure that the decrease in contrast temperature were somewhat equal. If the temperature decrease affected diagnostic image length is uncertain, but this is clearly a bias. The numerically highest diagnostic image lengths were observed for Visipaque 270, which is less viscous than the reference. This can be a result of chance due to the small sample size, but it may also imply that the effect of viscosity in diagnostic image length is of less importance than previously suggested. It is also possible that different chemical compositions decrease differently in temperature. Overall, the lack of temperature and viscosity measures is a clear limitation for the data collection.

To achieve diagnostic image length a subjective image quality of 2 (good) or 3 (optimal) had to be present. We observed when compared to reference a numerically, non-significantly overall better subjective image quality for the contrast-containing fluids. However, in clinical settings, is the diagnostic information necessary to evaluate the anatomy and pathology given by good image quality.

The alternative hypothesis was rejected for all the fluids in the current study. However, due to the underpowered study should caution be made when interpreting the results. The results indicate that there might be a potential for other fluids to replace the reference contrast. No complications or arrhythmias occurred during data collection, this may indicate that the fluids are well tolerated in porcine. Still, even if porcine share several physiological characteristics with human, caution should be taken in a potential investigation in human coronary anatomy. We believe that the potential for contrast saving alternatives should be investigated further. If the sample size had been greater, the results might have looked different. It would give a greater statistical power leading to detect small in group differences, and the measures done on diagnostic image length would maybe have given a more robust reflection of the flushing ability of the fluid by

limiting that the measures were a result of chance. The sample size clearly is a limitation in the present study, since most statistical methods are fitted for a greater sample size.

5.2 What can the current study contribute to?

With the current OCT generation, the coronary artery is cleared from blood by injecting a flushing fluid through the artery during OCT image acquisition, and viscous contrast has been recommended as flushing agent for optimal clearance of blood (6). Contrast-containing fluids have some potential side effects, and high volumes increase the chance for achieving CIN (7). Therefore, should the volume of contrast containing fluids administered be kept as low as possible, especially since some subsets of patients examined at the coronary catheterization laboratory often have risk factors that will increase the chance for CIN (9). Though, even if OCT can provide valuable diagnostic information of the coronary arteries, optimizing the technique to limit the risk for complications like CIN should be favorable, like reducing iodine-based contrast for OCT flushing. An OCT image recording is typically a supplementary examination during a SCA and PCI, in which a great volume of contrast often is already administered, especially during complex procedures.

In most coronary intervention practices, the kidney function of the patient is always taken into account before performing an OCT recording. After a risk-benefit consideration, if we consider that the information from an OCT would be beneficial for planning the procedure, the examination would be performed. For patients with the risk factors for achieving CIN, as well as all patients, it would be beneficial to replace the use of contrast for OCT recordings, especially if several OCT recordings are required.

Previous studies performed by Ozaki et al. and Frick et al (10, 11) showed a promising result for replacing contrast with lmw-dextran, a finding that was supported in the current study. We suggest that this is a field that should be investigated further, since it may decrease contrast fluid volume, and therefore decrease the chance for complications of the kidney function.

Even if this is an underpowered pilot study, our findings suggests there is a trend for several available fluids to serve as contrast saving alternatives. We want to get an increased focus on the subject regarding contrast saving alternatives, and believe that there is a possibility to reduce the use of contrast fluids in OCT imaging. Flushing fluids are a modifiable factor in the image generation in OCT that can be controlled by the operator. If further investigation is done on this topic, with greater studies, showing that there are non-contrast or diluted-contrast that are appropriate for OCT flushing, the operator could consider using one of these fluids instead of contrast. This could lead to a decreased total amount of contrast of the whole procedure, which again would be beneficial for the patient. Our findings suggest that it is a possibility to achieve good quality diagnostic OCT images with non-contrast fluids and diluted contrast, and therefore hypothetically lead to less complications of the kidney function. However, for further investigation registration of sCR and eGFR should be performed baseline -and post procedure to register to which degree, if any, replacing contrast affects the kidney function in the selected population. In addition, by using a non-contrast fluid for image acquisition, the limitation of OCT will also be decreased, and can potentially also lead to an increase in examinations of the coronaries with this imaging modality.

5.3 Discussion of the methods

The current study was performed in a blinded fashion, with a standardized protocol of how the data collection should be performed. The sample size was small, but we still consider that the dataset may provide valuable information.

5.3.1 The data collection

A study protocol was made before the collection of data. This was to ensure that equal procedures were used through all the data collection. Although, care was taken to standardize the procedures, since biological variation in the porcine could contribute to some of the measured variation. For example, though careful placement of the coronary

guide catheter, small differences in anatomy could lead to movement of the equipment during the injections and also movement of the OCT catheter. The coronary guide catheters have standardized models, and therefore not a customized fit for small differences in anatomy. However, it is of great importance that the catheter is placed selectively into the coronary artery for OCT flushing to ensure that the fluid is injected into the artery. This to avoid that the injection goes out in the ascending aorta, which would had led to inaccurate flushing of the fluids and therefore measures of diagnostic image length. Because of this issue a well experienced operator performed the procedure, and made sure that the coronary catheter was placed selectively into the coronary artery before the injections of the flushing fluids. -Movement of the equipment could also be affected by the breathing (thorax movement) and the heart beats. Therefore, in addition to recording ECG for detection of possible arrhythmias, we ensured stable hemodynamic conditions by continuous registration of heart rate and blood pressure. However, even if the coronary angiograms confirmed stable position of the coronary catheters, microscopic movements of the catheter in the coronary ostium or the OCT catheter in the coronary artery is unavoidable.

Both the RCA and LAD in both porcine had side branches that were easy to recognize on the SCA. Therefore, side branches were used as anatomical landmarks to ensure OCT recordings from the same coronary segment during each image acquisition. However, the OCT catheter is not anchored to the coronary artery wall, and can therefore move across the axial lumen and can lead to image artifacts. However, small axial movements do not affect the longitudinal imaging, but in curved vessels motion artifacts can decrease the length measures (1). However, these artifacts are rare and was not observed when measuring diagnostic image length in the current study, where the coronary arteries were of normal anatomy.

A previous study (11) administrated different volumes of the flushing fluids for comparison (contrast volume: 12.9 ± 2.3 mL, dextran volume: 18.2 ± 2.5 mL). Using such a protocol makes it problematic to differ between possible effects of volumes versus the effects of flushing fluids with different inherent properties. In the present study a mechanical injector was used to ensure identical flushing conditions for each flushing fluid during each OCT recording; equal volume of fluid was injected at equal flow rate and injection pressure. Thus, possible variation introduced by differences in flushing

conditions was restricted to a minimum. However, because of differences in temperature and/or viscosity discrete variations in flow dynamics may have been present in the injected fluids. We decided, however, to test the fluids in a manner as close to the clinical setting as possible, since our primary goal was to examine the image quality as observed by the clinician. As our study was not aimed for determining optimal flushing protocol for each fluid should this issue be determined in a future study, and preferable for each kind of artery.

5.3.2 Experimental study design – ethics in animal research

Performing OCT image acquisitions in the same segment of interest, with several fluids, made human research not an option. It was considered to be unethical to inject multiple fluids in a row that possible could harm the kidney function, cause serious arrhythmias that would require treatment, or use equipment that could have led to vessel damage without a strong clinical indication. Therefore, an alternative to humans had to be found. Porcine are a very experimental common model in medical research, because of their relatively close resemblance to human heart anatomy (62). Though, there are several ethical considerations performing an animal model. Even though there is documentation that repeated injections during OCT imaging is well tolerated in porcine (63), it was unclear if as many as 14 injections per subject carried the risk for serious arrhythmias that would require treatment. Before performing the data collection, the study was approved from the Norwegian Animal Research Authority. Obviously, animals cannot give a consent, it is therefore of great importance that a federal authority is present to be the voice of the animals. In our research application, we described the procedures as accurate and equal to the data collection protocol as possible. We emphasized possible negative effects such as discomfort due to the first intramuscular injection of sedation, as well as the aim for the study.

5.3.3 Sample size

Due to reasons described previously only two porcine were used, and we decided for the current study to consider the RCA and LAD in each subject as independent from each other in order to increase the number of observations to four. We made this decision despite that the RCA and LAD are within the same subject, probably violating the principle of independence for the statistics. It may also have lead to difficulty detecting real differences. This do raise some concerns, leading to invalidation of confidence intervals and significance tests since standard error relies on independence (79). We acknowledge that a clustering of the data may be present for each subject, which may violate the principle of independence. Importantly, in order to treat the subject as dependent as well as accounting for repeated measurements within each subject advanced statistical methods should be performed, which our sample size does not allow. However, we believe that it is possible to treat these two arteries as independent due to the great differences in their flow-dynamics. The heart does not have the same contraction pattern for the whole muscle and the different chambers have different blood pressure. Therefore, the arteries supplying the left and right heart territories have different flow and pressure profiles. Due to these differences between the location of RCA and LAD, we assumed it was possible to treat them as independent from each other for the pilot study, even if they are located in the same subject. The differences in flow-dynamics between the coronary arteries may also have led to more variation in our dataset by using both the coronary arteries. However, the treatment of the coronary arteries as independent is a clear bias for the current study, and should had been taken under further consideration before the study was performed. If thought further trough before the collection of data, the design might have been different regarding number of coronary arteries used or how the data was handled. Though, even if it might raise ethical questions in animal research one might speculate if future studies could be performed using one artery for each subject to erase this bias or analyze the arteries separately, and therefore not violate the principle of independence.

The post-hoc analysis revealed that the current study had a low statistical power (0.61). A problem with small sample sizes is type II errors, by incorrectly accepting the null-hypothesis, when an actual difference is present (73). For all the fluids in the pilot study, the alternative hypothesis was rejected. However, the alternative hypothesis might have

been incorrectly rejected, due to the underpowered statistics. The questions are whether this is a type II-error, if we should have considered higher test strength? The overall aim for the study was to investigate if other fluids had the potential to serve as contrast alternatives in OCT flushing. An increase in power would have decreased the chances of type II errors and therefore decreasing the risk of incorrectly rejecting the alternative hypothesis. Underpowered studies may lead to missing the significant effects and, therefore, failing to document the real relationship between the independent and dependent variables (80). A solution to decrease the chance for type II errors would have been to increase the sample size by searching for more funding in order to perform more animal experiments. This would have provided a better-powered study where firmer conclusions could be drawn, since adequate sample size is essential for meaningful interpretation of most statistical tests. However, due to change to next generation OCT equipment after the first two experiments, subsequently experiments would have introduced additional sources of possible variation to the measurements. Therefore, we decided upon restricting this part of the project to a pilot study utilizing data from the two porcine. Since few studies on this topic have been published we explored if some of the fluids had a potential for replacing contrast in OCT flushing. In addition, we evaluated the feasibility of the method for data collection, the measures/outcome, as well as post-hoc calculation of sample size to be used in future larger studies.

The post-hoc sample size calculation indicated that a minimum of two more porcine (still considered that the RCA and LAD are treated as independent from each other) was needed to reach a statistical power of 80% when using an α -level at 0.05. Though, caution should be taken when calculating a post-hoc sample size from a pilot study, since this may be biased due to the small sample (61). If the post-hoc analysis was performed with a larger statistical power of 90-95% the required sample size would probably have been greater, leading to a calculated sample size that would limit the chance to incorrectly accept the null-hypothesis and limit the chance for type II errors.

Because of the low sample size, one must exercise caution in interpreting the results. However, this does not mean that the results are without value. Even if there is a decreased statistical strength, there is a numerical trend for the fluids on diagnostic image length that underscores the null-hypothesis and also supports the previous research done

regarding this topic. The present study, despite a low sample, confirms the need for further exploration of potential contrast alternatives in OCT flushing.

5.3.4 Selecting the OCT analyzer

Originally, we planned to use measurements from three observers for the main analysis, as well as testing inter-observer agreement. However, due to the small sample size it was difficult to perform these analyses. Therefore, we decided upon for the pilot study, to restrict the analysis to data from one observer, and postpone the inter-observer analysis until a greater study could be performed. Before assessing the OCT measurements, the study group discussed which observer's data to select for the analysis. Factors as analysis experience (in both OCT and research) and involvement in the study were taken under consideration. Observer #1, who was an initiative taker for the pilot study, has many years of experience with OCT and also experience regarding research. But the question the study group had to ask regarding this observer was if the strong engagement for the project could be a bias in itself. Also, observer #2 has a broad OCT experience, and additionally has lectured about OCT in workshops and cardiological meetings. Moreover, this observer has large research experience. Observer #3 had recently started an OCT project as part of his PhD program, and was also very well experienced with OCT, but has less experience in clinical research. After a discussion the research group agreed upon performing the analysis with the data from Observer #2.

One of the reasons three observers were chosen for performing the measures of diagnostic image length and subjective image quality was with the intention to test the overall agreement between the three interobservers. However, the study group should have foreseen these statistical methods would be difficult to perform based on the small sample size. Though, it would had been of interest to analyze the diagnostic image length and subjective image quality based on measures completed by the two remaining observers, to see if their measures provided a somewhat alike result as the result from the chosen observer. Nevertheless, we decided on for the current study to use measurements completed by the selected observer.

5.3.5 Measurements

All measures performed in the present study were done subjectively. Even if we defined criteria for the categories for subjective image quality, subjective judgement has the final call, and decides in which category each image frames belong in. Categorizing image quality has been done before in a somewhat like fashion (14), but there is no standardized definition on categories of OCT image quality as far as we are aware of. Rather than just defining clear image segments as visible layers and a clear border between the lumen and vessel wall in $\geq 270^\circ$ as done in a previous study (10), we categorized the overall image quality of the diagnostic image length, resulting in good (category 2) and optimal (category 3). By having these two categories instead of one for good image quality, we were able to get a detailed information about the overall acceptable image quality. We are not aware of OCT software allowing for automated measurements of diagnostic image lengths consisting of frames of good image quality. Potentially, this could have reduced bias of subjective opinions or subconscious effects, and led to more standardized measurements. However, in clinical practice subjective assessment and measurements are performed on the OCT recordings. The subjective assessments performed by the clinician or staff is the foundation for clinical decision-making based on OCT-recordings. Anyhow, to limit the bias regarding the subconscious impact that may appear during OCT analysis, the fluids were anonymized throughout the OCT analysis process. This does not remove the potential bias, but we agreed on that this was an acceptable strategy for limiting possible bias as much as possible. Subconscious effects and subjective opinions may have affected the measurements, both for the diagnostic image length, as well as the overall subjective image quality.

The primary variable, diagnostic image length, was measured as a continuous length (in millimetres) of image frames with good or optimal image quality. We chose diagnostic image length in millimetres as the measure outcome, which is a familiar approach for OCT recordings clinical practice. In order to be included in diagnostic image length we demanded that every consecutive millimetre along the OCT recording of the coronary artery had to consist of at least one frame of good or optimal image quality. A similar approach has been used in another study performed by Ozaki et al. (10), in which they defined clear image segments as a segment with at least one frame within the segment (1

millimetre/5 frames) with good image quality (figure 5). However, the previous study measured the total number of clear image segments in the groups compared (10), and did not take account for if the clear image segments were continuous along the recorded vessel. We suggest that a continuous length may give a more predictable assessment of the flushing properties of the fluids, since image quality contains an approximately blood free artery lumen, and poor image quality appears when the fluid is mixed with blood. Residuals of blood that decreases image quality, and can lead to unpredictability whether the segment of interest will be of good image quality. Great variation of image quality within one segment/millimetre is hypothetically possible, but is probably very rare. Still, we believe that frame by frame was an adequate measuring method for diagnostic image length, since blood clearance leading to diagnostic image quality was the main goal. The calculation of OCT image segments (one millimetre) as the rate of pullback (mm/sec) times the frame rate (sec) has been previously validated, and is being used for clinical decision making of stent length and diameter (1).

The OCT system used in the current study has a standardized recording length of 54 mm. Therefore, we are restricted to information of flushing within the first 54 mm, and a possible flushing providing conditions for adequate OCT recordings of more than 54 mm will not be detected. We observed a measured length of 54 mm for 8 of the recordings in various fluids. The reference fluid and Visipaque 270 had both three diagnostic image lengths measured to 54 mm, whereas Iomeron 350 and Macrodex had one diagnostic image length measured to 54 mm. This may indicate that the diagnostic image length could have been above 54 mm if the OCT equipment could have detected it.

Hypothetically, if the true diagnostic image length for the reference fluid had been 100 mm, this could have affected our results. Therefore, the interpretation of the current study is restricted to the first 54 mm of the OCT recording, and does not take into account a possible diagnostic image length beyond 54 mm. However, in principal, the limited recorded length introduces an artificial cutoff in the true distribution of diagnostic image lengths for each fluid and therefore may cause challenges in the data interpretation. Further development of the OCT technology may address this issue by increasing the recording length.

5.4 The flushing fluids – independent variables

In total, seven different fluids were examined in the present study. Four of the fluids, including the reference, are contrast fluids containing different concentrations of iodine, whereas three of the fluids investigated are non-contrast fluids. If these fluids were the accurate choice to answer the null-hypothesis is not certain. Visipaque has been recommended as a fluid of choice for OCT flushing (6), therefore, we agreed to use Visipaque 320 as reference fluid. Since high viscosity was an argument for the recommendation of Visipaque (6), we tested two different iodine-concentrations of Visipaque due to differences in viscosity. In own clinical practice, a different contrast agent, Iomeron 350 is used both for SCA and OCT-flushing, and the OCT image quality have never been considered to be limited by using this contrast agent. Studies have showed that a 50/50 mixture of contrast and a non-contrast fluid (Voluven) can provide adequate OCT image quality (14). Iomeron is also available as a diluted-iodine solution (Iomeron 150), and therefore we tested this fluid for OCT flushing as well.

There are several non-contrast fluids for human use available on the coronary catheterization laboratory at all times. In an important study by Ozaki et al OCT flushing by a mixture of lmw-dextran and Ringer's lactate was compared to contrast (10). Both Voluven (a colloid like lmw-dextran), Ringer Acetat (closest to lactated Ringer's attainable), as well as Macrodex 60 (dextran-70), are easily available in the coronary catheterization laboratory and were therefore tested for OCT flushing. Various Ringer's solutions have also been used during the first generation of coronary OCT, and is therefore well tolerated (15). Compared to the lmw-dextran used in the studies by Ozaki et al. (10) and Frick et al. (11), contains Macrodex 60 a slightly different chemical composition. The average molecular weight for Macrodex 60 (dextran 70) is 70000 Da, while lmw-dextran (dextran 40) has an average molecular weight of 40000 Da (12, 81). In order to make our study more comparable to other studies it would have been beneficial using identical flushing fluids. However, we selected as similar fluids as possible based on the availability in our hospital.

As the aim of the study was to investigate if diluted-contrast or non-contrast could replace contrast in OCT flushing, we considered replacing the two contrast fluids investigated with non-contrast fluids. Visipaque 270 and Iomeron 350 could have been

replaced with other non-contrast fluids, however, we decided to investigate how the contrast agent used in our own clinical practice compared to the reference, as well as testing a different iodine-content of the reference brand. Even if this issue could make the findings more directly relevant to our daily clinical work, the testing of these two contrast fluids do not contribute to answering the null-hypothesis or the aim of the study. For this reason, we probably should have decided using non-contrast fluids instead.

5.5 Further investigation

Even if OCT has proven to be safe in porcine (63), one of the issues feared was that a total of 14 injections on each subject could lead to complications. In principal, there is a risk for life threatening arrhythmias caused by ischemia due to several large coronary injections in a row replacing the blood. In the current study, the porcine tolerated the injections well and were stable throughout the whole procedure, and no arrhythmias or procedural complications were observed. The sample size calculation gave a result of $n = 7$ (α -level 0.05, power 80%), which means that two more porcine in the study would have provided an adequate sample size of $n = 8$, if still using the combined measurements of LAD and RCA. After the post-hoc sample size calculations we were eager to perform further data collection, to achieve analysis with enough statistical power to make more valid conclusions. In the response from NARA they accepted five porcine in case the two used needed to be euthanatized before the data collection was completed. After the data collection for the pilot study, the OCT modality had been replaced with a newer model with software upgrades and a new OCT-catheter. We contacted the OCT vendor (St. Jude Medical), to investigate if we could have access to the OCT equipment used in our study (C7-XR FD-OCT) to be used in further experiments. Unfortunately, the OCT system was no longer available and had been replaced with the new OCT system.

We also considered performing future experiments with the new OCT system. However, performing a supplementary data collection with the new OCT system would lead to using two different software, as well as different machines and catheters, which would increase the risk of introducing more variation to our measurements. Therefore, we decided to not perform further data collection with the new OCT system.

Due to ethical as well as funding issues we decided not to apply to the NARA for more porcine to run a completely new data collection with the new OCT system. We do believe that our design for measuring fluids clearance of blood in the artery is possible to recreate. In both a new porcine model as well with a human model if approved from the Regional Committees for Medical and Health Research Ethics. If a human model is to be used, we suggest to choose just a few of the fluids and only one of the coronary arteries should be considered to limit the risk of arrhythmias and to limit the contrast administrated. Optimizing the flush protocol for fluids with different properties would also be of interest. If performed on humans, the OCT image acquisition should be performed on patients already having a clinical indication for OCT, to prevent damage on a healthy vessel. However, if several fluids should be investigated in the same segment of interest, greater systematic studies should be performed in an animal model.

Therefore, in this master thesis we decided to present the important results generated from the two porcine that were sacrificed in the pilot study. We believe that our small dataset carries valuable information of OCT flushing by several fluids being able to provide OCT recordings with adequate quality for clinical use. There is a need for larger studies before determining if contrast fluids may be replaced by alternative flushing fluids in OCT.

6. Conclusion

The null-hypothesis for this pilot study was that there was no statistical significant difference between the use of the standard contrast agent and the other contrasts, low-contrast or non-contrast fluids regarding OCT diagnostic image length and overall image quality. No statistical difference was observed for any of the fluids examined. However, a trend towards poorer diagnostic image length was observed for Ringer Acetat and Iomeron 150.

The findings in current study should be interpreted with caution due to the small sample size. All flushing fluids tested in the current study provided adequate OCT image quality for clinical use, however, the length of vessel with this image quality was variable. Therefore, our findings in this underpowered study suggests that future larger studies may identify non-contrast fluids that can replace contrast for OCT flushing. Our findings suggest that especially colloids should be examined more closely as a contrast alternative. We would strongly recommend further investigation on this issue, and we do believe there is a possibility to replace contrast flushing and still obtain good diagnostic image quality OCT recordings.

7. Reference list

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Appendix 1 - OCT Theory

Physical principles of optical coherence tomography (OCT)

OCT is a light-based modality based on interference between light waves, and provides high resolution cross-sectional images of tissue microstructure (2), or more specific the changes in the optical properties of the tissue/sample (30). The modality consists of an imaging catheter, a motorized pullback device and an imaging console. The image console contains the display, the light source, data storage and signal processing units (Figure 1).

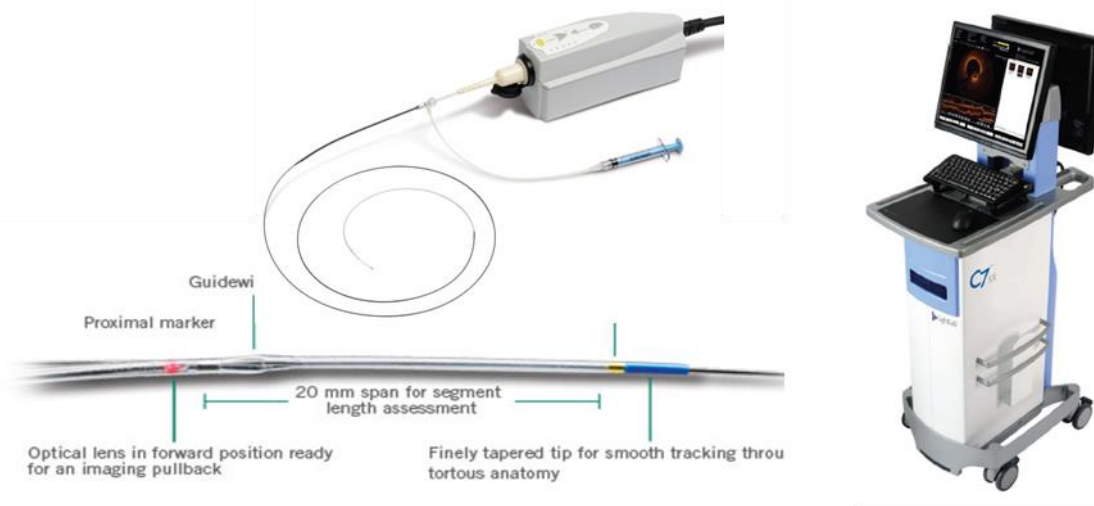


Figure: To the right is the optical coherence tomography (OCT) modality, with a double screen for live view and measurement. On the bottom to the left is an illustration of the Dragonfly OCT catheter, and up to the left is the docking station with a connected Dragonfly OCT catheter. Images reprinted from St. Jude Medical, with permission from St. Jude Medical.

To perform OCT image acquisition an optical fiber is inserted into the vessel lumen. This optical fiber emits light and also records the reflections simultaneously while being rotated and pulled back along the artery (15). The background for image creation is the comparison of

the back-reflected intensities from the two arms (Figure 2) (31). The intensities of the light is measured and digitally converted into a grey scale creating an image (32).

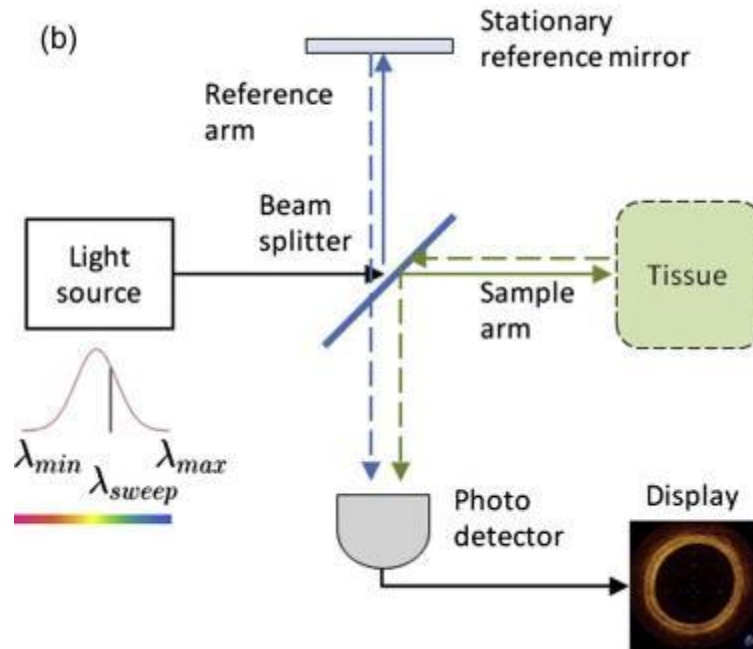


Figure 2: Illustration of optical coherence tomography. The near-infrared light is sent out from the light source at different wavelengths into the beam splitter. The beam splitter divides the light into two, travelling to the reference arm and the sample arm. The returning light is then sent to the photo detector for identification of differences in intensity (33). Image is reprinted from Medical Image Analysis, Vol 18, In-vivo segmentation and quantification of coronary lesions by optical coherence tomography images for a lesion type definition and stenosis grading, Simona Celi, Sergio Berti, p. 1157-1168, 2014, with permission from Elsevier

The OCT catheter consists of a light source, a detector and a fixed mirror (Figure 2) (34). A swept-source laser, with wave lengths between 1,250-1,370 μm (NIR-light) is emitted at any instance from a diode. The NIR-light is then divided in two different arms; the sample arm that goes into the coronary artery wall, and the reference arm that travels to a fixed mirror in the OCT catheter. The NIR that goes out to the sample arm will reflect at different depths due to the different properties of the variety of cells/tissues, and generate interference signals with different frequencies. Back-reflected NIR-light from both arms will enter back to an interferometer and a photo detector. The distances the lights have traveled from the arms are almost equivalent, but the difference in intensity between these generates the images. The

light reflected back from the reference arm has a known intensity, whereas the back reflected light from the sample arm varies, due to different anatomy and tissues, this leads to differences in intensities. These intensities are further transported to a computer where a mathematical calculation with Fourier transformation is done, and image generating is completed and turned in to a grey scale (15), where different tissue gets a different shade of grey. Figure 3 shows a single depth profile (one-dimensional), and a series of these (500) are captured together for the cross-sectional images (2-dimensional image).

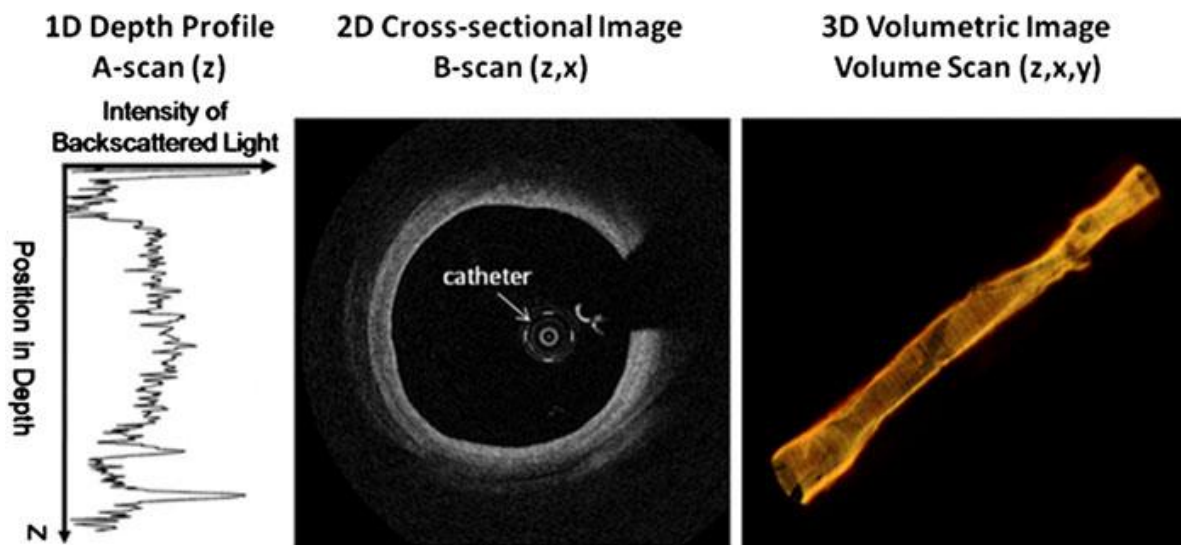


Figure 3: This figure shows the scanning schemes. To the left is a A-scan with the different intensities. The picture in the middle is a grey scale B-scan of a coronary artery. And due to the rotation of the catheter a 3-D profile of the artery is possible(26). Image is reprinted from International Journal of Cardiovascular Imaging, Intracoronary optical coherence tomography, basic theory and image acquisition techniques, vol 27, p 251-258, F. Prati, original copyright notice as given in the publication in which the material was originally published- Appendix 2), with permission of Springer.

Before the image acquisition, eventual presence of blood in the OCT catheter has to be removed to avoid attenuation of the NIR-light. A small syringe is attached to the catheter purging blood out of the catheter. An automatic image acquisition begins when the artery is cleared from blood, and clearance is made possible by using a mechanical injector or hand injection for injection of a blood replacing fluid. Before performing measurements of the created OCT images, calibration is necessary. Calibration is completed by adjusting the Z-

Appendix 1

offset around the catheter, which is the zero-point setting for the system (26), since the catheter has a standardized size used as reference (35).

As with any device introduced to an artery, caution is also necessary with OCT. The coronary wires and catheters into a coronary artery always contains a small risk of complication as coronary artery dissections, intramural hematoma, coronary artery perforation and occlusion of side branches (36). Care should be taken while advancing the OCT catheter into the artery, since intravascular modalities include a risk in itself since it is an invasive procedure (21). During clearance of blood and image acquisition chest pain and electrocardiogram changes may occur, this may be due to coronary spasms, caused by the presence of the catheter or the injection of contrast dye. This might be removed by intracoronary injection of nitroglycerine prior to insertion of the OCT catheter. Though, OCT has proven to be safe (37, 38), and these eventual discomforts are showed to resolve immediately after the image acquisition. There has been a theoretical concern regarding local heating caused by NIR-light, but this has not been observed to be an issue in studies this far (21).

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Lengdemål (lengde med diagnostisk kvalitet)

	oppgitt i millimeter	I en lengde: ja/nei
LAD dag 1		
LAD dag 2		
RCA dag 1		
RCA dag 2		

Kvalitetsmål

0 = ubedørbart

1 = så vidt bedørbart

2= Lett limitert kvalitet (diagnostik godt bilde, men ikke optimal bildekvalitet)

3 = Optimalt

LAD dag 1	
LAD dag 2	
RCA dag 1	
RCA dag 2	

Referanse bilde

	Diameter1	Diameter2	Areal	BorderID
LAD dag 1 prox ref.				
LAD dag 1 dist ref.				
RCA dag 1 prox ref.				
RCA dag 1 dist ref.				
LAD dag 2 prox ref.				
LAD dag 2 dist ref.				
RCA dag 2 prox ref.				
RCA dag 2 dist ref.				

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Søker og medarbeidere

Institusjon	055 UiO - Ullevål Sykehus, Seksjon for komparativ medisin, IEMF
Adresse	N-0407 Oslo Oslo
Telefon	+47 23 01 68 00
E-post	- - -
Ansvarshavende	Gro Furset Flatekval
Ansvarlig søker	Magne Brekke - Forsker (Ansvarlig søker/Prosjektansvarlig) (Kurs i forsøksdyrlære)
Medarbeider 1	Morten Eriksen - Forsker (Ansvarlig søker/Prosjektansvarlig) (Kurs i forsøksdyrlære)
Medarbeider 2	Anders Opdahl - Prosjektdeltaker (Kurs i forsøksdyrlære)
Søknadsdato	28.02.2013

Generelle opplysninger**Offentlighet**

Inneholder søknaden opplysninger som ønskes unntatt fra offentlighet? Nei

Hvis ja, angi relevante lover og paragrafer (f. eks. Offentlighetsloven, § 13, 1. avsnitt og Forvaltningsloven, § 13, 1. avsnitt, 2. punkt). - - -

Hvis ja, beskriv hvilke opplysninger som ønskes unntatt fra offentlighet.

- - -

Id	5111
Søkerens ref.nr.	- - -
Forsøkets arbeidstitel	Optimalisering av flushteknikk og flushvæsker ved Optisk koherens tomografi (OCT)
Dyreart	Pattedyr - svinedyr (Susidae) - Gris (Sus scrofa domesticus)
Institusjon	055 UiO - Ullevål Sykehus, Seksjon for komparativ medisin, IEMF
Type søknad	Nytt forsøk
Akutt forsøk	Ja
Tidligere erfaring med tilsvarende Ja forsøk	
Forskningen er finansiert av	Annen finansieringskilde
Planlagt start	21.02.2013
Planlagt slutt	21.02.2013

Beskrivelse

Vi ønsker å stille spørsmål om vår fremgangsmåte ved bruk av 350 mg I/ml røntgenkontrast er den væsken som gir best bilder ved Optisk Coherense Tomografi (OCT), eller om andre væsker kan gi like god eller bedre bildekvalitet, noe som hadde vært gunstig for pasienter ved nedsatt nyrefunksjon. Ved å legge OCT kateteret i fremre gren av venstre koronararterie (LAD) vil vi kjøre ett opptak ved flushteknikk for hver av væskene til utprøving i samme område i koronararterien. Det tilsvarende vil også bli gjennomført i hjertets høyre kransarterie (RCA).

Bakgrunn og hensikt

Gi en kort presentasjon av bakgrunn og hensikt med forsøket (maksimalt 500 ord), i en allment tilgjengelig språkform. Angi eventuell hypotese som skal testes. Angi særskilt hvis spesielle lovbestemmelser/krav fra offentlige myndigheter krever at forsøket skal utføres.

OCT er en relativt ny imaging teknikk for bildetakning inne i blodårene. Dette er en lysbasert bildeteknikk hvor bilder blir dannet ved bruk av nær-infrarødt lys med oppløsning ned i mot 10 mm. OCT gir billediagnostikk fra karetts innside og kan gi svært nytting informasjon, da en får en bedre mulighet for å få en nøyaktig kartlegging av koronararterienes anatomi og patofysiologi.

Vi bruker Fourier Domain OCT (FD-OCT) som er den det nyeste og tryggeste OCT teknikken, og er den samme som brukes i vanlig klinisk medisin.

Det brukes interferometri metode for å skape bildene. Lyset kateteret sender ut blir delt i to, hvorav en del blir sendt ut i pasienten (sample arm) og den andre halvparten til en forhåndsbestemt avstand (reference arm). Nærinfrarødt lys absorberes fullstendig av blod. Selve bildeopptaket må derfor foregå i et blodfritt lumen. Dette betyr at blodet må erstattes med annen væske. Hos oss brukes Iomeron 350 mg I/ml som er et lav-osmolært, ikkeionisk kontrastmiddel med lav viskositet. Om kontrastvæske med så sterk konsentrasjon er det beste eller om andre væsker kan gi like god eller bedre bildekvalitet er ikke avklart. Røntgenkontrast har noen få bivirkninger og det ansees som en generell fordel å redusere kontrastbruken. Det er uklart hvordan forskjellige væsker påvirker det nær-infrarøde lyset, og hvilken betydning viskositet og andre fysiske egenskaper har for evnen til å fortrenge blodet i kransårene. Væskelinjeksjonen gjøres med trykksprøyte eller manuell injeksjon gjennom ledekateret som står proksimalt i karet som undersøkes. Opptaket starter automatisk når lumen er blodfritt og fylt med annen væske. Det finnes et fåtall studier hvor lav-osmolære og iso-osmolære, non-ionisk kontrastmidler sammenlignes med andre væsker. Et studie sammenligner (low molecular weight) dextran og kontrast. Resultatet i studien viser at dextran har potensiale til å erstatte kontrast under OCT ved flushteknikk.

Med bakgrunn i faglitteratur og erfaring med OCT i klinisk human bruk har vi valgt 8 alternative væsketyper som skal testes

Vi vil studere alternative væsker under en studie, og kjøre en blindtest på disse. Alle OCT-opptakene skal analyseres, måles etter gitte parametere og settes opp mot hverandre.

Vil det vise seg at en av de andre væskene (ikke-kontrast eller kontrast med lavere lavere) gir like gode opptak som ved dagens rutiner vil dette være gunstig for pasienter med nedsatt nyrefunksjon. Mange av dagens pasienter har ganske kompleks sykehistorie, og det bør være et mål å kunne redusere sjansen for kontrastindusert nefropati, da en kjent risikofaktor ved bruk av røntgenkontrast er nettopp dette hos pasienter med nedsatt nyrefunksjon. Sjansen for nefropati henger sammen med mengden kontrast som blir anvendt. Det er også av interesse å finne ut av om væskens viskositet har betydning for erstatning av blodet.

Beregning av antall dyr

Gi en begrunnelse for antall forsøksdyr. Ved usikkerhet om populasjonsstørrelse skal det gjennomføres pilotforsøk, jf.

forskriftens § 13. Søk hjelp hos statistiker dersom du er i tvil.

Det er i alt 8 alternative væsker som skal testes, alle har vært brukt i klinisk human bruk og det er rapporter på bruk av disse væskene også på gris. Det er forskjellig flowforhold i høyre og venstre kransåre. Væskene bør derfor testes i begge karnsårer. Det er godt dokumentert at 8 injeksjoner med OCT opptak tåles godt. Det er imidlertid usikkert om 8+8 injeksjoner med OCT opptak vil tåles uten hemodynamisk fortyrrelse. Vi bør derfor ha minst to forsøksdyr hvor det ene tests med 8 injeksjoner/OCT opptak i venstre karansåre og det andre tilvarende i høyre kransåre. Faren for alvorlig hjerterytmeforstyrrelse er liten, men reell. Det vil ikke være mulig å gjennomføre forsøket etter f.eks ventrikkelflimmer. På grunn av anatomiske forskjeller kan det være vanskelig å oppnå tilfredstillende kateterposisjon hos enkelte forsøksdyr. Vi søker derfor om inntil 5 forsøksdyr. Skulle vi komme i mål med færre griser er selvsagt dette å foretrekke.

Hvis ingen komplikasjoner oppstår bør forsøket kunne gjennomføres med 2 forsøksdyr.

Gi en oversikt over samtlige forsøksgrupper og gruppestørrelser. Legg gjerne ved en tabell som vedlegg til søknaden.

ikke relevant.

Hvilken metode er brukt for beregning av antall dyr. Annen metode

Hvis "Power analyse"/"Ressursligning": Hvilke input er lagt inn?

Hvis "Annen metode": Gi en detaljert beskrivelse av den metoden som er benyttet.

Hvis "Ikke aktuelt": Beskriv hvorfor statistiske metoder ikke kan benyttes.

De prosjektansvarlige i studiet har tidligere god erfaring med lignende kateterisering av gris. Samt har flere av prosjektdeltakerene til vanlig arbeidsplass ved koronarangiologlab noe som gir en god kompetanse innenfor koronarangiografi og OCT.

Alternativer/3R

Erstatning ("replacement"): Hvorfor kan man ikke oppnå forsøkets hensikt uten å benytte levende dyr? Hvilke alternativer er vurdert og hvorfor er de forkastet?

Vi har hatt kontakt med fysikere og medisinsk teknisk både ved OUS Rikshospitalet og OUS Ullevål for å se om de har utstyr som kan være tilgjengelig for invitro forsøk med nær-infrarødt lys og som passer med vår problemstilling, dette har de ikke.

Hvilke databaser ble det søkt i og hvilke søkeord ble benyttet for å finne alternativer?

Egen litteratur og søk på MEDLINE. Søkeord MEDLINE: OCT, OCT-coronary arteries, OCT – coronary arteriescontrast. Søket gir få treff, og kun to artikler er relevante for hva vi er ute etter. Det ene er som nevnt et studie der en sammenligner dextran med 350 mg l/ml Omnipaque som viser at dextran har muligheten til å erstatte kontrast, og det andre er et OCT forsøk på gris hvor en i oppsummeringen mener kontrast (ikke nevnt hvilken) er bedre enn saltvann.

Reduksjon ("reduction"): Når bruk av dyr er uunngåelig: Hvilke tiltak, steg og forholdsregler har du brukt for å minimalisere antall dyr og fremdeles oppnå valide vitenskapelige resultater?

Forsøksdeltakerne driver selv med koronarangiografi og OCT daglig, så oppsett og prosedyre vil dermed være kjent og dermed vil det ikke benyttes flere griser enn nødvendig.

Raffinering ("refinement"): Når bruk av dyr er uunngåelig: Hvilke forbedringer av stell og prosedyrer er gjort for å minimalisere smerte, lidelse, ubehag og varig skade og for å øke dyrevelferden i forhold til tidlige lignende forsøk?

(Stikkord:

anestesi, analgesi, endepunkter, miljøberikelse, operasjonsteknikk, prøvetakningsteknikk osv).

Grisene vil bli lagt i narkose og få smertelindrende underveis.

Metodebeskrivelse

Forberedelsen av dyrene før inngrep:**For feltforsøk: Beskriv evt. sporing, innfangning, fikseringsmetode, transport osv.****For labforsøk: Beskriv evt. innkjøp, transport, karantene/akklimering, oppstalling, miljøberikelse, fôringsregime, merking, veiing osv.**

Forsøksdyrene går fritt i en forsøksavdeling som er godkjent, og vil faste med fri tilgang på vann fra kvelden før forsøket.

En kanyle settes i nakkemuskelen, dette kan gi et ubehag på opptil 30 sekunder. Gjennom denne setter en sederende. Sedasjon som blir brukt er Narketan/Ketamin 33mg/kg.

Hvilke inngrep (kirurgi, administrasjon av teststoff, merking av villlevende dyr, fysiske behandlinger m.m.) skal gjøres på dyret under selve forsøket? Legg evt. ved tegninger, protokoller, tidslinjer (aktivitetskart) eller lignende som vedlegg til søknaden.

Forsøksdyret/ene sederes ved hjelp av 11-33 mg/kg Narketan/Ketamin. Etter sedasjon legges en intravenøs tilgang i en ørevene for administrasjon av narkosemidler. Det kobles til sprøtepumper for infusjon av Fentanyl 30-100 µg/kg/h og propofol 12-20µg/kg/h. Forsøksdyret vil intuberes og legges på respirator. Et trykktateter plasseres i venstre ventrikel gjennom høyre arteria carotis, samt et kateter i venstre vena jugularis for evt. infusjon av væske. En 6 F innføringshylse legges ved hjelp av gjennomlysning i arteria femoralis, dette for å ha en innfører å føre koronarkateteret opp gjennom. Det vil også under forsøket gis Heparin etter vekt (100IE/kg) for å forhindre blodpropp.

Flushvæskene som skal testes og gis intrakoronart under prosedyren er: dextran, lomeron 350 mg //ml, lomeron 150mg l/ml, Visipaque 320mg l/ml, Visipaque 270mg l/ml, Hemaccel, Ringer Acetrat, Voluven. Det vil bli gitt 10-20 ml for hver injeksjon avhengig av kransarteriens diameter.

Hvilke registreringer skal gjøres og hvilke prøver skal tas i løpet av forsøket?

Blodtrykk og EKG skal registres under hver flush med væskene som skal testes ut.

Mengde, hastighet og trykk vil bli registrert for injeksjonen av flushvæskene,

Angi oppfølging og overvåkning av dyrene under hele forsøket (før, under og etter aktuelle inngrep). Legg gjerne ved relevant scoringsskjema:

Det vil være en kontinuerlig infusjon av anestetiske og analgetiske medikamenter. Blodtrykk og EKG vil også overvåkes kontinuerlig.

Angi avlivingsmetode og hvorfor denne metoden er valgt. Ved bruk av preparater oppgi generisk navn, preparatnavn og dosering:

Forsøksdyret vil avlives under anestesi. Erfaringsmessig vil en bruke 20 ml kcl 1M.I.V da dette er en meget rask og human avlivningsmetode under full narkose.

Angi kriterier for humane endepunkter (dvs. kriterier for å avbryte forsøket for det enkelte dyr/grupper av dyr fordi belastningen for dyret er større enn det som er nødvendig for å oppnå formålet med forsøket).

Dyrene har kontinuerlig anestesi og monitoreres intermitterende for respons på smertestimuli. Det gis ikke muskelrelaxerende medikamenter under forsøket for å muliggjøre en slik monitorering. Skulle uforutsette hendelser inntreffe under forsøket før det er ferdig avlives dyret ved hjelp av 20 ml kcl 1 mol i.v. Skulle i.v tilgangene bortfalle vil en ny i.v tilgang etableres umiddelbart.

Hvilke tiltak vil bli aktuelt å iverksette hvis dyrene når humant endepunkt (f. eks. behandling av symptomer, redusere eksponering, avliving)?

Avliving.

Forsøksdyr (art, medikamentbruk og smertevurdering)

Dyreart	Pattedyr - svinedyr (Susidae) - Gris (Sus scrofa domesticus)
Linje/Stamme	- - -
Kjønn	Begge
Antall	5
Vekt ved oppstart	25-30 kg
Vekt ved avslutning	25-30 kg
Alder	3-6 mnd
Antall dyr ved gjenbruk (jf. § 15)	Gjenbruk er ikke relevant
Erfaring med denne dyreart	Ja
Beskriv fordeling av antall dyr i forhold til kjønn, vekt og alder	lik

Varighet av hele forsøket for det enkelte dyr (d, t, min).

Dyr med en avvikende fenotype (se prinsipputtalelse).

Har dyrene arvelig sykdom/lidelse som kan påvirke deres velferd (eksempler: diabetes, autoimmun sykdom, økt forekomst av tumor, lidelser i bevegelsesapparatet, tanndefekter m.m.)?

Slike lidelser er ikke kjent/beskrevet i litteraturen

Hvilke tiltak/behandling skal iverksettes for å sikre velferden for dyr med arvelig/medfødt sykdom/lidelse nevnt over, og når regner du med at det blir nødvendig?

Slike tiltak vil ikke bli nødvendig

Sedasjon, analgesi og anestesi

Periode (mg/kg)	Type Administrasjonsmåte	Preparat	Induksjonsdose (mg/kg)	Vedlikeholdsdose
Før	Sedasjon	Narketan (ketamin)	33	0 IM
Under Anestesi	Propofol	15	20	IV
Under Anestesi	Fentanyl	20	50	IV

Annen medikamentering (alle andre medikamenter/testsubstanser som anvendes)

100 IE/kg/t heparin under prosedyre, ACT målinger vil bli utført underveis 20 ml KCL mol i.v til avlivning

Testsubstanser (flushvæskene): 10-20 ml per injeksjon intrakoronart per injeksjon med: lomeron 350 mg l/ml, lomeron 250 mg l/ml, Visipaque 320 mg l/ml, Visipaque 270 mg l/ml, Hemaccel, Voluven, Dextran, Ringer Acetat.

Neuromuskulære blokkere vil bli benyttet

Begrunnelse for bruk av neuromuskulær blokker:

Begrunnelse for valg av dyremodell

Gi en begrunnelse for valg av dyremodell, jf. forskriftens § 8 - dyreart, linje, kjønn, alder, spesielle egenskaper, genmodifikasjoner

Det er store fordeler ved bruk av gris da de og mennesket deler lignende anatomi og fysiologiske karakteristikk som i dette tilfellet ved kardiologien. Den kardiovaskulære anatomien til griser er lik menneskets med tanke på blant annet størrelsen, morfologi, kollateral arterie tilførsel og tilgangen av veletablerte vasa vasorum. Den lokale flow dynamikken til kransårene hos en gris er tilnærmet lik menneskets. Det er innenfor OCT-forskningen gjort flere studiemodeller med bruk av gris, hvorav en blant annet har bekreftet ved hjelp av histologi at OCT er trygt å bruke hos gris.

Smerte og ubehag

Forsøket innebærer smerte, men analgesi må utelates

Begrunnelse for at analgesi unnlates

Forsøket anses å innebære betydelig/vedvarende smerte eller ubehag.

Begrunnelse for vurderinger

Sedasjon gis med intramuskulær injeksjon som kan gi kortvarig ubehag (stikksmerte). Intravenøs tilgang for anestesi etableres etter sedasjon, men kan medføre kortvarig og sannsynlig lite ubehag for forsøksdyret (stikksmerte)

Styrke av smerte/ubehag Lite

Varighet av smerte/ubehag Sekunder

Vedlegg



FORSØKSDYRUTVALGET

Deres ref:

Vår ref:

Dato:

05.03.2013

magbre@ous-hf.no

VEDTAK OM BRUK AV FORSØKSDYR - FOTS ID 5111

Behandlet av lokalt ansvarshavende, 05.03.2013.

Dokumenter i saken:

Behandling:

Vedtak:

Søknaden er godkjent.

Begrunnelse:

Forsøket er av en art og natur slik at det kan godkjennes av ansvarshavende, jvf Forskrift om forsøk med dyr, nr 23, 15/1, 1996, § 11.

-Forsøket godkjennes i hht. søknaden jmf. forskriftens §§ 7 og 8. Forsøket vurderes å være av vitenskapelig og samfunnsmessig relevans og interesse og oppfyller dermed forsøksdyrforskriftens generelle vilkår for dyreforsøk, gitt i § 8, første ledd. Det foreligger ikke anvendelige alternativer til bruk av levende dyr i hht. kravet beskrevet i forskriftens § 8, tredje ledd.

-Det forutsettes at alle som deltar i forsøket er angitt i søknaden, jvf forskriftens § 12, tredje ledd og § 13 første ledd.

-Eventuelle avvik og endringer fra den godkjente søknad må meddeles skriftlig til ansvarshavende og evt. som søknad om endring av forsøket.

-Søker er ansvarlig for hvert år innen 1. mars å levere årsrapport til FDU over antallet dyr som er brukt i forutgående kalenderår, jvf. forskriftens § 24. Etter forsøkets avslutning leveres sluttrapport til FDU.

-Søker må opplyse om HMS-tiltak som kreves i forbindelse med bruk av spesielle legemidler/kjemikalier etc. i gjeldende prosjekt.

Vedtak kan påklages til Mattilsynet, jfr. lov 10 feb 1967 om behandlingsmåten i forvaltningssaker (forvaltningsloven) § 28. Klagefristen er 3 uker fra mottak av dette brev, jfr. forvaltningsloven § 29. Klagen stiles til Mattilsynet, Hovedkontoret, men sendes via Forsøksdyrutvalget.

Med hilsen for Forsøksdyrutvalget

sign

Gro Furset Flatekval

Ansvarshavende 055 UiO - Ullevål Sykehus, Seksjon for komparativ medisin, IEMF

Kopi: ansvarshavende/koordinator
postmottak@mattilsynet.no

Adresse: Forsøksdyrutvalget Telefaks: **23 21 68 01** Sekretariat: Gunvor Knudsen **23216663** c/o Mattilsynet, Felles postmottak e-
post: postmottak@mattilsynet.no Johanne Holmen **23216692**
Postboks 383 Hjemmeside: www.fdu.no
N- 2381 Brumunddal