Microvascular Function Assessment after Mastectomy and Radiation Therapy in Breast Cancer Patients
From Methodology to Clinical Application

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To my family
# Table of contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>7</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>9</td>
</tr>
<tr>
<td>LIST OF ORIGINAL PAPERS</td>
<td>11</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>13</td>
</tr>
<tr>
<td>THESIS AT A GLANCE</td>
<td>17</td>
</tr>
<tr>
<td>THE FEMALE BREAST</td>
<td>19</td>
</tr>
<tr>
<td>SURGICAL TREATMENT OF BREAST CANCER</td>
<td>21</td>
</tr>
<tr>
<td>POSTMASTECTOMY RADIOTHERAPY</td>
<td>23</td>
</tr>
<tr>
<td>RADIATION-INDUCED FIBROSIS</td>
<td>24</td>
</tr>
<tr>
<td>RADIATION-RELATED COMPLICATIONS</td>
<td>26</td>
</tr>
<tr>
<td>RECONSTRUCTIVE ALTERNATIVES</td>
<td>27</td>
</tr>
<tr>
<td>AUTOLOGOUS RECONSTRUCTION</td>
<td>27</td>
</tr>
<tr>
<td>IMPLANT-BASED RECONSTRUCTION</td>
<td>29</td>
</tr>
<tr>
<td>AUTOLOGOUS FAT GRAFT</td>
<td>31</td>
</tr>
<tr>
<td>TIMING</td>
<td>31</td>
</tr>
<tr>
<td>PATIENT-CENTERED DECISION MAKING IN BREAST RECONSTRUCTION</td>
<td>33</td>
</tr>
<tr>
<td>MICROCIRCULATION</td>
<td>37</td>
</tr>
<tr>
<td>NON-INVASIVE TECHNIQUES TO ASSESS MICROVASCULAR FUNCTION</td>
<td>39</td>
</tr>
<tr>
<td>LASER DOPPLER FLOWMETRY</td>
<td>39</td>
</tr>
<tr>
<td>LASER SPECKLE CONTRAST IMAGING</td>
<td>40</td>
</tr>
<tr>
<td>DIFFUSE REFLECTANCE SPECTROSCOPY</td>
<td>42</td>
</tr>
<tr>
<td>OTHER NON-INVASIVE IMAGING TECHNIQUES</td>
<td>43</td>
</tr>
<tr>
<td>HYPERSONTRIAL IMAGING</td>
<td>43</td>
</tr>
<tr>
<td>CAPILLAROSCOPY</td>
<td>43</td>
</tr>
<tr>
<td>THERMAL IMAGING</td>
<td>44</td>
</tr>
<tr>
<td>FUNCTIONAL MAGNETIC RESONANCE IMAGING</td>
<td>44</td>
</tr>
<tr>
<td>PHOTOCOUCISTIC IMAGING</td>
<td>44</td>
</tr>
<tr>
<td>METHODS FOR PROVOKING MICROVASCULAR RESPONSES</td>
<td>47</td>
</tr>
<tr>
<td>LOCAL HEATING</td>
<td>47</td>
</tr>
<tr>
<td>POST-OCCULUSSIVE REACTIVE HYPEREMIA</td>
<td>47</td>
</tr>
<tr>
<td>DRUG-INDUCED MICROVASCULAR PROVOCATIONS</td>
<td>48</td>
</tr>
<tr>
<td>Methyl nicotinate</td>
<td>48</td>
</tr>
<tr>
<td>Niacin</td>
<td>49</td>
</tr>
<tr>
<td>EMLA</td>
<td>49</td>
</tr>
<tr>
<td>AIM OF THESIS</td>
<td>51</td>
</tr>
<tr>
<td>METHODS</td>
<td>53</td>
</tr>
<tr>
<td>SUBJECTS AND ENVIRONMENTAL CONDITIONS</td>
<td>53</td>
</tr>
<tr>
<td>DRUGS</td>
<td>54</td>
</tr>
<tr>
<td>RADIOTHERAPY</td>
<td>54</td>
</tr>
<tr>
<td>EQUIPMENT</td>
<td>55</td>
</tr>
<tr>
<td>Laser speckle contrast imaging</td>
<td>55</td>
</tr>
<tr>
<td>Enhanced perfusion and oxygen saturation</td>
<td>55</td>
</tr>
<tr>
<td>Iontophoresis</td>
<td>56</td>
</tr>
<tr>
<td>DATA PROCESSING AND STATISTICS</td>
<td>57</td>
</tr>
</tbody>
</table>
REVIEW OF THE PAPERS AND RESULTS................................................................. 59
PAPER I: THE MICROVASCULAR RESPONSE IN THE SKIN TO TOPICAL APPLICATION OF
METHYL NICOTINATE: EFFECT OF CONCENTRATION AND VARIATION BETWEEN SKIN
SITES .................................................................................................................. 59
  SUMMARY .................................................................................................... 59
  METHODS ................................................................................................. 59
  Experiment I ............................................................................................. 59
  Experiment II ........................................................................................... 60
  RESULTS ................................................................................................... 61
  CONCLUSIONS ......................................................................................... 62
PAPER II: SKIN BLOOD FLOW RESPONSE TO TOPICALLY APPLIED METHYL NICOTINATE:
POSSIBLE MECHANISMS ............................................................................ 63
  SUMMARY ................................................................................................ 63
  METHODS ................................................................................................. 63
  Experiment I ............................................................................................. 63
  Experiment II ........................................................................................... 63
  Experiment III .......................................................................................... 64
  RESULTS ................................................................................................... 65
  CONCLUSIONS ......................................................................................... 65
PAPER III: MICROCIRCULATORY CHANGES IN THE SKIN AFTER POSTMASTECTOMY
RADIOTHERAPY IN WOMEN WITH BREAST CANCER...................................... 66
  SUMMARY ................................................................................................ 66
  METHODS ................................................................................................. 66
  RESULTS ................................................................................................... 67
  CONCLUSION ............................................................................................ 68
PAPER IV: SKIN PERFUSION AND OXYGEN SATURATION AFTER MASTECTOMY AND
RADIATION THERAPY IN BREAST CANCER PATIENTS .................................... 69
  SUMMARY ................................................................................................ 69
  METHODS ................................................................................................. 69
  RESULTS ................................................................................................... 70
  CONCLUSION ............................................................................................ 70
DISCUSSION ..................................................................................................... 71
  THE STUDY COHORT ............................................................................. 72
  USING MN TO ASSESS MICROVASCULAR RESPONSIVITY .......................... 74
  ARE LSCI AND EPOS RELIABLE MEASUREMENT METHODS TO PREDICT OUTCOME OF RECONSTRUCTIVE
  SURGERY? .................................................................................................... 75
  CLINICAL RELEVANCE OF THE STUDY RESULTS ................................. 77
  ETHICAL ASPECTS .................................................................................. 78
  THE FUTURE OF TISSUE MONITORING PRIOR TO RECONSTRUCTIVE SURGERY ................................. 79
  CONCLUSION ......................................................................................... 80
ACKNOWLEDGEMENTS .................................................................................. 81
POPULÄRVETENSKAPLIG SAMMANFATTNING .................................................. 83
REFERENCES ............................................................................................... 85
Abstract

Post-mastectomy radiotherapy (PMRT) is an important part of the treatment of breast cancer. It reduces the risk of recurrence and improves overall survival. Scaring and fibrosis of the skin and subcutaneous tissue of the chest wall or remaining breast are among its side-effects. These late side-effects of PMRT may in turn affect skin microcirculation and oxygenation, although this connection is not completely established. In patients that later require breast reconstruction, it is difficult as a plastic surgeon to evaluate if the microcirculatory changes have been affected by PMRT, and how such effects should have an impact on the choice of reconstructive method. In the work presented in this thesis, laser speckle contrast imaging (LSCI), laser-doppler flowmetry (LDF) and diffuse reflectance spectroscopy (DRS) have been used with a strong vasodilator, methyl nicotinate (MN) to study the microcirculatory changes after PMRT.

In studies I and II, we aimed to find the optimal concentration of MN and its main mechanisms of action. In healthy volunteers, the microvascular response to different concentrations of MN was evaluated on the forearm using LSCI. It was found that a concentration of 20 mmol/l resulted in a quick vasodilatory response with a long plateau phase, minimal tissue edema and no non-responders. In study II, we utilized locally administered drugs to block the three main pathways responsible for skin vasodilation. Subsequently, we provoked the skin with MN and assessed its effect with LSCI. From this study we could conclude that MN’s mechanism of action is largely mediated by prostaglandins and partly by local sensory nerves.

In study III, we examined the skin microcirculatory response in breast cancer patients before, immediately after, and at two and six months following unilateral PMRT, using the contralateral breast as a control. A significant increase in basal skin perfusion and perfusion after application of MN was observed on the irradiated chest wall immediately after RT compared to the contralateral breast and compared to before RT. At six months after RT, there was no longer a difference in basal skin perfusion or after application of MN in the irradiated chest wall compared to the contralateral breast and compared to before RT was given. The results from this study concluded that skin perfusion in the irradiated chest wall had returned to normal when measured six months after RT.

In study IV, the late effects on skin microvascular function were studied in women who had undergone mastectomy and PMRT several years prior to the study. Skin perfusion and oxygen saturation was measured with white light diffuse reflectance spectroscopy (DRS) combined with Laser Doppler Flowmetry (LDF) before and after application of MN on the irradiated chest wall with the contralateral non-irradiated breast as control.
In this study we found that skin perfusion and oxygenation in the breast are affected several years after radiotherapy and that our method could be a valuable clinical tool prior to deciding surgical procedures after PMRT.

To conclude, MN can be topically applied to the skin to reliably assess microvascular function and the microvascular capacity. LSCI and LDF have different strengths and drawbacks, with LSCI having the advantage of having a large spatial resolution that allows for measurements of control areas in the same field of view as the provoked areas. LDF in combination with DRS enabled us to further assess perfusion and oxygenation simultaneously which could be an advantage in fibrotic skin where skin perfusion and oxygen saturation may not correlate with each other. Although the study groups differed between the study examining the early effects of PMRT with the late effects of PMRT, we have been able to non-invasively visualize changes in microcirculation in relation to the acute and chronic phase after PMRT. Future studies are needed to investigate the value of pre-operative measurements with MN provocation for predicting surgical outcome.
# List of abbreviations

- **ANOVA**  
  Analysis of variance  
  *A statistical method used to analyze the differences among group means in a sample.*

- **DIEP**  
  Deep inferior epigastric perforator flap  
  *A type of flap consisting of skin, fat and vascular structure fed by the epigastric vessels.*

- **DRS**  
  Diffuse reflective spectroscopy  
  *A method to measure oxygenation in the skin.*

- **EI**  
  Expander implant  
  *A method to create a “tissue pocket” in the reconstruction of a breast.*

- **HSI**  
  Hyperspectral imaging  
  *A method to measure oxygenation in the skin.*

- **IBR**  
  Implant-based reconstruction  
  *A method to reconstruct a breast using an implant.*

- **ICG**  
  Indocyanine green  
  *An intravenous dye.*

- **LAP**  
  Lumbar artery perforator flap  
  *A type of flap consisting of skin, fat and vascular structures fed by the lumbar vessels.*

- **LD**  
  Latissimus dorsi  
  *A type of flap consisting of skin, fat, muscle and vascular structures fed by the thoracodorsal vessels.*

- **LSCI**  
  Laser speckle contrast imaging  
  *A method to visualize and measure perfusion.*

- **LDF**  
  Laser doppler flowmetry  
  *A method to measure perfusion.*

- **MN**  
  Methyl nicotinate  
  *A vasoactive substance.*
| **MRI** | Magnetic resonance imaging | An imaging technique. |
| **PAI** | Photoacoustic imaging | A method to measure oxygenation. |
| **PAP** | Profunda artery perforator flap | A type of flap consisting of skin, fat and vascular structure fed by the femoral vessels. |
| **PORH** | Post-occlusive reactive hyperemia | An increase blood flow caused by temporary occlusion of a feeding artery. |
| **PU** | Perfusion units | Units used in perfusion measurements in this thesis. |
| **RIF** | Radiation-induced fibrosis | A process of scarring after radiotherapy. |
| **ROI** | Region of interest | An area marked in the LSCI software from which a perfusion average is given. |
| **SIEA** | Superficial inferior epigastric perforator flap | A type of flap consisting of skin, fat and vascular structure fed by the epigastric vessels. |
| **TDAP** | Transverse dorsal artery perforator flap | A type of flap consisting of skin, fat and vascular structure fed by the thoracodorsal vessels. |
| **TRAM** | Transverse rectus abdominis myocutaneous flap | A type of flap consisting of skin, fat, muscle and vascular structure fed by the epigastric vessels. |
| **TUG** | Transverse upper gracilis flap | A type of flap consisting of skin, fat, muscle and vascular structure fed by femoral vessels. |
List of original papers

I

Elawa S, Mirdell R, Tesselar E, Fanebo S. The microvascular response in the skin to topical application of methyl nicotinate: Effect of concentration and variation between skin sites
Microvascular Research 124 (2019) 54–60

II

Elawa S, Mirdell R, Fanebo S, Tesselar E. Skin blood flow response to topically applied methyl nicotinate: Possible mechanisms

III

Elawa S, Mirdell R, Stefanis A, Tesselar E, Fanebo S. Microcirculatory changes in the skin after postmastectomy radiotherapy in women with breast cancer
Scientific Reports 2024 (2024) 14:4149

IV

Elawa S, Fredriksson I, Steinvall I, Zöterman J, Fanebo S, Tesselar E. Skin perfusion and oxygen saturation after mastectomy and radiation therapy in breast cancer patients
The Breast 75 (2024) 103704
Introduction

This thesis is about the effects of radiotherapy on microvascular function after breast cancer treatment, and the evaluation of a new method to test on the chest wall using two different non-invasive methods.

Breast cancer is the most common malignancy to affect women. It was probably described for the first time 3,500 years ago, by ancient Egyptians, as documented in the Edwin Smith’s Surgical Papyrus \(^1\). In 2020, more than two million breast cancer cases were diagnosed globally, which accounted for 27.5% of all cancers in women. Surgery plays a prominent role in breast cancer treatment and is, for most patients, usually the first step in the multimodal treatment offered \(^2\).

In 2020 about 10,000 women in Sweden were diagnosed with breast cancer, of which 67 % were treated with breast-conserving surgery (BCS), the rest with mastectomy. Of those who were treated with mastectomy for breast cancer with subsequent spread to axillary lymph nodes, almost 100 % received radiotherapy (RT). The average 5-year relative survival rate for women with breast cancer in Sweden was 85 % in 2023.

Many patients want to proceed with a breast reconstruction after the cancer is removed, aiming to recreate shape and symmetry of the breast. The current goal, according to Regional Cancer Centers in Sweden, is that at least 20 % of women who are treated with mastectomy due to breast cancer should be offered immediate reconstruction (i.e., simultaneous with breast cancer surgery), with the rest potentially being offered delayed reconstruction (i.e.,

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Figure 1. Incidence of breast cancer per year and age-specific incidence rate per 100,000 women in Sweden. Reprinted with the permission of the Swedish National Board of Health and Welfare.

Figure 2. Relative 5-year survival per 100,000 women in Sweden. Reprinted with the permission of the Swedish National Board of Health and Welfare.
performed as a separate procedure). According to data from a nationwide study, implant-based reconstructions (IBR) have been used more frequent than autologous breast reconstruction (58 vs. 31%) in Sweden.

It is well known that patients that have received postmastectomy radiotherapy (PMRT) are at risk for tissue morbidity if they are subject to a secondary reconstructive procedure. Such a procedure typically aims at reconstructing the breast with either the patient's own tissues through a tissue transfer, or by using an implant. Despite surgical and technical improvements over the last decades we still, however, face complication rates of up to 40 % in women with breast reconstruction after PMRT.

The mechanisms behind morbidity are not fully known, but it has been hypothesized that altered skin microcirculation plays a key role.

The microcirculation may be defined as vascular networks interconnected and regulated by several mechanisms that ultimately control the nutritive pathways through the smallest vessels in a tissue compartment. The easiest way for the surgeon to evaluate the local microcirculation is by gently pressing on the skin and releasing, whereafter the smallest capillaries refill with blood as the skin becomes red again. If the skin remains white, it is likely that blood flow is poor, and the surgeon and patient may face upcoming complications. Various patient-specific factors may contribute to poorly functioning microcirculation. It has been known for a long time that radiotherapy affects local blood flow when administered, but its effects on microcirculation over time are less studied. Despite the interest in blood flow among surgeons, knowledge regarding blood flow before and after breast reconstructive surgery remains limited. By evaluating microvascular function prior to a surgical procedure, we could potentially identify patients at risk for tissue morbidity after surgery and by choosing reconstructive technique dependent on the function also reduce tissue morbidity.

There are several ways of measuring skin microcirculation. Laser Doppler Flowmetry (LDF) is a laser-based well-established technique used to non-invasively monitor perfusion in reconstructive surgery. Laser Speckle Contrast Imaging (LSCI) is a progression of LDF that is image-based and has the advantages of illuminating and therefore examining a larger area compared to LDF, that provides us with the same technique but with a one-point measurement. When using LSCI in the tissue, the light is scattered by moving particles and will form a speckle pattern that contains information about concentration and speed of moving particles. When skin tissue is illuminated, the red blood cells will affect the signal and tissue perfusion will therefore be assessed. After processing the signal, LSCI does not provide us with an absolute value, instead it presents an arbitrary value that is believed to be a function of the concentration of red blood cells and their velocity. This arbitrary value is referred to as the tissue’s perfusion. Even though tissue perfusion is believed to be linear with blood flow under normal physiological
circumstances, the two are not the same thing. When measuring tissue perfusion in the skin after radiotherapy one must bear in mind that the fibrosis caused by radiotherapy could affect skin perfusion and oxygenation differently. A combined measurement method could therefore give us a clearer picture of the pathological state in a radiated tissue. A recently developed technique enables us to do that by combining LDF and Diffuse Reflectance Spectroscopy (DRS). DRS, which uses white light non-invasively, measures skin oxygenation and when combining both techniques, a more comprehensive microvascular measurement can be done. The perfusion and oxygenation of the resting skin may however be difficult to detect, because the basal blood flow is low, and due to the large capillary reserve in the skin. Results based on the skin microcirculation in the resting state may therefore be misleading. To overcome this, it is common that vasoactive agents are used. The dynamics of blood flow may then be assessed, and if a strong vasodilator is used one may be able to assess if the maximum microvascular capacity is affected.

In the following sections, a brief summary of the female breast, surgical treatment of breast cancer, radiotherapy and its effects on the breast after mastectomy and impact on subsequent reconstruction will be presented. The LSCI, LDF and DRS technique will also be described in more detail as well as other techniques for tissue monitoring. Various techniques for increasing local blood flow will be highlighted. Finally, a review of the four papers included in this thesis is presented, including the used methods and how they could be used to potentially assist the surgeon in deciding which reconstructive alternative should be used in an individual patient after PMRT.
Thesis at a glance

The studies described in this thesis are summarized in the table below

<table>
<thead>
<tr>
<th>Study</th>
<th>Aim</th>
<th>Subjects</th>
<th>Site</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>To study the dose-response, time to response, reproducibility, plateau phase and site-to-site differences of MN</td>
<td>Healthy Volunteers</td>
<td>Volar side of forearm, dorsal side of the hand, lower back, and epigastric region</td>
<td>LSCI+MN</td>
</tr>
<tr>
<td>II</td>
<td>To study the mechanism of action of MN</td>
<td>Healthy Volunteers</td>
<td>Volar side of forearm</td>
<td>LSCI+MN</td>
</tr>
<tr>
<td>III</td>
<td>To study the dynamics in microcirculation in the early phase of RT (before RT, directly after RT, two and six months after RT)</td>
<td>Breast cancer patients after mastectomy prior to PMRT</td>
<td>Above and below the areolar region/mastectomy scar of the chest wall and the contralateral breast</td>
<td>LSCI+MN</td>
</tr>
<tr>
<td>IV</td>
<td>To study the dynamics in microcirculation in the late phase after PMRT (more than two years after RT)</td>
<td>Breast cancer patients after mastectomy and PMRT</td>
<td>Below the areolar region/mastectomy scar of the chest wall and the contralateral breast</td>
<td>LDF and DRS (EPOS)+MN</td>
</tr>
</tbody>
</table>
The female breast

The female breast, also called the mammary gland, is a highly evolved and specialized organ. The breasts usually weigh between 500 and 1000 gram each and are divided into 3 parts: skin, parenchyma and stroma (fig. 3). The breasts extend on the chest wall vertically from the 2nd to 6th rib, horizontally from the lateral sternal border to mid-axillary line and has a segment “axillary tail of Spence” that pierces the deep fascia and lies in the axilla up to 3rd rib level. In young adults the form is hemispherical but becomes pendulous later in life. The breast parenchyma, as well as its blood flow, decreases with age and is transformed into fatty tissue with atrophied glands. This is important to bear in mind when comparing breast surgery on healthy premenopausal women with postmenopausal women that have decreased blood supply, and thus increased risk of complications.

The breast skin with its quality, thickness and elasticity affects its appearance. It is subject to hormonal, expansible, weight, gravitational and aging influences. The thickness and elasticity of the breast skin are important when considering surgical results.

The glandular tissue consists of branching ducts and terminal secretory lobules. One lactiferous duct drains 15 to 20 lobes. Each duct enlarges to form the lactiferous sinus before they open separately into the nipple. Milk connects in the lactiferous sinuses before its released in response to baby’s sucking. The ducts are arranged radially, which is important to bear in mind when incisions are made in this area.

The stroma is the supporting framework of breast parenchyma. The stroma is divided into a fibrous stroma, suspensory ligaments of Cooper, which separate the lobes and suspend the gland and the fatty stroma, which stand for the bulk of the mammary gland. This fat also is responsible for the contour, softness, consistency and shape of the breast. Fat is selectively deposited within

Figure 3. The female breast. (A) Chest wall (B) Pectoralis major muscle (C) Mammary gland (D) Fatty stroma.
the breast and is influenced by genetic and hormonal factors. In immediate breast reconstruction one of the most important risk factors for postmastectomy skin ischemia is breast morphology. Breast ptosis, heavy breast and mastectomy weight have all been associated with higher risk of complications.

The blood supply to the skin originates primarily from the subdermal plexus with communications to the underlying anteromedial intercostal and anterolateral intercostal perforators, the external mammary artery and the internal mammary perforators. When performing immediate reconstruction after mastectomy, mastectomy skin flap perfusion is important. A flap thickness of 8 mm seems to be a threshold for ischemic complications with significantly higher complications compared to thickness greater than 8 mm. The blood supply to the breast parenchyma derives from several major sources, the internal mammary perforators medially, thoracoacromial vessels superiorly and the lateral thoracic and lateral intercostal branches. The internal mammary perforators are estimated to provide 60% of breast vascular supply. The location of the main blood supply is constant. Partial or complete absence of branches from the main source may occur and should be considered in surgical planning, especially in BCS and immediate breast reconstruction. However, due to a substantial collateralization of arterial blood flow within the breast it is possible for the entire normal breast to survive on a fraction of its usual arterial input. Venous drainage typically follows the arterial supply, with most of the drainage extending toward the axilla. Principal venous drainage is through the perforating branches of the internal thoracic vein, tributaries of the axillary vein, and perforating branches of the posterior intercostal veins. For surgeons, a thorough knowledge of venous drainage is essential, as the lymphatic channels typically follow the course of blood vessels. The lymphatic drainage is relevant as it is the pathway for potential cancer metastasis via lymphatic and venous channels.
Surgical treatment of breast cancer

Surgical treatment of breast cancer may be in the form of partial mastectomy, a procedure where the breast cancer is removed with small amount of healthy tissue surrounding it or mastectomy, a complete removal of breast tissue (table 1). Skin sparing mastectomy involves complete removal of all breast tissue, sometimes including the nipple, with preservation of as much breast skin as possible. This may be done in patients that have adequate tumor margins and wish for immediate breast reconstruction.

Breast cancer surgery has improved substantially over the past decades. Up to the 1980s radical mastectomy, usually the Halsted mastectomy, or modified radical mastectomy, was the standard treatment for most patients regardless of the stage of their disease. The Halsted mastectomy originally included en bloc resection of the affected breast with overlying skin, underlying chest muscles and ipsilateral axillary lymph nodes. This resulted in a large chest wall defect and the wound was closed under tension or left open to secondary healing so that cancer recurrence would not be concealed. Halsted later used an axillary flap or skin grafts to cover the defects and wrote “Beware of the man with the plastic operation”7. With time and improved knowledge on what tumors are of risk for higher morbidity, the surgical techniques have evolved to be increasingly tissue sparing without compromising on survival rates. More important is the success of moderate-dose radiotherapy for elimination of subclinical foci of disease in the ipsilateral breast allowing the concept of breast-preserving therapy to move forward. Several prospective randomized trials, with 20 years of follow-up have shown that BCS followed by radiotherapy, is as effective as mastectomy for the treatment of breast tumors up to 5 cm 8-10. Partial mastectomy with radiotherapy is the standard of care today, and with the evolvement of oncoplastic surgery an aesthetic approach has been integrated in the concept of BCS with better surgical outcome.

Figure 4. A Halsted Mastectomy. License: CC-BY 4.0.
Mastectomy may be indicated in patients:

- with disease that is multifocal or multicentric within the breast.

- with a relation between breast volume and distribution of disease that gives a poor aesthetic outcome.

- with advanced locoregional disease, including large primary tumors (T2 lesions greater than 5 cm) and skin or chest wall involvement.

- with inflammatory breast cancer, in addition to systemic chemotherapy and radiotherapy, due to tumor burden within the dermal lymphatic channels and more diffuse involvement of the underlying breast parenchyma.

- with contraindications for postoperative radiation therapy.

- who initially undergo breast-conserving surgery and have margin involvement with tumor cells, if margin re-excision is not successful or is not technically or cosmetically feasible.

- with recurrent breast cancer who previous underwent treatment with partial mastectomy and radiotherapy.

- that wish to have a total mastectomy instead of partial mastectomy after thorough information. These patients are usually not subject to breast reconstruction afterwards.

Table 1. Indications for mastectomy.
Postmastectomy radiotherapy

Most patients with breast cancer will have other treatments after surgery, such as radiotherapy, chemotherapy, hormone therapy or molecular targeted therapy. Local and regional recurrence after primary breast cancer treatment is typically associated with an increased risk of concurrent and future spread of cancer elsewhere in the body.\textsuperscript{11,12} The 10-year relative survival is, 25% to 50% after local and regional recurrence even after attempts to surgically remove the cancer recurrence. The most common area of local and regional recurrence after mastectomy is the chest wall (53%), followed by lymph nodes above and below the collar bone (26%), and in the armpit (13 %). The risk of local and regional recurrence after mastectomy increases with the number of axillary lymph nodes containing breast cancer.\textsuperscript{13}

Radiotherapy has been an important part of breast cancer care for almost 80 years. Its use peaked in the 1970s but soon declined due to complications and failure to demonstrate improved breast cancer survival. In 1997, researchers documented a significant improvement in survival among women with node positive breast cancer who received radiotherapy, which radically changed its use in breast cancer care.\textsuperscript{14,15} Radiotherapy remains a well-established additional treatment (adjuvant) to date. Its primary aim is to reduce local and regional recurrence in breast cancer.

PMRT is today indicated after BCS where the risk for recurrence is reduced by 48 % as well as breast cancer specific mortality by 18 %\textsuperscript{12}. After mastectomy for tumors >50 mm PMRT reduces the risk for recurrence with 17 % and improves overall survival.\textsuperscript{15,17} In Sweden, RT is also recommended if there is macro-metastasis in one or several lymph nodes or metastasis after preoperative treatment. Regional node radiotherapy significantly reduces breast cancer mortality and all-cause mortality.\textsuperscript{18}

RT can be given in different doses measured in Gray (Gy), in different number of fractions, with extra boost (extern fractioned boost of 10-16 Gy) and bolus (to increase skin dose for the photon beams). The trend in RT is towards a lower total dose, a higher dose per fraction, and with a smaller number of fractions. The most common regime is RT according to START B: 2.67 Gy, 15 fractions to a total of 40.05 Gy or according to Whelan: 2.67, 16 fractions to a total 42.5 Gy.\textsuperscript{19}

While PMRT is effective, it carries a risk of complications, which vary based on the radiation dose and include breast necrosis, chest wall necrosis, coronary atherosclerosis, brachial plexus pain and paresis, and lymphedema of the arm.
Radiation-induced fibrosis

In radiotherapy, ionizing radiation is used for the treatment of cancer because it has the ability to damage tumor cells, resulting in their inability to survive and proliferate further. Ionizing radiation causes damage to cells via two main mechanisms: direct interaction with DNA and indirectly damage through the generation of reactive oxygen species (ROS) that subsequently cause DNA breaks which in turn induce cellular events such as apoptosis, necrosis and abnormal mitosis. Radiation-induced fibrosis (RIF) is a late side effect of radiotherapy. Since radiation also affects the healthy cells in the skin and subcutaneous tissue, radiotherapy causes progressive functional and cosmetic impairment. The skin is sensitive to these effects due to its high proliferative capacity and oxygenation requirements of its basal epidermal cells.

Radiation gives rise to both acute and chronic effects (fig. 5). The acute effects are presented as erythema, dry or moist desquamation and possibly ulcers of the skin.

Acute erythema appears from an increased blood volume due to capillary dilatation in the dermis, which results from an inflammatory response 21,22. These acute changes in skin microcirculation following RT have been previously studied with LSCI. The non-invasiveness of the technique makes longitudinal objective observations of skin reactions possible 23.

Chronic effects are changed pigmentation, fibrosis of the skin, telangiectasia, sebaceous and sweat gland malfunction. Radiation induced fibrosis (RIF) usually occurs 4–12 months after radiotherapy and progresses over several years. The mechanism of RIF is similar to that of any chronic wound healing process. Morphologically, RIF involves progressive interstitial and microvascular deposition of extracellular matrix (ECM) protein 24. An initial injury incites an acute response that leads to inflammation, followed by fibroblast recruitment and activation with extracellular matrix deposition. Proinflammatory cytokines are released due to exposure to various molecules known as damage-associated molecular patterns (DAMPs). Among the chemokines and cytokines that are released due to this inflammatory process transforming growth factor (TGF) β1 and connective tissue growth factor (CTGF) is important and heavily implicated in RIF. TGF-β is responsible for several functions, with activation of myofibroblast as the most crucial part in the contribution of excess collagen formation and reduces vascularity over time. Myofibroblasts originate from dermal fibroblasts. In a quiescent state, fibroblasts produce few proteins and interact minimally with their surroundings. On exposure to fibrinogenic cytokines they differentiate to myofibroblasts 25. Radiation injury also causes vasculitis that participates in fibrogenesis 26. Intravascular and perivascular matrix deposition results in hypoxia and ischemia 27. This makes fibrotic areas susceptible to gradual ischemia. Depending on the radiation regime (dose, number of fractions, volume
of tissue, and duration of complete treatment), and concomitant treatments such as surgery and chemotherapy, fibrosis may be more or less pronounced.

Fibrosis may be quantified with MRI, but this device is not portable and difficult to access for routine clinical use, which makes it less suitable for clinical evaluation. Skin hardness may be measured by durometer and skin elasticity may be measured by suction-based methods as Cutometer® (MPA 580; Courage and Khazaka, Cologne, Germany). These devices have been used to evaluate tissue changes after burns and contractures.

Cutaneous RIF may after autologous fat grafting go into regression, according to several pilot studies. The mechanisms underlying this phenomenon remains unknown.

Figure 5. Schematic overview of the effects of PMRT.
Radiation-related complications

Despite its therapeutic advantages, PMRT increases the risk of complications and often provides poor cosmesis in women with breast reconstructions. Capsular contracture is a complication that manifests as a periprosthetic tightening (around an implant) and thickening that affects the shape, consistency, and position of the implant. The capsule around an implant is initially thin and soft, with no effect on appearance of the breast. With time the capsule undergoes a progressive thickening and becomes harder and shrinks in such a way that it may alter the breast contour and could cause severe pain. The exact etiopathogenesis behind capsular contracture is still unclear. PMRT is, however, a strong risk factor for the development of capsular contracture \(^{34}\). As all patients undergoing IBR or even breast augmentation develop at least grade I contracture, it may be difficult to address the exact cause of capsular contracture in patients with a history of PMRT \(^{35}\).

The degree of contractures is classified by Baker (Classification of capsular contracture after prosthetic breast reconstruction) from grade I to IV and is based on a clinical and subjective assessment (table 2).

Not only can PMRT adversely affect the aesthetic outcome of IBR, but it also increases the risk of implant extrusion, incidence of infection, mastectomy flap necrosis and re-operation due to complication \(^{36-39}\). When an autologous reconstruction is done there is an increased incidence of hematoma and seroma formation \(^{40}\).

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>Soft but visible implant</td>
</tr>
<tr>
<td>Class II</td>
<td>Implant with mild firmness</td>
</tr>
<tr>
<td>Class III</td>
<td>Implant with moderate firmness</td>
</tr>
<tr>
<td>Class IV</td>
<td>Excessively firm and symptomatic breast with a poor aesthetic result</td>
</tr>
</tbody>
</table>

Table 2. Classification of capsular contracture
Reconstructive alternatives

William Halsted, the founder of breast surgery, focused on the issue of saving the lives of the patients, leaving aside the “body image” and disabilities related to removal of the breast. In this regard he wrote: “The disability is irrelevant in comparison to the life of the patient. In addition, these patients are old, they have an average of nearly 55 years.” Today, this statement seems outdated, especially as the mean age of patients have changed radically, and survival among breast cancer patients have drastically increased (fig. 1 and 2).

Today, some patients describe that the mastectomy caused more trauma than the cancer illness itself. By restoring the breast, reconstruction aims to improve the psychosocial and physical consequences of undergoing a mastectomy. In reconstructive surgery, it is important to understand how radiotherapy affects the tissue to be able to decide a suitable reconstructive alternative.

Autologous reconstruction

Autologous breast reconstruction refers to the use of a patient's own tissues, taken from a different part of the body where there is excess fat and skin, sometimes also muscle, to restore the volume and skin (mostly in delayed reconstruction) of the breast after mastectomy. The technique is based on the identification of vessels and sometimes nerves that supply the tissue that is transferred to the chest wall. This technique is technically very demanding and requires a good knowledge of vascular anatomy, both at the donor site, and at the area that is to be reconstructed. Tissue transfer can either be a free flap, or a pedicled flap. In free flaps the tissue is completely detached from its blood supply and the circulation in the tissue re-established by surgically connecting the artery(s) and vein(s) at the site of reconstruction. This distinguishes them from pedicled flaps, such as latissimus dorsi (LD) myocutaneous flap from the back or transverse rectus abdominus myocutaneous (TRAM) flap from the abdomen, which always have their original vascular supply preserved. The most common free flap for breast reconstruction is the deep inferior epigastric perforator (DIEP) flap based on perforator from the deep epigastric artery and composed of skin and subcutaneous fat from the lower part of the abdomen (fig. 6). Other less common perforator-based tissue flaps may also be used as the superficial inferior epigastric artery (SIEA) flap from the abdomen, the lumbar artery perforator (LAP) flap from the lower back, the transverse upper gracilis (TUG) flap and the profunda artery perforator flap (PAP) from the upper inner thigh. DIEP flaps are today considered by many plastic surgeons the golden standard for autologous breast reconstruction.
In general, autologous tissue tends to be superior to IBR in the setting of PMRT, as autologous tissues do not become enveloped in fibrous capsules and cannot be extruded. In a prospective multicenter study, complications were examined two years after surgery in patients undergoing immediate and delayed breast reconstruction using expander/implant, pedicled TRAM flap and free TRAM flap. Immediate reconstruction showed significantly higher complication rates across all reconstructive methods compared to delayed reconstruction. Furthermore, there was evidence of a trend toward higher total and major complication rates in patients with IBR who received radiotherapy, compared to the other subgroups. TRAM flaps have been replaced with DIEP flaps due to the higher risk of abdominal hernias associated with TRAM flaps. In a study focusing on DIEP flaps after immediate reconstruction, Rogers et al. could show higher grades of shrinkage, flap contracture, and fat necrosis in irradiated DIEP flaps compared to non-irradiated DIEP flaps. Similar results have been shown for free TRAM flaps, which have strengthened the rationale of delaying autologous reconstruction after PMRT. Contrary to this, a more recent study on immediate reconstruction with DIEP flaps after preoperative radiotherapy found the incidence of complications such as open wounds, mastectomy skin necrosis, fat necrosis, and unplanned returns to the operating theatre low, with no reported DIEP flap failures. Most importantly, patients from the same study reported high satisfaction 12 months after...
surgery, with a very good aesthetic outcome observed on panel assessment 45. Moreover, a prospective multicohort study conducted by the Mastectomy Reconstruction Outcomes Consortium (MROC), involving 11 North American centers and 57 plastic surgeons evaluated the outcomes of immediate or delayed autologous reconstruction in patients undergoing PMRT. There was no significant difference in complication rates after one year between groups, suggesting that immediate autologous reconstructions tolerate radiotherapy better than previously anticipated, with a minimal level of morbidity 46. However, as irradiation protocols differ between centers, results are difficult to interpret and compare. Nevertheless, there is an increasing interest among patients and caregivers in immediate autologous reconstruction for patients who may later undergo PMRT.

**Implant-based reconstruction**

Implants remain the predominant form of reconstruction offered to women who receive PMRT. For some patients complete autologous reconstruction is not possible due to limited tissue available for transfer, or technical contraindications, whereas in other patients they want to avoid donor site morbidity. In the US, approximately 80% of patients seeking breast reconstruction undergo IBR, as opposed to 20 % undergoing autologous reconstruction (American Society of Plastic Surgeons, Plastic Surgery Statistics Report 2022). In Sweden, IBR is increasingly common, especially among patients offered immediate reconstruction or those undergoing prophylactic mastectomy (Swedish Breast Implant Register, 2022).

IBR can occur either in a single stage or in two stages. Single-stage reconstruction refers to the placement of a permanent implant at the time of mastectomy. In two-stage reconstruction, a tissue expander is placed underneath the skin and muscle and is gradually filled with sterile saline every week (fig. 7). The tissue expander is usually overexpanded by 15–20%. At the time of the exchange procedure, circumferential capsulotomy may be done, which entails incision of the scar tissue lining the pocket around the expander. The purpose of the capsulotomy is to improve contour, particularly lower pole projection. The expander is thereafter changed to a permanent implant 47.

![Figure 7. Schematic figure of expander implant reconstruction. (A) Expander implant at inset (B) Expander implant after gradual filling with saline.](image-url)
The effectiveness of IBR in the setting of radiotherapy has been called into question because of high rates of failure and adverse events, mainly implant extrusion and capsular contracture. Published data on the outcomes of implant reconstruction of the irradiated breast have, however, been inconclusive and contradictory. Postoperative complication rates following IBR are known to be higher in irradiated breasts when compared to similar non-irradiated breasts. In a study by Nava et al., complication rates were as high as 40%.

A few possible explanations for these discrepancies include surgical technique, differences from institution to institution, the advancement of breast implants, differences in the delivery and dose of radiotherapy and the limited subset of patients undergoing radiotherapy in one form or another as part of their oncologic treatment. Another important fact, applicable for both autologous reconstruction and IBR, is that there is little consensus on exactly what constitutes a surgical complication. The Clavien-Dindo Classification (CDC) provides a standardized definition of surgical complications and has previously been applied in breast reconstruction research. Nevertheless, according to a large Swedish population-based cohort study, implant removal after IBR was significantly associated with PMRT.

Whether there is a difference in risk for capsular contracture if PMRT is to be given to a permanent implant or to a tissue expander is also a matter of debate. Radiotherapy on a tissue expander is associated with higher rates of complications compared to a permanent implant. However, in the long term, two-stage IBR seems more favorable compared to one stage IBR when evaluating capsular contracture. Cordeiro et al.’s contribution has been important in this debate and in assessing the role of IBR after PMRT. In their study of 319 patients receiving PMRT and reconstructed with a two-stage expander implant, 91% who received PMRT successfully completed two-stage expander–implant reconstruction, compared with 99% who did not receive PMRT. Among patients who underwent radiotherapy, 53% had acceptable reconstructions, with Baker grade I or II capsular contracture. Furthermore, 90% with irradiated implants reported being satisfied, of whom 94% would choose the same method of reconstruction again. Another study by the same group, also on two-stage IBR, with long-term follow up of 2,326 breast implant reconstructions (12.5% with a history of PMRT), revealed severe capsular contracture grade IV in 7.4% of the irradiated implants and 0.6% of the non-irradiated implants. Implant loss occurred in 9.1% after irradiation, compared to 0.7% in the non-irradiated group. Seventy percent of irradiated patients had good to excellent aesthetic results, and 95% would choose implants again.

Apart from the dilemma on when and how to reconstruct a patient that received PMRT, there is a clinical dilemma on how to best handle capsular contracture when it occurs. In patients with severe contracture with extremely thin and damaged skin, autologous reconstruction is recommended. However, in some cases, the patients opt to try to keep
an IBR. By offering secondary procedures with capsular release (i.e. capsulotomy or capsulectomy), implant exchange and fat injection, some patients with severe capsular contractures may be treated. Haran et al. report that post-irradiation capsular contracture may be treated successfully to up to 86 % with these procedures 56.

Autologous fat graft

Autologous fat graft (AFG) may also be an option when offering breast reconstruction. This may be done with or without prior tissue expansion. The technique is based on harvesting fat from a donor site (usually abdomen, or thighs) with liposuction, processing of the fat graft and injecting it into the recipient site. One problem with autologous fat transfer is graft survival and retention postoperatively. Many studies are published demonstrating a retention rate between 25 % and 90 % 33,57,61. The survival of fat grafts after RT is significantly reduced compared to non-irradiated tissue due to the compromised blood supply in the recipient areas because of radiation damage. Achieving total breast reconstruction with autologous fat requires multiple procedures under anesthesia, which may pose challenges from a cost perspective.

AFG, even in lesser amounts, may be used in the treatment of chronic changes after RT with improved skin and subcutaneous quality. By preconditioning with AFG, complications could be reduced in subsequent IBR in patients with a history of PMRT 30,31,62-65.

Timing

Another reconstructive challenge is to find the optimal timing in relation to PMRT for the breast reconstructive procedure, because we know that irradiation after reconstruction may be associated with greater risk of complications. The temporal changes in the skin microcirculation secondary to radiation exposure may be of importance for this morbidity, however, these effects were poorly described previously. Historically, most surgeons have recommended a delayed reconstruction if PMRT was required. Compared to delayed reconstruction, immediate reconstruction protects women from negative body image, psychosocial distress, and diminished sexual wellbeing 66-68. Delayed reconstruction following radiotherapy treatment may also be much more technically challenging due to scarring and damage to small vessels and the potential to adversely affect microvascular anastomoses. Immediate reconstruction with both IBR and autologous tissue is, however, described to be associated with higher risk of complication compared to delayed reconstruction, and radiotherapy increases that risk 46,69. For irradiated implants the risk for infection, as well as capsule formation and reconstructive failure, is greater compared to non-irradiated implants 70,71. This trend is consistent across
various studies, including large multicenter populations were significantly higher complication rates were observed in immediate reconstructions, and in patients receiving radiotherapy before/during IBR \(^{72}\). The results for procedure timing is, however, inconsistent were some studies have reported higher complication rates for delayed procedures \(^{42}\), while others have not found any differences \(^{73}\).
Patient-centered decision making in breast reconstruction

For the plastic surgeon, timing must accommodate adjunctive therapy for systemic breast disease and the consideration of quality of life. The patient can be presented as a candidate for immediate breast reconstruction or delayed reconstruction (fig. 8). The decision regarding timing should be deferred until after completion of pathological evaluation and staging of the patient’s disease. Women with breast cancer who have been treated with neoadjuvant chemotherapy and in whom lymph node status (the main determinant for requiring PMRT) is either not known or has changed because of response to treatment before mastectomy can be especially challenging. In these cases, a delayed-immediate reconstruction may be an alternative. An expander implant is placed at the same time as the mastectomy. If the patients do not need PMRT, the expander is later replaced by an implant or autologous tissue.

For women requiring immediate reconstruction the surgical options are autologous procedures or IBR. The latter being the simplest method of breast reconstruction comprising either one- or two-stage procedures, various anatomical locations of implants (either above or beneath the pectoralis major muscle), and with or without biological or synthetic meshes. Women with unilateral IBR, however, will typically develop an asymmetry that will become more pronounced over time. Autologous reconstruction, on the other hand, behave more like a natural breast, developing ptosis over time and changing with the weight of the patient. IBR could still be a good option for thin patients with few autologous tissue donor sites, patients who do not wish to compromise physical function because of flap harvest, elderly patients, or those with comorbidities and into whom a long surgical procedure is contraindicated. Additionally, IBR seems to offer stable and satisfactory results over time for patients in need of bilateral reconstruction, particularly given the ease of achieving symmetry when implants are used on both sides.

In delayed reconstruction after PMRT, patients are presented with the choice between autologous reconstruction or expander implant (EI) due to the lack of skin after mastectomy. Most of the studies comparing EI and autologous procedures have primarily focused on immediate breast reconstructions. From these studies, it is consistently observed that complications tend to be higher after EI, and that patients who undergo autologous reconstruction are less likely to experience reconstructive failure. This conclusion is supported by studies specifically examining patients with delayed reconstruction and long-term follow up. Moreover, long-term patient-reported outcomes, including health-related quality of life, and aesthetic outcomes have demonstrated significant superiority following autologous reconstruction, especially
when abdominal based methods are used, compared to IBR. Clough et al. could illustrate that patient satisfaction after IBR declined from 86% at two-year post-reconstructive follow-up to 54% at five-year follow-up. For TRAM, the proportion of patient satisfaction was 96% at two-year follow-up and remained 94% at the five-year follow-up. Hu et al., reached similar conclusion in their study.

Despite varying findings in previous studies, using non-irradiated autologous tissue is the standard of care for delayed breast reconstruction after PMRT, offering the most predictable results. Autologous reconstruction, however, faces several barriers including the fact that it is a considerably more technically complicated surgery and that it may be relatively less reimbursed in some healthcare systems. Health care cost and total cost of care was compared between autologous reconstruction and IBR in a study including 12,296 women in the US, from a large private insurance company database, that had undergone mastectomy with immediate breast reconstruction. At two-year follow-up, the total cost of care was similar between autologous reconstruction and IBR. Similar results have been shown in a cohort of 15,154 women, also in the US. Several factors contribute to rising cost of IBR as use of acellular dermal matrix, pre-operative perforator imaging and intraoperative fluorescent angiography and subsequent need for revisional procedures or fat grafting. Due to the advancement of surgical techniques, autologous reconstruction with DIEP flaps may in long-term be financially advantageous for the health care system compared to IBR.

When discussing the possible donor site for autologous reconstruction the surgeon describes the donor site scar, the donor deformity, and any functional impact. The DIEP flap has become more popular with more surgeons offering the procedure and more patients desire to be reconstructed with that alternative. Prior to deciding, DIEP perforators may be assessed using CT angiography. A patient is not a candidate if she has an abdominal donor site that cannot be closed primarily, because she is too thin or has a severe pot-belly habitus. Other contraindications include a previous TRAM/DIEP flap or abdominoplasty, previous abdominal surgery where the deep inferior epigastric vessels were divided or damaged, or significant medical co-morbidities that make the patient a poor surgical candidate. The LD flap is the most widely used pedicled flap for breast reconstruction, which may be supplemented with an implant in patients with a low BMI, or, if using an extended harvesting approach, may be entirely autologous. The combination of LD flap and expander or implant is a well-accepted, reliable and safe method for postmastectomy reconstruction. This is a very robust flap, and has a failure rate of less than 0.5 per cent, with a good cosmetic result and high satisfaction rates in appropriately selected patients, especially those whose BMI is too high for consideration of the use of a free flap, or not suitable for a free flap but in need of autologous reconstruction. Its main drawback is seroma formation on the back.
Some patients harbor skepticism regarding the utilization of the body’s largest muscle. TDAP (thoracodorsal artery perforator) flap uses only the skin and subcutaneous tissue of the back, and comprising the function of the LD. TDAP, and other pedicled perforator flaps may replace the skin or enhance the skin envelope after PMRT. Together with an implant, it may be a good alternative in the reconstructive arsenal postmastectomy.

Understanding the patient’s expectations is key to producing a surgical result that satisfies the patient. Unfortunately, little is known about the decision-making process regarding patients’ choices of type and timing of breast reconstruction. Most studies suggest that these choices are ultimately based on personal and/or individual surgeon preference. Choosing the right reconstruction is important, as women who experience postoperative complications after breast reconstruction may also experience elevated anxiety, depression, and distress. When setting a goal for breast reconstruction it is important to remember that there is a significant variability among patients and between patients and surgeons on the important facts and goals to guide decision making about reconstruction. In a prospective study in women undergoing mastectomy for treatment or prevention of breast cancer, a survey was done before surgery regarding information about breast reconstructions. Patients answered approximately half of the knowledge questions incorrectly, with particularly low knowledge about the risk of complications (14.3 %), despite the fact that more than 90 % reported that they had discussed breast reconstruction, and their preference on the reconstructive procedure with their surgeon before surgery.

The use of photographs of other patients’ reconstructive results may help the patients in their decision making. Various options may be explored, and the patients may get an idea of what a realistic result may look like. It is important that the presented pictures give a realistic projection of what may be expected, and that it is not a “show off” the surgeon’s best result. In the future, AI algorithms and augmented reality features may be used to help the patients get an idea of what can be expected from different reconstructive solutions.
Immediate reconstruction

- Mastectomy + Autologous reconstruction → PMRT
- Mastectomy + Permanent implant reconstruction → PMRT

Delayed-immediate reconstruction

- Mastectomy + EI → PMRT → Autologous reconstruction
- Mastectomy + EI → PMRT → EI exchanged for permanent implant

Delayed reconstruction

- Mastectomy → PMRT → Autologous reconstruction
- Mastectomy → PMRT → Implant-based reconstruction
- Mastectomy → PMRT → Autologous reconstruction + implant

Figure 8. Timing and choice of reconstruction in relation to PMRT.
Microcirculation

The human circulation can be regarded as a closed system of vessels that connect all cells in the body. Its smallest part is called the microcirculation and accounts for about 99% of all blood vessels in adults. This is where the arterial and venous system connects, both structurally and functionally, and it includes vessels with a diameter of less than 150 μm. These include arterioles, venules, arteriovenous anastomoses, and capillaries. The microcirculation's main function is capillary exchange, which is delivery of oxygen and nutrients to tissues, and removal of carbon dioxide and waste products. This process is carried out by the capillary network that branches extensively to bring blood within close reach of every cell. The total surface area of the capillaries is approximately 1000 m², which is roughly equal to the size of two tennis courts, and it is estimated that no cell in the human body is further away than 0.1 mm from a capillary. This distance is often referred to as the Krogh length. Capillaries are passive vessels at rest that is recruited when in need to elevate tissue oxygen levels. The microvasculature has a complicated three-dimensional spatial structure, and its structural complexity leads to heterogeneity in microvascular perfusion. Heterogenous perfusion in turn leads to a heterogeneous oxygen supply to the tissue.

The exchange of compounds across capillary walls is accomplished primarily by diffusion. Capillaries are ideally suited to enhance diffusion as their thin walls minimize diffusion distances and their extensive branching maximizes surface area and time available for exchange. Because the red blood cell movements in the capillary network is stochastic with a velocity that is very slow (~0.5 mm/sec) the exchange is facilitated. Capillary exchange is the entire purpose of the circulatory system, i.e., all other activities of the system are directed toward ensuring an adequate distribution of replenished blood to capillaries for exchange with all cells throughout the body. The inflow to the capillaries is regulated by the circumference of the feeding arterioles. The walls of arterioles have a thick layer of smooth muscle that is richly innervated by sympathetic nerve fibers. The smooth muscle is also sensitive to many local chemical changes and to some circulating hormones. The smooth muscle layer runs circularly around the arteriole and upon contraction, the vessel’s circumference (and diameter) becomes smaller, thus decreasing the flow through that vessel. This process is called vasoconstriction. Vasodilatation, on the other hand, refers to enlargement in the circumference and diameter of a vessel due to relaxation of its smooth muscle layer. A variety of factors, locally in the organ as well as sensory stimuli (e.g., temperature, touch, pain), influence the activity of the arteriolar smooth muscle with resulting change in vessels wall resistance and blood flow through the vessel.

The skin microcirculation consists of two vascular plexuses; the superficial plexus at a depth of 400–500 μm, and deep plexus located at a depth of approximately 2 mm below
the skin surface. From the upper layer to the basal one, arterial capillaries with cross-
section of 10 μm loop away from the deep plexus to the superficial plexus through
ascending arterioles and descending venules. These are in turn paired when they connect
the two plexuses by arteriovenous anastomoses 99.

In clinical practice, blood pressure and flow measurements are used for circulatory
assessments, but central hemodynamic parameters may be satisfactory despite critical
insufficiency at the microcirculatory level. The skin and sublingual microcirculation are
the only microvascular beds readily available for human examination at the bedside.
Several methods may be used to analyze the microcirculation of human skin and to
measure its perfusion. In reconstructive surgery it has been used to predict wound
healing, to assess tissue morbidity in complex wounds, in scald injuries and after flap
reconstructions 100-102.
Non-invasive techniques to assess microvascular function

Assessment of blood flow in skin is usually done by observation of subjective signs, such as capillary refill time, color, skin temperature. These observations are at best highly subjective, but also much dependent on the surgeon's experience. It is therefore generally believed that more objective techniques are needed to assess skin blood flow and viability. In a pre-operative planning, before a reconstructive procedure, a pre-operative assessment of the skin microvasculature and its reactivity may assist the surgeon to make wiser and more patient-centered decisions on what reconstructive procedure should be used. By choosing a reconstructive procedure that is safe in a patient with suboptimal microcirculatory function, tissue morbidity may be prevented. There are various invasive and non-invasive ways of measuring microcirculation. I have in my thesis focused on non-invasive clinically compatible methods to measure skin microvascular function and will continue to describe the methods I have used and methods that I could have used as alternative.

Laser doppler flowmetry

The doppler effect was discovered by the professor Christian Johann Doppler (1803-1853). He noted that a whistle from a railroad train had a higher pitch when the train was approaching him, and the pitch diminished when the train passed and moved away. He noticed the same phenomena when he observed a star from the earth that shifted towards the red spectrum (lower frequency, longer wavelength) if the star recedes, and towards the violet (higher frequency, shorter wavelength) if it approaches. In 1842 he published his most famous paper “Über das färbige Licht der Doppelstern und einer anderer Gestirne des Himmels” (On the Colored Light of the Double Stars and Certain Other Stars in the Heavens). The doppler effect is important to understand when studying LDF.

The laser doppler instrument uses near-infrared monochromatic light to illuminate the tissue. It measures perfusion or microcirculatory blood flow in a volume of one cubic millimeter. An optical fiber leads the laser light to a probe attached to the tissue surface. The laser light is scattered by moving blood cells in the microvasculature, and this causes the laser light to change frequency due to Doppler shift. The frequency change depends on the number of blood cells in the illuminated area and the speed of those blood cells and will therefore be a measurement of blood perfusion - Blood perfusion = Concentration of moving blood cells x Mean velocity of the cells. Another optical fiber picks up the scattered light caused by blood cells moving within the volume and returns it to a photodetector in the instrument. The reading in the photodetector is electronically
processed and the signal converted into perfusion value. As the microvascular anatomy in the measured volume is unknown, it is not possible to give an exact value of the perfusion in milliliter per second per cm$^3$ with the method, instead it is presented as the arbitrary value (Perfusion Units, PU).

LDF in reconstructive surgery has been used for tissue assessment. Changes in perfusion may be followed in real time on a bedside monitor, which makes it suitable for monitoring of flaps.

Laser Speckle Contrast Imaging

Laser speckle contrast imaging (LSCI) uses a slightly different method than the Doppler effect to measure perfusion in a tissue. A divergent near-infrared 785 nm laser beam is used to make sure the laser is spread out over a large area, typically 20×20cm when images are taken from a 30 cm distance (fig. 9). The measurement depth is related to the wavelength of the laser, with longer wavelengths penetrating deeper. For the specific system used in this thesis, the penetration depth is approximately 300 μm, where the ROI has its strongest impact. Based on simulation models, it is estimated that the majority of the detected LSCI signal derives from the upper 700 μm of tissue $^{104}$.

The concept of LSCI is based on coherent light scattered from a collection of randomly distributed particles producing an interference pattern. Whenever coherent light interacts with a random scattering medium, a photodetector will receive light that has scattered from varying positions within the medium. The phenomenon occurs because small irregularities in a surface cause the distance between the surface and the image plane (the eye or a camera) to vary. If the distance difference between two irregularities corresponds to a multiple of the wavelength of light, the waves will interfere constructively. If the distance instead corresponds to half a wavelength, the waves will interfere destructively (fig. 10). This will create a pattern of lighter and darker areas on the surface, called a speckle pattern. The phenomenon may be observed with the naked eye if a laser pointer is used.

If the light is scattered from a moving object the optical path differences in light travelling from the various particles to the image plane will be constantly changing. This is called a “time-varying” speckle and can be seen in real time as a fluctuation in the speckle pattern $^{105}$. If these fluctuations are captured by a camera with a finite exposure time, there will be a blurring on the exposure caused by the movements of the speckles. The higher the velocity, the faster are the fluctuations and the more blurring occurs in each integration time. With a charged-couple device (CCD) camera the spatial blurring of the speckle pattern may be quantified.
The speckle contrast $K$ is calculated using the formula:

$$K = \frac{\sigma}{\langle I \rangle}$$

where $\sigma$ is the standard deviation of the intensity $I$ and $\langle I \rangle$ is the mean intensity, calculated over a window in space or time. Spatial contrast uses an area of multiple pixels in one frame. The number of pixels over which the speckle contrast is computed will affect the result: too few, and the statistics will be questionable, too many and spatial resolution will be lost. Usually, a square of $7\times7$ or $5\times5$ pixels is used.

Figure 9. The LSCI system. The camera house (A) contains an infrared laser that creates the speckle pattern, a CCD camera that capture the speckle pattern, a visible red laser that projects a positioning pattern on the assessed tissue and a color camera used for documentation. The speckle pattern is analyzed by a computer (B) and the image is shown on a monitor (C). (Illustration by Johan Zöttman)
LSCI has been evaluated and used as a tool for perfusion assessment in many studies but is still not widely used in everyday clinical situations. Initial studies were on retinal blood flow in various diseases including glaucoma, retinopathy, and macular degeneration\textsuperscript{106}. Its use has continued, both in clinical and research applications. In trauma care, LSCI has been used in burn wound assessment to discriminate between deeper and more superficial burns\textsuperscript{107}. LSCI has also been used to assess blood perfusion in various flaps\textsuperscript{108-112}.

**Diffuse reflectance spectroscopy**

Because biological molecules have unique absorption spectra as a function of the wavelength of light, it is possible to detect a precise concentration of these molecules by spectroscopy. Diffuse reflectance spectroscopy (DRS) estimates the tissue fraction of red blood cells (RBCs) and their oxygen saturation, by utilizing the characteristic absorption properties of different chromophores (e.g., oxy- and deoxyhemoglobin), for the decomposition of tissue bulk absorption from a measured diffuse reflectance spectrum. A more detailed description of the technique used in this thesis can be found in the method section.

Besides assessing microcirculatory changes in the skin, DRS has been used to differentiate between cancerous and non-cancerous tissue, to classify tumours, to decide tumour margins and to monitor diabetes foot ulcers\textsuperscript{113-116}.
Other non-invasive imaging techniques

Hyperspectral Imaging

Hyperspectral Imaging (HSI) is a method of wide-field diffuse reflectance spectroscopy that utilizes a spectral separator to vary the wavelength of light, typically a halogen lamp or a white LED, that is entering a digital camera and provides a diffuse reflectance spectrum for every pixel. Unlike traditional imaging systems that capture three bands of color (red, green, and blue), hyperspectral imaging captures a multitude of bands across a wider range of wavelengths. These spectra are then compared to standard transmission solutions to calculate the concentration of oxy- and deoxyhemoglobin in each pixel, from which spatial maps of these parameters are constructed.

HSI is still not implemented in clinical practice but has been useful in the analysis of oxygen mapping in flap surgery \(^{117}\), in predicting haemorrhagic shock \(^{118,119}\), evaluating microcirculatory changes in diabetic foot ulcers \(^{120,121}\) and in various animal models to monitor oxygenation \(^{122}\). Chin and co-workers studied irradiated mice with HSI and found it useful as biomarker for dose reconstruction and for monitoring acute skin reactions before any are visible \(^{122}\).

Capillaroscopy

Capillaroscopy is a non-invasive diagnostic technique designed to evaluate small vessels of the microcirculation. Various devices may be used as the wide field microscope, the dermatoscope, the videocapillaroscope, and the ophthalmoscope. The original device was transillumination videomicroscopy which may be used to measure capillary density, heterogeneity, and microvascular blood flow. A digital videocapillaroscope combines a microscope with a digital video camera and it is considered as the main tool for measuring and evaluating capillaroscopic parameters \(^{123}\). The main drawback is that despite the abundance of capillaries in the skin, they run perpendicular to the skin surface, and only the tip of the loop is visible. Capillaroscopy is therefore mostly used in the nailfold were terminal rows of capillaries run parallel to the skin surface available for examining morphological details and blood flow. It has, however, even been studied in free flap surgery. Matsui et al. have studied how videocapillaroscopy behaves in free flaps. They noticed when the pedicle artery was clamped, the number of visualizable blood vessels decreased and flap colour became relatively white. When the pedicle vein was clamped, the number of visualizable blood vessels increased, and flap colour tone had a tendency toward red \(^{124}\).
A development of capillaroscopy is the Sidestream Dark Field (SDF) imaging which is a validated intravital microscopic imaging technique for imaging capillaries on organ surfaces and for measuring red blood cell velocities in individual capillaries. It contains multiple diode flashes that encircle the optics of the camera. Superficial reflections do not reach the central optics, but light that is scattered against deeper layers such as capillaries does.

**Thermal Imaging**

In thermal imaging an infrared camera is used to detect the thermal radiation from the tissue. It can present a full field colour coded image showing warmer and cooler areas of, for example, skin. The system offers smartphone-compatible thermal imaging (FLIR Systems, Inc., Wilsonville, Ore.).

In reconstructive surgery it has been used to detect perforators prior to free flap planning and post-operative monitoring. According to data collected in patients with RIF there is a difference between fibrotic and healthy skin that could be measured with this technique.

**Functional Magnetic Resonance Imaging**

FMRI is based on Magnetic Resonance Imaging (MRI) which is a non-invasive imaging technique developed to demonstrate regional and time-varying changes in brain metabolism. BOLD (blood oxygen level–dependent) contrast MRI is a fMRI technique sensitive to vascular oxygenation since deoxyhemoglobin blood is paramagnetic, while oxyhemoglobin blood is diamagnetic. The magnetic properties of oxygenated and deoxygenated blood create differences in the local magnetic field, which may be detected by the MRI scanner.

To our knowledge the technique has still not been used in measurement of oxygenation in skin and subcutaneous tissue due to its difficulties to access in routine clinical use, being not portable and dependent on trained medical staff to interpret its results. In the area of breast cancer its potential current use is as an indirect measurement of breast tumour perfusion and hypoxia to monitor response to treatment.

**Photoacoustic imaging**

Photoacoustic imaging (PAI) is a medical imaging technique that combines principles from both optics and ultrasound to create detailed images. A short-pulsed laser is used to deliver light into the tissue of interest. This laser light is absorbed by endogenous
chromophores (oxy- and deoxyhemoglobin) or exogenous contrast agents (Indocyanine green (ICG), Methylene Blue Dye). When the absorbed laser energy is rapidly converted into heat, it induces a thermoelastic expansion in the tissue, leading to the generation of ultrasound waves. Ultrasound transducers are used to detect the generated acoustic waves. These transducers pick up the signals and convert them into electrical signals. The collected signals are processed and reconstructed to form detailed images representing the distribution of optical absorption within the tissue. This allows visualization of structures based on differences in the absorption properties of various tissues or contrast agents. Photoacoustic imaging offers several advantages in clinical applications, including high-resolution images and the ability to visualize both anatomical and functional information.
Methods for provoking microvascular responses

Blood flow measurements in the human skin is difficult in the resting state, as the basal blood flow is low. For this reason, it is common to use a provocation to visualize physiological and pathophysiological responses through an increase in perfusion. The vast increase in perfusion that may be gained through such a provocation indicates that the human skin has a large reserve of capillary capacity, and that this capacity may vary among individuals. We therefore hypothesize that a simple method to increase the basal perfusion may be used for meaningful assessment of microvascular function or capacity. Most commonly provocations of skin microcirculation are done by a physiological provocation or by local use of vasoactive drugs.

Local heating

Applying local heat is a common method to study microvascular function in the skin. Local heating to 42–43°C, is usually sufficient to cause maximal vasodilation. The microvascular response to a sudden increase in temperature is known to depend on at least two different mechanisms and depending on how fast heat is applied. It typically consists of an early peak followed by a decrease in perfusion and a late plateau. The early peak, known as the axon reflex, is observed after three to four minutes, lasts for about 10 minutes, and is mediated by local activation of cutaneous sensory nerves. The late plateau, which is reached within 20-30 minutes, is primarily mediated by the release of nitric oxide from the endothelium that causes a smooth muscle relaxation and increase of local microvascular blood flow. The main disadvantage with heat is that the effect is somewhat hard to control if a non-contact delivery such as heat fan is used. Other methods, such as heating glasses and heat pads have the disadvantage that they complicate or affect the accuracy of optical measurements. This has been solved in EPOS (PeriFlux 6000, Perimed AB, Järfalla, Sweden) which has an integrated heating element in the probe.

Post-occlusive reactive hyperemia

Post-occlusive reactive hyperemia (PORH) is the physiological reaction of limb reperfusion following a brief period of ischemia induced by arterial occlusion. It is a well-established technique for noninvasive assessment of peripheral microvascular function and a powerful predictor of all-cause and cardiovascular morbidity and mortality. Its exact physiological mechanisms are still not totally understood. The fundamental stimulus driving PORH is tissue hypoxia. Various mechanisms behind vasorelaxation mediated hyperemia have been studied where nitric oxide, prostaglandin, adenosine and potassium
may contribute. For PORH the main disadvantage is that it can only be used on limbs as it requires circular compression.\textsuperscript{137}

Drug-induced microvascular provocations

Several different vasoactive drugs and ways to apply them to the skin microcirculation have been investigated over the years. Most commonly drugs have been delivered transdermally, either topically (for example: Methyl nicotinate, EMLA, and Niacin), or by iontophoresis or microdialysis (for example: Acetylcholine, Nitroprusside, Noradrenaline, NG-nitro-L-arginine methyl ester, or Insulin). The obvious benefit of using a topical administration of the vasoactive drug is that it does not require any technical equipment and that it is completely non-invasive.\textsuperscript{138,139}

Methyl nicotinate

Methyl nicotinate (MN) is a methyl ester of nicotinate (niacin or Vitamin B3) that is mainly used by dermatologists to assess skin barrier function. MN reaches its receptor by epidermal diffusion and possibly by other mechanisms involving transport by the blood flowing in the dermis, due to its rapid rate of spread. The function of stratum corneum is important to MN’s uptake as it is the major barrier to diffusion of penetrating substances. The perfusion increase occurs from the first few minutes from application and reaches a stable plateau 5 min after application, which lasts for at least another 15 min (fig. 11). Its mechanism is primarily mediated through the prostaglandin pathway, but also involves neurogenic components.

The effect of topical application of MN at the forearm and foot levels of diabetic neuropathic patients has been compared with vasodilation induced by iontophoresis of ACh and SNP, with comparable vasodilatory responses.\textsuperscript{140}
Niacin

Besides its ability to induce skin vasodilation, niacin has been available as a vitamin supplement and to regulate lipid levels. Its use has been limited by its main adverse effect, flushing \(^{141}\). Flushing consists of a cutaneous vasodilation accompanied by a burning sensation mainly affecting the upper body and face \(^{142,143}\). On a cellular level niacin stimulates Langerhans cells to activate a specific receptor (GPR109A) that increases arachidonic acid and prostaglandins in capillaries, causing vascular smooth muscle cell relaxation and cutaneous vasodilatation \(^{144,145}\).

EMLA

Eutectic Mixture of the Local Analgesics lidocaine and prilocaine (EMLA) was introduced as percutaneous analgesia before venous puncture, removal of genital wart or molluscum contagiosum. In reconstructive surgery it has been used as an analgesic prior to harvesting split thickness skin graft. The cream is applied on the skin surface under a plastic film occlusion for at least 1 h before a painful procedure. After cutaneous application, EMLA has a biphasic vascular response. An initial vasoconstriction, maximal after 1,5 h is followed by a vasodilation after >3h. This effect is presumably caused by smooth muscle relaxant effect of the analgesics \(^{146}\).
Aim of thesis

The overall aim of this thesis was to describe how the microvascular effects in the skin changes over time after PMRT, and to evaluate a new model for assessment of microvascular capacity with the use of MN and non-invasive methodology. Specifically, the aims of the papers were:

- To evaluate MN as a reliable method to increase basal perfusion and assess skin microvascular capacity. Also, to study MN’s optimal concentration when applied to the skin, the reproducibility of the microvascular response, its plateau phase and how it behaves on different sites on the human body in healthy individuals.

- To explore MN’s involvement in the nitric oxide, local sensory nerves and prostaglandin-mediated pathways in healthy individuals.

- To evaluate early microcirculatory changes with LSCI before and after MN provocation during and after PMRT in breast cancer patients.

- To investigate if chest wall skin perfusion and oxygen saturation is affected late after PMRT in breast cancer patients, measured with LDF and DRS before and after MN provocation.
Methods

Subjects and environmental conditions

In studies I and II, none of the subjects used regular medication, except for oral contraceptives. All subjects recruited were non-smoking. Female subjects were included regardless of their menstrual phase. All subjects were asked to abstain from caffeine and strenuous exercise for at least 24 h before the measurement, which was recorded with the subjects lying in a supine position. They were acclimatized for 10 min before the start of the measurements. Room temperature was kept at 21.0 ± 1.0 °C. The study cohort varied between the experiments.

Study I, was divided into two experiments. To study different concentrations and reproducibility twelve subjects were included (five females) with a mean age of 28 years, and in the part investigating the variation between different sites twelve subjects were included (five males) with a mean age of 27 years.

Study II, was divided into three experiments. Experiment I had ten subjects (five females) with a mean age of 28 years, Experiment II had nine subjects (three females) with a mean age of 26 years, and Experiment III had five subjects (one female) with a mean age of 27 years.

In study III and IV, measurements were made at a room temperature of 21.0 ± 1.0° C with the participants lying in a supine position.

In study III, 22 women with a mean age of 62 years, who had undergone macroscopically complete surgical removal of their breast cancer were consecutively enrolled from the department of oncology prior to PMRT.

In study IV, 31 women with a mean age of 50 years who had been referred to our reconstructive plastic surgery department for delayed breast reconstruction two years or more after unilateral mastectomy and PMRT, were consecutively enrolled.

In studies I and II, no formal sample size calculations were made since the variability in responses between participants was unknown. Instead, a sample size was chosen that was in line with the number of participants included in previous, similar studies by other researchers. In study III the number of participants was based on a sample size that allows for detecting a 20% change in microvascular perfusion, accounting for a 10% loss to follow up. In Study IV the number of participants was based on a sample size that allows for detecting a 10% change in oxygen saturation, accounting for a 10% loss to follow up.
Drugs

Methyl Nicotinate (Sigma-Aldrich) and Chlorhexidine ethanol (5 mg/ml, Fresenius AB, Uppsala, Sweden) was used in all studies.

In study II, a non-steroidal anti-inflammatory drug (NSAID) gel (Voltaren Gel, GlaxoSmithKline Consumer Healthcare Gel 23.2 mg/g) was used to investigate the role of the prostaglandin mediated pathway. To assess the role of local sensory nerves a lidocaine/prilocaine mixture (EMLA, Aspen Nordic 25 mg/g + 25 mg/g) was used. L-NMMA (Cayman Chemical), a nonspecific NO synthase inhibitor was administered using transdermal iontophoresis to block the NO-mediated pathway.

Radiotherapy

In studies III and IV, we measured microcirculatory changes in breast cancer patients that had received PMRT.

In study III, five women (23%) received a conventional regimen of 25 x 2 Gy, while 17 (77%), underwent a hypofractionated regimen of 16 x 2.66 Gy. Additionally, boost radiation was administered in two (9%) of the cases. Various energy levels were used, with 6 MV being the most common, followed by a combination of 6 + 15 MV. About one fourth of the patients received 15 MV alone. All the patients received external radiotherapy.

In study IV, the median radiation dose was 40.05 Gy with median time since last fraction was 44 months.
Equipment

Laser speckle contrast imaging

In studies I-III, a laser speckle contrast imager was used (PeriCam PSI System, Perimed AB, Järfalla, Sweden). It uses a divergent near infrared (NIR) laser beam at a wavelength of 785 nm to create the speckle pattern and a visible red laser (650 nm) for positioning. A monochrome CMOS camera captures the speckle image for the analysis and a separate color camera is used for documentation (fig.12).

The instrument was calibrated in accordance with instructions from the manufacturer. The same acquisition parameters were used for all perfusion recordings. The measurement distance was between 20 and 30 cm. The sampling rate was set to 21 images per second. Averaging of every 5 images was done to reduce signal to noise ratio, to improve image quality, and to reduce data size, resulting in an effective frame rate of 4.2 images per second.

Enhanced perfusion and oxygen saturation

In study IV, microcirculatory measurements were performed using a multimodal optical instrument (PeriFlux 6000 EPOS (enhanced perfusion and oxygen saturation), Perimed AB, Järfalla, Sweden) that integrates white light diffuse reflectance spectroscopy (DRS) and laser Doppler flowmetry (LDF) for comprehensive microcirculatory assessment. The EPOS system (fig. 13) acquires DRS and LDF data noninvasively using a fiberoptic probe placed in contact with the skin tissue. The fiber optic probe consists of three optic fibers, one for light delivery (source) and two for
light collection (detector). The distance between the source and detector is important because it dictates the sampling depth. The two detectors are each connected to a separate spectrometer. A spectrometer divides the collected light into various wavelengths and further analysis of the resulting spectrum is done in the CPU of the instrument. The acquired data are analyzed by the EPOS system using an adaptive model-based algorithm that automatically accounts for intra- and inter-individual variations in the optical and geometrical properties of the skin. This allows for a quantitative and simultaneous estimation of the skin microcirculatory oxygen saturation ($S_{02}$), speed resolved perfusion ($\text{perf}_{\text{tot}}$, $\text{perf}_{0.1}$, $\text{perf}_{1.0}$, and $\text{perf}_{10}$, respectively), and RBC tissue fraction ($C_{\text{RBC}}$) in real-time.

Using an inverse Monte Carlo method the accuracy of DRS has been evaluated in a multilayer skin model, with an accuracy of 3-5 % \cite{148}.

Iontophoresis

With a battery-powered iontophoresis device containing a power source and two electrode compartments, L-NMMA was delivered in study III to the underlying tissue through the skin. Drugs is typically charged substances, either positively charged (cation) or negatively charged (anion). The drug is placed in the electrode compartment bearing the same charge; for example, a negatively charged drug such as L-NMMA would be placed in the anion compartment for example. The indifferent electrode, that is neutral or opposite of charge, compartment is placed at a distal site. A small current is then applied, with the positive charges in the anodal compartment moving towards the cathode whereas anions move in the opposite direction. Charged drugs may therefore be moved into the skin, facilitating their penetration through the skin's barrier. \cite{147}
Data processing and statistics

LSCI images were processed using the system analysis software (PSIWin, Perimed, Järfalla, Sweden). The software allows for regions of interest (ROI) to be selected in the LSCI images and mean perfusion for each region is calculated and presented as mean PU ± SD. Matlab (R2019a, The MathWorks, Natick, MA, USA) was used for the multiple regression. Other statistical calculations were made using GraphPad Prism (version 6 for Windows, GraphPad Software, San Diego, CA, USA), and a probability of less than 0.05 was accepted as significant. For the stepwise regression method, p-values are generally considered invalid, and were therefore not reported.

In study I, correlation analysis and Bland-Altman plot was used for the analysis of the day-to-day and the site-to-site variability. Normal distribution of the data was tested using D’Agostino & Pearson omnibus normality test. Differences between concentrations were analyzed with Dunn's multiple comparisons test. The data from different anatomical sites were analyzed with a one-way ANOVA with multiple comparisons using the Tukey correction method. A two-tailed paired Student's t-test was used to compare the difference in perfusion between the control and MN area.

In study II, paired, two-tailed, Student's t tests were used. Relative change in perfusion was calculated as absolute increase from baseline divided by the absolute increase from baseline in the control area.

In study III, a two-way ANOVA for repeated measures was used to evaluate the difference in perfusion across different time points, between different radiation doses and between different measurement sites. Multiple linear regression was used to explore the influence of potential factors associated with the microvascular response after RT. The backward stepwise selection method was used to produce an initial screening of these potential factors.

In study IV, Wilcoxon signed rank tests were used to analyze two matched samples and Mann-Whitney U tests were used for unpaired data to test for differences between groups. Spearman correlation coefficients were used to describe the association between variables.
Review of the papers and results

Paper I: The microvascular response in the skin to topical application of methyl nicotinate: Effect of concentration and variation between skin sites

Microvascular Research 2019

Summary

The capacity of the cutaneous blood flow is far greater than needed for epidermal nutrition and the excess is primarily to regulate heat loss and temperature control. Due to the inherent low blood flow in the skin’s resting state, and a large reserve of capillary blood flow, a simple method to increase the basal perfusion would be desirable for assessment of its capacity. In this study we evaluated the reproducibility of such a provocation with the aim of determining the microvascular response with a strong vasodilator, at different body sites.

Methyl Nicotinate (MN) is a harmless methyl ester of nicotinate, commonly known as niacin. It is easy to use with minimal patient discomfort and may be stored in a refrigerator. Its optimal concentration when topically applied to the skin, the reproducibility of the microvascular response and its variation in different sites on the human body was studied in Paper I.

Methods

The study was divided into two experiments (fig. 14). A Laser Speckle Contrast Imager (PeriCam PSI System, Perimed AB, Järfälla, Sweden) was used to measure the perfusion of the skin after topical administration of MN. Baseline measurements were recorded for each experiment.

Experiment I

Different concentrations of MN were studied on different days. Two series of concentrations were tested and after initial analysis of the response in perfusion, a set of concentrations were selected for further testing the day-to-day, and site-to-site reproducibility (0, 1, 2.5, 5, 10, 20 and, 40 mmol/L). Blood pressure, saturation, pulse,
and room temperature were also measured. Tissue edema was repeatedly evaluated through visual assessment.

Experiment II

The variation in the microvascular response to MN between different anatomical sites was investigated with MN applied to four different body sites: the lower back, the epigastric region, the volar side of the forearm and the dorsal side of the hand.

Figure 14. Schematic overview of the experimental setup, including typical response to topical application of MN as visualized and quantified by LSCI. A high perfusion is characterized by bright green colors that contrast the black/blue low perfusion areas.
**Results**

The concentrations of 5, 10 and, 20 mmol/L of MN proved to be the most reliable in day-to-day ($r > 0.90$) and site-to-site reproducibility ($r > 0.90$), with no significant differences observed when comparing measurements taken at 5 minutes or 20 minutes after provocations (fig. 15). When comparing different anatomical sites, the forearm showed a significantly larger relative increase in perfusion compared to all other sites ($p < 0.03$) (fig. 16). MN concentration of 10 mmol/L produced equal or better responses than 20 mmol/L with respect to day-to-day and site-to-site reproducibility. However, we noticed a higher interindividual variation (17% non-responders) in this group. This phenomenon was not evident with the concentration of 20 mmol/L (table 3).

![Figure 15. Mean (SD) perfusion response on the volar side of the forearm after topical application of different concentrations of methyl nicotinate (MN).](image1)

![Figure 16. Perfusion in the skin after topical application of methyl nicotinate (MN) in different anatomical sites.](image2)

<table>
<thead>
<tr>
<th>MN Concentration</th>
<th>5 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mmol/L</td>
<td>165.1 (21.3) PU</td>
<td>160.9 (33.6) PU</td>
</tr>
<tr>
<td>10 mmol/L</td>
<td>158.0 (27.0) PU</td>
<td>135.1 (35.7) PU</td>
</tr>
<tr>
<td>5 mmol/L</td>
<td>154.7 (20.5) PU</td>
<td>129.1 (37.7) PU</td>
</tr>
</tbody>
</table>

Table 3. Mean (SD) perfusion after 5 and 20 min of topically applying methyl nicotinate in two non-responders. Note how the perfusion remains increased with 20 mmol/L and how it decreases over the course of time with the lower concentrations 5 and 10 mmol/L.
Conclusions

In this study we found that the optimal concentration range for MN was between 5 mmol/L and 20 mmol/L, with a slight preference for 20 mmol/L due to a less inter-individual variation. A physiological plateau phase appeared roughly 5 min after application of MN and lasted for at least 15 min. The responses varied between measurement sites on different body parts.

Topically applied MN, in an optimal dose, provides a quick vasodilatory response, with a long plateau phase with minimal tissue edema. MN is both chemically and biologically stable and easy to apply, making it a promising agent for safe and reliable evaluation of microvascular vasodilatation and reactivity in the skin. We concluded that future studies should evaluate its role in predicting outcomes after surgery in terms of tissue morbidity.
Paper II: Skin blood flow response to topically applied methyl nicotinate: Possible mechanisms

Skin Research and Technology 2019

Summary

Generally, vasodilation in the skin is believed to be controlled by three different mechanisms: prostaglandin-mediated vasodilation, nerve-mediated vasodilation, and the release of nitric oxide from the endothelium. The aim in this study was to explore how MN functions by locally blocking the prostaglandin-mediated pathway, local sensory nerves, and the nitric oxide pathway in different experiments.

Methods

The study was divided into three different experiments (fig. 17). Each experiment aimed to block one of three pathways by using pathway-specific drugs, followed by the application of MN to both the pretreated areas and the untreated control sites. LSCI was used to measure perfusion of the skin at these locations.

Experiment I

NSAID gel was applied topically to the forearm and covered for 60 minutes to allow the drug to reach its maximum effect. Then, 50 µL 20 mmol/L MN was immediately applied on the area treated with NSAID gel. This procedure was repeated after at least 48 h in the same subjects. Perfusion was measured at baseline before application of the drug and 15 minutes after application of MN. Additionally, we investigated the possibility that the NSAID gel could limit the penetration of MN in the skin in a separate measurement by applying NSAID immediately after MN.

Experiment II

EMLA cream was applied on the forearm and covered for 60 minutes to allow the drug to reach its maximum effect. After removing the cream 50 µL of 20 mmol/L MN was applied. Perfusion was measured at baseline before application of the drug and 15 minutes after application of MN.
Experiment III

L-NMMA was delivered using transdermal iontophoresis with a constant current of 0.02 mA for 10 minutes. After the drugs had been delivered, the electrodes were removed. Perfusion was measured at baseline after the drug had been delivered and 15 minutes after application of MN.

Figure 17. Schematic overview of the experimental setup
Results

In Experiment I, an 82% (20%) relative decrease in perfusion was observed after pretreatment with NSAID compared to the control site following provocation with MN (p<0.001). Conversely, when NSAID was applied to the skin after MN, the relative decrease in perfusion was only 48% (24%) compared to the control site (p=0.003) (fig. 18).

In Experiment II, pretreatment with EMLA resulted in a relative decrease in perfusion of 32% (32%) compared to the control site following provocation with MN (p=0.028).

In Experiment III, pretreatment with L-NMMA resulted in a relative decrease in perfusion of 7% (23%) compared to the control site following provocation with MN (p=0.55).

The interindividual variation (%CV) was highest after pretreatment with MN in the NSAID group 49%, 38% after lidocaine/prilocaine and 22% after L-NMMA.

![Figure 18. Box plot of the skin perfusion in the forearm at baseline, and after 15 minutes of application of methyl nicotinate in pretreated (T) and control (C) sites.]

Conclusions

The main finding of this study was that the vasodilatory actions of MN are largely mediated by prostaglandins and partly by local sensory nerves.
Paper III: Microcirculatory changes in the skin after postmastectomy radiotherapy in women with breast cancer

Scientific Reports 2024

Summary

RT is important for reducing recurrence of breast cancer and improves overall survival. Its side effects include acute and late skin reactions, with a high morbidity rate among patients that undergo IBR. The precise mechanisms of ionizing radiation-induced skin injury remain unclear. With the aid of LSCI and MN, we aimed to study the vasoactive response before, directly after, and at two and six months after RT. Most of the patients included in the study had undergone a partial mastectomy prior to RT. Our hypothesis was that immediately after RT, we would observe an increased vasoactive response on the irradiated chest wall compared to the non-irradiated breast when provoked with MN, followed by a decline in this response two to six months after RT. Multiple linear regression was used to explore the influence of potential factors associated with the microvascular response after RT.

Methods

Skin perfusion was measured with LSCI. Measurements were done above and below the areolar regions of both chest wall and contralateral breast (fig. 19). Initially, measurements were taken at rest, followed by measurements 15 min after application of MN. Perfusion was measured for one min at four different time points: before RT (baseline), directly after RT, and two months and six months after the last fraction of RT. Age, BMI, blood systolic and diastolic pressure, adjuvant medical treatment, hypertension, smoking, diabetes mellitus, and radiation dose were documented to investigate their potential association with the microvascular response after RT.

Figure 19. Overview of the measurement sites
Results

There were no significant differences in skin perfusion between measurement sites above or below the areolar complex/mastectomy scar before or after MN provocation at any of the time points. Immediately after RT, a significant increase in skin perfusion was noted in the irradiated chest wall (p<0.0001), whereas the perfusion in non-irradiated breast remained unchanged. The increased skin perfusion due to RT was dependent on radiation dose and fraction. Women who received a prolonged radiation protocol had a significantly larger increase in perfusion (p<0.0001). After the application of MN, a significant increase in skin perfusion was measured at all time points, both in the irradiated chest wall and non-irradiated breast. In the irradiated chest wall, the perfusion after application of MN was significantly higher immediately after RT compared to before RT (p=0.033). At two and six months after RT there were no significant differences, after the application of MN, between the irradiated chest wall and the contralateral breast. However, at two months, there was still a slight but significant increase in basal perfusion in the irradiated chest wall compared to the same side before RT (fig. 20). Diabetes mellitus was associated with a reduced vasodilatory response after radiation, whereas hypertension and a higher radiation dose were factors that were related to increased vasodilatory response.

![Figure 20](image_url)

Figure 20. (A) Mean perfusion at rest in irradiated and non-irradiated skin before RT and at three time points thereafter. (B) Mean perfusion in irradiated and non-irradiated skin after application of MN before RT and at three time points thereafter.
Conclusion

In the irradiated chest wall, skin perfusion after MN provocation returned to baseline levels as early as two months after RT. By six months after RT, perfusion both at rest and after provocation with MN showed the same results as before RT. These findings suggest that the microcirculatory function in the skin remains intact six months after RT, and that RIF has not yet impaired the capillary bed. However, tissue oxygenation was not measured, so microvascular function was not completely evaluated.
Paper IV: Skin perfusion and oxygen saturation after mastectomy and radiation therapy in breast cancer patients

The Breast 2024

Summary

In this study, we utilized a multimodal optical instrument that integrates white light diffuse reflectance spectroscopy (DRS) and Laser Doppler Flowmetry (LDF) to assess skin microcirculation and oxygen saturation (\(SO_2\)) in patients with a history of mastectomy and PMRT. We evaluated the potential late effects on skin microvascular function in patients who had received their last radiation dose two years or more before their visit to discuss delayed breast reconstruction. The aim of the study was to evaluate how RIF affects skin microvascular function when skin perfusion and oxygenation was measured. Our hypothesis was that reduced oxygen saturation with MN provocation could be correlated with the severity of RIF in the irradiated chest wall.

Methods

Using EPOS (PeriFlux 6000) we measured skin perfusion and oxygen saturation in 31 patients with a history of mastectomy and PMRT. Measurements were done below the areolar region/mastectomy scar at rest (baseline) and after MN provocation (fig. 21). Each measurement lasted 20 seconds, with simultaneous measurement of microcirculatory oxygen saturation (\(S_{\text{O2}}\) and speed resolved perfusion (\(\text{perf}_{\text{m}}\)).
Results

The median time since last fraction of RT was 44 months. There was no significant difference in baseline skin perfusion between the irradiated chest wall and the non-irradiated breast. However, RBC oxygen saturation during baseline was significantly higher in the irradiated chest wall compared to the non-irradiated breast (p=0.01). With MN provocation, the perfusion was significantly higher in the non-irradiated breast compared to the irradiated chest wall (p<0.001). The same phenomenon was noted for saturation (p=0.001) (fig. 22).

A majority, 81 % (25/31) of the participants showed a ≥10 % difference in skin perfusion between the non-irradiated breast and the irradiated chest wall, while 26 % (8/31) showed a ≥10 % difference in skin oxygenation between the non-irradiated breast and the irradiated chest wall. Correlation analysis revealed a stronger association on the irradiated chest wall (r = 0.58; p<0.001) than in the non-irradiated breast (r = 0.40; p=0.02) between skin perfusion and oxygenation after MN provocation.

![Image](image.png)

Figure 22. Oxygenation (%) in irradiated and non-irradiated skin before and after application of methyl nicotinate (MN). Perfusion (% RBC × mm/s) in irradiated and non-irradiated skin before and after MN.

Conclusion

In this study, we have shown that skin perfusion and oxygen saturation was significantly higher in the non-irradiated breast compared to the irradiated chest wall when MN was used. Oxygen saturation was higher in the irradiated chest wall compared to non-irradiated breast at rest, illustrating the insidious scenario of skin measurement of microvascular function at rest. Most of the patients had an obvious decrease in skin perfusion in the irradiated chest wall after PMRT, but only a fourth had more than 10 % decline in oxygen saturation.
Discussion

As plastic surgeons we often have time to plan our surgical procedure based on what we believe is subjectively best suitable for the patient. Women that require mastectomy and PMRT were until recently usually recommended a delayed reconstruction, but treatment algorithms are constantly being updated and a more person-centred decision making, with tailored solutions to everyone is likely to be the future. To help us and the patient in the decision making, we collect information about patients’ medical history, oncological history, family history, social history, allergies, and medication history. The effects of RJF on the skin overlying the chest wall are assessed through a thorough physical examination, including status on how the scarred tissues affect the chest wall and quality of the skin and subcutaneous tissue. We then provide the patients with thorough information about the different procedures that we offer, including pros and cons of each technique. For most patients we can show relevant photographs of expected outcomes and inform about the possibility of complications. A missing puzzle piece has, however, always been regarding how the patients’ tissue will behave during and after our reconstructive procedure. This knowledge gap made us think of how we could develop a test that would give an objective view on the tissue status, to help us estimate risk for tissue morbidity, with regards to the skin microcirculation. We have in our department been using laser-based techniques for microcirculatory assessment to follow healing of burns, to study venous congestion in flaps and in studying various microcirculatory phenomena for many years. For this thesis we used our knowledge on these non-invasive tools to investigate microcirculation in a study population that is previously less explored, i.e., patients that are eligible for breast reconstruction but are at risk for tissue morbidity because of a previous surgery (mastectomy) and PMRT. The effects of radiation therapy on the irradiated chest wall and reconstructed breast are widely feared but poorly understood. There is support in the literature for how blood flow and microcirculation is affected after PMRT, but to our knowledge there were no previous studies on the long-term dynamics of microcirculatory changes in the skin after PMRT. Our hypothesis was thus that, to give patients correct information about timing, choice of reconstruction and to address causes of surgical complications, we would need a better understanding of their skin microcirculation prior to surgery. The overall aim of my thesis was therefore to find a user-friendly tool, that is easy to understand for both the doctor and the patient, non-invasive and affordable to measure microcirculatory changes after PMRT. We also aimed at exploring the temporal characteristics of blood flow changes after PMRT to better understand how blood flow in the skin is affected in the early (two to six months) phase, up to more than two years after the radiotherapy. We used MN for our test because it has previously been shown to be a promising compound with the ability to increase capillary capacity, and to make a more accurate measurement of the skin’s capacity to increase blood flow and O₂ delivery.
LSCI is often considered one of the most promising noninvasive techniques for clinical perfusion monitoring in reconstructive surgery. Its main benefits are in its ability to reproducibly measure perfusion in a large area of the superficial dermis. When compared to LDF the better spatial resolution comes with a lower temporal resolution. The better spatial resolution is of importance as the microcirculation is highly heterogenous in the skin in general, and various parts of a skin area may have drastically different perfusion, especially when subject to a reconstructive procedure. We, and others, have in several previous papers demonstrated LSCI’s ability to accurately detect areas in the skin at risk for morbidity, but this is the first time we have used it to pre-operatively assess the skin’s microcirculatory capacity. To enhance the assessment of microcirculation and to be able to relate skin perfusion with oxygen saturation, a rather new combination of noninvasive techniques has been included in our arsenal of methods in this thesis, with the ambition to in future studies relate measurement results with complications and tissue morbidity after PMRT.

In the following sections I will discuss the dilemmas of our study cohort, MN as a provocation model, methodological concerns with LSCI, LDF and DRS as measurement tools for microvascular function, clinical relevance and ethical aspects of our studies. I will also discuss the future of non-invasive measurements and their role in breast reconstruction planning.

The study cohort

There are a few important differences between the study subjects in the first two studies (studies I and II) and the subsequent clinical studies (studies III and IV). Firstly, all subjects in the first studies were healthy and significantly younger with a mean age of 29 years compared to 64 years in study III and 50 in study IV. We know age could affect the microvascular response. For example, Tsuchida and his co-workers demonstrated that skin blood flow in men is reduced by 40% between age 20 and 70\textsuperscript{149}. This difference may be explained by difficulties in accessing the microvasculature reserve, that is non-perfused during rest, due to reduction in arteriolar reactivity with increasing age\textsuperscript{150}. As in most aging organs there is also an increased vascular stiffness that may impact these effects\textsuperscript{151,152}. To contradict this there are studies that show that the response to MN is less dependent on age. Roskos et al. studied cutaneous erythema on the forearm after MN application in young (20-34 years) and old (64-86 years) individuals by using LDF. They evaluated time of onset of action, time to peak, magnitude of the maximum response, area under the response-time curve and the time to decay to 75% of the maximum response. Statistical analysis of all data showed no significant differences between the age groups for the same concentrations\textsuperscript{153}. Similarly, a study by Guy and co-workers was unable to observe any age or racial differences in the response of human
skin to the topically applied MN. Altogether, despite the fact that microcirculation changes throughout life, these studies strengthen the rationale for using MN in our studies where we have patients with age differences.

There are also differences between the study subjects in our two clinical studies (studies III and IV). Age-wise, the cohorts are similar, but the surgical treatment for their cancers differs. Most of the patients in study III had undergone a partial mastectomy, with a substantial amount of skin, breast tissue and blood vessels left to preserve the remaining breast. Partial mastectomy is currently the most common surgical procedure for women with breast cancer who receive PMRT, accounting for approximately 85% of all patients with tumors that are less than 30 mm in diameter. On the other hand, all the patients in study IV had undergone a mastectomy with complete removal of all breast tissue with its overlying skin. It is likely that this difference in surgical procedure produces a substantial difference in microcirculation between these groups and that direct comparison is not possible. One must realize that mastectomy substantially reduces collateral vessels supplying the dermal plexus of the mastectomy skin flaps, whereas there are a lot of remaining nutrient vessels surrounding the area of radiation after partial mastectomy.

Due to the differences in our study groups (studies III and IV), it is not fair to say that we followed microcirculatory dynamics with MN-test from an early phase to a late phase (two years or more). Our initial ambition was to follow the cohort from study III when they came back for their yearly follow-up, however, due to circumstances following the COVID-19 pandemic this was not possible. This would have helped us to better understand the process from early phase to late phase of RIF, especially in the cases that later need a reconstruction. Our ambition was also to correlate the amount of remaining tissue in the breast (from routine CT scans of the breast) with the radiation dose, frequencies, boost, bolus dose and microvascular response as all these factors seems to correlate with the severity of RIF. As some of these patients later would require reconstructive surgery to the breast, we would compare microvascular response to MN with surgical outcome. To our knowledge this has not been studied yet.

Another important limitation related to our study cohorts, and that is related to inclusion, is the lack of relevant control groups. For both study III and IV, we consecutively included all available patients that came to our oncology department for PMRT (study III) and to our plastic surgery department for reconstructive evaluation (study IV). Because most patients that undergo PMRT have been subject to partial mastectomy, and all patients that were presented for reconstruction had a mastectomy it is difficult to compare them (see above). Optimally our study IV would have included both patients that were eligible for inclusion in study III (mastectomy and PMRT), but also patients that had a mastectomy without receiving PMRT. As is now, we can only evaluate our results relative to a control area on the healthy breast, however we cannot be completely certain that the decreased perfusion and oxygenation measured was all related to the
PMRT given. It is likely that a combination of factors, also including the scar tissue, the amount of breast tissue resected at the mastectomy, and co-morbidity play an important role. In the best of worlds, we would have included such controls in our study, but unfortunately the majority of patients that come to our department for breast reconstructive evaluation have been subject to mastectomy and PMRT. This is because many patients who are eligible for mastectomy without PMRT and suitable for breast reconstruction are offered immediate reconstruction, usually with prosthesis. A multicentric study could therefore be needed to be able to include more patients, including relevant controls. Furthermore, we did not relate the locations of the measurements to how the RT was dose-planned, especially in the patients that received bolus dose. This would have enabled a more robust evaluation of the results but also added complexity to the study design.

Using MN to assess microvascular responsivity

The first two studies aimed to create a method that we could use to assess microvascular changes in chest wall skin to PMRT. We needed a potent vasodilator that was easy to use, with minimal discomfort for the patient, safe, with a reliable and reproducible response. Because the patient group that we intend to use it in predominantly consist of rather young patients, some in distress because of their cancer diagnosis, the test needed to be completely non-invasive and easy to perform. MN was chosen based on these basic requirements and after careful survey of drugs available on the market that could be given non-invasively. In total we used MN in 101 subjects in this thesis project. The results from healthy volunteers in study I and II made it possible to use MN in 53 patients without any observed patient discomfort and with adequate response among all patients.

As previously discussed, there are several pathways involved in vasodilation. The vasodilating mechanism of MN is mainly mediated by prostaglandin release. MN’s role in skin capillary recruitment is, however, not completely elucidated. PORH and local heating have both been reliable models to predict vascular dysfunction and one could therefore argue that we should have compared the response of 20 nmol/L MN with local heating and PORH on the volar aspect of the forearm to elucidate their different responses. This may be of importance if MN should be recommended as a reliable test for microvascular function in a broader context, but for evaluation on the breast the convenience of applying a topical ointment directly on the affected area was most important. Also, because we used LSCI, we could measure the response in several areas, including control areas simultaneously. A heated LDF probe would not have been able to do this, and a PORH-design would not have been possible on the breast. In our clinical tests of the MN model, we found that one major benefit with our study setup is that we were able to use the contralateral non-irradiated breast as control, and that we
therefore could specifically study the microvascular reaction to PMRT and not MN’s ability of assessing total capillary capacity. In study III, there was, however, a worry that MN saturated the capillary bed. As the response to PMRT showed a dose-dependent variation in increase in perfusion on the irradiated chest wall after stimulation with MN, this suggested that the response to MN did not saturate the capillary bed.

When studying the differences in skin perfusion and oxygen saturation at rest, a minor difference in oxygenation was observed, characterized by higher oxygenation levels in irradiated chest wall compared to the non-irradiated breast. However, following the application of MN, there was a significant reduction in oxygenation observed in irradiated chest wall as opposed to the non-irradiated breasts. This indicates the subtlety and complexity of skin evaluation under resting conditions.

In our experience, topical application of MN has been a safe, reliable, easy to handle method to provoke the vascular bed. It has many advantages and future studies should evaluate how well the response to MN correlates to outcome in terms of tissue morbidity. Ideally, an MN test could have role in pre-operative examination prior to reconstructive surgery, by providing a cut off value that could be used for clinical guidance regarding the choice of reconstruction technique.

Are LSCI and EPOS reliable measurement methods to predict outcome of reconstructive surgery?

The blood flow that mainly contributes to the measured perfusion value in both LDF and LSCI is found within the upper 0.5 mm of skin. This corresponds to the superficial dermal plexus in the papillary dermis. The deeper plexus in the junction between dermis and the subdermal tissue does not contribute to any extent to the measured perfusion value. The consequence of this is that both techniques may fail to assess the perfusion in the arteriovenous shunts located in the deeper dermis, that account for a large part of the dermal circulatory regulation. The dermal blood flow can, if needed, increase from a baseline of 250 ml/min to 8 L/min to meet an increased need 155. Another important aspect is that LSCI measures perfusion, concentration of red blood cells and their velocity, and not blood flow. PU will therefore not have a linear relative to absolute flow, mL/kg/min, instead it is an indirect measure of blood flow. When interpreting the PU value, we also must take into consideration that the same vessel, with the same blood flow will give rise to different perfusion signals depending on its depth in the tissue from the measurement surface. The measurement depth depends on how deep the photons penetrate and how many photons are reflected and captured by the light detector. This in turn depends on several factors, such as the wavelength of the laser, concentration and distribution of RBCs in the assessed volume and in the case of LDF, the fiber separation in the probe.
Both LDF and LSCI have methodological concerns that affect the measurement. Mahé et al. showed in their study on LSCI that perfusion measured as PU dropped to 9.1 during complete arterial occlusion of the forearm in healthy subject, which should be considered as the approximation of biological zero\textsuperscript{156}. Zötterman et al. studied how distance from the camera, curvature of the tissue, how high and low perfusion states, and motion of the examined tissue affect the result measured with LSCI. Of the factors examined only motion of the examined tissue had a significant effect on measurements\textsuperscript{157}. LDF is also motion sensitive. Manipulation of the optical fibers gives rise to output signals related to the fiber movement and not to the tissue perfusion. Similarly, tissue movements, such as muscular contraction, cannot easily be separated from the blood flow related portion of the signal\textsuperscript{158}.

In clinical practice the standard device for flap evaluation in reconstructive surgery is indocyanine green (ICG). MN with a non-invasive measurement technique has the advantage over ICG because it may be used pre-operatively for microvascular assessment. ICG is dependent on an injection of ICG, that binds to plasma proteins and protein-bound ICG emits light when illuminated with near-infrared light\textsuperscript{159}. Compared to LSCI, which presents perfusion as an arbitrary unit, ICG evaluation is based on a qualitative scoring of ICG fluorescence intensity with the surgeon estimating the speed and degree of filling of ICG in the tissue and correlating this information to the microvascular blood flow or perfusion of the tissue. Despite their differences, measurement results could be correlated between LSCI and ICG\textsuperscript{160,161}

As we previously assumed, and later could show in study IV, the relationship between perfusion and oxygen saturation is not constant in radiation injured skin. To increase the sensitivity and specificity in microvascular measurements after PMRT by measuring both parameters we may be able to estimate risk of subsequent tissue morbidity more accurate compared to only measuring perfusion. This is possible with EPOS. A major drawback with EPOS and all single point measurement systems is that it does not take the spatial heterogeneity in the dermal perfusion into account\textsuperscript{162}. This can be seen when LDF is used as a higher variability in the assessment of dermal perfusion compared to LSCI\textsuperscript{108}

As we wanted to evaluate EPOS as an easy test of microvascular function during patient consultation, we choose to make one measurement on the irradiated chest wall to compare to one measurement on the non-irradiate breast. From our previous study (Study III) we knew that there were no differences between the measurement above or below the mastectomy scar/areolar areas, wherefor we choose to measure below the mastectomy scar because this area is the most susceptible part in breast reconstruction. The results from study III were, however, only based on skin perfusion and in a study group were the patients had more skin, breast tissue and related vessels left. To overcome the question if oxygen spatial heterogeneity or if different parts of the chest wall is unequally affected by RIF, we could have used a large field technique such as HSI instead
of EPOS to overcome these problems. However, MN has the ability to recruit capillaries, and this could potentially be in favor of single point measurements as spatial heterogeneity no longer would be a problem.

Clinical relevance of the study results

The patients in study III represented a group with early microvascular changes due to PMRT. The results from this study concluded that skin perfusion returned to normal six months after PMRT. This could indicate that, with regards to the microcirculation of the tissue in the area of the reconstruction, a surgical procedure such as a two-stage IBR could be done after six months when the microcirculation had returned completely to normal. However, our results were not correlated with any surgical reconstructive procedure, and we can therefore not draw any definitive conclusions regarding this. There are, however, studies looking at reconstructive timing after PMRT that seem to correspond well to our results. Peled and colleagues, for example, compared expander-implant exchange within three to six months after PMRT with reconstruction more than six months after PMRT. Delaying expander exchange for at least six months after PMRT significantly reduced expander-implant failure (22.4 % versus 7.7 %) 163.

Whether RIF induces tissue hypoxia or not is debated 164. In the healthy, non-irradiated skin, the transfer of oxygen from blood to tissue is dependent on both flow-limited exchange and diffusion-limited exchange 165. Perfusion could therefore be intact in radiation-injured tissue, but oxygen delivery be diminished. In study IV, EPOS could provide us with a perfusion and oxygen saturation values with high accuracy. The aim of this study was to elucidate whether this could be an easy tool to evaluate the severity of radiation effect of the skin. With this study, we were able to show a significant decline in skin perfusion and oxygen saturation after MN test. The absolute difference in oxygen for the whole study group was low, with 26 % of the patients having a 10 % or more decline in oxygen saturation in the irradiated breast compared to the non-irradiated breast. One major drawback of our studies is that the clinical significance of this decline could not be evaluated in our study group, as all the patients were offered autologous reconstruction. When incorporating healthy, well vascularized tissue into an area with previous RIF, the skin below the mastectomy incision is often severely fibrotic and needs to be replaced with healthy skin from a donor site to adequately reconstruct the breast contour, and the chronic change in the remittent tissue goes partly or totally into regression 166. In some cases, the caudal part of the irradiated breast envelope is spared when performing autologous reconstruction. This is usually done if the surgeon believes the remaining skin is of good quality and provides an aesthetical advantage compared to removing the skin. This evaluation can be difficult, and our current method could be an
interesting tool in this context, as well as following the subsequent changes in perfusion and oxygenation after autologous reconstruction in an area affected by PMRT.

Ethical aspects

There are several studies describing the physical distress women with breast cancer are in during their cancer treatment. In each of our clinical studies we tried not to add extra visits to the patients and combine all visits with routine checkups or at the same time as other scheduled consultations at the hospital. As previously mentioned, all measurements were performed non-invasively ensuring patient comfort. The devices we used were not connected to the internet or to any other network. The data was stored on a separate storage device as backup, with all the measurement encrypted. None of the measurements could be traced to a specific patient without the responsible researchers' permit. Demographic data on patients was securely stored in a locked compartment at the hospital's safe.

When designing the studies in this thesis we wanted to be able to correlate the findings done with our measurement methods to surgical procedures. The panorama of breast reconstruction is changing. The number of patients in need of mastectomy and subsequent breast reconstruction is declining due to evolvement of BCS and RT. Most of the patients in our department that need immediate or delayed reconstruction before or after PMRT are offered autologous reconstruction. We therefore have few patients with IBR after PMRT, which would be the most interesting group to study with our method.

By inspiration of Chin et al. we initially designed and planned an animal model were radiotherapy was to be given to rat skull were skin was previously excised, all under anesthesia. Later an expansion prothesis was to be inserted and gradually filled while monitoring the effect on skin microcirculation with LSCI. The purpose of the study would be to induce RIF and measure how tissue microcirculation was affected compared to non-irradiated rats with expanded skin, simulating the same scenario as in expansion of chest wall skin. The need for an animal study was due to the small number of patients that were referred to our department with a radiated chest wall in need of tissue expansion and later permanent implant. When considering the ethical issues and the "four Rs" principle (Reduction, Refinement, Replacement and Responsibility) we had difficulties motivating our study, despite approval from the ethical committee. Instead, we redesigned our clinical studies and adjusted them to the patients that we see in our department and the surgical options we offer them.
The future of tissue monitoring prior to reconstructive surgery

To be able to quantify the risk for complications and tissue morbidity in breast reconstruction patients with a history of PMRT we would need to include more patients, collect more data about potential risk factors, follow microvascular changes, correlate to previous surgical procedures, oncological treatments and RT regimes. To further be able to analyze how these factors impact outcome we would need to correlate them to surgical results, morbidity and patient satisfaction after breast reconstruction. Enormous amounts of data could with the help of AI help us to quantify a risk score for each patient when all parameters were to be given, and hopefully guide us in surgical planning and decision-making to improve patient safety.

To make measurements more accurate combining different techniques in one device would be favorable. If the response to MN test could be measured by LSCI and HSI, we could address the variability in microvascular function when assessing chest wall skin after PMRT.

However, for clinicians, surgeons, and patients the software of the techniques used in this thesis must be more interactive and easier to interpret for medical staff as for patients. To be able to rely on results given, our measurement tools need to be integrated in our clinical practice. The current systems are still too bulky even if the discrete components of LSCI are relativity small. There are several groups working on handheld devices which is a step in the right direction 168-170.

An emerging technique is PAI that can image structures several centimetres inside the tissue 171. With multispectral PAI different chromophores may be distinguished and blood oxygen levels can be estimated. Since Rf causes changes in tissue composition PAI could have the potential to visualize and quantify these changes in comparison with the healthy surrounding tissue.
Conclusion

One in eight women will develop breast cancer during their lifetime. Current treatment most often includes radiotherapy in combination with removal of part of the breast or the entire breast tissue (approximately one third of women treated for breast cancer). The opportunity to restore the removed tissue and regain cosmetic appearance is provided by reconstructing the breast tissue with an implant or the patient’s own tissue transferred to the chest wall. Patients who have received radiotherapy are thought to have a higher risk for complications, such as poor wound healing, fibrosis formation, and infection. The pathogenesis behind these complications is not fully understood, but altered microcirculation of the remaining tissue is likely to play an instrumental role. This thesis addresses how the skin microcirculation, after partial and total mastectomy, reacts over time when exposed to radiation therapy, and if a provocation test can be used to assess its competence.

Although many surgeons may prefer autologous reconstruction after PMRT, it is a clinical reality that a significant number of patients end up undergoing both implant-reconstruction and radiotherapy. Unfortunately, as there is no current register that covers all breast reconstructions in Sweden, the prevalence of complication after PMRT in relation to different types and timing of breast reconstruction is not known. For our pre-operative test, patients who have undergone an immediate or delayed-immediate IBR and later develop capsular contracture and wish for new IBR, as well as those who wish for a delayed IBR after mastectomy and PMRT, would be of special interest to study.

While deciding whether to offer surgery to a patient in need of reconstructive surgery can be difficult, determining which surgical procedures to offer may be an even greater challenge. With today’s available technologies, decisions need to be based on clinical aspects and cannot be reduced to a machine. LSCI and EPOS have been studied by many groups and for many years, and their reliability cannot be questioned, although it remains to be proven whether the relatively small changes observed are clinically relevant or not. Still, the MN test remains a promising tool that, combined with other methods for tissue evaluation, could give us an enhanced vision and help us to understand the expected development of the future wound caused by surgical procedures. Further studies on MN’s role in reconstructive surgery are, however, needed especially clinical studies with measurable surgical outcomes.
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Populärvetenskaplig sammanfattning


minsta kärlbädd befinner sig i vila. Den har då en stor reservkapacitet som kan rekryteras vid behov. Vill man bedöma hur väl kärlbädden fungerar och vilken kapacitet den har måste man därför stimulera den så att den maximalt öppnas.

I samtliga studier har patienter på Onkologiska kliniken Universitetssjukhuset i Linköping, Hand- och plastikkirurgiska kliniken Universitetssjukhuset i Linköping samt friska försökspersoner inkluderats efter att de lämnat sitt muntliga och skriftliga samtycke. Mätningar har gjorts i särskilt anpassad forskningslokal på Hand- och plastikkirurgiska kliniken (Studie I-II), vid kliniska forskningsenheten Universitetssjukhuset Linköping (Studie III) och i samband med besök på Hand-och plastikkirurgiska kliniken för bröstrekonstruktion (Studie IV).

I studie I undersöcktes om Methyl Nicotinate (MN), vilket kommer från Vitamin B3, kan vara en pålitlig metod för att tillfälligt öka hudens genomblödning i de ytliga blodkärlen. På friska försökspersoner kunde vi konstatera att MN är enkelt att använda genom att det smörjs på huden, ger pålitligt svar i form av ökad genomblödning och så pass länge att det fanns ett tidsfönster på 15 minuter att måta effekten. Med en framprövd, optimal koncentration, kunde ingen av deltagandena av ett obehag och rodnaden som uppstod i huden försvar vid en timme.

I studie II studerades hur MN verkar på hudens ytliga kärl. Ökad genomblödning i huden sker i huvudsak genom tre olika verkningssätt. Genom att blockera respektive sätt, med ofarliga ämnen, kunde vi finna dess huvudsakliga verkningssätt.

I studie III följde vi patienter innan, vid sista dosen, samt två och sex månader efter avslutad stråle behandling. Vi mätte blodcirkulation i vila och efter användandet av MN. Blodflödet i det strålade bröstet jämfördes med det friska bröstet. Här kunde vi se att blodflödet återgick till sitt normala flöde både i vila, och efter att ha ökat blodflödet lokalt med MN, sex månader efter stråle behandling.

I Studie IV mätte vi blodcirkulation och syresättning i blodet hos kvinnor som tidigare hade genomgått borttagande av hela brösten och fått efterföljande stråle behandling. Här var det tydligt att efter längre tid (mer än två år) sjunker blodflödet och hos vissa även syresättningen betydligt i huden. Detta kunde bara påvisas med hjälp av MN, men inte i vilande hud.

Den kunskap som framkommit i denna avhandling har lärt oss mer om hur blodförsörjningen påverkas efter stråle behandling hos kvinnor med tidigare bröstcancer. Vår förhopningar är att denna kunskap och det test vi framtagit för att bedöma hudens blodcirkulation ska kunna användas efter stråle behandling, inför återskapande ingrepp, för att identifiera patienter som är i riskzonen för att få problem vid kirurgin och därmed minska antalet komplikationer.
References


41. Halsted WS. The results of operations for the cure of cancer of the breast performed at the Johns Hopkins Hospital from June, 1889, to January, 1894. Annals of surgery 1894;20:497–555.


93. Lee CN, Hultman CS, Sepucha K. Do patients and providers agree about the most important facts and goals for breast reconstruction decisions? Annals of plastic surgery 2010;64(5):563-566.


143. Goldsmith GA, Cordill S. The vasodilating effects of nicotinic acid (relating to metabolic rate and body temperature). American Journal of Medical Sciences 1943;205:204-208.


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Microvascular Function Assessment after Mastectomy and Radiation Therapy in Breast Cancer Patients
From Methodology to Clinical Application

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