

ORIGINAL ARTICLE

Changes in skin microcirculation during radiation therapy for breast cancer

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ABSTRACT

Background: The majority of breast cancer patients who receive radiation treatment are affected by acute radiation-induced skin changes. The assessment of these changes is usually done by subjective methods, which complicates the comparison between different treatments or patient groups. This study investigates the feasibility of new robust methods for monitoring skin microcirculation to objectively assess and quantify acute skin reactions during radiation treatment.

Material and methods: Laser Doppler flowmetry, laser speckle contrast imaging, and polarized light spectroscopy imaging were used to measure radiation-induced changes in microvascular perfusion and red blood cell concentration (RBC) in the skin of 15 patients undergoing adjuvant radiation therapy for breast cancer. Measurements were made before treatment, once a week during treatment, and directly after the last fraction.

Results: In the treated breast, perfusion and RBC concentration were increased after 1–5 fractions (2.66–13.3 Gy) compared to baseline. The largest effects were seen in the areola and the medial area. No changes in perfusion and RBC concentration were seen in the untreated breast. In contrast, Radiation Therapy Oncology Group (RTOG) scores were increased only after 2 weeks of treatment, which demonstrates the potential of the proposed methods for early assessment of skin changes. Also, there was a moderate to good correlation between the perfusion ($r = 0.52$) and RBC concentration ($r = 0.59$) and the RTOG score given a week later.

Conclusion: We conclude that radiation-induced microvascular changes in the skin can be objectively measured using novel camera-based techniques before visual changes in the skin are apparent. Objective measurement of microvascular changes in the skin may be valuable in the comparison of skin reactions between different radiation treatments and possibly in predicting acute skin effects at an earlier stage.

ARTICLE HISTORY

Received 20 December 2016
Revised 14 February 2017
Accepted 18 February 2017

Introduction

Breast cancer is the most common cancer form among women, with more than 1.7 million new cases diagnosed worldwide in 2012 [1]. Postoperative radiotherapy has been shown to reduce the risk of local recurrence and distant metastasis, and to increase the overall survival of breast cancer [2]. The aim of radiotherapy is to deliver a high dose of ionizing radiation to a defined target volume with the smallest possible damage to the surrounding normal tissue. Ionizing radiation produces lesions in the DNA of the cells which subsequently leads to mitotic cell death, cell death via apoptosis or terminal growth arrest senescence [3]. While these are expected effects in tumor cells, unfortunately, the unavoidable irradiation of normal tissues and the cellular changes it introduces lead to acute and late side effects. Acute side effects are caused by damage to cells that divide both frequently and rapidly. This is the case of the skin, because of the rapid proliferation of the basal keratinocytes [4], resulting in acute erythema in 95% of the patients undergoing radiotherapy [5,6].

Acute erythema appears from an increased blood volume due to capillary dilatation in the dermis, which results from an inflammatory response [6,7]. A redness is usually visible between 10 and 14 days after the start of the radiation treatment and continues to increase in severity during treatment [5], with a maximum effect of 1–2 weeks after completed treatment. However, transient erythema may develop already during the first day of treatment [8].

Acute erythema during and after radiotherapy is usually assessed by visual grading scales, such as the Radiation Therapy Oncology Group (RTOG) assessment scale which classifies skin reactions ranging from mild erythema to necrosis [9]. This subjective assessment is prone to interobserver variability which may complicate analysis and interpretation of side effects, for instance in the case of comparison between clinical treatment regimes. Therefore, objective and robust methods to assess acute skin reactions are needed.

Non-invasive, camera-based techniques are increasingly being used to objectively assess changes in skin microcirculation. Currently, clinical applications of these techniques

Table 1. The modified RTOG-scale.

0	1	2	2.5	3	4
No change	Follicular, faint or dull erythema/epilation/dry desquamation/decreased sweating	Tender or bright erythema	Patchy moist desquamation/moderate edema	Confluent, moist desquamation other than skin folds, pitting edema	Ulceration, hemorrhage, necrosis

include objective measurement of erythema in psoriasis [10], or induced by ultraviolet radiation [11], and in the assessment of burn perfusion for prediction of healing time [12]. Such techniques may, therefore, be adapted to monitor changes in skin microcirculation that may be induced by radiation.

This study aimed to measure radiation-induced microvascular changes in the skin using laser Doppler flowmetry, and two camera-based techniques, Laser Speckle Contrast Imaging (LSCI) and polarized light spectroscopy imaging (PLSI) during adjuvant radiation treatment for stage I–II breast cancer after breast-conserving surgery, and to compare these changes with weekly visual scoring using the RTOG scale. The working hypotheses were that it is feasible to objectively and sensitively measure radiation-induced skin changes using these techniques and that the changes are related to the cumulative target dose.

Material and methods

Patients

Between December 2014 and March 2016, women who were scheduled for adjuvant radiation treatment for stage I–II breast cancer after breast-conserving surgery at Linköping University Hospital were approached for participation in the study by their radiation oncologist. Fifteen women, mean (SD) age 61.2 (11.4) years, range 38–75 years, were included.

Patients were treated with three-dimensional conformal radiotherapy (3D-CRT) using a tangential photon irradiation technique. The energy of the photons was generally 6 MV, although 15 MV photons were also used depending on the anatomy of the patients. The prescribed dose to the target was 42.56 Gy in 16 fractions in all cases, according to national guidelines. The treatment was delivered in daily fractions, five fractions per week. Four patients (27%) with hormone receptor-positive breast cancer received tamoxifen. Patients with lymph node metastases received chemotherapy, according to national guidelines. Patients not administered adjuvant chemotherapy began radiotherapy within 9 weeks after surgery.

The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the regional ethics board at Linköping University (Dnr 2014/299-31). All patients were given written information about the study and gave their written informed consent before being included in the study.

Visual assessment

The radiation therapists assessed the skin of each patient once every week and graded any skin reactions using the

RTOG scale [9]. The criteria for the RTOG scale are given in Table 1. Mean RTOG scores were calculated for each patient for week 1, week 2, and week 3. As no skin reactions were graded before the start of the radiation therapy, a score of zero was assumed as baseline.

Laser Doppler flowmetry

In LDF, low-power laser light from a laser diode contained in a small probe impinges on the skin surface and the individual photons migrate through the tissue in a random fashion, the exact statistical pattern of which is determined by the tissue optical properties. Interaction with one or more moving objects such as blood cells shifts the scattered photons in frequency. The backscattered light carries information about both the speed of the red blood cells and their mean concentration, the product of which is defined as perfusion. In this study, a laser Doppler perfusion monitor (Periflux 5000, Perimed AB, Järfälla, Sweden) with a thermostatic laser Doppler probe (Probe 457, Perimed AB, Järfälla, Sweden) was used to measure baseline perfusion and local thermal hyperemia response in the skin. The probe has a fiber separation of 0.25 mm and the measurement volume lies mainly in the dermal skin, with an estimated depth of measurement of 0.5 mm. The bandwidth of the system is 15 kHz. The system was calibrated before the start of the study according to the guidelines of the manufacturer.

Laser speckle contrast imaging

LSCI is a camera-based imaging technique for blood flow measurement [13], in which reflected light from a divergent laser beam that illuminates the skin is analyzed. This reflected light forms a speckle pattern on the camera sensor, the dynamics of which depend on the movement of red blood cells in the skin. The measurement depth is estimated to be 300 µm, corresponding to the dermal layer [14]. LSCI is able to scan a relatively large area of skin (typically 15 cm × 15 cm) in a time frame of a few seconds while at the same time maintaining a high spatial resolution (0.2 mm/pixel at 25 cm distance). The technique has been shown to have a better reproducibility than LDF [15,16], and this has been attributed to the fact that blood flow changes can be assessed in an area instead of in a single point.

In this study, a laser speckle contrast imager (Pericam PSI, Perimed AB, Järfälla, Sweden) was used to measure skin perfusion. The system uses a laser beam with a wavelength of 785 nm to illuminate the skin. The perfusion image is calculated by analyzing the reduction in speckle contrast in a 3 × 3 window surrounding each pixel, which is proportional to the movement and concentration of red blood cells in the skin.

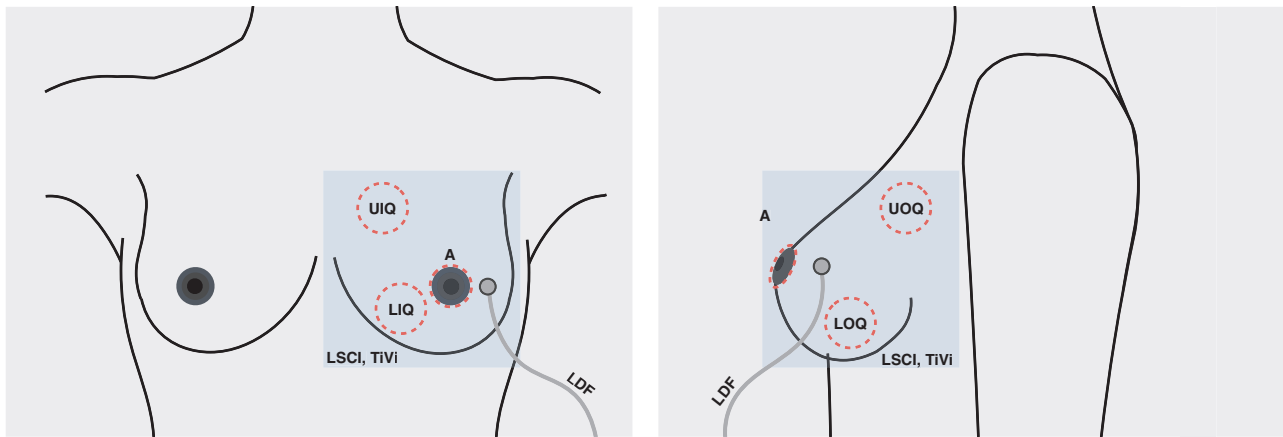


Figure 1. Area of measurement. A laser Doppler probe was placed 2 cm lateral of the areola of the irradiated breast. The image areas used with LSCI and PLSI are marked in blue. Mean perfusion and RBC concentration were calculated in five regions of interest: the areola (A), upper inner quadrant (UIQ), upper outer quadrant (UOQ), and lower outer quadrant (LOQ). Identical images were acquired from the non-irradiated breast.

The image size corresponds to a 12 cm × 12 cm area of skin and the spatial resolution of the perfusion image is 0.2 mm/pixel at a measurement distance of 25 cm.

Polarised light spectroscopy imaging

A polarized light spectroscopy imager (TiVi600, Wheelsbridge AB, Linköping, Sweden), based on a digital camera (EOS 550D, Canon Inc., Tokyo, Japan), was used to measure changes in RBC concentration in the upper dermis. The camera was equipped with a custom-made ring of bright white light emitting diodes and with perpendicular polarizing filters in front of the light source and the lens. The broad-spectrum white light becomes linearly polarized after it has passed the first polarizing filter. Reflected light from the skin consists of both polarized (directly reflected) and randomly polarized (subsurface) light. The polarizing filter in front of the lens prevents any directly reflected light from reaching the photoarray in the camera. The measurement depth of the technique in human skin is estimated to be around 0.5 mm, which corresponds to the dermal layer. The concentration of red blood cells in the skin is estimated by analyzing the pixel values in the different color planes of the RGB images acquired by the camera, using an algorithm that is based on the characteristic absorption spectrum of hemoglobin [17].

Measurement protocol

Baseline measurements were made on the first day of radiation treatment, before irradiation. Measurements were repeated once a week during the radiation treatment, either directly before or directly after irradiation. For practical reasons, measurements were made after a different number of fractions for each patient, but at least once a week. Therefore, measurement time was pooled into the following categories: before treatment, after 1–5 fractions, after 6–10 fractions, after 11–15 fractions, and after treatment (16 fractions). The protocol was the same with each measurement.

During measurements, the patients were lying supine in a temperature-controlled room ($T = 23 \pm 1^\circ\text{C}$).

Measurements were made using LDF, LSCI, and PLSI. The laser Doppler probe was carefully mounted 2 cm lateral of the areola of the irradiated breast using transparent double-sided adhesive. After a 5-min acclimatization period, the probe was heated to 39°C for 20 min to measure endothelium-dependent vasodilatation [18]. Meanwhile, LSCI and 2 PLSI images were acquired of both the irradiated and the non-irradiated breast, one frontal and one lateral (Figure 1). The LSCI-camera was positioned between 20 and 30 cm from the skin, according to the guidelines of the manufacturer. Within this distance interval, there is no significant variation in measured perfusion [19]. Each LSCI image was calculated as the average of 21 consecutive image frames, acquired with a frame rate of 21 frames/s. Thus, the total image acquisition time was 1 s per image. The PLSI camera was held between 15 and 20 cm from the skin, to allow for imaging of the whole breast and simultaneously maximizing the intensity of the illuminating light. PLSI images were acquired using autofocus, at ISO speed 400 and at fixed white balance setting ("shade"). Room lights were dimmed while the images were acquired to prevent interference with the measurement.

Data analysis

Laser Doppler measurements were made at a sample rate of 33 samples per second, and averaged over 1-min intervals using Perisoft for Windows, version 2.5.5 (Perimed AB, Järfälla, Sweden). The mean perfusion and the RBC concentration were measured with LSCI and PLSI, respectively, in five regions of both the irradiated and the non-irradiated breast: the upper inner quadrant (UIQ), lower inner quadrant (LIQ), upper outer quadrant (UOQ), lower outer quadrant (LOQ), and areola (A) (Figure 1). The mean perfusion in these regions was calculated by selecting regions of interest in the perfusion images using the LSCI system's software (PimSoft 1.3, Perimed AB, Järfälla, Sweden). Mean PLSI indices (RBC concentrations) were calculated from PLSI image files in the same manner as above using ImageJ (version 1.49t, US National Institutes of Health, Bethesda, MD). The inner quadrants and areola were measured from the frontal images,

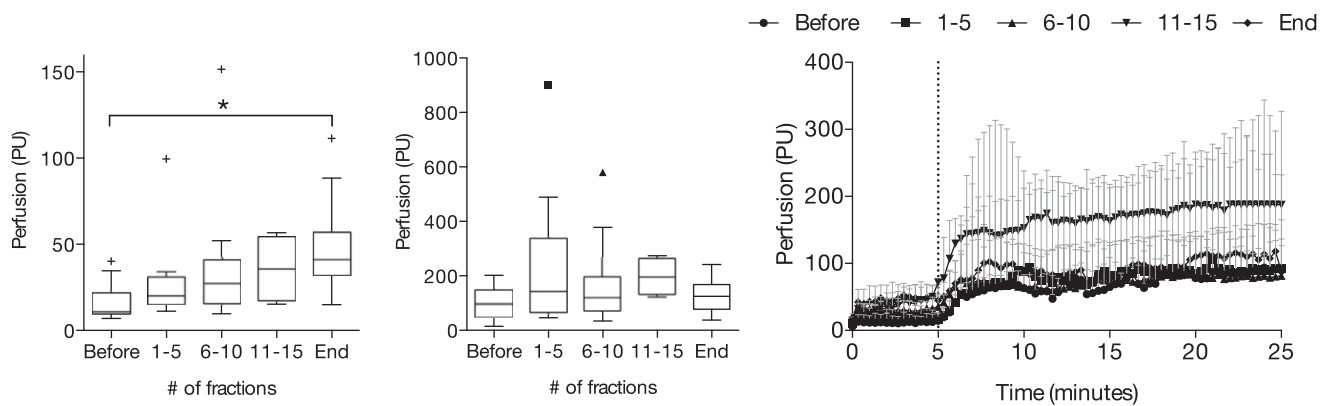


Figure 2. Skin perfusion in the breast before, during and after radiation treatment as measured with laser Doppler flowmetry. (A) Basal skin perfusion, (B) peak hyperemic response after 20 min of local heating to 39 °C, and (C) perfusion time-response curves during local heating. Data in box-and-whisker graphs show with Tukey whiskers. Time response data are plotted as median $\pm$ interquartile range. * indicates $p < 0.05$.

while the outer quadrants were measured from the lateral images.

Statistical analysis

Changes in perfusion as measured using LSCI and RBC concentration over time and difference between skin sites were analyzed using two-way analyses of variance with Dunnett's correction for multiple comparisons. Since perfusion as measured using LDF was not normally distributed, as determined by a d'Agostino & Pearson omnibus normality test, a Kruskal-Wallis test with Dunn's correction for multiple comparisons was used to analyze differences in perfusion over time. Values in text and figures are presented as mean $\pm$ SD (for LSCI and PLSI) or median (interquartile range) for LDF data. Statistical calculations were done using Microsoft Excel 2011 and GraphPad Prism version 6.0h for Mac OS X (GraphPad Software, San Diego, CA, "http://www.graphpad.com"). For all analyses, probabilities of less than 0.05 were accepted as significant.

Results

Visual assessment

Patients developed various degrees of skin reactions. The RTOG score after 1 week of treatment was 0.21 (RTOG 0: $N=11$; RTOG 1: $N=3$). After 2 weeks, it had increased to 0.73 (RTOG 0: $N=5$; RTOG 1: $N=9$; RTOG 2: $N=1$). After 3 weeks, the RTOG score was 1.5 (RTOG 0: $N=0$; RTOG 1: $N=9$; RTOG 2: $N=5$; RTOG 3: $N=1$).

Laser Doppler flowmetry

The median (interquartile range) skin perfusion before treatment was 11 (12) PU (Figure 2). This increased to 21 (16) PU after 1–5 fractions, 28 (25) PU after 6–10 fractions, 36 (37) PU after 11–15 fractions, and 41 (25) PU after the treatment was completed. The increase from baseline was only significant after the treatment was completed ($p < 0.001$).

After heating the skin locally to 39 °C, perfusion increased gradually and reached a plateau during the following 20 min

(Figure 2). The peak hyperemic response before treatment was 121 (98) PU, 145 (269) PU after 1–5 fractions, 121 (124) PU after 6–10 fractions, 196 (132) PU after 11–15 fractions, and 125 (90) PU after the treatment was completed. There were no significant changes in peak hyperemic response during radiation treatment.

Laser speckle contrast imaging

Before the treatment, the highest perfusion in the irradiated breast was measured in the areola (87 ± 24 PU). It increased significantly already between 1 and 5 fractions of the radiation treatment and reached a final value of 182 ± 41 PU, the highest of all regions (Figure 3). Perfusion in the four quadrants of the irradiated breast ranged from 71 ± 15 PU (LOQ) to 80 ± 27 PU (LIQ) before the treatment. During treatment, perfusion increased significantly in UIQ (after 11–15 fractions, $p=0.02$, and after treatment, $p=0.04$) and in LIQ (after 11–15 fractions, $p=0.02$), but did not change in the outer quadrants. Between 6 and 15 treatments, i.e. during the second and third week, a plateau was observed in which the perfusion in the areola was relatively unchanged.

In the non-irradiated breast, the skin perfusion at baseline was similar to the perfusion in the radiated breast, with the areola showing the highest perfusion (84 ± 20 PU before treatment). There was a significant variation in perfusion between different quadrants ($p=0.03$), as a result of the outer quadrants having a higher perfusion than the inner quadrants, but no significant change in perfusion was seen during the treatment in any of the skin regions ($p=0.48$).

Polarized light spectroscopy imaging

Similar to the perfusion, the highest RBC concentration in the irradiated breast was measured in the areola (75 ± 28 AU before treatment). During treatment, it was significantly increased after six fractions of radiation treatment, with a final value of 190 ± 26 AU (Figure 4). RBC concentration in the irradiated breast was lowest in the lower outer quadrant (LOQ: 20 ± 13 AU) and highest in the upper inner quadrant (UIQ: 28 ± 17 AU) before the treatment. During treatment, RBC concentration was significantly increased in UIQ after

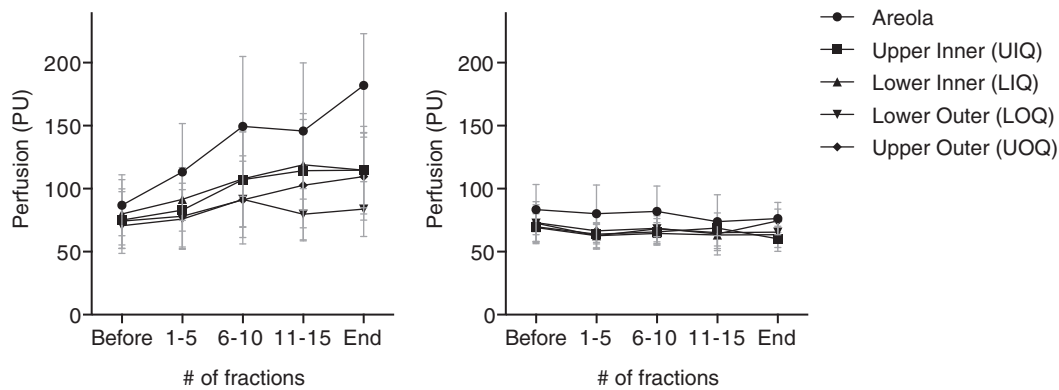


Figure 3. Changes in mean (SD) perfusion, as measured using laser speckle contrast imaging (LSCI) during radiation treatment in different skin regions of the irradiated breast (left) and the non-irradiated breast (right).

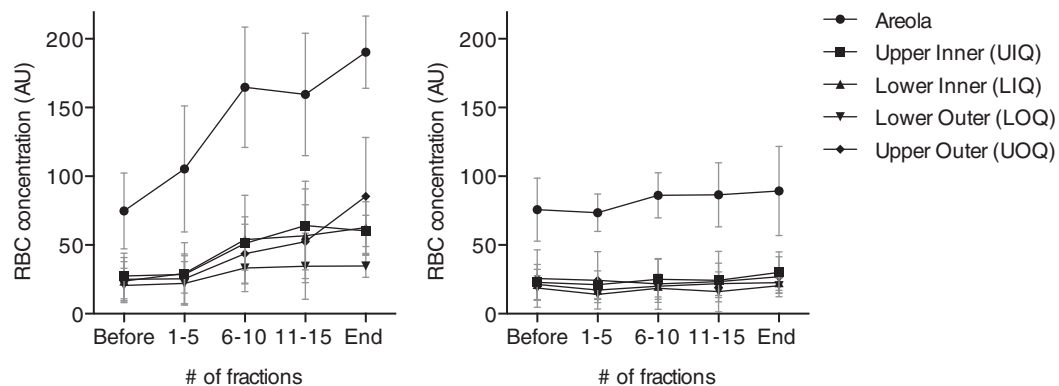


Figure 4. Changes in mean (SD) RBC concentration, as measured using polarized light spectroscopy imaging (PLSI), during radiation treatment in different skin regions of the irradiated breast (left) and the non-irradiated breast (right).

11–15 fractions, $p=0.01$, in LIQ (after 11–15 fractions, $p=0.03$ and after treatment, $p=0.02$), and in the upper outer quadrant (after treatment, $p<0.001$). Although a slight increase in RBC concentration was observed in the lower outer quadrant (LOQ), no significant change from baseline was observed in this quadrant at any time during treatment. Similar to the perfusion, RBC concentration was relatively stable between the 6th and 15th fraction.

The RBC concentration in the non-irradiated breast was similar to the perfusion in the irradiated breast at baseline, with the areola showing the highest perfusion (76 ± 23 PU before treatment). Contrary to the perfusion, RBC concentration did not vary significantly between different quadrants ($p=0.06$) and no significant change in RBC concentration was seen during the treatment in any of the skin regions ($p=0.34$).

Relation between RTOG and microvascular measures

The correlations between the different measures by which skin changes were assessed (RTOG score, perfusion, and relative RBC concentration defined as the RBC concentration in the irradiated breast minus the RBC concentration in the non-irradiated breast) are visualized in Figure 5. There was a moderate correlation between skin perfusion as measured using LDF and visual assessment as measured using the RTOG scale (Spearman $\rho: 0.57$, $p<0.001$). There was also a

moderate correlation between perfusion, as measured with LSCI, and RTOG score (Spearman $\rho=0.56$, $p<0.001$) and between relative RBC concentration and RTOG score (Spearman $\rho=0.55$, $p<0.001$). There was a good correlation between perfusion, as measured with LSCI, and relative RBC concentration ($\rho=0.64$, $p<0.001$).

The correlation between the current week's measurement and the following week's RTOG score is shown in Figure 6. The best predictability for next week's RTOG score was found to be the relative RBC concentration ($\rho=0.59$, $p<0.02$), followed by perfusion, as measured with LSCI ($\rho=0.52$, $p=0.001$), and perfusion, as measured with LDF ($\rho=0.43$, $p=0.01$).

Discussion

When the skin is exposed to radiation, some of the basal cells are damaged. This leads to a disturbance in the balance between production of cells in the basal layer and shedding of cells from the stratum corneum, resulting in a temporary thinning of epidermis [4]. The damage of the basal cells results in an inflammatory response, in which immune cells are activated with subsequent secretion of cytokines, chemokines, histamine, and serotonin [4]. These alterations in the skin may present clinically as erythema, desquamation, and necrosis, but also result in symptoms such as discomfort, itching, and pain [5]. Many of the substances that are

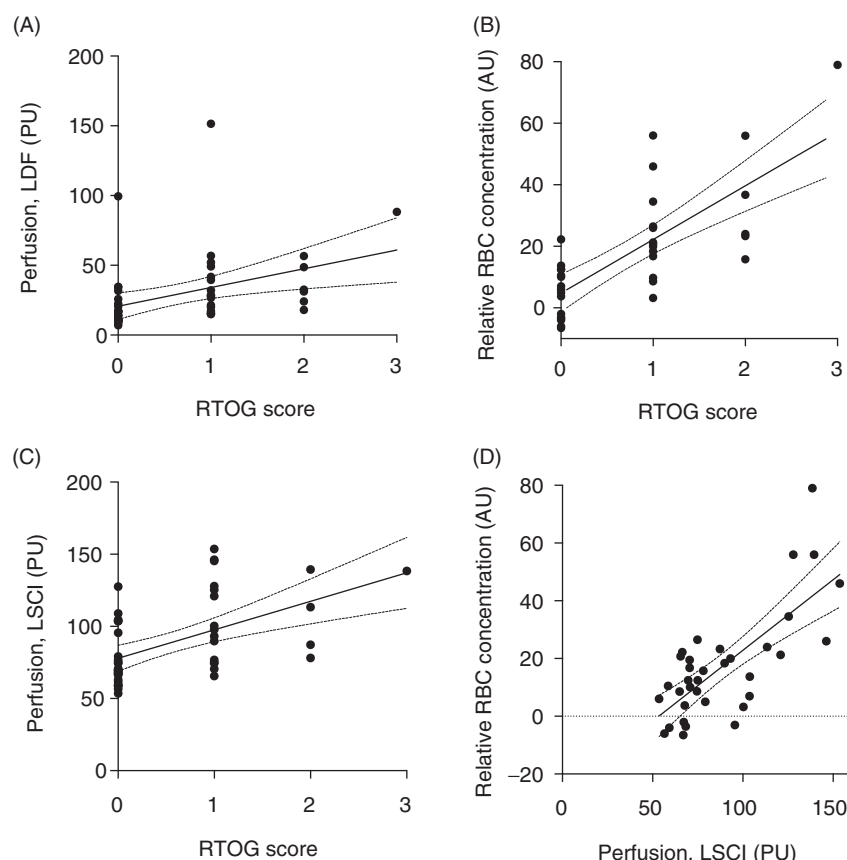


Figure 5. Correlations (and regression lines with 95% CI) between RTOG score and (A) perfusion as measured with LDF, (B) perfusion as measured with LSCI, and (C) relative RBC concentration. (D) shows the correlation between perfusion as measured with LSCI and relative RBC concentration.

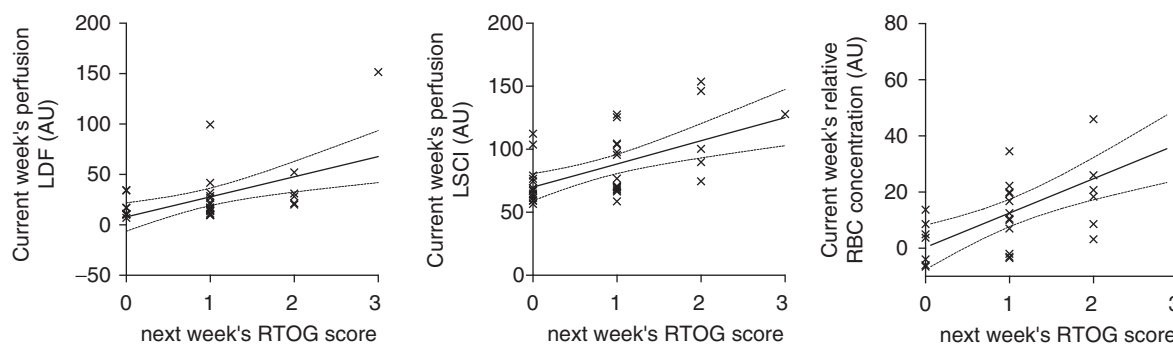


Figure 6. Correlation (and regression lines with 95% CI) between the current week's measurement and the following week's RTOG score. (A) perfusion as measured with LDF, (B) perfusion as measured with LSCI, and (C) relative RBC concentration.

secreted in response to cell damage during radiation therapy have vasoactive properties, and therefore, changes in skin microcirculation may be a good indicator of skin toxicity.

Clinical assessment of skin reactions during radiation therapy is commonly done by visual scoring. The difficulties with this method of assessment include the variations in chromatic acuity of the observers, the variation in ambient lighting and the lack of quantitative means to describe the intensity of the reactions [20]. The subjectivity and temporal delay of visual grading scales to characterize acute skin reactions could interfere with comparisons of reactions between different treatments or patient groups. Furthermore, it has been suggested that the appearance of early complications could modulate the late effects in the same organ [21]. Therefore, robust methods which provide an objective

evaluation of tissue functions, especially at earlier stages during the treatment, should be sought to quickly identify the patients at risk of developing acute reactions. This mirrors the use of functional imaging to early characterize the tumor response before morphological changes become evident [22,23].

After the first week of treatment, we observed an increase in RTOG score in only four out of 17 patients, and those patients were all given a score of 1, while changes in perfusion and RBC concentration were observed in 16 and 15 patients, respectively. This indicates the relatively low sensitivity of visual assessment. It is important to predict and follow up on skin reactions early during radiotherapy to be able to provide adequate treatment and minimize future complications for the patients. The risk for infiltration of pathogens

or bacteria in the skin is highly increased when the skin barrier is damaged, which can lead to bacterial superinfections [4]. Radiation skin injury can also negatively affect the quality of a patient's life because of the pain, discomfort, itching, and burning these reactions can give [6].

In this study, we have used three non-invasive techniques to measure skin alterations during and after radiation therapy. One of the techniques, laser Doppler flowmetry, was used to measure the reaction of the skin microcirculation to local heating. The response of the skin microcirculation to local heating at 39°C can be described as a biphasic reaction. Initially an activation of the cutaneous sensory nerves is seen, also known as the axon reflex, which causes an increase in perfusion during the first 10 min, followed by a decrease in perfusion. Secondly, nitric oxide is released generating a smooth muscle vascular relaxation and increase of the local microvascular blood flow, which is dependent on endothelial function. A plateau of the response is reached after 20–30 min [18]. The laser Doppler probe measures perfusion in one spot, and is known to be affected by the large heterogeneity of the dermal microvasculature [24]. The results of this study indeed showed extreme variations in skin perfusion during treatment, with maximum hyperemic responses after local heating ranging between 34 and 900 PU. This variation was larger than we have typically have observed in studies in healthy individuals. This may be related to the structural changes in the dermal microcirculation caused by radiation injury. It is probably because of this variability, and the fact that perfusion measurements are limited to a single skin site, that a significant increase was only observed at the end of the treatment.

This is the first study in which two novel camera-based techniques, LSCI and PLSI, are used to objectively assess radiation-induced changes in the skin microcirculation. In the majority of patients, microvascular changes already occurred during the first week of fractionated radiotherapy with breast cancer, before they were visible by eye. The strongest effects were seen in the areola, where the increase in perfusion and RBC concentration after 1–5 fractions was significant at the group level (only one patient showed a decreased perfusion). In the other parts of the breast, increases in perfusion and RBC concentration were less consistent. In contrast, RTOG scores after one week were 0 (13 patients) or 1 (three patients). In many cases, RTOG scores of 0 were given while perfusion had measurably increased. By the end of the treatment, all patients had increased perfusion in the areola and in the inner quadrants of the treated breast, while RBC concentration was increased in all quadrants by the end of the treatment.

The relative sensitivity of the microvascular measures compared to the RTOG score is underlined by the fact that the perfusion and RBC concentration were correlated to next week's RTOG values. This, together with the fact that changes in microvascular measures often were not paralleled by an increased RTOG score, indicates that the techniques used in this study may be used to earlier predict skin toxicity. In this regard, RBC concentration turned out to be the best predictor, which is to be expected as it is related the of degree

erythema, which is one of the aspects (although not the only one) that is assessed by the RTOG score.

While correlations with RTOG were similar for LSCI and PLSI, the latter technique is from a practical perspective a more convenient technique to use for assessing skin toxicity. Because it is based on a digital single-lens reflex (D-SLR) camera, it is portable and easy-to-use. It has the additional advantage of providing a high-quality photograph of the irradiated skin area, which can be used to document visual skin assessment, and which, beside the measure of RBC concentration, may be used to more objectively grade other skin alterations. These may include patchiness, the degree of desquamation, edema, ulceration, hemorrhage, and necrosis, all of which are properties that are included in the RTOG assessment. This is valuable since severe skin damage may confound the measurement of RBC concentration due to the changes in skin optical properties [7]. In the future, techniques based on digital imaging may be further developed to utilize mobile technology and automatic analysis and transfer of images to the hospital's image archive.

In previous studies, several different measurement techniques have been used to assess skin alterations during radiation therapy. Reflectance spectrophotometry measures skin color, and the reflectance values have been shown to correlate with the RTOG score [25–28]. Ultrasonic imaging has been proposed for evaluation of post-radiation changes in epidermal thickness [29,30] and dielectric measurements have also been found to correlate to clinical erythema scores [31]. Near-infrared (NIR) spectroscopy, laser Doppler perfusion imaging, and digital photography have all been found to provide measures of erythema that correlated with the radiation dose to various degrees and it has been suggested that digital photography provides the most promising technique, because it is easy to use and could detect changes as soon as after a treatment dose of 8 Gy [7]. This is in agreement with the results of this study.

Microvascular measurements for prediction of clinical outcome are already used today in burn assessment, where skin perfusion at 3–5 d after the injury accurately predicts the healing time of the wound. It can thus be used as a diagnostic tool to decide treatment strategy. While routine measurements of skin alterations by LDF are probably too time-consuming in a typical radiation therapy department, LSCI and PLSI may yield a faster and more accurate and complete assessment than visual scoring, provided that the infrastructure for image analysis, distribution, and storage is put in place. Both techniques are easy-to use and do not require much training. The interobserver variability is low [32,33]. Future studies may focus on the usability aspects of objective techniques for measurement of skin reactions during and after radiation therapy. Also, radiation-induced skin reactions at other skin regions may be assessed in future studies, including the head and neck region.

This study has several limitations. The number of patients included was relatively small and patients were limited to those who had received breast-conserving surgery. While skin reactions were seen in the majority of patients, an RTOG score of 3 was only observed in one patient at the end of the treatment. Future studies should therefore have a larger

number of patients, and could even include other patient groups such as mastectomy patients, and patients undergoing different fractionation schemes, including boost doses.

The actual dose absorbed in the skin was not determined. Instead, all changes were related to the cumulative number of fractions and thus the cumulative target dose. The radiation dose to the skin is dependent on many factors including the beam quality, the incident beam angle, and field size. As a result of the lack of charged particle equilibrium, the dose is difficult to calculate by planning systems and the dose gradient is steep. Further studies may measure the dose to different skin areas in individual patients and aim to correlate this to the microvascular changes observed during the treatment.

The visual assessment using the RTOG score was not always done on the same day as the microvascular measurements, which may cause a slight temporal mismatch in the data. However, the difference between visual and microvascular measurements was never more than 3 d.

In conclusion, the results in this study suggest that measurement of microvascular changes, including changes in perfusion and RBC concentrations in the skin may make it possible to objectively assess skin reactions and may help predict severe reactions at an earlier stage.

Acknowledgments

The authors thank Johanna Stark, Felicia André, and the nursing staff at the radiotherapy department at Linköping University Hospital for their assistance with the measurements.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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