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2017-05-18

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Original Research Article

# Association between cumulative radiation dose, adverse skin reactions, and changes in surface hemoglobin among women undergoing breast conserving therapy

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ABSTRACT

*Introduction:* Radiation therapy is crucial to effective cancer treatment. Modern treatment strategies have reduced possible skin injury, but few clinical studies have addressed the dose relationship between radiation exposure and skin reaction. This prospective clinical study analyzes skin oxygenation/perfusion in patients undergoing fractionated breast conserving therapy via hyperspectral imaging (HSI).
*Methods:* Forty-three women undergoing breast conserving therapy were enrolled in this study. Optically stimulated luminescent dosimeters (OSLDs) measured radiation exposure in four sites: treatment breast, lumpectomy scar, medial tattoo and the control breast. The oxygenation/perfusion states of these sites were prospectively imaged before and after each treatment fraction with HSI. Visual skin reactions were classified according to the RTOG system.
*Results:* 2753 observations were obtained and indicated a dose-response relationship between radiation exposure and oxygenated hemoglobin (OxyHb) after a 600 cGy cumulative dose threshold. There was a relatively weak association between DeoxyHb and radiation exposure. Results suggest strong correlations between changes in mean OxyHb and skin reaction as well as between radiation exposure and changes in skin reaction.
*Conclusion:* HSI demonstrates promise in the assessment of skin dose as well as an objective measure of skin reaction. The ability to easily identify adverse skin reactions and to modify the treatment plan may circumvent the need for detrimental treatment breaks.

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## Introduction

Radiation therapy remains a critically important and valuable component for oncologic patient care. Modern equipment and refined treatment algorithms have improved normal tissue effects from treatment particularly in breast. However, the use of increased radiation dose to tumor targets and compressed radiation treatment fractionation schedules still lead to significant ischemic effects of radiation therapy on dermal circulation and

subcutaneous tissue in other parts of the body. It is still unclear whether or not there is a dose–response relationship between radiation to both skin oxygenation and adverse skin reactions. Furthermore, from a surgical perspective, since reconstruction often requires operating within irradiated tissue, better understanding the underlying changes in skin microcirculation is paramount.

Encouraging earlier clinical studies demonstrated that laser Doppler flowmetry (LDF) could be used to study acute changes in cutaneous microvasculature [1,2], but LDF is based on the capillary flow velocity and may not be reflective of physiological tissue oxygenation. Our lab has focused on hyperspectral imaging (HSI) for evaluation of skin oxygenation and perfusion after a variety of injuries. HSI derived oxygenation has been shown to correlate with transcutaneous $PO_2$ measurements as well as intravascular volume

[3–5]. We have utilized a commercially produced HSI device, which provides near real-time analysis of oxygenated and deoxygenated hemoglobin (OxyHb and DeoxyHb, respectively) content in skin. In previous animal studies, we successfully demonstrated that this technology reliably detects and assesses skin changes after exposure to both ionizing and thermal radiation [6–8]. Our translational studies on the acute phase suggest that cutaneous changes in oxygenation may be dose related and that this may be a potential surrogate marker for radiation exposure [9]. Subsequent studies by other investigators have used similar spectroscopic techniques and validated dose related oxygenation changes [10,11]. However, these previous studies have all been based on single fraction exposures of radiation and therefore have limited external validity to clinical fractionated radiation schedules.

In this prospective clinical study, we utilize fractionated dose therapy in breast cancer patients undergoing breast conserving therapy (BCT) radiation as a model for radiation exposure. By measuring their received dose at the skin level and assessing their skin oxygenation at those sites using our previous HSI methodology, we evaluate any correlation between dose and skin oxygenation level in the acute treatment phase. We designed this study around breast cancer patients since their standardized treatment maps provide consistent sites for comparison. Based on our previous studies, we hypothesize that breast skin OxyHb level will rise during the period of irradiation.

## Methods

Under IRB approved protocol, all patients ($n = 43$) were recruited from the Radiation Oncology Clinic at the University Campus of UMass Memorial Healthcare Center. Women aged 18–85 who were planning on having breast radiation as part of breast conserving therapy (i.e. lumpectomy plus radiation) and were able to provide informed consent were invited to participate in the study. However, patients with previous breast irradiation in situations where radiation therapy prescription doses may be altered (excluding boost dosage), inflammatory skin diseases (e.g. psoriasis, eczema) over the breast area to be irradiated, and collagen vascular disease and/or other systemic vasculitides were excluded from the study. Additionally, patients who were unable to sit or lie down comfortably for 20 s for image acquisition were excluded.

Clinical information including patient age, BMI, smoking status, significant past medical history, and history of chemotherapy were recorded. All patients were enrolled and consented to the study either by their radiation oncologist or the study coordinator.

### Radiation measurement

Breast irradiation for breast conserving therapy involved two opposing X-ray beams from a megavoltage medical linear accelerator. Radiation dose measurements at the skin surface were assessed by use of optically stimulated luminescent dosimeters (OSLD). Calibrated OSLDs were placed on each patient's chest in four areas during the initial treatment fraction. Three measured areas were in the irradiated field: middle of treatment breast (TB), lumpectomy scar site (SCAR), and medial tattoo (MT). TB and SCAR sites were selected since they were always within the irradiated field. MT was chosen as a readily identifiable site in each patient that could demonstrate a dose gradient as it is offset from the target site. One OSLD was placed on the nipple of the untreated breast as control (CONTROL). (Fig. 1) All subsequent fractions were assumed to have the same skin dose, unless a boost was noted. In this case, an additional OSLD was placed and measured that session's additional doses. Each OSLD was developed and read 24 h after radiation exposure. Cumulative dose measurements were calculated for each treatment site as number of treatment fractions times OSLD measurement, with additions accounting for any boost.

### Hyperspectral imaging

Our group has previously established the use of monitoring of irradiated skin using HSI [8,9]. A commercial FDA-approved hyperspectral device (OxyVu-2™ (Hypermed, Inc., Waltham, MA)) was used to obtain images at each OSLD measurement site (TB, SCAR, MT and CONTROL) at baseline before therapy as well as before and after each treatment fraction. The OxyVu-2™ analyzes the skin content of oxygenated and deoxygenated hgb at a depth of 2 mm, which corresponds anatomically to the subdermal plexus. The system was calibrated to a reference card for all acquisitions, and pixel reflectance was determined relative to this standard reflectance. A target dot was used to correct for motion artifact from respiratory effort. Patients were asked to position themselves prone on stretcher during the imaging. Spatial maps of tissue oxygenation were then generated using the device's proprietary algorithms. The general optical properties of this device have been previously described [12]. A narrow band-pass, liquid–crystal tunable filter (LCTF-10-20, CRI, Inc. Hopkinton, Massachusetts) was used to vary the wavelength of light passed on to a digital imaging detector (Guppy F-146B, Allied Vision Technologies, Stadtroda, Germany) to provide many images at 15 select wavelengths between 500 and 660 nm. Broadband light-emitting diodes were used to illuminate the sample (LUXEON, Philips Lumiled, Inc. San Jose, California). Twenty-second scans of tissue samples were obtained at approximately a 17-inch focal distance. Color images were created from the scans to demonstrate tissue oxygenation spatially. The spatial resolution of the OxyHb and DeoxyHb images was 60 μm.

### Skin reaction assessment

At each HSI acquisition, a concurrent digital photograph was obtained of each measured site. These photographs were graded by two independent technicians using the RTOG skin toxicity scale on a categorical scale of 0 through 4 (0 = no reaction, 1 = slight erythema, 2 = bright erythema or patchy desquamation, 3 = confluent desquamation, or 4 = ulceration) [13]. The average grading score between the two technicians was used for analysis. Due to a computer hard drive failure there were 10 women with lost data for at least one skin reading; in this case the single technician's reading was used in lieu of a mean.

## Statistical methods

We examined the unadjusted dose-OxyHb association by computing mean change in OxyHb in each successive cumulative dose interval, in 200 cGy dose increments. We computed the linear slope as cumulative dose increased (adjusted for woman), to help assess the general shape of association, and to identify at what cumulative dose the association becomes detectable.

We used a mixed model, in which individual woman was treated as a random effect, to examine the association between cumulative radiation dose and change from baseline OxyHb or DeoxyHb at skin surface. Cumulative radiation dose was the cumulative sum of each day's dose, including any boost doses. Changes in hemoglobin were differences between measurements immediately after a daily dose, and baseline hemoglobin before treatment, where each woman had a different baseline at each location (TB, SCAR, MT, and CONTROL, where CONTROL received minimal or insignificant radiation).
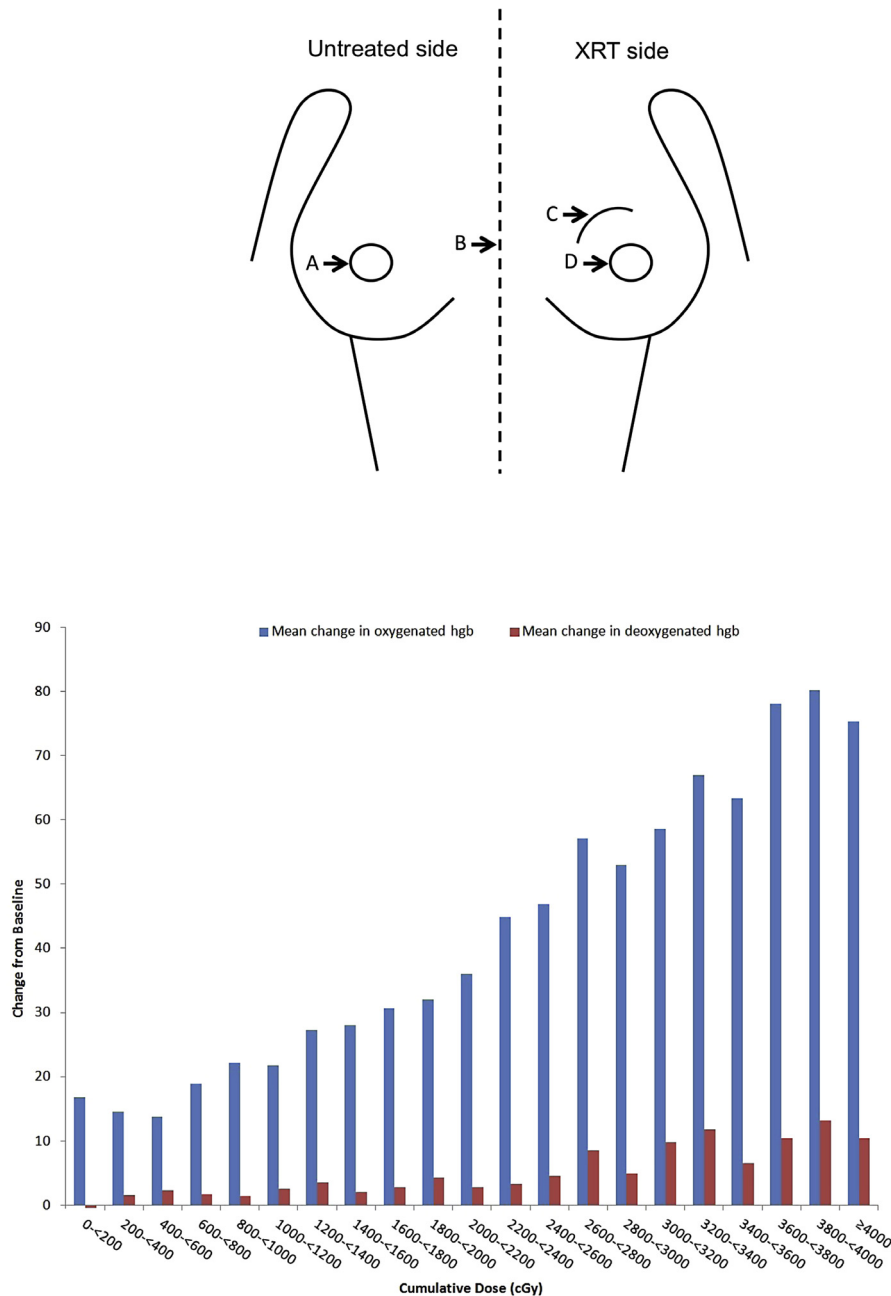
**Fig. 1.** (Above) Schematic of CONTROL (A), MT (B), SCAR (C) and TB (D) sites. Arrows indicate OSLD placement. (Below) Mean unit change since baseline in oxygenated and deoxygenated hemoglobin by cumulative radiation interval ($n = 1770$ observations at scar and treatment breast).

The method of fractional polynomials was used to examine possible non-linearity between dose and hemoglobin change [14]. Associations were assessed independently for each radiation location. We tested for possible modifications to the dose-hemoglobin associations by adding the following covariates to our final models, one at a time: age, body mass index, Fitzpatrick score, and history of chemotherapy, smoking, hypertension and type II diabetes. Because the effect of cumulative dose might differ depending on how rapidly it was attained, we included day since baseline in final models, to help control for time (the dose-hemoglobin estimate may then be interpreted as being at a random day since baseline).

Differences in dose-hemoglobin associations at the 3 main locations (TB, SCAR, and MT), were evaluated by comparing model likelihoods for models with additional terms for location-specific estimates to models without the additional terms. To obtain a sense of how much of the variation in OxyHb is due to cumulative dose plus the individual woman, we computed coefficients of correlation ($R^2$) for final adjusted models by ordinary least squares regression, treating individual woman as a fixed effect.

A two-sided $\alpha$-level of 0.05 was used to determine statistical significance and report confidence intervals. All analyses were conducted using the SAS software package, version 9.3 (SAS Institute). Categorical variables are shown as frequencies with proportions; continuous variables are shown as means with minimum, maximum, and standard deviation.

## Results

Our study sample consisted of 43 women of mean age 58 years (range 42–82), mean BMI 29 kg/m$^2$(range 20–46), mean Fitzpatrick scale 2.6 units (range 1–5), 33% had a past history of chemother-

apy, 42% had past or current smoking, 28% were hypertensive, and 4.7% had type 2 diabetes (Table 1).

*Relationship between radiation dose and change in hemoglobin*

Forty-three women having 2753 observations at TB, SCAR, and MT were included in analyses. Data from the control breast were ignored, as radiation and changes in hemoglobin were minimal there. Mean treatment time since baseline was approximately 16 days, with most treatments extending to approximately 31 days with a maximum of 36 days.

For OxyHb response to dose changes, we analyzed 41 women with data at MT, 36 at SCAR, and 43 at TB (Table 2). Eight observations with a total dose >5000 cGy were excluded, since there was not enough information at such high doses to reach firm conclusions. Observations below a dose level of 2 cGy for OxyHb were also excluded, as the noise from the hyperspectral measurements becomes dominant. The mean dose at scar and TB was approximately 2000 cGy, with maxima 4942 cGy and 4547 cGy respectively. The mean dose at MT was approximately 1000 cGy with a maximum of 3366 cGy. Mean changes in OxyHb since baseline were larger and similar at SCAR and TB than at MT, and uniformly larger than mean changes in DeoxyHb.

Unadjusted mean changes in OxyHb and DeoxyHb at successive cumulative radiation dose intervals up to 4942 cGy, for 36 women with a total of 1770 observations at SCAR ($n = 868$) and TB ($n = 902$), appear in Table 3. For this analysis, we omitted 7 women who had TB but no SCAR data, and MT because of its narrower dose range. Linear slopes were estimated (adjusted for random woman) between change in cumulative dose and change in hemoglobin,

where each interval extends from 0 cGy to the interval endpoint (eg, for the interval 200–<400 cGy, the slope estimate includes the 138 observations between 0 and <400 cGy). The cumulative linear slope estimate becomes quite statistically significant ($p < 0.0001$) between 600 and 800 cGy cumulative dose for OxyHb, and between 2600 and 2800 cGy for DeoxyHb. The linear association appears relatively weak for DeoxyHb, but strong and increasing with higher dose for OxyHb to a maximum slope of 1.8 hgb units per 100 cGy.

A plot of mean change in hemoglobin per cumulative radiation interval to be linear for OxyHb; a lesser, possibly linear association is seen for DeoxyHb (Fig. 1). We did not analyze DeoxyHb further because of this relatively weak association.

*Estimating a model based on SCAR and TB locations*

The mixed model was used to examine the association between cumulative radiation dose and change in OxyHb for the 36 women with both SCAR and TB data, since the range of cumulative dose was similar at each location.

The final model was of the form:

Change in oxygenated hgb since baseline = cum dose/100 cGy + (cum dose/100 cGy)$^{0.5}$,
where cum dose is the cumulative radiation dose in cGy.

The first 2 terms were derived from the method of fractional polynomials ($p < 0.0001$ vs linear cum dose). No other covariates (see Statistical Methods) were statistically significant in the above model, except for day of measurement ($p = 0.0003$). Models for SCAR and TB had the same functional form (as above), and location was not statistically significant ($p = 0.09$). Since SCAR and TB had the same model form, we have 2 independent pieces of evidence for the shape of association up to cumulative dose <5000 cGy.

In ordinary least squares regression, the $R^2$ (% variation in OxyHb explained by a covariate(s)) for a model containing cumulative dose as specified above (and no other covariates) was 41%; 66% after adding woman as a fixed effect. For cumulative dose modeled as a single linear term, $R^2$ were 40% and 65%, respectively. Comparable $R^2$ for linear DeoxyHb were 6% (cumulative dose alone) and 37% (adding patient).

*Comparison of MT, SCAR, and TB locations using the final model*

We refit mixed models for the 29 women who had information at MT (448 observations), SCAR (445 observations), and TB (443 observations), to see if MT, with its lower cumulative dose, had a similar association between OxyHb and dose as SCAR and TB; and to compare the association found in the previous section for SCAR and TB at their full dose range (Fig. 2) to the association at a more restricted dose range, 383–2686 cGy. This range represents the 5th percentile for SCAR and 95th percentile for MT dose, respectively, ensuring a good degree of dose overlap at all 3 locations.

Best-fitting models were:

MT change in OxyHb = (cum dose/100 cGy)$^{-0.5}$ + ln(cum dose/100 cGy).
SCAR and TB change in OxyHb = (cum dose/100 cGy)$^{-2}$ + (cum dose/100 cGy)$^2$.

These models were statistically significantly better than a linear cumulative dose association ($p = 0.0005$ for MT, $p = 0.004$ for SCAR + TB vs linear). SCAR and TB had similar non-linear individual associations, although not of the same exact form; the form shown above is for the 2 locations combined. When other covariates were

**Table 1**
Demographics and medical history ($n = 43$ women).

|  | n (%) |
| --- | --- |
| Lumpectomy | 42 (100) |
| Prior Chemotherapy | 14 (33) |
| *Dose Prescribed (cGy)* | |
| 4256 | 5 (12) |
| 4500 | 4 (9.3) |
| 4600 | 5 (12) |
| 4680 | 3 (7.0) |
| 5000 | 2 (4.7) |
| 5040 | 23 (53) |
| 5220 | 1 (2.3) |
| *Number of treatment Fractions* | |
| 16 | 5 (12) |
| 20 | 1 (2.3) |
| 23 | 4 (9.3) |
| 25 | 4 (9.3) |
| 26 | 3 (7.0) |
| 28 | 25 (58) |
| 29 | 1 (2.3) |
| *Fitzpatrick Skin Score* | |
| 1 | 2 (4.7) |
| 2 | 18 (42) |
| 3 | 17 (40) |
| 4 | 5 (12) |
| 5 | 1 (2.3) |
| *BMI* | |
| <25 | 13 (32) |
| 25.0–29.9 | 14 (34) |
| 30+ | 14 (34) |
| *Comorbidities* | |
| Diabetes | 2 (4.7) |
| History of smoking | 18 (42) |
| Vascular disease | 12 (28) |
| Anemia | 6 (14) |
| Hypertension | 12 (28) |

**Table 2**
Summary of cumulative radiation dose and changes in hemoglobin (hgb), by treatment location.

| Location | # of observations | Minimum | Maximum | Median | Mean |
|---|---|---|---|---|---|
| *Medial tattoo (41 women):* | | | | | |
| Cumulative dose, cGy | 828 | 23 | 3366 | 798 | 1013 |
| Change in oxygenated hgb, units | 828 | 2 | 141 | 16 | 25 |
| Change in deoxygenated hgb, units | 828 | −26 | 316 | 2 | 3.3 |
| *Scar (36 women):* | | | | | |
| Cumulative dose, cGy | 868 | 125 | 4942 | 2044 | 2073 |
| Change in oxygenated hgb, units | 868 | 2 | 158 | 32 | 40 |
| Change in deoxygenated hgb, units | 868 | −36 | 124 | 5 | 7.0 |
| *Treatment breast (43 women):* | | | | | |
| Cumulative dose, cGy | 1057 | 8.6 | 4547 | 1945 | 1967 |
| Change in oxygenated hgb, units | 1057 | 2 | 176 | 32 | 40 |
| Change in deoxygenated hgb, units | 1057 | −51 | 78 | 3 | 2.6 |

**Table 3**
Unadjusted mean changes since baseline in oxygenated and deoxygenated surface blood hemoglobin, at increasing cumulative radiation dose interval, and cumulative linear slopes (36 women with 1770 observations at scar and treatment breast).

| N | Median since baseline | Days Cum dose | Mean Oxy hgb | Cum linear slope* (*p*-value) | Mean Deoxy hgb | Cum linear slope* (p) |
|---|---|---|---|---|---|---|
| 53 | 7 days | 0–<200 cGy | 16.8 units | 1.31 units (.67) | −.43 units | 2.54 units (.20) |
| 85 | 2 | 200–<400 | 14.5 | 1.31 (.20) | 1.5 | 1.33 (.04) |
| 82 | 4 | 400–<600 | 13.8 | 0.64 (.19) | 2.3 | 0.59 (.11) |
| 81 | 5 | 600–<800 | 18.9 | 1.17 (**) | 1.7 | 0.39 (.08) |
| 92 | 7 | 800–<1000 | 22.2 | 1.50 (**) | 1.4 | 0.15 (.33) |
| 99 | 9 | 1000–<1200 | 21.8 | 1.26 (**) | 2.5 | 0.22 (.05) |
| 95 | 10 | 1200≤1400 | 27.3 | 1.40 (**) | 3.6 | 0.23 (.01) |
| 100 | 12 | 1400–<1600 | 28.0 | 1.35 (**) | 2.1 | 0.17 (.02) |
| 98 | 13 | 1600–<1800 | 30.6 | 1.32 (**) | 2.8 | 0.14 (.02) |
| 99 | 15 | 1800–<2000 | 32.0 | 1.29 (**) | 4.3 | 0.20 (.0002) |
| 102 | 16 | 2000–<2200 | 36.0 | 1.32 (**) | 2.8 | 0.17 (.0003) |
| 93 | 18 | 2200–<2400 | 44.9 | 1.43 (**) | 3.3 | 0.14 (.0003) |
| 90 | 19 | 2400–<2600 | 46.9 | 1.50 (**) | 4.5 | 0.13 (.0003) |
| 98 | 21 | 2600–<2800 | 57.1 | 1.60 (**) | 8.5 | 0.18 (**) |
| 89 | 22 | 2800–<3000 | 53.0 | 1.61 (**) | 4.9 | 0.17 (**) |
| 88 | 24 | 3000–<3200 | 58.6 | 1.65 (**) | 9.8 | 0.20 (**) |
| 91 | 26 | 3200–<3400 | 66.9 | 1.74 (**) | 11.8 | 0.26 (**) |
| 73 | 26 | 3400–<3600 | 63.3 | 1.75 (**) | 6.6 | 0.24 (**) |
| 64 | 28 | 3600–<3800 | 78.0 | 1.80 (**) | 10.4 | 0.25 (**) |
| 40 | 29 | 3800–<4000 | 80.2 | 1.84 (**) | 13.2 | 0.26 (**) |
| 58 | 31 | 4000–4942 | 75.3 | 1.84 (**) | 10.4 | 0.26 (**) |

N – Number of observations in interval (combines observations from different women).
Cum – cumulative.
Oxy hgb – change in oxygenated hemoglobin, since baseline.
Deoxy hgb – change in deoxygenated hemoglobin, since baseline.
*Increase/decrease (if negative) in oxy or deoxy hgb units per 100 cGy increase over the cumulative dose interval which begins at 0 and ends at the stated Cum dose interval (ie, 0 < 200 cGy ($n$ = 53), 0–<400 cGy ($n$ = 138), ..., 0–4942 cGy ($n$ = 1770)), controlling for woman as a random effect.
**$p < 0.0001$.

added, they were not statistically significant, except for day of measurement ($p = 0.03$ for MT and $p = 0.03$ for SCAR plus TB).

Fig. 2 shows the general shape of association between predicted OxyHb and cumulative radiation dose at MT, and at SCAR and TB combined, evaluated at 16 days after baseline. As with the broader dose range for SCAR and TB (2–4942 cGy), the association is mainly linear (700–2700 cGy), with some upward curvature for SCAR and TB. Between 400 and 700 cGy the MT association is negative, the SCAR plus TB association positive.

*Comparison of skin reactions to dose and hemoglobin changes*

Skin reactions during treatment from 2531 scans of 42 women were evaluated by two independent study technicians. Due to lost data, however, there were 29 scans with only one skin reaction score recorded. Among this subset of 2502, complete agreement in assessed skin reaction category was 68% between the 2 technicians (radiation therapists); 0.6% differed by >1 category. Including all scans for which there was at least one reading recorded there

were a total of 2531 readings: 27% were 0 to <1, 61% were 1 to <2, 11% were 2 to <3, and 1% were 3 or higher. There was a strong correlation between cumulative radiation dose and severity of skin reaction at each of the measurement sites (Fig. 3). SCAR and TB sites have similar mean cumulative doses for a given skin reaction, with overlapping confidence intervals, while MT sites have smaller mean doses for a given skin reaction.

Additionally, there was a strong correlation between change in mean OxyHb and skin reaction (Fig. 3). Mean increases are seen from skin reactions Null to 1 to 2, with a smaller increase from 2 to 3 and overlapping confidence intervals. As with cumulative dose, mean change in OxyHb was similar at SCAR and TB, for a given skin reaction.

## Discussion

Despite the longstanding use of radiation therapy, there have not been any definitive clinical studies which demonstrate the relationships between skin dose, oxygenation, and adverse skin
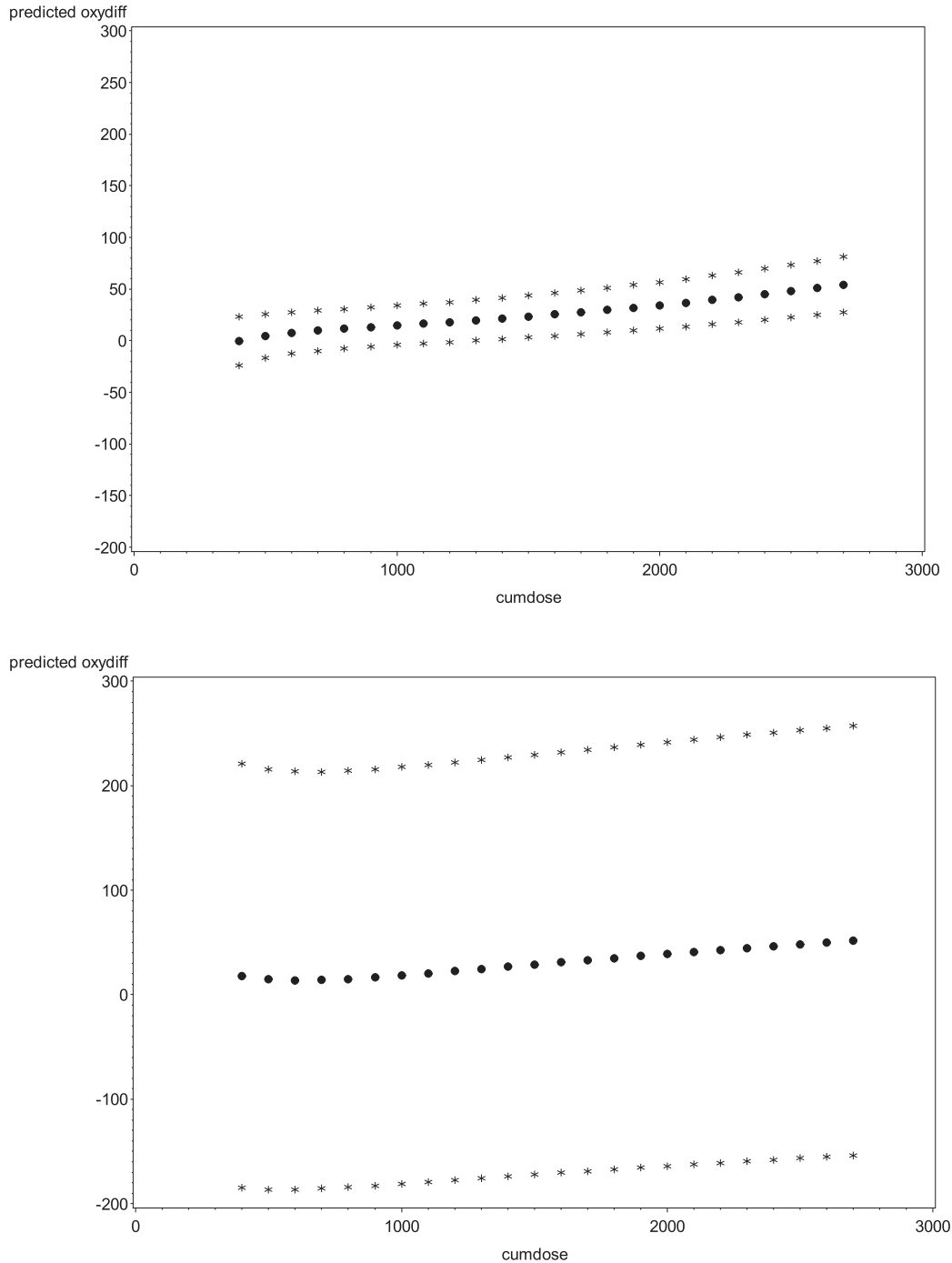
**Fig. 2.** Model-predicted change since baseline in oxygenated hemoglobin at combined scar and treatment breast (above), and medial tattoo locations (below), by cumulative radiation dose (cGy). Model includes 29 women, 1336 observations, (asterisks indicate 95% CI).

reactions [2]. Our current clinical results suggest relationships between all three in the setting of acute radiation exposure.

*Near linear relationship of OxyHb change and dose*

Our previous animal study demonstrated a strong indication for a dose-dependent phenomenon. We had found that hemoglobin had a dose-related change after increasing single fraction exposure (500–5000 cGy) of beta-radiation [9]. Indeed, our current fractionated irradiation findings are consistent with other animal studies demonstrating a dose-dependent effect on the microvasculature [15–17]. Interestingly, it appears from this clinical data that there

is a threshold dose (around 600–800 cGy) at which OxyHb's relationship to skin dose becomes statistically significant.

Because this clinical study measured the precise skin dose received using OSLD's, we had the ability to carefully correlate dose with skin hemoglobin changes. Between all the sites measured (SCAR, TB, and MT), there appeared to be largely linear associations between cumulative dose and oxygenated hemoglobin change. We demonstrated that between 2 and 5000 cGy, 40% of the change in oxygenated hemoglobin was accounted for by dose. Although we tested for other factors such as age, smoking, BMI and chemotherapy, none helped explain the dose effect; much of the remainder of variability may be accounted for by inter-patient differences, as it
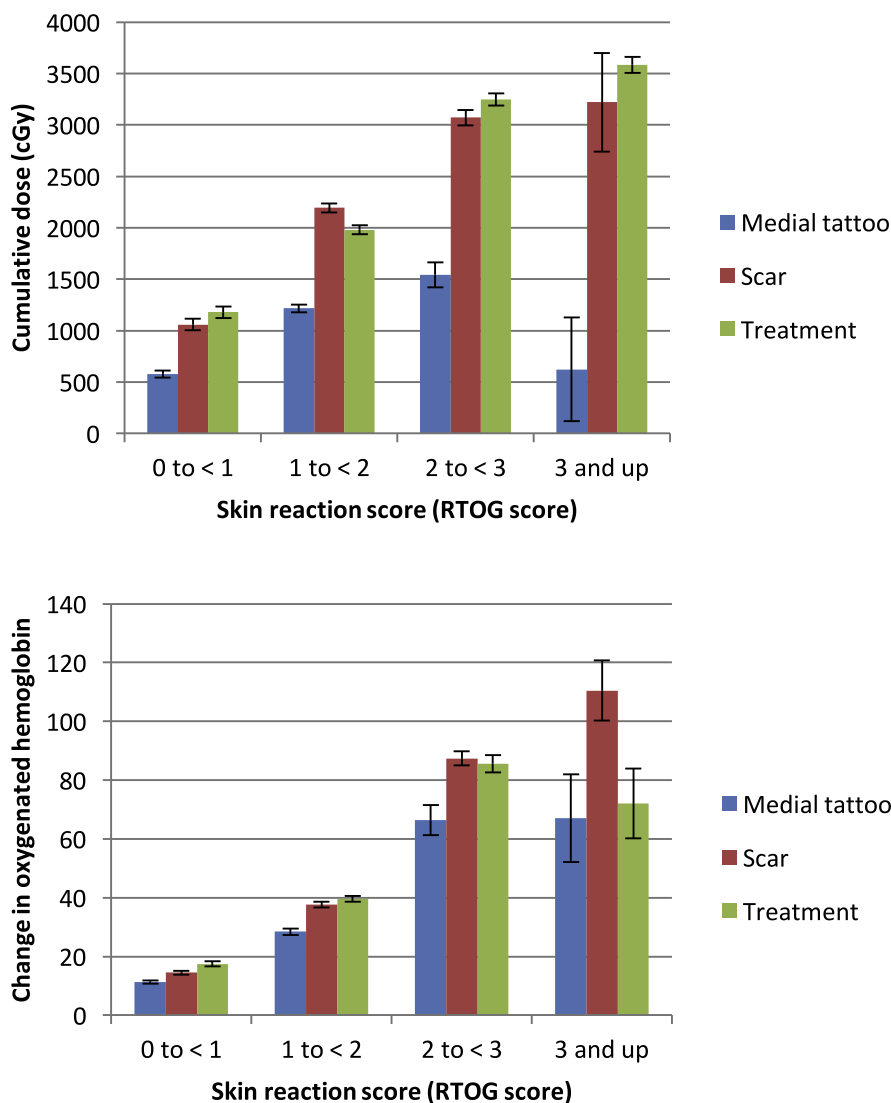
**Fig. 3.** (Above) Mean cumulative dose by skin reaction score, separated by radiation location site (*n* = 42 women, 2531 observations). Spearman correlations for TB, MT and SCAR were 0.56861, 0.37381, and 0.58996, respectively (*p* < 0.0001). (Below) Mean change in oxygenated hemoglobin since baseline by skin reaction score, separated by radiation location. Spearman correlations for TB, MT and SCAR were 0.64868, 0.54609, and 0.77246, respectively (*p* < 0.0001).

is known that there is a significant degree of skin response variation among patients [18,19]. The greater variability in the MT site relative to the combined SCAR and TB model suggests that MT skin may have a different response. We hypothesize that this difference is due to the different nature of the sternal skin, which may undergo significantly more chronic UV sun exposure than either of the primary breast sites.

There was also a significant but much weaker association of deoxygenated hemoglobin with cumulative dose. We hypothesize that this less robust deoxygenated hemoglobin relationship differs somewhat from our previous animal findings due to the physiologic differences between single and fractioned dosing, which involve lower radiation doses. Lower doses have been suggested to induce angiogenesis while higher doses have been associated with vessel destruction and endothelial cell inhibition [20–25].

The dose-dependent nature of OxyHb change seen in our patients appears to have a temporary decrease in OxyHb over the first 4 or 5 days. After this time, the change in OxyHb begins to rise to a near-linear relationship. The initial drop may be explained by the temporary increase in DeoxyHb seen in our previous animal study in the first 3 days after single fraction exposure [9]. Explana-

tions for this acute drop in OxyHb (or conversely, increase in DeoxyHb) may be due to the histamine release and succeeding inflammatory response that leads to increased vascular leak [26,27]. Previous studies have demonstrated a relationship between increasing dose and vascular permeability [15,28,29], and we believe this phenomenon may explain the brief initial drop in oxygenated hemoglobin.

### Skin reaction and OxyHb relationship to dose

Currently, the development of acute skin reactions during radiotherapy usually signals a treatment break to allow the tissue to recover. However, treatment breaks of more than a week during breast cancer radiotherapy can negatively impact recurrence rate and overall survival [30,31]. Treatment interruptions in other sensitive areas of the body due to skin reactions, such as head and neck, have similarly shown decreased locoregional control and lower overall survival [32]. The ability to identify high-risk areas or high-risk patients who develop skin reactions earlier and modify the treatment plan accordingly may circumvent the need for these

detrimental treatment breaks in not only breast but other radiosensitive areas.

One of the remaining questions regarding radiodermatitis is whether skin reactions have a predictable relationship with dose received, and how this could be assessed [33–35]. Although limited to moist desquamation as the highest form of skin reaction we observed in our study population, our data indicates that there is a correlation between degree of skin reaction and increasing dose. This finding is supported by our previous study of radiodermatitis in the animal model in which increasing single fractions of beta radiation led to increased skin reactions and damage to the microvasculature [9]. Other preclinical studies have supported the dose dependent effect on the dermis as well [16,17].

Our previous animal study suggested that HSI analysis of hemoglobin could predict formation of acute skin reactions [9]. Another spectral imaging technology (DermaScope) has been studied as a potential objective assessment tool for radiation-induced erythema; however, its correlation with clinical assessments was not strong [36]. Our preclinical data appears to be confirmed by the current clinical study.

We have observed that OxyHb response corresponds to the degree of acute skin injury in TB and SCAR sites. If further validated, the OxyHb response may prove to be a valuable assessment tool for the improved skin monitoring of patients receiving both therapeutic and diagnostic radiologic procedures. Interestingly, although treatment and scar sites reacted to radiation similarly, MT differed from the other two sites with a lower skin response. Similar to the difference in OxyHb-dose model results, we hypothesize that this is due to increased UV sun exposure in MT skin.

### Limitations

Despite a significant association, dose was not a perfect predictor of OxyHb. Dose plus patient accounted for about 65% of its variability, leaving 35% unexplained. The unexplained portion may in part be due to systematic factors we did not measure, or entirely to individual patient variability.

The RTOG score agreement had only 68% perfect agreement between the 2 technicians. We hoped to achieve a better representation by averaging the two technicians' scores. This lack of consistency highlights the subjective nature of the scoring system and the need for an objective gold standard.

### Conclusions

Use of HSI hemoglobin analysis may prove useful for the real-time assessment of patient's skin response to radiation dose. Although breast-related skin reactions have significantly decreased due to modern fractionation schemes, our findings might still be applicable to other radiosensitive parts of the body, such as head and neck. We suggest future correlational studies of our breast model predictions to other areas of the body. Our study demonstrates strong evidence of radiation dose-response relationship, however, inter-patient variability remains a challenge, as approximately 40% of the variability in change in oxygenated hemoglobin is accounted for by dose, 25% by individual woman, and 35% by causes we could not model. An upcoming analysis of this patient cohort will correlate these early adverse reactions and OxyHb responses to late skin morbidity.

### Disclosures

HyperMed Imaging, Inc. provided the OxyVu-2 device for use in this study. No renumeration or grant funding was received from the company.

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