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Using Quantitative Imaging Techniques to Assess Vascularity in AIDS-Related Kaposi's Sarcoma

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Abstract: **Three quantitative and non-invasive techniques were used to monitor angiogenesis in Kaposi's sarcoma patients: thermography, laser Doppler imaging (LDI), and near-infrared spectroscopy. Before and after combination cytotoxic and anti-angiogenesis therapy, blood volume, oxygenated hemoglobin, temperature, and blood flow were analyzed. These three techniques are objective, easy to perform, and appear to be very sensitive in assessing changes in the lesions upon administration of therapy.**

Keywords: Multi-modality imaging, laser Doppler imaging, thermography, near-infrared spectroscopy

I. INTRODUCTION

Kaposi's sarcoma (KS) is a highly vascular tumor that is a frequent cause of morbidity and mortality among people infected with acquired immunodeficiency syndrome (AIDS). Angiogenesis and capillary permeability play important roles in the development and progression of KS [1, 2]. Visually inspecting and palpating skin lesions have long been used to assess the course of cutaneous disease in patients with KS. However, reliable assessment requires a highly trained evaluator and evaluations made by different observers or by the same observer at different times can be inconsistent. Quantitative instrumental methods offer a potentially more objective means of assessing skin health to supplement the visual clinical observations. Moreover, such approaches can be used to provide early markers for tumor responses and to learn about the pathophysiology of the disease and its changes in response to treatment.

The purpose of this study is to investigate the applicability of three quantitative and non-invasive methods for the assessment of vascularity and vascular changes associated with KS lesions: thermography, Laser Doppler imaging, and near-infrared spectroscopy.

Thermography graphically depicts temperature gradients and has been used to study biological thermoregulatory abnormalities that directly or indirectly influence skin temperature. Thermography provides an integrated thermal signature that combines deep and surface sources and in general can be related to increased blood flow associated with increased metabolic activity. LDI can more directly measure the blood perfusion as the blood supply increases during angiogenesis or changes in therapy. By combining thermography with LDI, it may be possible to differentiate the near surface sources from the deeper infrared sources, thus providing a useful means to assess local changes in tissue vascularization.

Near-infrared spectroscopy is most closely related to visual assessment. With S. Demos at the Lawrence Livermore National Laboratory, a spectral imager at six wavelengths (700, 750, 800, 850, 900, and 1000 nm) was designed. Local variations in melanin, oxygenated hemoglobin (HbO_2) and blood volume can be found. The spectroscopic data was combined with 8-12 μm thermal and 780 nm laser Doppler images obtained of the same area.
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II. METHODOLOGY

1. Subjects

The study protocol was approved by the institutional review board of the NCI and written consent was obtained from all subjects. Lesions that were considered for the measurement were at least 0.2 cm in diameter. Most of the lesions studied were nodular. Immediately prior to imaging, each patient removed sufficient garments and accessories to expose the entire area to be recorded including the contralateral side. Prior to and during imaging, the subjects were seated at rest in a closed room around 23°C. Measurements were obtained prior to therapy and after receiving combination therapy with a cytotoxic and an anti-angiogenic drug for 18 weeks.

2. Clinical Assessment

Clinicians assessed the patients using a minor modification of the AIDS Clinical Treatment Group KS parameters [3, 4]. Overall tumor responses were based on changes in the total number of KS lesions, the number of nodular KS lesions, the sum of the products of the larger perpendicular diameters of five "marker" lesions selected at entry onto the protocol, tumor associated edema effusions, and visceral disease. Viral loads, CD4 count, and other laboratory parameters were also assessed. After clinical assessment, one or more lesions of

each patient were imaged by thermal, laser Doppler and multi-spectral imaging.

3. Imaging Techniques

A. Near-Infrared Spectroscopy

Near-infrared spectroscopy is a non-contact and noninvasive method of monitoring changes in concentrations of blood volume and oxygenated- and deoxygenated-hemoglobin. Assessing these analytes is complicated by other pigments in the skin, i.e. melanin and hemosiderin. However, it is possible to correct for such pigments, and NIR spectroscopy has the potential to aid in assessing the pathogenesis of the status and changes of KS lesions during therapy.

The system used here captures images with a high-resolution CCD portable camera at six near-infrared wavelengths (700, 750, 800, 850, 900 and 1000 nm). A white light held approximately 15 cm from tissue illuminates the tissue uniformly. Using optical filters, images are obtained at the six wavelengths and the intensity images are used in a mathematical optical model of skin with epidermis and much thicker, highly scattering dermis. Each layer contains major chromophores that determine absorption in the corresponding layer and the layers together determine the total reflectance of the skin. A multivariate analysis allows for the reconstruction of physiological parameters based on absorption coefficients of skin parameters.

For the mathematical optical skin model used in this paper, the effect of the thin epidermis layer on the intensity of the diffusely reflected light was determined by the effective attenuation of light, *Aepi*:

$$A_{epi}(\lambda) = e^{-\mu_{a(epi)}(\lambda)t}$$

where $\mu_{a(epi)}(\lambda)$ was the epidermis absorption coefficient [mm^{-1}], λ was the wavelength [nm], and t was the thickness of the epidermis [mm]. The epidermis absorption coefficient was determined by the equation:

$$\mu_{a(epi)}(\lambda) = V_{mel}\,\mu_{a(mel)}(\lambda) + (1 - V_{mel})\,\mu_{a(skin)}(\lambda)$$

where *Vmel* was the volume fraction of melanin, $\mu_{a(mel)}(\lambda)$ [mm^{-1}] was the melanin absorption coefficient, and $\mu_{a(skin)}(\lambda)$ was the absorption coefficient of normal skin (baseline) [mm^{-1}]. Researchers have used different equations to calculate the melanin [5-6] and baseline skin [5-8] absorption coefficients. This model used the equations used by Meglinski and Matcher [9] and Jacques [6] for the melanin and baseline skin absorption coefficients, respectively. The influence of the much thicker, highly scattering dermis layer on the skin reflectance should be estimated by a stochastic model of photon migration, e.g., random walk theory. Fitting the known random walk expression for diffuse reflectivity of the turbid slab [10] gives the following formula:

$$A_{dermis}(\lambda) = 1.06 - 1.45\left(\frac{\mu_{a(dermis)}(\lambda)}{\mu'_s(\lambda)}\right)^{0.35}$$

where $\mu'_s(\lambda)$ was the reduced scattering coefficient [mm^{-1}], $\mu_{a(dermis)}(\lambda)$ was the dermis absorption coefficient [mm^{-1}], depending on the volume of blood in the tissue and hemoglobin oxygenation, i.e. relative fractions of HbO_2 and deoxygenated hemoglobin (Hb). At wavelengths greater than 850 nm, the contribution of water and lipids should be taken into account. The dermis absorption coefficient was:

$$\mu_{a(dermis)}(\lambda) = V_{blood}\,\mu_{a(blood)}(\lambda) + (1 - V_{blood})\,\mu_{a(skin)}(\lambda)$$

where V_{blood} was the volume fraction of blood in the dermis layer and $\mu_{a(skin)}(\lambda)$ was the same absorption coefficient of skin discussed above. The absorption coefficient of blood was calculated by the volume fraction of HbO_2 times the absorption coefficient of HbO_2 plus the volume fraction of Hb times the absorption coefficient of Hb.

In the dermis, large cylindrical collagen fibers are responsible for Mie scattering, while smaller scale collagen fibers and other micro-structures are responsible for Rayleigh scattering [6]. The reduced scattering coefficient, $\mu'_s(\lambda)$, was calculated combining Mie and Rayleigh components [11]:

$$\mu'_{s(mie)}(\lambda) = 2 * 10^4 * \lambda^{-1.5} \text{ and}$$

$$\mu'_{s(rayleigh)}(\lambda) = 2 * 10^{11} * \lambda^{-4}$$

Each multi-spectral image was corrected for the light intensity, light source and camera. Then each was divided by a weight factor to bring the intensity of the images into the physiologically acceptable range. A best-fit procedure was used to reconstruct for the melanin volume, HbO_2 fraction, and blood volume fraction. For this preliminary study, the epidermis thickness was assumed to be constant at 60 μm [11] and the melanin content was based on [12].

B. Thermography and Laser Doppler Imaging

Thermography provides a two-dimensional image of superficial skin temperatures [13]. The concept is that higher temperatures occur in the skin superficial to veins that are involved in activee transport of blood. Thermal patterns were recorded using an infrared camera with a uniform sensitivity in the wavelength range 8-12 μm and temperature resolution of 0.05^{o}C. The instrument is sensitive to heat changes from blood flow changes. Thermograms were recorded immediately after uncovering the lesion area including contralateral side. The experiment was repeated after 15 minutes when the body temperature had stabilized with ambient temperature.

LDI provides a two-dimensional image of blood velocity over a defined area [14]. LDI flux value is assessed in the lesion and contralateral areas using a laser Doppler imager (MoorLDI).

III. RESULTS

A typical set of comparative multi-modality images of a non-KS subject is shown in Fig. 1 including thermal, laser Doppler and spectral images. Also shown in Fig. 1 are tissue

blood volume and oxygenated hemoglobin images reconstructed by the mathematical optical skin model described above. One can observe higher temperature, blood flux, blood volume, and oxygenated hemoglobin in the vein than the surrounding tissue.

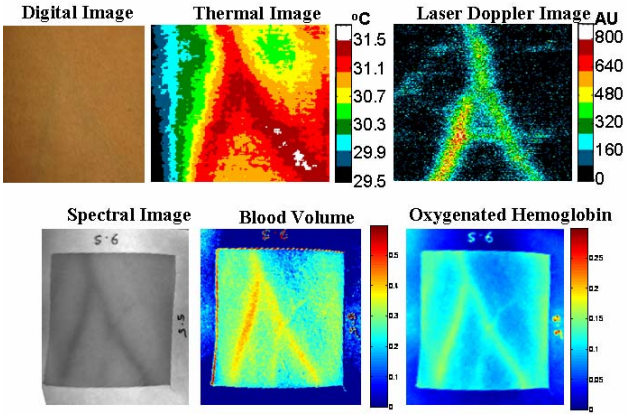

Fig. 1 Typical example of images obtained from a non-KS patient using multi-imaging modality imaging techniques: thermal, laser Doppler, spectral, reconstructed tissue blood volume and reconstructed oxygenated hemoglobin.

Previously published results on the use of thermography and laser Doppler imaging demonstrated that KS lesions generally showed increased temperature and blood velocity as compared to normal skin in surrounding tissue and the contralateral site [15]. Patients were studied after treatment with a combination of cytotoxic chemotherapy (Doxil) and anti-angiogenesis therapy (IL-12), a regimen with substantial anti-KS activity, followed by IL-12 alone [16]. After 18 and 42 weeks of this drug treatment, the temperature and blood velocity of the lesions were found to be significantly reduced from the baseline.

An example of multi-modality images obtained from a patient with KS before the treatment is shown in Fig. 2. Skin temperature is significantly elevated in the skin overlaying the tumor. Relatively high contrast of blood volume and oxygenated-hemoglobin are observed in the tumor region, which is expected for a metabolically active tumor. The normal tissue blood volume fraction is approximately 5%. This follows previous research that the volume fraction of blood in tissue is 0.2-5% [12].

It is interesting to note the differences between the top and bottom halves of the lesion. In the NIR spectroscopy and laser Doppler images, the top part of the lesion shows higher blood volume (Fig. 2c) and blood flux (Fig. 2e) than the lower half. However, this variation is not apparent in the thermal (Fig. 2d) or oxygenated hemoglobin (Fig. 2b) images. There is also significant blood flux and higher temperatures in areas surrounding the visible lesion. These results show that the visible region of the lesion (shown in black outline) does not always correspond to the full physiological area of the lesion.

Fig. 3 shows the same patient after 17 weeks of treatment. This patient, who was receiving cytotoxic/anti-angiogenesis treatment of Doxil/IL-12, showed a partial response by week 17 according to the clinical assessment. At week 17, more centralized blood flux is seen in the laser Doppler images. In addition, the lesion shows a decrease in HbO_2 saturation in the top portion of the lesion.

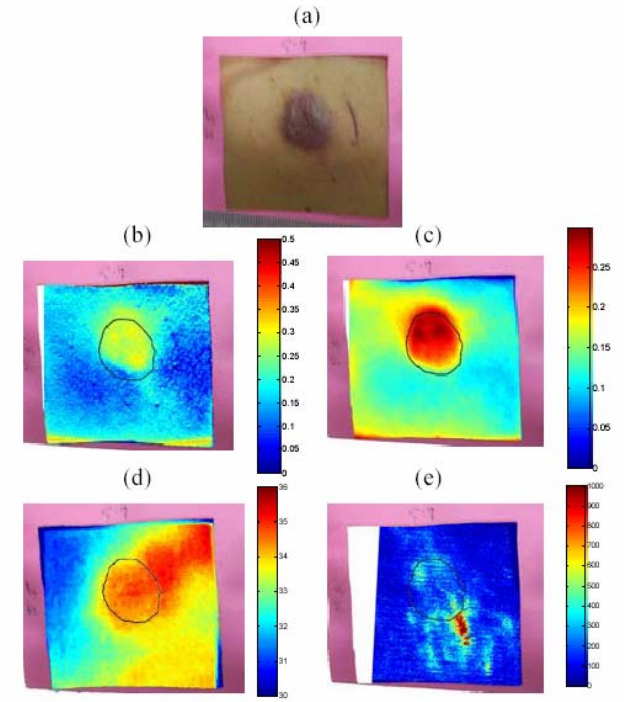

Fig. 2 Example of multi-modality images obtained from a KS patient before cytotoxic/antio-angiogenesis treatment: (a) digital photograph, (b) reconstructed oxygenated hemoglobin, (c) reconstructed tissue blood volume, (d) thermal, and (e) laser Doppler.

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IV. DISCUSSION

KS is a highly vascular tumor predominantly involved in the skin. Therefore, it can be investigated by non-invasive methods that show vascular changes. The thermal signature of the skin not only reflects superficial vascularity, but also provides deep tissue activity. LDI enables a detailed analysis of blood flow patterns in skin up to an approximate depth of 1 mm. Near-infrared spectroscopy provides a spatial map of blood volume and oxygenated-hemoglobin in tissue. In this study, changes in the vasculature and blood-related appear significant and may be an indication of the anti-angiogenesis drug treatment. The present study demonstrates that thermography, LDI and near-infrared spectroscopy can be used to detect functional vascular abnormalities in KS lesions and document improvements with therapy.

We will present comparative images and quantitative information about the temperature, blood flow, HbO_2 fraction and tissue blood volume fraction of AIDS-associated KS patients before and after treatment with anti-retroviral therapy alone or combination cytotoxic/anti-angiogenesis therapy. Over 30 patients have been monitored to date and our results are in the process of being compared with the

clinical assessments. These three non-invasive imaging techniques are objective, easy to perform, and appear to be very sensitive in assessing KS lesion progress upon administration of therapy

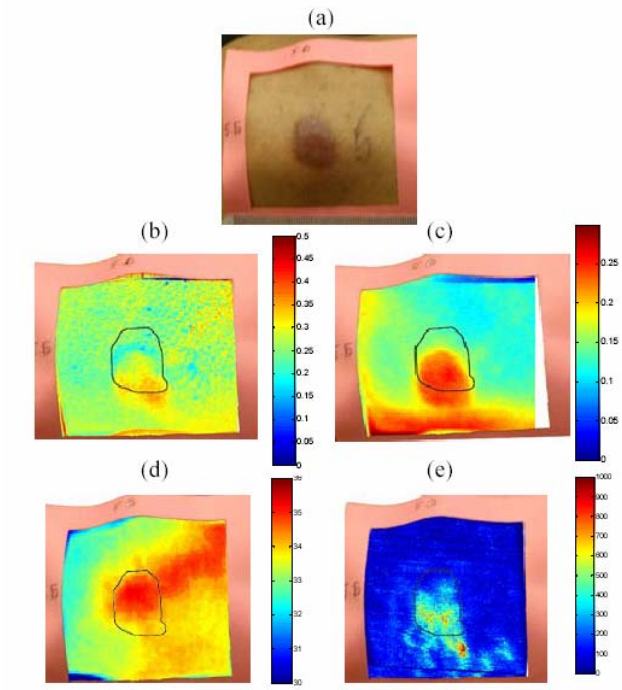

Fig. 3 Example of multi-modality images obtained from a KS patient after cytotoxic/anti-angiogenesis treatment: (a) photograph, (b) reconstructed oxygenated hemoglobin, (c) reconstructed tissue blood volume, (d) thermal, and (e) laser Doppler.

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