ONLINE DATA SUPPLEMENT

Our Technique for Sublingual Microcirculatory Image Acquisition, Processing, and Analysis:

A. About the Instruments

Orthogonal Polarization Spectral (OPS) imaging (Cytoscan, Cytometrics Inc., Philadelphia) uses optical filtration of polarized light that is absorbed by hemoglobin so that red blood cells in the microvasculature appear dark. The technique has been validated in both experimental models and human studies.[45-48] The newer Sidestream Dark Field (SDF) imaging technique (Microscan, Microvision Medical, Amsterdam) is very similar but uses a ring of light emitting diodes (LEDs) instead of a halogen light source, producing a higher contrast image with improved visualization of the smallest capillaries.[49]

B. Image Acquisition

We follow the following image acquisition Standard Operating Procedure[38]:

1. Remove secretions from the surface of the sublingual mucosa with a suction catheter or gauze.
2. Place a sterile disposable cap on the videomicroscope probe.
3. Position the probe on the sublingual mucosal surface.
4. The following steps are to be followed for proper image collection and must be repeated prior to the recording of images at each sublingual site:

    a. Position the probe so that the microcirculation comes into view.

    b. Gently advance the probe into the sublingual area until the flow is partially or completely occluded.

    c. Retract the probe from the mucosal surface until contact with the tissue is lost.

    d. Just before contact is lost, you will see what the flow looks like with no pressure. This represents an acceptable image quality for recording data.

    e. Advance the probe again slowly until contact is regained and the microcirculation comes into view (as in 4d above).

f. Focus the image.

5. Record 5 video clips of 20 seconds each.

a. Obtain two of the clips from the left side of the frenulum of the tongue and two from the right side. The fifth clip can be obtained from either side depending on where the clearest images can be obtained (operator's discretion).

C. Image Processing:

We capture the video sequences on digital tape cassettes that are stored by code without source patient identifiers so that data can be analyzed off-line in a blinded fashion. We convert videos from tape to audio video interleaved (AVI) file format with video processing software and use a random number generator (1 to 10,000) to assign random number codes to each video clip, so that image analysis is blinded to both the identity of the subject as well as the time point that the video was obtained.

D. Image Analysis:

We determine the microcirculatory flow index (MFI) with a semi-quantitative technique after Spronk *et al* (0=absent flow; 1=non-continuous flow; 2=continuous flow; 3=brisk flow).[101, 150] The method for determining MFI has good inter-rater agreement among multiple raters ($\kappa = 0.77 – 0.85$).[38, 150] We determine the MFI for all four quadrants of the image and calculate the mean of the four quadrants to give a single MFI value for each video sequence. We then average the MFI from each of the five video sequences to give a discrete MFI value for each time point that the subjects are imaged.

To determine heterogeneity of flow between different sublingual sites, we calculate the Heterogeneity Index as the highest site MFI minus the lowest site MFI divided by the mean value for MFI across all sublingual sites.[38]