36 Analysis of Vascular Tissue Using NIR Spectrometry

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36.1 INTRODUCTION

Assessment of tissue oxygen saturation by near-infrared spectrometry was one of the earliest medical uses of NIRS, and studies employing the method continue to be published in the literature. Measurement of tissue oxygen saturation can be accomplished easily using only two short-wavelength NIR peaks, which helped accelerate the widespread acceptance of the method. On the other hand, papers describing NIR imaging in vascular tissue for atherosclerosis were published decades before medical instrumentation became available for that purpose. Hyperspectral imaging requires instrumentation that simply was not widely available 30 years ago. In the past few years, however, such instrumentation has become commercially available from many sources, and medical instruments using NIR imaging have been cleared for marketing by the US Food and Drug Administration. As a result, there are now many publications on NIR imaging of vascular tissue for atherosclerosis.

Atherosclerosis is not the only application of NIRS in the vascular area. NIRS is still used in agricultural assays of meat. NIR illumination is also being investigated for phototherapy of wounds, but such phototherapy is not yet accepted by conventional medicine. In a novel approach to NIR imaging, researchers continue to pursue X-ray-activated near-infrared persistent luminescent probes for deep-tissue in vivo bioimaging. These new optical probes are free of autofluorescence and provide self-sustained emission after X-ray excitation with a high signal-to-noise ratio.

36.2 NEAR-INFRARED IMAGING IN ATHEROSCLEROSIS

Imaging and autopsy studies have described atherosclerotic plaque in different vascular beds including varying degrees of lipid, fibrosis, and calcification. Recently, near-infrared spectroscopy has been validated as an accurate method for detecting lipid-core plaque in the coronary circulation. Invasive evaluation of plaque composition using near-infrared spectroscopy and intravascular ultrasound has not been reported in different peripheral arterial circulations. Invasive angiography and near-infrared spectroscopy in combination with intravascular ultrasound have been assessed in peripheral artery disease subjects prior to percutaneous revascularization (Abbas, 2017).

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Imaging evaluation included measurements from angiography, intravascular ultrasound, and near-infrared spectroscopy. Results were compared among various vascular beds with regard to the appearance and degree of calcification and lipid-core plaque. One hundred twenty-six peripheral artery disease subjects were enrolled in the trial with a total of 149 lesions in their blood vessels, including the internal carotid, subclavian/axillary, renal, iliac, femoropopliteal, and infrapopliteal arteries. Plaque morphology was calcified in 89% of lesions and fibrous in 11%. Calcification ranged from 100% of renal artery stenoses (blockages) to 55% of subclavian/axillary artery stenoses. Lipid-core plaque was present in 32% of lesions, and prevalence ranged from 60% in carotid artery stenoses to 0% in renal artery stenoses. Lipid-core plaque was only noted in fibrocalcific plaque and was longitudinally and circumferentially surrounded by calcification. Near-infrared spectroscopy with intravascular ultrasound in stable peripheral artery disease subjects showed a high occurrence of calcified plaque and statistically significant differences in the occurrence of lipid-core plaque in various arterial beds. In the peripheral circulation, lipid-core plaque, when present, was always attendant with calcified plaque. The strong association of calcified plaque with lipid-core plaque in severe peripheral artery disease may help stabilize these plaques. Additional studies may help confirm this mechanism.

Near-infrared spectroscopy has been shown to be capable of identifying the constituents of lipid-rich plaques, and near-infrared imaging of lipid plaques has been used with computational modeling of low-density lipoprotein (LDL) transport in coronary arteries to good effect (Bampali, 2017). In this pilot study, five subjects received coronary angiography, intravascular ultrasound, and NIRS imaging. The borders of the lumen and adventitia were detected in the Intravascular Ultrasound (IVUS) images, and the 3D centerline was identified from the angiographic images. 3D reconstruction of the coronary arteries was accomplished by the integration of the detected borders with the 3D arterial centerline. Blood flow and LDL transport were modeled in the reconstructed sections in order to estimate the endothelial shear stress (ESS) and LDL accumulation in the arterial wall. ESS and LDL concentration maps were compared against the corresponding chemogram sections using NIRS imaging that marked the lipid-rich plaques. The mean ESS was 3.36±3.03 Pa, and the normalized LDL concentration was 0.093±0.005. The Pearson correlation coefficient of ESS with lumen area was −0.538 (p<0.0001) and with plaque area was 0.111.

An elevated coronary artery calcium score (CACS) suggests a heightened risk for subsequent coronary events. However, it is not known whether subjects having a high CACS actually have large lipid-rich plaques characteristic of lesions giving rise to myocardial infarction (Bundy, 2017). Bundy et al. completed multi-vessel intracoronary near-infrared spectroscopy and intravascular ultrasound in 20 asymptomatic subjects with no previous history of coronary artery disease who had a screening CACS ≥300 Agatston units. (The Agatston score is named after its developer Arthur Agatston and is a measure of calcium on a coronary CT calcium scan. The score is calculated using a weighted value assigned to the highest density of calcification in a given coronary artery. The density is measured in Hounsfield units, and scores of 1 for 130–199 HU, 2 for 200–299 HU, 3 for 300–399 HU, and 4 for 400 HU and greater. This weighted score is then multiplied by the area (in square millimeters) of the coronary calcification.) A lipid-rich plaque was defined as a lesion having ≥1 bright yellow block on the near-IR chemogram. To measure the lipid burden of each lesion, the maximum lipid-core burden index in 4 mm (maxLCBI_{4mm}) was recorded. The average CACS of the study population was 764±465 Agatston units. Lipid-rich plaque was not ubiquitous, and 40.0% subjects had no lipid-rich plaque discovered by near-infrared spectroscopy in any of their arteries. Large lipid-rich plaques having a maxLCBI_{4mm} ≥400 (lipid-core burden index) were infrequent in general, found in only 25.0% of individuals and in only 1.9% of 268 10-mm coronary segments examined. This pilot study was the first to report near-infrared results in asymptomatic subjects with a high CACS, and showed that a high CACS is not necessarily suggestive of underlying lipid-rich plaque. The differences in coronary lipids suggest that near-infrared spectroscopic imaging might be able to improve risk stratification among asymptomatic individuals with a high CACS.
Serum lipids play an important part in the development of atherosclerosis. It is widely known that low-density lipoprotein cholesterol (LDL-C) level is an independent predictor of cardiovascular events, and reducing LDL-C markedly reduces the risk of major cardiovascular events (Honda, 2017). Other cholesterol fractions can have different effects. There is an inverse relationship between high-density lipoprotein cholesterol (HDL-C) levels and prospective risk of cardiovascular disease, in spite of LDL-C levels. But little is known about the connection between serum lipid measurements and change over time in plaque composition using in vivo coronary imaging. The aim of the Honda et al. study was to examine the association between serum lipids and change in coronary plaque lipid burden assessed by near-infrared spectroscopy (NIRS).

Serial near-infrared spectrometry–intravascular ultrasound studies were conducted in 49 subjects who received coronary angiography for an acute coronary syndrome (ACS) or stable ischemic symptoms. Autopsy studies have demonstrated that plaque rupture is commonly observed in subjects with acute coronary syndromes. These vulnerable plaques are distinguished by the existence of a large lipid pool. Sequential IVUS research has shown that LDL-C lowering with statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors slows the rate of atherosclerotic plaque progression, and provided evidence that the LDL-C levels were associated with the change in plaque volume.

Near-infrared/intravascular ultrasound imaging was carried out within the same coronary artery at baseline and at follow-up in Honda et al. After intracoronary administration of nitroglycerine (100–200 µg), a 3.2-Fr IVUS/NIRS (InfraReDx, Burlington, Massachusetts) catheter was moved forward into the target coronary artery and then withdrawn by automated pullback at 0.5 mm s⁻¹ until arriving at the aorta. Intravascular ultrasound grayscale images and near-IR spectra were coregistered and stored together. LCBI and maxLCBI₄mm were calculated automatically by the LipiScan analyzer software (LipiScan, InfraReDx, Burlington, MA, USA). The primary endpoint for the study was the change in maxLCBI₄mm. Univariate and multivariate linear regression analyses were used to explore the association between the serum lipid parameters and the change in lipid-core burden index at the 4-mm maximal segment (maxLCBI₄mm).

The average age of the subjects in the Honda study was 61 ± 9 years, 29% were female, and 35% presented with an acute coronary syndrome. The median low-density lipoprotein cholesterol was 101 mg dL⁻¹, the median high-density lipoprotein cholesterol (HDL-C) was 43 mg dL⁻¹, the median total cholesterol was 174 mg dL⁻¹, and the median triglyceride level was 133 mg dL⁻¹. Through the median follow-up period of 13 months, maxLCBI₄mm declined significantly from 277 to 194. The percent change in HDL-C was negatively associated with the change in maxLCBI₄mm in univariate analysis. There were no significant associations among the other lipid parameters and change in maxLCBI₄mm. Percent change in HDL-C remained significantly associated with the change in maxLCBI₄mm in multivariate analysis.

The Honda study suggested that change in HDL-C is inversely correlated with the change in lipid-core plaque measured by NIRS. This correlation was independent of the use of statins by the subjects. Furthermore, none of the subjects whose HDL-C increased experienced growth of lipid-core plaque, while almost one-third of the subjects without an increase in HDL-C displayed plaque lipid growth even when using statin therapy. No significant correlations were found between other lipid parameters, including LDL-C level and change in lipid-core plaque. This finding emphasizes the influence of HDL on change in plaque composition. LDL-C lowering is a key therapeutic approach to reducing the risk of coronary artery disease, and the amount of LDL-C lowering is key. In addition, a larger drop in LDL-C by statin therapy and new PCSK9 inhibitors is correlated with a larger drop in plaque burden. However, the correlation between LDL-C levels and change in coronary plaque lipid burden over time is disputed. Some intracoronary imaging studies have communicated a positive correlation between LDL-C levels and lipid content of plaque. However, the Honda experiment and some other studies using radiofrequency intravascular ultrasound or near-IR spectroscopy demonstrated that serum LDL-C levels during statin therapy were not correlated with
change in lipid burden of plaque. While the explanation for this difference is uncertain, a possible reason is that the majority of the study subjects (78%) were already on treatment with statins and the median baseline LDL-C levels were relatively low (101 mg dL$^{-1}$). Prior statin use may have weakened the correlation between LDL-C levels and change in coronary lipid burden over time. For this reason, the results suggest there are restrictions on the ability of LDL-C targeting therapy to alter the lipid composition of plaque in statin-treated subjects. Other therapeutic targets may be necessary to attain regression of lipid constituents within plaque.

IVUS and near-infrared spectroscopy were used to investigate characteristics of lesions that were determined to be histopathologically thin-cap fibroatheromas (TCFAs) (Inaba, 2017). Pathological studies have demonstrated that a TCFA is the crucial predecessor lesion of plaque rupture in subjects dying from acute myocardial infarction. A TCFA has been defined as a big necrotic core with an overlying thin fibrous cap less than 65 µm in thickness. Clinical analysis of characteristics of TCFAs in vivo has encompassed several imaging modalities in the last 10 years: intravascular ultrasound, angioscopy, virtual histology or integrated backscatter-IVUS, and optical coherence tomography. But no single intravascular imaging method can detect all the features that histopathologically describe a TCFA. Near-infrared spectroscopy was developed and validated in human coronary artery autopsy studies for the in vivo identification of lipid-rich plaques (LRPs). Near-infrared spectroscopy has been combined with grayscale intravascular ultrasound in an integrated intravascular imaging device, with the capacity to increase the number of properties of a histopathologically defined TCFA that can be analyzed in vivo. The goal of the Inaba in vitro study was to investigate the recognition of pathologically defined TCFAs using grayscale intravascular ultrasound combined with near-IR spectrometry.

An Atlantis SR Pro 40-MHz catheter connected to an iLab system (Boston Scientific, Fremont, California) was moved forward along a 0.014-in. guidewire through the coronary artery mounted in a fixture. Intravascular ultrasound imaging was accomplished using motorized pullback at 0.5 mm s$^{-1}$ to include proximal and distal luer connectors.

Employing the identical protocol as for intravascular ultrasound imaging, a 3.2-Fr InfraReDx (Burlington, Massachusetts) near-infrared spectrometric catheter was advanced into the distal coronary vessel. Automated mechanical pullback was performed at a speed of 0.5 mm s$^{-1}$ and the light rotated at 240 rpm. The custom fixture’s guideposts were marked during pullback to ensure registration with histology and intravascular ultrasound. Raw spectra were obtained at a rate of 1 spectrum every 25 ms.

The probability of lipid-rich plaque was depicted as a chemogram, which is a digital color-coded map of the location and probability of lipid as if observed from the luminal surface, with the X-axis designating the pullback position (1 pixel every 0.1 mm) and the Y-axis designating the circumferential position (1 pixel every 1°) after spatial filtering and image processing of the raw data as though the coronary vessel had been cut open along its longitudinal axis and positioned flat. Spectroscopic data at each pixel were constructed into a probability of lipid-rich plaque that was then mapped to a 128 (7-bit) red-to-yellow color scale with the lowest probability of lipid depicted as red and the highest probability of lipid depicted as yellow. Near-IR image analysis was accomplished offline by employing in-house, MATLAB-based software.

Intravascular ultrasound, near-infrared spectrometry, and histopathology evaluation included 39 left anterior descending arteries, 36 right coronary arteries, and 32 left circumflex arteries from 54 autopsied hearts. The median age of the subjects was 65 years, 80% had a cardiovascular cause of death, and 70% were male. A set of 271 IVUS-defined lesions were assessed. Of the 271 intravascular ultrasound-identified lesions, 73% of the lesions were mild to moderate with plaque burden less than 70%. Two hundred seventy-one lesions were histopathologically categorized and classified as 26 adaptive intimal thickenings, 65 noncalcified thick-cap fibroatheromas, 62 prefibroatheromas, 66 calcified thick-cap fibroatheromas, 42 calcified fibrous plaques, and 10 TCFAs.

Lesions identified as TCFAs showed the largest plaque burden, the highest remodeling index, and the greatest maxLCBI$_{4mm}$. Plaque burden equal to or greater than 69% (90% sensitivity,
75% specificity, and area under the curve 0.87), remodeling index equal to or greater than 1.07 (80% sensitivity, 79% specificity, and area under the curve 0.84), and maxLCBI\textsubscript{4mm} equal to or greater than 323 (80% sensitivity, 85% specificity, and area under the curve 0.84) predicted a histopathologic TCFA. Simply put, a large plaque burden and a high remodeling index assessed by intravascular ultrasound and lipid-rich plaque ascertained by near-infrared spectroscopy maxLCBI\textsubscript{4mm} are practical predictive markers of TCFA. Larger LCBI (maxLCBI\textsubscript{4mm} ≥ 323) is correlated with the TCFA in a nonruptured plaque. Real-time quantitative analysis of lipids within coronary artery plaques has become possible using NIRS.

Coronary artery disease is expected to remain the major cause of mortality and morbidity worldwide (Schuurman et al., 2018). Subjects with a history of coronary artery disease are at increased risk of later dangerous cardiovascular events, such as acute coronary syndrome. In about three-fourth of all cases, an acute coronary syndrome is produced by rupture of the fibrous cap covering vulnerable, lipid-rich core plaque in the coronary arteries. Coronary angiography is incapable of distinguishing lipid-rich core-containing plaques in the coronary artery wall, but lipid-rich plaques can be pinpointed by near-infrared spectroscopy. Using a catheter for intracoronary imaging via diffuse reflectance spectroscopy is becoming useful in recognizing subjects at increased risk of unfavorable cardiovascular outcome.

The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis (ATHEROREMO) and the Integrated Biomarker and Imaging Study 3 (IBIS-3) were created to probe phenotypes and vulnerability of plaque as determined by intravascular ultrasound and near-infrared spectroscopy. In the Schuurman study, long-term follow-up of both studies was accomplished with the intent to scrutinize the long-term predictive value of lipid-rich plaques as measured by near-infrared spectroscopy in subjects with coronary artery disease undergoing coronary angiography.

The most recent study (Schuurman et al., 2018) combined subjects from the ATHEROREMO-NIRS and IBIS-3-NIRS substudies. Both of these studies were executed in the Netherlands and had similar enrollment criteria and baseline study procedures. Subjects receiving diagnostic coronary angiography or percutaneous coronary intervention for acute coronary syndrome or stable angina pectoris (SAP) received baseline invasive imaging by near-infrared spectrometry and intravascular ultrasound and were followed up later on adverse cardiovascular events. The images collected were reviewed offline and were not utilized for subject care. ATHEROREMO-NIRS enrolled 203 subjects between April 2009 and January 2011, and IBIS-3-NIRS enrolled 131 subjects between January 2010 and June 2013. A total of 286 subjects were available because 48 subjects participated in both studies. Of these subjects, 275 subjects had baseline data available for both near-infrared spectroscopy and intravascular ultrasound and were therefore included in the Schuurman analysis.

Near-infrared spectroscopy was completed in a nonculprit artery of 275 subjects undergoing coronary angiography for acute coronary syndrome or stable angina. Lipid-core burden index was measured by an independent lab for the area of interest (LCBI\textsubscript{ROI}) and the 4- and 10-mm-long sections with the maximum LCBI (maxLCBI\textsubscript{4mm} and maxLCBI\textsubscript{10mm}). The primary endpoint was major adverse cardiac events (MACE), which was defined as the composite of all-cause death, nonfatal acute coronary syndrome, or unscheduled revascularization. Hazard ratios were adjusted for age, clinical risk factors, gender, and section plaque burden calculated from intravascular ultrasound. During a median follow-up of 4.1 years, 28.7% of subjects had a MACE. There was a statistically significant and independent continuous relationship between higher maxLCBI\textsubscript{4mm} values and a higher risk of MACE. Each 100-unit increase of maxLCBI\textsubscript{4mm} was associated with a 19% increase in MACE (hazard ratio 1.19, 95% confidence intervals: 1.07–1.32, \(P=0.001\)). Continuous maxLCBI\textsubscript{4mm} continued to be independently associated with MACE after exclusion of target lesion-related events as well as after exclusion of adverse events connected to the near-infrared spectrometry-imaged coronary segment.

Three study limitations are worthy of special discussion. First, the study population also contained subjects from IBIS-3, who were given large doses of rosuvastatin following the index procedure.
This cholesterol-lowering drug may have influenced the effect estimates. Nevertheless, a post hoc analysis did not reveal significant effect changes according to the study (ATHEROREMO or IBIS-3). Second, the follow-up questionnaire was finished by 90% of the subjects. Follow-up data were retrieved for most of the remaining subjects from accessible hospital records. However, the chance that loss to follow-up was at least in part biased cannot be completely excluded. On the other hand, a post hoc analysis of clinical and near-infrared spectroscopy attributes of the nonresponders as contrasted with those of the responders did not show any distinctions that suggested selective loss to follow-up. Third, the sample size of the single-center study was comparatively low. The authors examined LCBI as quartiles and as a continuous variable to study the association with adverse cardiac outcome after exclusion of target lesion-related and image section-related events. The Schuurman study concluded that LCBI, as measured by near-infrared spectroscopy in one nonculprit coronary artery segment, predicts adverse cardiac outcome independent of clinical characteristics and intravascular ultrasound, during extended follow-up over 4 years in subjects referred for coronary angiography because of acute coronary syndrome or stable angina pectoris.

The subcutaneous vein network serves an important role in maintaining human skin tissue (Seker, 2017). In spite of advancements in optical imaging technologies, problems still remain with imaging of veins deep in the skin. Differing technologies have been applied to solve subcutaneous vein imaging issues. Venographic guidance supplies adequate contrast by employing special contrast agents for radiographic imaging. On the other hand, ultrasound-based imaging systems avoid exposing the subject to ionizing radiation, but much more training is required to use and interpret the data. For these reasons, diffuse optical methods are now being investigated as tools to replace the typical methods in some specific diagnostic studies.

Intravascular near-infrared fluorescence imaging (NIRF) can offer high-resolution observation of the human coronary artery. Animal studies have demonstrated feasibility of using NIRF for identifying inflammation and fibrin deposition in coronary arteries and other blood vessels of similar size. Near-infrared fluorescence (NIRF) molecular imaging offers an interesting new medical imaging implement that has more sensitivity than diffuse reflectance, and lacks the ionizing radiation of positron emission tomography and X-rays. Clinical applications of NIRF include using indocyanine green (ICG) as a nonspecific NIRF contrast agent to demarcate the blood and lymphatic vasculature. The literature contains an increasing number of publications on functional near-infrared imaging (fNIRI). Progress should continue in this nascent field. Soon fNIR should offer increased spatial and temporal resolution. fNIRI can be potentially integrated with additional imaging or sensing methods, for instance, fMRI, EEG, PET, and MEG. fNIRI can be reduced in size and made wearable to increase its scope of applications to such things as monitoring effects of drugs for depression, schizophrenia, or stroke patients. Real-time visualization of peripheral veins is also a potential use. Venipuncture is a medical procedure for gaining intravenous access. While a peripheral vein can be accessed in a single attempt, usually between 2 and 10 attempts are necessary. Excessive venipunctures are time-consuming, are uncomfortable for patients, and can increase cost through the use of extra supplies. Currently, there are a number of commercially available medical devices that utilize NIR to ease vascular access procedures.

Seker et al. (2017) assembled a near-infrared light source to gain the advantage of lower absorption of hemoglobin in this optical region. A reflectance geometry was employed to obtain vascular images. Vascular network analysis utilized recorded images for calculation of the width of vessels of interest and connectivities of selected regions. Seker et al. proposed that their imaging system had great advantages over other commercially available products, including the use of nonionizing radiation, an acceptable penetration depth of 0.5–3 mm, cost-effectiveness, and straightforward procedures for analyses.

The Seker et al. general optical system comprised an NIR LED-based light source, polarizers, infrared (IR) filter, and a camera (Xenics Infrared Camera: Xeva-1.7-320). The LED light source was implemented in both a disk and ring layout. Each LED module (disk/ring type) had 17 LEDs, some emitting 870 nm and some 940 nm.
Seker et al. devised two kinds of phantoms: one liquid-based phantom to imitate human blood and one pork gelatin-based solid phantom to imitate skin tissue. The absorbance of tissue and blood was imitated with 0.2% India ink, and 0.1% intralipid solution was used for scattering properties. Optical absorption and scattering coefficients were determined from the phantom media, using spectrometer and integrating sphere system-based measurements. Then, NIR optical imaging was performed on the solid (gelatin) phantoms in which artificial veins (plastic tubes) were inserted into the phantom base material. Different tube diameters were employed to imitate veins at a certain depth level (3.5 mm). The liquid phantom devised to imitate human blood was injected into the tubes to obtain vascular images. The Seker et al. method provided good results compared to the ultrasound-based methods employed in clinical practice.

Lipid-core plaque has been analyzed by near-infrared spectroscopy and compared to microcirculatory resistance (IMR) (Yang, 2017). Microvascular damage arising from distal embolization in the course of percutaneous coronary intervention is a significant source of periprocedural myocardial infarction, which is connected with poor clinical outcomes. Yang et al. evaluated the relationship between lipid-core plaque analyzed by NIRS and microvascular dysfunction analyzed invasively by the index of microcirculatory resistance (IMR). Yang et al. enrolled 36 subjects with left anterior descending artery plaques. NIRS was carried out before PCI, and fractional flow reserve (FFR), thermodilution coronary flow reserve (CFR), and IMR were carried out following PCI. The maximum value of LCBI for any of the 4-mm sections was computed, and the big-lipid-core plaque group was designated as those with maxLCBI_{4mm} over 500. Microvascular dysfunction was designated as post-stent IMR over 25.

The mean age of the enrolled subjects was 60 years. Seven subjects (19.4%) had LCP that fell into the big group. The diameter of stenosis was similar in all subjects. The recorded intravascular ultrasound parameters of minimal lumen area (2.56±0.58 vs. 2.34±0.37 mm^2) and lesion length (24.6±10.2 vs. 29.7±11.8 mm) were not significantly different. Pre-PCI FFR (0.73±0.09 vs. 0.74±0.08), post-PCI FFR (0.87±0.05 vs. 0.86±0.04), and post-PCI CFR (3.07±1.83 vs. 2.06±1.00) were not significantly different between the two groups. Post-PCI IMR was larger in the big-LCP group (19.6±15.5 vs. 42.6±17.6). Microvascular dysfunction was more frequent in the big-LCP group than in the small-LCP group (85.7% vs. 17.2%). In the course of elective PCI, large LCPs at the site of the target lesion were connected with post-procedural microvascular dysfunction.

### 36.3 TISSUE OXYGEN SATURATION

Intraoperative cerebral (SctO\textsubscript{2}) and muscle tissue oxygen saturation (SmtO\textsubscript{2}) established by NIRS are associated with postoperative complications and length of hospital stay (LOS) in subjects undergoing major spine surgery (Heringlake, 2017). They signed up 102 subjects undergoing lumbar or thoracolumbar spinal surgery and tracked tissue oxygen saturation bilaterally on the forehead and on a designated spot on both legs for ascertaining average SpO\textsubscript{2} and SaO\textsubscript{2}, respectively. A ForeSight EliteVR (CAS Medical Systems, Branford, CT, USA) oximeter was used for the measurements. All measurements began when the subjects were anesthetized and moved to the horizontal position, and ended after a varying period of surgery.

Subjects were organized into classes with many or few complications and longer or shorter LOS using the number of complications and LOS registered in the hospital computer system. In spite of antecedent research in the surgical milieu, univariate and multivariate statistics uncovered no relationship between alterations in SctO\textsubscript{2} and complications or LOS. On the other hand, a number of SmtO\textsubscript{2} indices revealed feeble but statistically significant relationships with poor outcomes. Despite what might be anticipated on the basis of pathophysiology, subjects exhibiting adverse outcomes had higher SmtO\textsubscript{2} measurements than those with a less convoluted postoperative course. This surprising discovery is in clear contrast to previous research and was not explained in the published study. The authors did address a critical matter: the relationship between cerebral desaturation and clinical outcomes in subjects undergoing non-cardiac surgery. While there are still few data
on perioperative cerebral oxygenation beyond the field of cardiac surgery, the study seemed to be acceptably powered.

From the perioperative time course of SmtO₂ classified by complications and LOS and the respective SDs, it was apparent that there was enormous variability in all observations (with SDs up to 20%) and a massive overlap among subject classes. Furthermore, the more lengthy the surgery, the higher were the variability of the results and the excursions of SmtO₂ values relative to baseline. Interestingly, the minimum SpO₂ values in subjects with a complicated course were measured when surgery in uncomplicated subjects had already completed. What it all means is not totally clear yet. Does somatic oxygenation really differentiate between subjects more than brain oxygenation between subjects with or without complications or longer LOS? Given the limitations of this study, it is hard to firmly draw such a conclusion. The data analyzed depict only the intraoperative time course and not excursions of cerebral and somatic oxygenation with regard to the non-anesthetized baseline. The data on the fractional inspired oxygen (FIO₂) administered during horizontal positioning of the subjects (when the measurements started) are not present; therefore, one cannot determine whether some subjects (i.e., those with cardiovascular risk factors or a high BMI) were preoxygenated with a greater FIO₂ before horizontal positioning, while others (i.e., with fewer risk factors or simply lean) were not, which is a facile way to inject confounders in saturation measurements. The relationship between bleeding and blood product use with poor outcomes was much more powerful than the association between alterations in SmtO₂ and complications or LOS. The publication does not state whether the anesthetists in charge used a greater FIO₂ in bleeding and unstable subjects than in stable subjects who were not bleeding. The lack of information on potentially important confounders means one can only speculate whether subject- or anesthesia-specific factors led to greater intraoperative SmtO₂, or the absence of outcome-relevant variations in SctO₂, or even both. Furthermore, the prominent changeability of SmtO₂, particularly in the class without complications, and the protracted assessments in the class with complications or prolonged LOS (with reduced SmtO₂ with reference to baseline) convey that it is arguable whether this experiment is frankly adequate to make consequential conjectures on the importance of somatic NIRS in this subject population. Meng and coworkers have communicated very weak, but statistically significant, correlations with outcomes. An enhanced multivariate prediction model might be extracted from such a data cloud, but whether anyone will be able to direct therapy in the operating room or at the bedside using the information contained within these noisy data is unclear. Nevertheless, even more can be learned from this experiment. The variability of the SmtO₂ measurements presents the question of whether it is suitable to use a technology that has been specially developed and calibrated for evaluating brain oxygen saturation somewhere else on the body, and then to require the assumption that these uncalibrated, off-label measurements provide more worthwhile information than those acquired from the measurement location for which the instrument was principally created and approved. The authors acknowledged that in spite of the results of many single-center experiments, a recent multicenter investigation in cardiac surgical subjects did not find any relationship between lowered perioperative cerebral oxygen saturation and poor outcomes. Even though NIRS sensors have been used upon different parts of the human body in observational studies with encouraging results, most of these investigations were conducted with instruments that had been specially designed for application at peripheral sites and not with cerebral oximeter probes. Prior to venturing further into somatic monitoring using cerebral sensors, it may be judicious to pay more thorough attention to the use of this technology for the indication for which it was primarily created and approved, applying the technology judiciously, and taking conscientious care of possible confounders.

Ultrasound-guided frequency-domain near-infrared spectroscopy (FD-NIRS) has been used to quantify placental oxygenation (Ko, 2017). Vigorous transport of oxygen across the placenta is vital for healthy fetal development. Noninvasive, transabdominal oximetry using continuous-wave near-infrared spectroscopy (CW-NIRS) has been tested in quantification of in vivo placental oxygenation in a small case study. Notwithstanding, the accuracy of NIRS techniques is highly dependent on
tissue depth and variability in the optical properties of superficial tissue. CW-NIRS studies so far have been incapable of dealing with uncertainties related to the intervening abdominal and uterine tissue. Eventually, frequency-domain near-infrared spectroscopy (FD-NIRS) employing radio frequency (RF)-modulated light permitted subject-specific characterization of optical properties, and as a result, upgrading physiological accuracy.

In Ko et al., ultrasound-guided FD-NIRS was employed to quantify placental oxygenation in a singleton gestation with an anterior placenta. Imaging was conducted at two positions with the probe rotated 0°, 45°, and 90° relative to the sagittal plane at each position. Optical source–detector separations varied from 3.5 to 7 cm in the instrumentation to characterize superficial vs. deep-tissue contributions to the total observed signal. Depth sensitivity to placental oxygenation was validated using finite element modeling of collected optical data from tissues.

Physiologic assessments of total hemoglobin concentration (THC, mM) and tissue oxygen saturation (StO2, %) were obtained at 20.4 weeks’ gestation in a healthy mother with a BMI of 22.3. Placental depth, calculated from the ultrasound probe to the surface of the placenta, varied from 1.8 to 3.1 cm in this research. Simulations using collected superficial and placental optical properties validated sensitivity to placental tissue at depths up to 3.5 cm. Ko et al. concluded that noninvasive quantification of placental oxygenation might assist in pinpointing abnormal placental evolution. Trial measurements showed the usefulness of point-of-care, ultrasound-guided FD-NIRS oximetry. Including the most modern RF generation and detection hardware and analytical algorithms should further enhance the specificity and quantification accuracy of FD-NIRS placental oximetry.

Near-infrared spectroscopy was used to predict organ failure and outcome in sepsis in the Assessing Risk in Sepsis using a Tissue Oxygen Saturation (ARISTOS) study (Macdonald, 2018). Sepsis, described as a dysregulated host response to infection resulting in organ dysfunction, is connected to significant morbidity and mortality. In the emergency department, it is crucial to recognize the subject with early sepsis to make certain that the subject receives expedient treatment and appropriate positioning in the treatment order. Recognizing sepsis is difficult, considering the dynamic and heterogeneous nature of sepsis and the absence of dependable objective diagnostic criteria. Near-infrared spectroscopy is already used as a noninvasive technique for determining tissue oxygen saturation (StO2), the percentage of oxygenated hemoglobin in skeletal muscle. NIRS can recognize subjects with reduced tissue perfusion and assist clinical identification of early sepsis. Sepsis leads to changes in the function of the microcirculation, and an abnormal StO2 (normal range 75%–90%) was thought to be a possible indicator of microcirculation dysfunction. Even though reduced StO2 is consistent with other indexes of oxygenation, the clinical value in the emergency department is still undetermined. Experiments have evaluated StO2 for the identification of sepsis and severity/mortality, and to forecast the requirement for intensive care admission. New-onset organ dysfunction is the hallmark of sepsis and is key to the newly revised consensus definition. As a practical matter, sepsis is established by a rise in the sequential organ failure assessment (SOFA) score of 2 or more points from baseline. Furthermore, there is a bedside tool, the “quick sequential organ failure assessment” (qSOFA) score, that allocates one point for each of systolic blood pressure (SBP) less than 100 mmHg, respiratory rate (RR) of at least 22/min, and altered mental activity, which has been advanced to distinguish a greater risk of poor outcome.

The objective of the Macdonald experiment was to study whether StO2 measurement is practical for diagnosis of sepsis, and to forecast clinical outcomes (mortality and prolonged ICU admission) among a group of emergency department subjects admitted to the hospital with infection. The inclusion criteria for study participants were constructed on the 2001 international consensus definition for sepsis that was valid at the time recruitment was initiated. The consensus definition necessitated the existence of at least two systemic inflammatory response criteria (temperature > 38°C or < 36°C; pulse rate 90 beats/min; respirations > 20 breaths/min; white blood cell count > 12 × 10^9/L or < 4 × 10^9/L) in the environment of suspected or authenticated infection. Subjects to be treated by administration of intravenous antibiotics and admission to hospital were evaluated for eligibility throughout the hours of the research personnel. Exclusion criteria included age less than 18 years,
taking intravenous antibiotics before presentation to research personnel (i.e., interhospital transfers), anticipated mortality by another cause (e.g., malignancy) within the next 90 days, preexisting limitation of care order, or other comorbidity prohibiting invasive organ support.

The primary endpoint was sepsis, as established by a SOFA score rise of 2 or more points within 72h of admission, and integrated in-hospital mortality/ICU admission of at least 3 days. A group of 323 participants, with a median age of 64 (interquartile range: 47–77) years, was enlisted at three Australian hospitals. Forty-four percent met the criteria for sepsis, and 7% perished within 30 days. The mean±SD StO2 was 74%±8% in sepsis and 78%±7% without sepsis. StO2 correlated with the peak sequential organ failure assessment score (Spearman’s 𝜌 = −0.27, 𝑃 < 0.0001). The area under the receiver operating characteristic curve was 0.66 (95% confidence interval: 0.60–0.72) for sepsis and 0.66 (0.58–0.75) for the composite outcome. StO2 < 75% had an odds ratio of 2.67 (1.45–4.94) for the composite outcome compared with StO2 at least 75%. NIRS-derived StO2 corresponds to organ failure and is connected with the result in sepsis. Still, NIRS’s ability to distinguish sepsis among emergency department subjects with infection is meager. NIRS cannot be endorsed for emergency department use in sepsis diagnosis. In logistic regression evaluation, a depressed StO2 lower than 75% is an independent risk factor for mortality and/or protracted ICU admission. The result obtained that StO2 combined with an increased lactate and the qSOFA score forecasts outcome is a potentially viable hypothesis and mandates independent prospective validation. The key is to identify the subject at risk of developing organ failure among those subjects appearing with characteristics of infection. Changes in microcirculation are a major constituent of the pathogenesis of sepsis. Having said that, the evaluation of microcirculatory dysfunction in the clinical setting is problematic. NIRS suggests a possible solution because NIRS can be implemented in a bedside test that is noninvasive and readily reproduced. Both an abnormally low StO2 (<75%) and a high StO2 (>89%) have been proposed to be connected with sepsis. The Macdonald data are predominantly consistent with preceding studies, which support a connection between abnormal StO2 and organ failure/outcome in sepsis. For all that, the ability of StO2 to distinguish between clinical outcome classes of interest is deficient, and NIRS cannot be endorsed in isolation for classification purposes on the basis of the Macdonald results.

36.4 RELATED TISSUES AND THERAPIES

Historically, near-infrared spectroscopy has been used in agriculture, and such uses persist to the present day. A recent paper (Roberts et al., 2017) described a feasibility study on the potential use of near-infrared reflectance spectroscopy to analyze muscles in living animals. Vascular tissues such as the aorta contains significant amounts of muscle. The reliability with which near-infrared spectroscopy penetrates living tissues was cited in the paper as a major reason for employing it to discriminate between fat and muscle tissue through the skin. In beef cattle, where postmortem carcass assessment or other meat composition analysis is conducted to assess the nutritional value of feeds on animal performance, a rapid, noninvasive, and nondestructive assay offers significant advantages to producers.

Short-wavelength NIR (SWNIR; 700–1100 nm; 13,900–9400 cm⁻¹) range can penetrate deeply into the skin, providing a possible spectral window for the analysis of animal and human tissues. A Fourier transform (FT) NIR instrument was used to collect data, and The Unscrambler software (version X; CAMO ASA, Oslo, Norway) was used for chemometric analysis (Roberts et al., 2017). The authors were able to differentiate between skin, muscle, fat, fat under skin, and muscle under skin. In the SWNIR region, spectral differences between these five groups were observed around the 10,750 and 10,288 cm⁻¹ regions associated with water (O-H bonds). Absorbance bands around 11,900, 11,655, 10,929, and 9911 cm⁻¹ were attributed to differences in water content (O-H bonds) and also associated with –CH2– third overtones. Absorbance bands between 10,929 and 10,800 cm⁻¹ were correlated with lipid content of the tissue analyzed, while further bands associated with the
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CH stretch harmonics were observed between 11,655 and 10,929 cm⁻¹. Absorbance bands between 10,400 and 9911 cm⁻¹ were attributed mainly to water (O-H bonds). The most applicable changes were noticed in the long NIR wavelength region, where differences between the five types of samples were observed at 7100 cm⁻¹ (O-H and C-H), 5263 cm⁻¹ (O-H, mostly related to water), and between 4762 and 4350 cm⁻¹ (CH combination tones). The results from the Roberts study indicated that the NIR spectrum has potential use to monitor some beef meat properties connected with fat or muscle noninvasively through the skin. Such a method would provide beef producers or industry with a useful tool to record the effects of health and nutrition on product characteristics with the goal of producing a consistent product and maximizing profits.

Spectral attenuation of brain and retina tissues has been determined in the near-infrared range using a fiber-based supercontinuum device (Saldaña-Díaz et al., 2017). The optical setup integrated a fiber-based supercontinuum light source with a straightforward fiber-optic collimator. This arrangement simultaneously permitted a broad spectral range of measurement and efficiently filtered the transmitted light while reducing the scattered light. The optical measurement of rat brain and retina tissues was provided to illustrate the performance. The attenuation coefficient for the brain and retina tissues was procured in the near-infrared region. The technique could be used in clinical research as a noninvasive method.

The many problems associated with chronic wounds have focused attention on the development of new painless, noninvasive, biophysical therapeutic interventions (Yadav, 2017). Near-infrared light-induced photobiomodulation (PBM) therapy is under investigation as a drug-free approach to promotion of wound healing, reducing inflammation and pain, and restoring function. The penetration power of near-IR light combined with its ability to beneficially modulate biochemical responses is potentially useful. Near-infrared light (800–830 nm) has been found to be the most effective and widely studied wavelength range, followed by red (630–680 nm) and near-infrared 904-nm superpulsed light.

PBM is a form of light therapy that can use coherent light sources (lasers), noncoherent light sources (comprising filtered lamps or light-emitting diodes), or sometimes a combination of both, generally in the visible and NIR spectral regions. It is a nonthermal process involving endogenous chromophores evoking photophysical and photochemical events (linear and nonlinear) at a mixture of biological scales. PBM is different from other light-based treatments in that it does not ablate tissue and it does not rely on heating. PBM is also different than photodynamic therapy (PDT), in which light excites endogenous chromophores or exogenously delivered nontoxic photosensitizers that then react with ambient oxygen to produce reactive oxygen species (ROS) that can kill infectious microorganisms and cancer cells or destroy undesirable tissues (e.g., atherosclerotic plaques in the arteries or choroidal neovascularization in the eyes).

An “optical window” exists in tissue between 700 and 1350 nm where the useful tissue penetration of light is maximized due to low absorption and scattering by the primary tissue components. The fact that NIR light can penetrate into a deep dermal tissue injury allows noninvasive treatment to be conducted without pharmaceuticals to improve healing processes. The effect of NIR light on tissue may depend mostly upon two factors: the amount of energy absorbed and the rate at which the energy is applied. The energy absorbed per unit volume of the tissue should be proportional to the product of the light intensity, the pulse duration, and the absorption coefficient of the tissue at the wavelength of the light. Successful therapeutic outcomes in PBM need ideal optical treatment protocols, including illumination parameters (e.g., wavelength, flux, power density, pulse intensity, and duty cycle) and the perfect treatment timetable.

NIR light is only slightly absorbed in the short-wavelength regions and has a substantial depth of tissue penetration (up to 30–40 mm), and so it provides increased accumulation of photons in the wound bed and more therapeutic healing efficacy. Either continuous-wave (CW) or pulsed-wave (PW) therapy can be used. Some report that PW is more effective than CW, because in PW there are pulse off-times of longer duration than the on-times, which lessens tissue heating. Superpulsed
low-level light therapy emits extremely short pulses on the order of nanoseconds \((10^{-9}s)\) or less, so very high-peak powers can be achieved without heating. These very short pulses may permit quick absorption at the cellular level, and Yadav et al. hypothesize that the period between pulses encourages better cell communication, leading to improved wound healing. The episodic nanosecond pulsing model in superpulsed low-level light therapy could build up multiple photodissociation events of NO from CCO, which in turn might boost mitochondrial respiration.

PBM is not yet accepted by conventional medicine. The major barriers to PBM utilization are as follows: (1) few well-controlled, randomized, double-blind clinical trials showing efficacy, (2) uncertainty regarding the cellular and molecular mechanisms transducing signals from the photons incident on the cells that exert the biological effects in the illuminated tissues, and (3) a large number of variables in dosing optically. There are different ways to dose for the same type of injury. To overcome these issues, researchers must focus on understanding of mechanisms, providing better guidelines and standardized protocols, and developing consistent illumination exposure parameters and well-controlled, randomized, double-blind clinical trials showing efficacy.

Researchers continue to pursue X-ray-activated near-infrared persistent luminescent probes for deep-tissue in vivo bioimaging (Xue, 2017). Near-infrared persistent luminescence nanoparticles (PLNPs) are new optical probes that are free of autofluorescence and provide self-sustained emission after excitation with a high signal-to-noise ratio. Unfortunately, most NIR-emitting PLNPs have a short decay time and demand excitation by ultraviolet or visible light that itself poorly penetrates tissue to only a small depth. This, in turn, greatly reduces their applications for in vivo long-term imaging and biomarking. For these reasons, improved NIR-emitting PLNPs with in vivo activation features using new excitation sources with deeper penetration depths present a significant improvement to the PLNP technology. Xue et al. are developing a new type of X-ray-activated \(\text{ZnGa}_2\text{O}_4:\text{Cr}\) PLNPs (X-PLNPs) with efficient NIR persistent emission and rechargeable activation features, in which both the excitation wavelength and emission wavelength are able to penetrate deeply through tissue in vivo. X-PLNPs display enduring (up to 6h) NIR emission at 700 nm after the discontinuation of the X-ray excitation source. Moreover, the devised X-PLNPs can be easily reactivated by a soft X-ray excitation source with little excitation power \((45 \text{kVp}, 0.5 \text{mA})\) to restate in vivo bioimaging signals at up to 20 mm depth. Renewable in vivo whole-body bioimaging was also demonstrated through intravenous injection/oral administration of X-PLNPs after in situ X-ray activation. This achievement is the first time that NIR-emitted PLNPs have been shown to be recharged by X-ray light for deep-tissue in vivo bioimaging. This finding clears the way for in vivo renewable bioimaging employing PLNPs and makes the PLNPs highly valuable in the bioimaging field.

36.5 CONCLUSION

Measurement of tissue oxygen saturation using near-infrared spectrometry continues to be a popular application. Simple, robust devices for determining tissue oxygen saturation are widely available. More complicated applications of NIR spectral imaging in vascular tissue are finally beginning to appear, decades after the technique was first described. Medical instruments using NIR imaging have been cleared for marketing by the US Food and Drug Administration. As a result, many publications on NIR imaging of vascular tissue for atherosclerosis appear annually.

NIRS is still employed in measurement of meat for agricultural applications. NIR light is also being studied for phototherapy of wounds, but such phototherapy is not yet accepted by mainstream medicine. In a recently developed approach to NIR imaging, researchers continue to pursue X-ray-activated near-infrared persistent luminescent probes for deep-tissue in vivo bioimaging. These new optical probes are free of autofluorescence and provide self-sustained emission after X-ray excitation with a high signal-to-noise ratio. NIRS will continue to be a growing method in medical applications and diagnostics.
REFERENCES


