NIH Workshop 2018: Towards Minimally Invasive or Noninvasive Approaches to Assess Tissue Oxygenation Pre- and Post-transfusion

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ARTICLE INFO

Introduction

Because blood transfusion is one of the most common therapeutic interventions in hospitalized patients, much recent research has focused on improving the storage quality in vitro of donor red blood cells (RBCs) that are then used for transfusion. However, there is a significant need for enhancing our understanding of the efficacy of the transfused RBCs in vivo. To this end, the NIH sponsored a one-and-a-half-day workshop that brought together experts in multiple disciplines relevant to tissue oxygenation (eg, transfusion medicine, critical care medicine, cardiology, neurology, neonatology and pediatrics, bioengineering, biochemistry, and imaging). These individuals presented their latest findings, discussed key challenges, and aimed to identify opportunities for facilitating development of new technologies and/or biomarker panels to assess tissue oxygenation in a minimally-invasive to non-invasive fashion, before and after RBC transfusion.

The workshop was structured into 4 sessions: (1) Global Perspective; (2) Organ Systems; (3) Neonatology; and (4) Emerging Technologies. The first day provided an overview of current approaches in the clinical setting, both from a global perspective, including the use of metabolomics for studying RBCs and tissue perfusion, and from a more focused perspective, including tissue oxygenation assessments in neonates and in specific adult organ systems. The second day focused on emerging technologies, which could be applied pre- and post-RBC transfusion, to assess tissue oxygenation in minimally invasive or noninvasive ways. Each day concluded with an open-microphone discussion among the speakers and workshop participants. The workshop presentations and ensuing interdisciplinary discussions highlighted the potential of technologies to combine global "omics" signatures with additional measures (eg, thenar eminence measurements or various imaging methods) to predict which patients could potentially benefit from a RBC transfusion and whether the ensuing RBC transfusion was effective. The discussions highlighted the need for collaborations across the various disciplines represented at the meeting to leverage existing technologies and to develop novel approaches for assessing RBC transfusion efficacy in various clinical settings.

Although the Workshop took place in April, 2018, the concepts described and the ensuing discussions were, perhaps, even more relevant in April, 2020, at the time of writing this manuscript, during the explosive growth of the COVID-19 pandemic in the United States. Thus, issues relating to maintaining and improving tissue oxygenation and perfusion are especially pertinent because of the extensive pulmonary damage resulting from SARS-CoV-2 infection [1], compromises in perfusion caused by thrombotic-embolic phenomena [2], and damage to circulating RBCs, potentially compromising their oxygen-carrying capacity [3]. The severe end organ effects of SARS-CoV-2 infection mandate even more urgency for improving our understanding of tissue perfusion and oxygenation, improve methods for measuring and monitoring them, and develop novel ways of enhancing them.

General Session I

Keynote: Framing the Challenge: Quantifying Impact of Anemia/Transfusion Upon O_2 Delivery Homeostasis (Allan Doctor, M.D., University of Maryland)

Transfusion decision-making should maintain hemoglobin levels above thresholds that limit O_2 delivery or that impose problematic or unsustainable stress on compensatory physiology. Historically, near-normal values were presumed necessary to achieve this goal, particularly during stress imposed by illness. We now better appreciate (1) the role of RBCs in O_2 delivery homeostasis, beyond simple O_2 transport [4], (2) the extent of physiologic

compensation for anemia (tolerance), (3) the damage to RBCs during storage [5], and (4) the impacts of noninfectious serious hazards of transfusion. As such, conservative transfusion approaches are emerging, based on noninferiority trials of permissive anemia (eg, restrictive hemoglobin-based thresholds) [6]. However, we lack a decision-making structure that identifies specific individuals for whom permissive anemia is unsafe (or, conversely specific individuals meeting transfusion "triggers" for whom transfusion is unnecessary) and guides transfusion timing and amount, based on outcome-linked, O_2 delivery-based targets. Significant knowledge gaps contribute to this lack, because we cannot reliably determine: (1) the onset and severity of inadequate O_2 delivery, or "prefailure" physiology that indicates impending inadequate O_2 delivery that would be prevented by transfusion, (2) the degree to which anemia contributes to inadequate O_2 delivery in patients with complex pathologies, (3) the likelihood that inadequate O_2 delivery will respond to transfusion, (4) the context-specific assessment of transfusion risk/benefit, and (5) the appropriate amount to transfuse, if initiated. Moreover, patients rarely have isolated anemia, and various comorbidities generate unique physiology requiring individualized approaches; examples affecting O_2 delivery include: low cardiac output (ie, heart failure), hypoxia (eg, cyanotic pulmonary or heart disease), vasculopathy (eg, stroke, shock), or altered metabolism (eg, hypothermia, burns, and sepsis).

Although RBC transfusion increases hemoglobin levels and blood O_2 content, it does not necessarily increase tissue O_2 delivery or relieve compensatory physiology, unless blood O_2 content is rate-limiting for transferring O_2 from lung to tissue. Currently, transfusion decisions aim to maintain RBC mass well above a threshold that may critically limit tissue O_2 delivery. Although traditional thresholds are currently being re-evaluated, a comprehensive paradigm shift is emerging that reconsiders the "hemoglobin trigger" strategy itself. Clearly, it is not feasible to define specific hemoglobin goals that account for all the complex developmental, disease-related, and stress-specific situations that complicate anemia. Ideally, transfusion decisions should be based on individual, context-specific considerations of the degree to which anemia contributes to tissue O_2 delivery constraints and/or reserves. This may involve "physiologic triggers" to improve perfusion sufficiency or reserve, rather than to maintain a specific hemoglobin level, irrespective of context [7].

To identify the best means to define and evaluate approaches to transfusion decision-making with patient-specific physiologic targets, several knowledge gaps must be addressed; as examples, there are no data supporting a physiologic goal-directed strategy, no consensus on what goals should be targeted, and no consensus on what thresholds are appropriate for these goals. Therefore, we must improve the current means to assess the functionality of the circulating RBC mass and its relationship to tissue O_2 delivery, an area of investigation ripe for translation [8]. Some potential focus areas include:

a. Studies examining physiologic **tolerance of anemia** in the critically ill.
b. Studies exploring accuracy, precision, and reliability of novel approaches **to quantify and monitor O_2 consumption and delivery.**
   i. Methods might include bedside VO_2 measurement, dynamic near infrared spectroscopy (NIRS) measurements, using nanoparticles that report real-time measurement of tissue O_2 levels or the abundance of biochemical markers of perfusion sufficiency (eg, pH, lactate, reactive oxygen species, cytochrome redox state).
   ii. Integrated approaches using real-time risk analytic engines that evaluate multiple data streams (eg, from monitors, laboratory results, and the electronic health record),

c. Studies exploring accuracy, precision, and reliability of novel approaches to **quantify and monitor "erythron" status** (eg, circulating blood volume; RBC mass, production, and clearance; RBC performance (eg, O$_2$ affinity, deformability, adhesion, vasoactivity).

d. Studies evaluating **transfusion decision making** during critical illness (eg, Bayesian modeling, application of systems dynamics, development of computerized decision support). These should address the issue of sequencing; for example, with the complex pathology commonly encountered in intensive care units, what is optimal timing of RBC transfusion in a therapeutic sequence that targets impaired O$_2$ delivery (eg, when anemia complicates impaired respiratory and cardiovascular performance or contributes to organ-specific threats)?

Global Perspective Session I (Session Chair: Steven Spitalnik, M.D., Columbia University)

Overview of Measuring Tissue Oxygenation in the Clinical Setting (Walter "Sunny" Dzik, M.D., Harvard University)

The basic concept of oxygen extraction as a measure of compensation for decreased oxygen delivery was reviewed. This highlighted the shortcomings of measuring oxygen extraction ratios as a reliable guide for adequate tissue oxygenation in settings of variable oxygen delivery (ie, microvascular dysoxia) and variable tissue oxygen demands. Oxygen extraction measurement is fundamental to several technologies, such as NIRS and blood oxygen level dependent (BOLD) imaging, and the reliance on extraction ratios represents a shortcoming of these techniques. In addition, hemoglobin is not the final molecule in the chain of oxygen delivery to tissues and additional attention should evaluate the role of myoglobin in transferring oxygen from hemoglobin to the mitochondria.

Three methods for measuring tissue oxygenation were reviewed: NIRS, BOLD, and assessment of hypoxia-inducible factor (HIF). In a meta-analysis of papers involving cardiac surgery [9], RBC transfusion decisions guided by NIRS measurements did not significantly improve rates of adverse end-organ clinical outcomes. However, NIRS monitoring did identify improved regional cerebral oxygenation following RBC transfusion in severely anemic children [10].

Because most RBC transfusions for non-bleeding patients aim to increase the reserve of tissue oxygenation, measurements of "compensation to reduced oxygenation" may be useful physiological triggers for RBC transfusion. In addition, some type of "stress test" that uncovers reduced oxygenation reserve may identify the patients most likely to benefit from RBC transfusion. BOLD imaging relies on detecting deoxy-hemoglobin, which has a faster T2 relaxation time due to the paramagnetic properties of iron in deoxyhemoglobin. Although BOLD imaging offers the advantage of deep tissue visualization of oxygen, its disadvantages include measuring hemoglobin saturation (rather than tissue oxygen content) and requiring a magnetic resonance imaging (MRI) instrument.

Although HIF measurements are not yet clinically available to guide transfusion, assessing a tissue's metabolic status with regard to adequate oxygenation would significantly and importantly complement assessing the presence of oxygen itself. Multiple candidate molecules, including HIF, lactate-pyruvate, NAD/NADH, mitochondrial enzymes, and local CO$_2$ production, might serve as biomarkers of locally inadequate tissue oxygenation. Ultimately, the ideal device would target deep tissues of interest (eg, heart, brain, liver, gut, kidney), measure real-time oxygen reserve at the bedside (ie, stress testing), and provide information on the quantity of oxygen present and the metabolic status of the relevant target tissue.

Metabolomics for Studying RBCs and Tissue Perfusion (John Roback, M.D., Ph.D., Emory University School of Medicine)

Although many groups are developing novel devices or imaging modalities to identify hypoxic patients who could benefit from RBC transfusion, this talk focused on RBC and plasma biomarkers that could improve the safety and efficacy of transfusion therapy.

Most published transfusion medicine metabolomics data aim to understand the metabolic changes that occur during RBC storage. However, one can also leverage the availability of RBC units from known good-storing and poor-storing donors to identify metabolic pathways that differ between these two storage phenotypes with the goal of identifying metabolic biomarkers that predict RBC storage behavior. Although the data set of pedigreed donors with these divergent phenotypes in one study was small (ie, 6 good-storing and 4 poor-storing donors), the data were robust and supplied clues to the biology underlying these storage phenotypes. For example, recent findings [11] demonstrated that, although RBC donor units were largely indistinguishable in their post-transfusion effects on the recipient if stored between 7 and 35 days prior to transfusion, dramatic differences were seen at 35 to 42 days of storage, suggesting that there is an inflection point in storage after Day 35, and that RBCs transfused at Day 42 of storage may have entered the steeply descending part of the quality curve. However, RBCs from some poor-storing donors may experience this inflection point earlier in storage, which could be a subject for future research.

Much less published information exists regarding metabolic biomarkers that could improve on the currently used clinical laboratory tests (eg, blood lactate levels [10]), along with clinical features, to identify patients who would benefit from transfusion. Although one informative paper [12] used metabolomics to identify blood biomarkers that increased or decreased after experimental hemorrhagic shock in rats, much more needs to be done, including determining whether transfusions can abrogate the observed metabolic changes.

Overview of RBC Biology in the Transfusion Setting (Narla Mohandas, Ph.D., New York Blood Center)

Much research has focused on the RBC "storage lesion." The described changes include a rapid early decrease in RBC 2,3-diphosphoglycerate levels followed by time dependent decreases in cellular ATP and increases in plasma hemoglobin levels and numbers of microparticles, starting at >4 weeks during storage. Although these changes are highly reproducible, their impact on the function of the transfused RBCs in vivo is not clear. In large part, this is because many of these studies are correlative and not mechanistic.

In addition, the cellular deformability of stored RBCs is normal until they become sphero-echinocytes and spherocytes due to the progressive loss of surface area. These changes occur later in storage (ie, >4 weeks). If the storage lesion is defined as decreased post-transfusion recovery of the donor RBCs, the reduced deformability of stored RBCs due to cell surface area loss and increased sphericity leading to splenic sequestration can account for decreases in 24-hour post-transfusion recovery [13]. Nonetheless, how different extents of loss of deformability influence tissue oxygen delivery by the transfused RBCs still remaining in the circulation is unclear.

Regarding future studies, a clearer definition of the storage lesion is needed that functionally relates to RBC life span and effective oxygen delivery. The biological variability between donors in RBC storage quality could also be important, but addressing this issue requires thoughtful and critically validated study designs. In addition, biological changes in RBCs during storage have been

well documented, but the physiological basis for their reduced survival post-transfusion has not been critically validated. Finally, developing noninvasive or minimally invasive strategies for measuring stored RBC efficacy in delivering oxygen to various tissues in vivo is the most important issue in RBC transfusion research; although this is a challenging problem, integrated, multidisciplinary approaches are likely to be successful.

Organ Systems Session II (Session Chair: Allan Doctor, M.D., University of Maryland)

Tissue-Integrating Sensors for Long-Term, Continuous Monitoring of Interstitial Oxygenation: Use in Limb Ischemia, Trauma/Resuscitation, Respiratory Function, and Brain Hypoxia (Natalie Wisniewski, Ph.D., Profusa)

Currently available wearable/external monitoring tools have faltered in providing clinical-grade, real-time, biochemical data at the tissue level. Tissue-integrating sensor technology may enable wearables to live up to their promise of improving healthcare [14]. These novel biosensors are composed of tissue-like hydrogel scaffolds that reside permanently under the skin and use existing mobile networks to provide real-time continuous wireless biochemical data for remote or cellphone viewing. These soft, porous, oxygen sensing scaffolds, functionalized with luminescent chemistries, perform in vivo for more than 4 years. In this setting, tissue grows in and throughout the porous sensor scaffolds, but avoids a foreign body response, and enables long-term sensing. Oxygen levels (or other analytes that the sensors can detect) trigger concentration-dependent, reversible changes in optical properties. These optical changes occur in the NIR range and are readily detectable through the skin; thus, a NIR detector is placed at the skin surface without needing to insert a fiberoptic probe. After the initial scaffold insertion, measurements are collected noninvasively, allowing long-term longitudinal tracking of tissue hypoxia.

The first such tissue-integrating sensor is approved for use in the European Union for continuous monitoring of tissue oxygen, and is currently seeking FDA approval. These sensors have been used in multiple clinical applications, including critical limb ischemia, trauma models of hemorrhage and resuscitation, pilot hypoxia and other high-altitude applications, wound healing, tumor hypoxia, exercise physiology, sepsis, and stroke models. Sensors remain subcutaneously and cannot be felt or seen, except by a specialty wearable optical reader to visualize them and record their signals. This same tissue-integrating sensor platform is currently being adapted to measure lactate, glucose, carbon dioxide, and various ions and other molecules, with the goal of measuring a continuous "Chem-7" panel that is relayed automatically to a cellphone and the cloud; this would allow remote monitoring and applying artificial intelligence algorithms to guide health and treatment decision-making. Thus, this approach would also be applicable for real-time monitoring of tissue health related to blood transfusion outcomes.

Oxygen Imaging in Tissue by Phosphorescence Quenching (Sergei Vinogradov, Ph.D., University of Pennsylvania)

Phosphorescence quenching relies on exogenous phosphorescent (optical) probes to measure dissolved oxygen [15]. It is accurate and versatile with many current research applications, ranging from simple-to-implement peripheral oxygen measurements with fiber optic oximeters to high-resolution pO_2 microscopy in the brain. Variants exist in which plastics or gels containing phosphorescent dyes are either implanted or laid over the target tissue to provide local oxygenation measurements; oxygen measurements

of the skin can also be made, which potentially correlate with oxygenation of the underlying tissue. Indeed, phosphorescence was effective in assessing the efficacy of RBC transfusion [16]. Key advantages of phosphorescence include that it is exquisitely sensitive to oxygen, is calibration free (with properly designed soluble probes), and provides absolute pO_2 measurements; in addition, such instrumentation can be compact and inexpensive. However, it has low resolution at a depth of several centimeters (similar to NIRS). In addition, for imaging over larger areas, volumetric imaging probes must be dissolved in the interstitial fluid; thus, there is direct contact between the probes and the body, which is similar to other exogenous contrast agents. Nonetheless, a variant of the latter method avoids direct contact of probes with tissues through the use of microscopic oxygen chambers, with the probe solution mounted at the tips of thin optical fibers, which are inserted into the target tissue.

Tissue Oxygenation With Novel Oxygen Carrier Therapeutics: Overcoming Hypoxia in Stroke, Cardiac Ischemia and Brain Tumors (Natacha Le Moan, Ph.D., Omniox)

Oxygen supply and diffusion into tissue are necessary for organ function and survival. Tissue oxygenation is disturbed in pathological situations, including cancer, diabetes, trauma, sepsis, heart disease, wound healing, and stroke. Omniox, a California-based company focused on biotherapeutics that target hypoxia, is developing oxygen carriers to treat diseases in which hypoxia drives poor outcomes [17]. They use direct and indirect methods to evaluate the effect of oxygen carriers on tissue oxygenation in preclinical models of cancer and ischemia. Direct measurements of tissue oxygenation with optical or polarographic sensor probes are often invasive or minimally invasive, which limits continuous and repetitive measurements over long periods of time; thus, they only provide a snapshot of tissue pO_2 in a fixed region. Indirect methods can measure a parameter related to oxygenation in a large tissue area, but do not directly quantify tissue pO_2. These indirect methods often use endogenous and/or exogenous markers of tissue oxygen content that can be detected by ELISA, immunohistochemistry, flow cytometry, or imaging techniques. Each method has advantages and disadvantages in sensitivity, specificity, reproducibility, and clinical applications. Thus, there is an important need to develop new approaches to assess oxygen levels continuously and repetitively in tissue using noninvasive or minimally invasive tools. These would be applicable in various clinical settings for diagnosing and managing hypoxic and ischemic disorders.

Tissue Oxygenation in the Kidney: Lessons From BOLD MRI (Stephen C. Textor, M.D., Mayo Clinic)

Renal circulation is complex, reflecting its roles in blood filtration, solute reabsorption, and blood pressure regulation. Although the cortical region is over-perfused with oxygenated blood, deeper medullary segments normally receive less blood perfusion and consume oxygen as a function of energy-dependent solute transport [18]. Hence, some regions function normally at hypoxic levels, as reflected by near complete transition to deoxygenated hemoglobin on the steep segments of the hemoglobin saturation curve. BOLD MRI uses the paramagnetic properties of deoxyhemoglobin to characterize local tissue oxygenation noninvasively; in addition, it does not require exogenous contrast agents. Studies using oxygen-sensing probes confirm the magnitude of changes during various maneuvers, including vascular occlusion, ureteral obstruction, and inhibition of solute transport. Experimental studies with lysed RBCs and varying hematocrit confirm that magnetic relaxation kinetics are affected by proximity to cell membranes,

depending in part on the magnetic field strength. Research applications require attention to (patho)physiologic determinants of oxygenation, including sodium balance, water intake, diuretics, antihypertensive medications, and hematocrit [19]. Multiple analytic strategies exist, ranging from regions-of-interest, fractional tissue oxygenation, and computer-generated concentric objects within coronal sections. Studies in experimental animals and human subjects under controlled conditions demonstrated changes in kidney oxygenation associated with vascular occlusive disease, contrast administration, revascularization, and angiogenic maneuvers using mitochondrial protection, mesenchymal stromal/stem cell infusion, and vascular endothelial growth factors. Taken together, BOLD MRI provides reproducible, noninvasive estimation of tissue oxygenation in vivo in vascular tissues, such as the kidney, in humans.

Wearable, Paintable, and Conformal Transcutaneous Oxygenation Sensors for Real-Time pO₂ Quantification and Imaging (Conor L. Evans, Ph.D., Massachusetts General Hospital)

Wearable, Paintable, and Conformal Transcutaneous Oxygenation Sensors for Real-Time pO_2 Quantification and Imaging (Conor L. Evans, Ph.D., Massachusetts General Hospital)

A new set of pO_2 imaging technologies was discussed for quantitative transcutaneous oxygenation measurements and their potential for sensing tissue oxygenation changes related to transfusion. The motivation for developing these oxygen sensors came from problems faced by wounded warriors and their caregivers, who required noninvasive, nondisruptive tools to visualize and quantify tissue pO_2 during surgery and throughout recovery. Several technologies were developed to address this, from perfusion imaging tools to TcPO₂ devices, but none were suitable. To address this, we initially developed a conformal, paintable, and sprayable material that changes color quantitatively in response to oxygen. This technology uses porphyrin molecules in which red emission is turned-off, or "quenched," by oxygen. By pairing these with green-emitting dyes, a wearable bandage material was created that changed from green, to yellow, to red with decreasing oxygenation. Precise visual readout of tissue oxygenation is measured by the material's green-to-red emission ratio using common imaging equipment, such as point-and-shoot and smartphone cameras.

These wearable sensors were evaluated using various animal models, including porcine skin burn, skin graft, and skin inflammation models, and mouse noncompressible trunk injury models. To overcome the weak emission properties of commercial porphyrins, a new class of ultrabright, "clickable," porphyrin molecules were developed that are readily functionalized for oxygen sensing applications [20,21]. A paintable bandage formulation of these new sensors was tested in a successful clinical trial of deep epigastric flap breast reconstruction surgery in post-mastectomy breast cancer paints. The rate change of tissue pO_2 of this thin film oxygen sensor was found to correlate well with StO_2 rate changes.

Neonatology Session III (Session Chair: Naomi Luban, M.D., Children's National Health System)

Oxygen Saturation Targets in Neonatal Patients (Waldemar A. Carlo, M.D., University of Alabama at Birmingham)

Many critically ill neonates need oxygen supplementation to survive and reduce their risk of serious morbidity. However, optimal tissue oxygenation in neonates has been elusive. Data before the recent oxygen saturation targeting trials suggested that oxygen toxicity in preterm infants may increase the risk for retinopathy of prematurity, bronchopulmonary dysplasia, cerebral palsy, and death. Several contemporary observational studies suggested that targeting a lower oxygen saturation may reduce death, retinopathy of prematurity, and bronchopulmonary dysplasia. These and other studies suggested that oxygen saturation targeting below 90% would safely improve outcomes.

Multicenter randomized clinical trials were conducted to determine if lower oxygen saturation targets (ie, 85-89%) would improve outcomes, as compared to higher oxygen saturations (91-95%) in extremely preterm infants (24-27 weeks at birth). The first was the Surfactant, Positive Pressure, and Pulse Oximetry Trial (SUPPORT) [22], and other trials were similarly designed. Meta-analyses, including 4965 infants, indicated that targeting a higher oxygen saturation decreased mortality and necrotizing enterocolitis. Even though higher oxygen saturation targeting led to more retinopathy of prematurity, the rates of blindness were not affected [23].

However, arterial oxygen saturation does not necessarily correlate well with tissue oxygenation. As such, NIRS is increasingly used as a noninvasive tool for continuously monitoring tissue oxygenation and hemodynamics, particularly in the brain. In a multicenter randomized clinical trial of extremely preterm infants, targeting cerebral hemoglobin oxygen saturation in the 55% to 85% range decreased the burden of hypoxia by ~70% with trends for reduced all-cause mortality and brain injury; the latter defined as intracranial hemorrhage, white matter abnormalities, ventricular dilation, and cerebral atrophy.

Ultrasound Imaging of the Human Placenta: A New Approach to the Diagnosis of Fetal Health (Alfred Abuhamad, M.D., Eastern Virginia Medical School)

Abnormal placental development in early gestation is associated with various maternal and fetal adverse outcomes, including preeclampsia, preterm labor, and fetal growth restriction. In addition, altered placental structure in early gestation produces placental dysfunction later in pregnancy, manifested by maternal and fetal disease. Alterations in placental structure include changes in placental biometry, tissue density, calcium content, and vascular supply. Abnormalities in placenta shear wave elastography also correlate with placental dysfunction, especially in diseased states, such as preeclampsia.

Ultrasound is the optimal imaging modality for placental evaluation in early gestation, because it is noninvasive, applied in real time, widely available, and relatively inexpensive. Furthermore, ultrasound is relatively safe during pregnancy because it does not involve ionizing radiation. Novel ultrasound tools, such as elastography, calcium contact, and microvascular assessment, can be applied in early gestation for noninvasively evaluating the human placenta. These ultrasound findings can be correlated with nonoptimal pregnancy outcomes, collectively defined as delivery at <37 weeks gestation for any indication. Preliminary data suggest a correlation between alterations in placental vascular structure and function and complications later during pregnancy. One main goal is to combine ultrasound findings and maternal biomarker measurements in early gestation to create a placental index that predicts pregnancy outcomes later in gestation [24].

Keynote: Controversies in Red Cell Transfusion in Preterm Infants: Opportunity for Tissue Oxygenation Monitoring (Ravi Mangal Patel, M.D., M.Sc., Emory University School of Medicine)

Preterm infants routinely receive RBC transfusions to improve oxygen delivery. Hemoglobin thresholds, which do not necessarily reflect adequate tissue oxygenation, often guide these transfusions. Therefore, the effect of conservative versus liberal RBC transfusion thresholds may differ for individual patients based on clinical context (eg, surgery, sepsis, degree of prematurity, and postnatal age) and adequacy of oxygen delivery in relation to oxygen consumption. Determining tissue oxygenation adequacy and related effects of RBC transfusion and anemia are relevant for key clinical outcomes, such as neurodevelopmental impairment and necrotizing

enterocolitis. In addition, few studies considered donor RBC and recipient factors in assessing the effects of transfusion on tissue oxygenation. NIRS enables continuous, noninvasive monitoring of regional tissue oxygenation, including mesenteric, renal, and brain tissues. It may be useful in guiding the need for, and response to, RBC transfusion in preterm infants [25]. However, NIRS use is limited by a paucity of studies evaluating its relationship to regional oxygen saturation measurements and relevant clinical outcomes. In addition, assessing important, but infrequent, outcomes requires large, costly, cohort studies with longitudinal measurements. Furthermore, methods for analyzing tissue oxygenation data that appropriately account for signal dropout, signal below or above detection limits, and variability in tissue oxygenation parameters, are not commonly used [26]. Study designs involving longitudinal measures across multiple RBC exposures could allow for understanding "within patient" variation in transfusion responses that could be related to variations in donor characteristics (eg, sex, age), product characteristics (eg, storage age, irradiation), or time-varying recipient characteristics (eg, anemia severity). Such studies could improve our understanding of optimal RBC product characteristics or identify patient factors that influence responses to transfusion and how these responses may relate to important clinical outcomes.

Emerging Technologies Session IV (Session Chair: Steven Spitalnik, M.D., Columbia University)

Breezing: A Noninvasive Device to Measure Overall Metabolism (Erica Forzani, Ph.D., Arizona State University)

Calorimeters can measure oxygen consumption rate (VO_2) and carbon dioxide production rate (VCO_2) using the indirect calorimetry method. This uses the gas exchange rate of oxygen and carbon dioxide to assess the energy produced by the individual performing the test at rest, known as Resting Energy Expenditure (REE) or Resting Metabolic Rate [27].

A novel indirect calorimeter device uses a single sensor chip allowing detection of both gases, which previously required electrochemical and infrared sensors. This Breezing sensing technology was validated against the "gold standard" method (ie, the Douglas Bag) and demonstrated near 100% accuracy.

Measurements are typically performed under resting conditions in a quiet environment, and assessing REE enables one to determine the nutritional needs for patient interventions related to weight management [28]. In addition, REE and the parameters associated with detecting VCO_2 and VO_2, such as exhalation rate (L/min), breathing frequency (breath/min), and end-tidal volume (mL), also indicate respiratory state and are useful for diagnosing pulmonary disease. Furthermore, indirect calorimetry can be used before and after exercise, dialysis, and meal supplements to evaluate the impact of these interventions on metabolic function. This approach could also be potentially useful for evaluating the physiological effects of RBC transfusions on overall metabolic function.

Role of NIRS to Guide Medical Care and Assess Transfusion Efficacy (Elliott Bennett-Guerrero, M.D., Stony Brook University School of Medicine)

NIRS, introduced in 1977 and commercially available for ~20 years, uses near infrared light (~700-900 nm) to detect the weighted average of hemoglobin saturation in arterial and venous blood in a small region under the sensor. This contrasts with pulse oximetry, which solely measures arterial oxygen saturation and venous oxygen saturation (ie, SvO_2 of pulmonary artery or "mixed venous" blood). Most clinical applications of NIRS in adults focus on cerebral and peripheral tissue monitoring of "tissue oxy-

genation" in surgical, intensive care, and other high risk hospitalized patients. Common sites for peripheral measurements, with or without a vascular occlusion test, are the hand (ie, the thenar eminence), forearm, and calf muscles.

NIRS has several possible clinical roles and could potentially aid in testing RBC transfusion "quality" [29]. For example, cerebral NIRS can detect unilateral cerebral ischemia in cardiac and vascular surgery, where rapid detection and correction of flow imbalances due to surgical manipulation can be lifesaving. NIRS may also have a role in assessing oxygenation/perfusion of flaps after reconstructive plastic surgery. Chronic nonhealing wounds heal better if oxygenation/perfusion can be optimized; therefore, NIRS measurements could potentially help in that setting. Several studies emphasized the potential for NIRS as an early warning indicator of impending decompensation from bleeding (eg, in surgery and trauma). In addition, NIRS might help optimize global oxygen delivery in critically ill patients. Finally, there is interest in using NIRS to do "spot checks" (ie, surveillance) in lower risk acute care patients who might have overt tissue hypoperfusion despite adequate arterial oxygen saturation.

In several studies, NIRS documented increased tissue oxygenation after RBC transfusion. However, increases in tissue oxygenation are usually subtle in patients who are not suffering from a critical level of anemia. Therefore, it is not clear what role NIRS should have in guiding whether a transfusion should be administered or in determining the efficacy of that transfusion. It is challenging to find patients with profound tissue hypoxia/anemia (eg, a hemoglobin of ~3 g/dL), where transfusion of multiple units of RBCs would dramatically change tissue oxygenation [10]. Nonetheless, inducing transient global hypoxia in healthy volunteers is possible; this was shown to be safe and, as such, NIRS could potentially be used in this context to assess RBC quality rigorously.

Multiscale Photoacoustic Tomography of Tissue Oxygen (Lei Li, Ph.D., California Institute of Technology, presenting on behalf of Lihong V. Wang, Ph.D.)

The life sciences and modern medicine can benefit from imaging technologies that bridge microscopic insights to macroscopic observations. Photoacoustic tomography (PAT) provides multiscale imaging over a range of spatial scales, including organelles, cells, tissues, whole bodies of small animals, and human organs [30].

PAT is a hybrid imaging technique that combines optical and acoustic energy via the photoacoustic effect, a natural phenomenon that converts absorbed photons into acoustic waves. PAT inherits the advantages of both optical imaging and ultrasound imaging. PAT directly detects acoustic waves induced by the excitation photons, regardless of whether they are ballistic photons or scattered/diffused photons; thus, PAT achieves far greater penetration than optical microscopy. More importantly, acoustic waves are much less scattered inside biological tissues (ie, ~3 orders of magnitude weaker than optical scattering on a per unit path length basis); therefore, PAT provides much higher spatial resolution in deep tissue (ie, from 1 mm up to several centimeters) than pure optical imaging technology.

PAT is sensitive to optical absorption and reveals anatomical, functional, metabolic, histological, and molecular information about biological tissues at high imaging speed. Taking advantage of the absorption spectral signatures of oxyhemoglobin and deoxyhemoglobin, PAT can map detailed blood oxygen saturation in mouse brains, mouse internal organs, and human finger cuticles [31].

Label-free functional imaging of single RBCs in vivo has the potential to uncover the fundamental mechanism of oxygen metabolism in cells. Photoacoustic microscopy (PAM) can noninvasively image oxygen delivery from single flowing RBCs in vivo with millisecond-scale temporal resolution and

micrometer-scale spatial resolution. Multiple single-RBC functional parameters, including total hemoglobin concentration, oxygen saturation, flow speed, oxygen release rate, and metabolic rate of oxygen, can be quantified simultaneously in real time, which enables numerous biomedical studies and clinical applications.

How to Measure the Effectiveness of Therapy by Measuring Oxygenation of the Target Tissues (Harold Swartz, Ph.D., Dartmouth University)

Because RBC transfusion typically aims to provide adequate oxygenation, evaluating the quality of RBC preparations should include direct demonstration of their effectiveness in improving target tissue oxygenation [32]. This should be done in both preclinical studies and human patients. Evaluating efficacy requires repeated direct measurements of oxygen in target tissues, the ability to measure continuously over clinically relevant periods of time, and repeating these measurements on multiple days. It is not sufficient to measure oxygen content in the circulation because it does not account for potential barriers to full equilibrium with cells in target tissues. Measurements in the circulation also do not account for the balance between oxygen delivery and consumption in target tissues. Therefore, it is desirable to make direct and repeated measurements of oxygen in target tissues to determine transfusion efficacy in each patient.

Electron paramagnetic resonance (EPR) oximetry can make direct and repeated measurements of tissue oxygen in both preclinical models and human subjects [33]. This involves a one-time, minimally invasive, injection of appropriate paramagnetic materials into the target of interest. Subsequently, oxygen measurements can be made noninvasively as frequently as desired, including continuous and repetitive measurements at any desired time interval.

Using EPR in vivo, oxygen was extensively and successfully measured in virtually all tissues in animals, ranging from mice to pigs. In the last 15 years, EPR studies in vivo were also extended to humans, with EPR dosimetry and oximetry measurements made at several institutions in the United States, Korea, Japan, and Belgium, confirming that EPR measurements in vivo can be made continuously and repeatedly. India Ink is an especially useful oxygen sensor, because it is minimally invasive (a simple needle stick, leaving a subcutaneous spot of India Ink similar to a tattoo) and the FDA determined that no special permission is needed for its use in human subjects. Therefore, this could be used clinically to monitor the effectiveness of an intervention to raise tissue pO_2 initially, and then remeasure it whenever necessary. The apparatus required is simple and can be operated by minimally trained individuals with a skill level similar to that for measuring blood pressure. EPR oximetry may also be used to determine when more widely available, but less intrinsically specific techniques (eg, MRI or positron emission tomography), can provide the desired information regarding tissue oxygenation.

Clinical Trial on Tumor Oxygenation Using EPR Oximetry With OxyChip (Periannan Kuppusamy, Ph.D., Dartmouth Geisel School of Medicine)

We developed an EPR oximetry-based method to measure absolute values of tissue oxygen (pO_2) in clinical settings. It uses a paramagnetic oxygen sensor (OxyChip) containing LiNc-BuO microcrystals in a biocompatible polymer matrix [34]. In preclinical studies, OxyChip made direct and repeated pO_2 measurements for a year or longer, without toxicity or changes in sensitivity. In clinical studies, OxyChip safely and effectively measured tumor pO_2 in cancer patients undergoing surgery, radiation, or chemotherapy. To this end, following implantation into tumor tissue within 20 mm of the skin surface, pO_2 measurements were performed noninvasively using an external radiofrequency coil at 1.2 GHz. Repeated pO_2 measurements were made for weeks/months after OxyChip implantation, with values ranging from severely hypoxic (~1 mm Hg) to normal (>20 mm Hg). Tumors had variable responses to 100% O_2 via a non-rebreathing face mask, with most showing a positive response to hyperoxyia intervention, whereas some showed no or negative responses. We also developed OxyChip as an adhesive film for noninvasive measurement of transcutaneous oxygen ($TcpO_2$) levels, to evaluate blood perfusion in tissue immediately beneath the skin. These data are valuable in assessing wound healing, diagnosing peripheral vascular/arterial insufficiency, and predicting disease progression or response to therapy. In a cohort of healthy human subjects, repeated $TcpO_2$ measurements were robust, reliable, and reproducible, with 7.8 ± 0.8 to 22.0 ± 1.0 mm Hg in the volar forearm ($N = 29$) and 8.1 ± 0.3 to 23.4 ± 1.3 mm Hg in the foot ($N = 86$). Thus, OxyChip can address a clinically relevant need, enabling reliable and repeated pO_2 measurements in tissues. This approach will support development and optimization of hypoxia modifiers to improve treatment outcomes.

Low Field Magnetic Resonance Imaging of Tissue Oxygen In Vivo: Metabolic and Physiologic Imaging Biomarkers of the Tumor Microenvironment to Guide Treatment in Tumor Bearing Mice (Murali Krishna Cherukuri, Ph.D., Radiation Biology Branch, National Cancer Institute, NIH)

The tumor microenvironment in solid tumors is characterized by regions of poor perfusion, hypoxia, and low pH. Biochemically, tumor cells, both in vitro and in vivo, display the aerobic glycolysis phenotype. Imaging techniques providing biomarkers reporting on these features would be useful in providing diagnostic/prognostic information and for developing appropriate treatments based on *a priori* information about the tumor microenvironment.

Data describing the metabolic and physiologic microenvironments of three tumor xenografts in mice were shown using EPR imaging, to noninvasively provide quantitative pO_2 maps, and metabolic MRI using hyperpolarized ^{13}C-labeled pyruvate, to provide biochemical profiling [35]. These tumor-bearing mice were treated with radiation therapy or hypoxia-activated prodrugs to evaluate the potential for the imaging biomarkers to predict treatment response. These data support the ability of these modalities to predict differences in the benefit of oxygen-dependent antitumor treatments in individual pancreatic tumor cell lines, which may help choose the best treatments for patients with pancreatic cancer. Three preclinical models using hyperpolarization MRI, with plans for clinical translation, were also presented: (1) glycolysis assessment as a biomarker for graft versus host disease; (2) in vivo pharmacodynamic assessment of a lactate dehydrogenase A inhibitor; and (3) monitoring the transition from low-grade glioma to glioblastoma in isocitrate dehydrogenase-1 mutant cells.

Conclusions

In conclusion, the Symposium brought together a highly diverse group of participants to discuss multiple perspectives about tissue perfusion and oxygenation, a relatively understudied area that was identified as a research priority during the 2015 Transfusion Symposium [36]. The presentations and discussions highlighted important research needs and challenges, and identified various opportunities; these are discussed in more detail below.

Research Needs and Challenges

The general consensus was that minimally invasive or noninvasive methods to measure tissue oxygenation accurately are needed

(1) pretransfusion to predict who would benefit from RBC transfusions, and (2) post-transfusion to determine if the patient actually did benefit from this intervention. These methods are also required to enable testing the differential effects of various RBC products with specific characteristics (e.g., whole blood versus packed RBCs). The discussion built on a previous initiative to develop assessment tools to evaluate dynamic changes in microvascular blood flow and tissue oxygenation in clinical research applications.

The presentations and panel discussions identified multiple promising biomarkers and assessment tools that could be applied clinically to assess tissue oxygenation pre- and post-transfusion. These included:

- NIRS as a noninvasive tool for continuously monitoring tissue oxygenation and hemodynamics, particularly in the brain. Nonetheless, rigorous testing in clinical studies is required to determine whether it can guide transfusion therapy to optimize care and improve outcomes.
- BOLD imaging to provide deep tissue visualization of oxygen delivery. However, it relies on measurements of hemoglobin saturation (rather than tissue oxygen content) and requires an MRI instrument. Nonetheless, it can provide reproducible, noninvasive estimation of tissue oxygenation in vascular tissues (eg, kidney).
- Multiple candidate molecules, including HIF, lactate-pyruvate, NAD/NADH, mitochondrial enzymes, and local CO_2 production, that might provide biomarkers of local tissue oxygenation. Although HIF measurements are not yet clinically available to guide transfusion therapy, assessing the metabolic status of the tissue response to oxygenation may be an important complement to measuring oxygen itself.
- PAM, a noninvasive, label-free method to image oxygen delivery from single flowing RBCs in vivo. It has millisecond-scale temporal resolution and micrometer-scale spatial resolution. Multiple single-RBC functional parameters can be quantified simultaneously in real time, suggesting its potential for clinically evaluating transfusion efficacy.
- EPR oximetry for direct and repeated measurements of tissue oxygen. This involves a one-time, minimally invasive injection of appropriate paramagnetic materials into an area of interest. Subsequently, oxygen measurements can be made noninvasively and repetitively, including continuously. Therefore, it could repetitively monitor transfusion efficacy in raising tissue pO_2 in clinically relevant settings.
- Oxygen tension (pO_2) imaging and sensing technologies to provide quantitative transcutaneous oxygenation measurements with potential applicability to sense transfusion-induced changes in tissue oxygenation. Ultrabright, "clickable" porphyrins, which can be functionalized for oxygen sensing applications, have now been used in clinical trials to measure transcutaneous pO_2.
- Novel ultrasound tools, such as elastography, calcium contact, and microvascular assessment. These can be applied in early gestation for noninvasively evaluating human placenta, including local tissue oxygenation.
- Phosphorescence quenching relies on the ability of nonbound, freely diffusing, dissolved oxygen in biological tissues to quench exogenous, phosphorescent, molecular probes. Because these measurements depend on phosphorescence decay time, as opposed to intensity, they are independent of local probe concentrations.
- Biosensors of tissue-like hydrogel scaffolds can permanently reside subcutaneously and use existing mobile networks to provide real-time, continuous, wireless, biochemical data for remote viewing. Functionalized soft, porous oxygen sensing scaffolds with luminescent chemistries were active in vivo for

years. The first such sensor is approved for use in the European Union and is awaiting FDA approval.

Research Opportunities

The following represent specific research opportunities:

- The ideal device would target deep tissues of interest (eg, heart, brain, liver, gut, kidney), provide real-time bedside assessment of oxygen reserve (ie, stress testing), and measure target tissue oxygen quantity and metabolic status.
- Light-based reporter molecules that penetrate deeply are desirable because measuring oxygen levels just beneath the skin does not provide meaningful tissue oxygen status of vital organs. Although current MRI-based methods can overcome this limitation, they require an MRI scanner, making it less useful for transfusion decisions. Nonetheless, a miniaturized, bedside, MRI scanner could be valuable.
- Transfusion efficacy would best be evaluated by simultaneously measuring SpO_2, pCO_2, pO_2, bicarbonate, noninvasive oxygen consumption (VO_2), carbon dioxide production (VCO_2), and lactate production; the availability of decision support tools to evaluate these data in an integrated way could enhance clinical decision making.
- Although most existing methods determine oxygen concentration, other parameters, such as measuring acidosis via carbon dioxide and lactic acid, are important. Thus, noninvasive methods that measure gas exchange (eg, indirect calorimetry), could determine oxygen consumption efficacy and elimination of carbon dioxide.
- New imaging detection methods, chemical sensors, photoacoustics, and metabolomic measurements of tissue oxygenation could be useful for assessing transfusion efficacy.
- Physical methods (eg, magnetic, photoacoustic, and infrared) are generally non-invasive, but have limited signal penetration depth. Although PAM is promising, further study is required to test its applicability to humans.
- Most noninvasive imaging methods detect tissue oxygenation by assessing hemoglobin saturation. Tools to measure tissue pO_2 directly would be useful for providing spatial and temporal information regarding the effects of oxygen modifying agents.
- Oxygen sensing probes that are accurate in low oxygen ranges would be useful. Most such probes have good sensitivity over 10 mm Hg, but few are sensitive at 0.1 to 5 mm Hg; the latter range of tissue hypoxia is where it is important to evaluate the effects of oxygenation agents.
- Studies assessing tissue oxygenation responses to RBC transfusion would be strengthened by evaluating donor RBC characteristics, using information from the supplier (eg, age, gender, storage duration, and irradiation) and biomarker assessment of the unit itself (eg, metabolomics).
- Limited data exist regarding metabolic biomarkers that could improve on currently available blood tests (eg, lactate), along with clinical features, to identify patients who would benefit from RBC transfusion. Research areas could include metabolomic analysis of the hypoxic patient:
  ○ To identify serum- and/or RBC-based biomarkers to categorize patients better regarding who would, or would not, benefit from transfusion
  ○ To determine the extent to which these biomarkers (and the underlying physiology) can be corrected by transfusion, and to identify therapeutic requirements (eg, when to transfuse, the transfusion goals, whether RBC quality affects whether transfusions correct underlying pathophysiology)
- To evaluate transfusion efficacy, markers of short-term and long-term tissue well-being after transfusion are required.

- Hemoglobin threshold-driven RBC transfusion practices in preterm infants currently do not incorporate measuring tissue oxygenation. Both donor and recipient factors should be considered to assess the effects of transfusion on tissue oxygenation.
- Current NIRS methods do not integrate data on systemic arterial oxygen saturation or blood flow, limiting understanding of the underlying causes of changes in tissue oxygen saturation.
- Study designs accounting for longitudinal measures across multiple RBC exposures could provide understanding of within-patient variation in transfusion responses that may relate to donor characteristics (eg, RBC storage age, donor sex and age) or time-varying recipient characteristics (eg, anemia severity).
- Multidisciplinary research programs, crossing multiple topical areas, could bring ideas together in a set of clinical studies where hypotheses and data could be addressed from various perspectives. Focusing on a specific patient population would narrow the scope and allow testing of specific ideas. Two important populations are models are neonates and patients with sickle-cell anemia. A consortium approach would enable testing multiple hypotheses by pooling scientific and technological expertise and resources, including donor RBC quality in vitro, donor RBC quality post-transfusion (i.e., in vivo), and deep tissue oxygenation post-transfusion with correlation to peripheral oxygenation.
- Opportunities for smaller cross-disciplinary studies were also discussed, including evaluating multiple tools simultaneously to compare and contrast measurements of blood oxygen saturation and tissue perfusion post-transfusion.

Author Contributions

All authors participated in the meeting, contributed talks, and prepared the initial drafts of the sections identified with them. Drs. Ochocinska, Glynn, and Spitalnik compiled and edited the texts, wrote the initial drafts of the Introduction and Conclusions, and organized the overall manuscript. All authors read and approved the final draft.

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Declaration of Interest

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