New methods for monitoring dynamic airway tissue oxygenation and perfusion in experimental and clinical transplantation

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Submitted 16 May 2012; accepted in final form 20 September 2012

Khan MA, Dhillon G, Jiang X, Lin Y, Nicolls MR. New methods for monitoring dynamic airway tissue oxygenation and perfusion in experimental and clinical transplantation. Am J Physiol Lung Cell Mol Physiol 303: L861–L869, 2012.—The Papworth Hospital Autopsy study revealed that an additional attrition of periairway vessels is associated with the development of chronic rejection, otherwise known as the bronchiolitis obliterans syndrome (BOS). Experimental studies subsequently demonstrated that airway vessels are subject to alloimmune injury and that the loss of a functional microvascular system identifies allografts that cannot be rescued with immunosuppressive therapy. Therefore, surgical and medical strategies, which preserve the functionality of the existent vasculature in lung transplant patients, may conceivably limit the incidence of BOS. Given these unique anatomic and physiological considerations, there is an emerging rationale to better understand the perfusion and oxygenation status of airways in transplanted lungs. This article describes novel methodologies, some newly developed by our group, for assessing airway tissue oxygenation and perfusion in experimental and clinical transplantation.

IN LUNG TRANSPLANT RECIPIENTS, bronchial artery revascularization surgery is usually not performed, and the pulmonary artery circulation is presumably the principal source of operational circulation in these allografts (17). Possibly for this reason, our group recently found that lung transplant airway tissue is relatively hypoxic in the first year following transplantation (5). Additionally, normal-appearing airways located immediately adjacent to chronically rejected airways exhibit a dropout in microvessels, suggesting a relationship between microvascular rarification and subsequent BOS (13, 14). Thus lung transplant airways start off with a compromised circulation that may become further compromised just before the onset of BOS.

We have studied the relationship between microvascular loss and subsequent airway fibrosis in orthotopic tracheal transplants (OTTs), which are functional grafts through which mice can breathe (1, 9, 10, 16). Although these allografts do not develop bronchiolitis obliterans, a pathology of terminal bronchioles, they do develop lymphocytic bronchitis (a large airway precursor of BOS) (19). Findings from the OTT model have been cautiously extrapolated to explain how airways become fibrotic in BOS terminal bronchioles (1, 9, 12, 16). Our group discovered that the loss of a functional microcirculation identifies all allografts that cannot be rescued from fibroproliferative remodeling (1). Functional microcirculation in this context is defined as the presence of perfused microvessels identified by injecting, before death, transplant recipients with fluorescein isothiocyanate (FITC) conjugated to lectin (FITC-lectin). FITC-lectin binds endothelial carbohydrates and effectively reveals all perfused blood vessels. (7). This technique shows all microvessels in continuity with the systemic circulation and is visualized by confocal microscopy of whole mount sections. We subsequently reasoned that, with frank ischemia, it should also be feasible to assess oxygenation and airway perfusion (Qaw) with emerging bioprobe technologies. The advantage of these new approaches is that they do not require that the animal be killed when making each assessment, and they can be also be used in humans. These new protocols also facilitate serial physiological evaluations of airway oxygenation and Qaw. Furthermore, the rationale for pursuing serial examinations made increasing sense, as there appeared to be a clear temporal pattern of vascular loss followed by vascular reinvestment, a pattern that had clear pathological consequences. Concomitantly, we also realized that such an approach should be possible in clinical lung transplantation, where the issues of microvascular loss and BOS had already been raised (13, 14). For this reason, we developed a new protocol to assess airway oxygenation in humans (5). This article presents, in detail, the FITC-lectin technique method adapted for use in the OTT model and describes the methods of several new techniques developed by our group to assess airway oxygenation and new applications of established methods to gauge Qaw in preclinical and clinical transplant studies.

MATERIALS AND METHODS

Orthotopic tracheal transplantation. All animal studies are first approved by the Palo Alto Veterans Administration Health Care System Institutional Animal Care and Use Committee. Tracheal transplants are performed according to our established procedure as described previously (16). All mice (4–6 wk) were purchased from Jackson Laboratory. C57BL/6 (B6;H-2b) mice are transplanted with C57BL/6 (syngeneic) or major histocompatibility complex (MHC)-mismatched BALB/c (H-2b) mice (allogeneic). After transection of the recipient trachea, the donor trachea is sewn in with 10–0 nylon sutures, and the overlying skin is closed with 5–0 silk sutures. After the procedure, mice are given buprenorphine (0.1 mg/kg sc) for postoperative analgesia and baytril (5 mg/kg sc) for postoperative antibiotics.

Lectin-binding assay to detect functional microvasculature. This method was originally provided to our group by Dr. Donald McDonal (UCSF) and adapted to OTT experiments (6). Tomato lectin...
(Lycopersicon esculentum) binds to the surface N-acetyl glucosamine oligomers of endothelial cells lining perfused vessels and delineates the microvasculature of transplants (6). This methodology is as follows: 1) 100 μl of 1 mg/ml FITC-conjugated Lycopersicon esculentum, tomato lectin (Vector Laboratories) is injected into the inferior vena cava of an anesthetized animal over 1 min. 2) After 3–5 min in systemic circulation, sternotomy is performed, and the transcardiac perfusion is performed by cannulating aorta via the left ventricle with an 18-gauge angiocatheter and perfused with 1% para-formaldehyde at the flow rate of 2 ml/min for 3–5 min using a peristaltic pump (Fisher Scientific). 3) The orthotopic tracheal graft, along with the adjoining recipient trachea, is then dissected from the surrounding tissues. The midanterior trachea is then cut in the center of C ring with a scalpel along the sagittal plane. With this approach, the membranous portion of the trachea is not transected, and the trachea is subsequently opened like a postage stamp. 4) The whole trachea mount is then pressed gently to expose the two-dimensional view by restraining all four ends using micro needles for 30 min. 5) After the opened trachea is stabilized in this manner, it is placed on a glass slide in Vectashield H1000 anti-quench mounting medium (Vector Laboratories) with a heavy coverslip (Fisher Scientific) placed over the tissue. 6) FITC-Lectin-perfused whole trachea mounts are then analyzed by the LSM 510 META confocal scanning microscope (Carl Zeiss) (×20). These mounted tracheas provide a clear planar view, and it is relatively easy to quantify microvasculature in different study groups by ImageJ software.

**Serial tissue PO2 monitoring by fluorescence quenching technique.** Optical fluorescence technology has been utilized to develop unique oxygen sensors (OxyLab pO2 system by Oxford Optronix UK) for quantitative monitoring of oxygen in tissue, physiological fluids, cell cultures, and in vitro samples. These sensors work on the principle that the presence of oxygen in tissue fluids can quench light emitted by a luminescent platinum dye. Light quenching is proportional to the PO2 in the vicinity of the dye. The OxyLab pO2 system consists of a portable monitor and probes. Each probe is comprised of a single optical fiber (Fig. 1, A and B) that conducts light from the monitor to the sensing tip, which contains the luminescent platinum dye held in a polymer matrix. When the dye is illuminated by source light from monitor, it luminesces. This signal is recorded by the monitor as luminescence decay time, and the calculated PO2 data are presented in units of mmHg. Optical fluorescence technology provides superior sensitivity across the physiologically relevant PO2 range (0–100 mmHg).

We have developed a technique to assess PO2s in OTTs (1, 9, 10). Our microsurgery operation platform is demonstrated in Fig. 2A. The detailed procedure is as follows: 1) With the grafted mouse under general anesthesia, tracheal grafts are carefully exposed using stay sutures to gently retract the strap muscles, and a 23-gauge needle is used to puncture a small hole in the anterior wall of the trachea. 2) The probes are connected to either the OxyLab PO2 for oxygen tension assessments or OxyLab laser Doppler flowmetry (LDF) monitor (Oxford Optronix) for perfusion monitoring (Fig. 2B). Both monitors provide a continuous digital readout of PO2s or blood perfusion units (BPU)s, respectively. The PO2 assessments have a detection range between 0–100 mmHg and an output resolution of 0.05 mmHg. The perfusion assessments with this system have a range between 1–10,000 BPU/s and an output resolution of 0.1 BPU/s. 3) A fiber optic PO2 probe is connected to a micromanipulator and inserted carefully through this hole at a 45° angle to contact the epithelium of the opposite wall of the trachea (Fig. 2, C and D). (The membranous portion of the trachea, which the probe would touch if directed straight down to the posterior trachea, is typically avoided, as we have noted that the vasculization pattern of this relatively small posterior region of the airway is frequently distinct from the vast majority of the airway contiguous with the C ring). 4) The probe is lowered very gradually via the micromanipulator until the PO2 levels decrease to 5 mmHg or less (indicating a zeroing effect induced by firm tissue compression). 5) The probe is then raised gradually in small increments until the PO2 reading plateaus and a consistent reading is obtained. The consistent PO2 will be lost as the probe continues to be lifted off the surface of the airway. The loss of contact of the probe with the airway epithelium is indicated by a rapid rise to at least 60 mmHg, indicating that luminal PO2 is now being sensed by the probe. For each sample, our group typically takes several readings and averages the values.

**Blood Qaw monitoring by laser Doppler flowmetry.** LDF provides real-time measurement of microvascular red blood cell perfusion in tissue. Laser Doppler signals from the tissue are recorded as relative...
units called BPUs that are defined using a carefully controlled motility standard. This technique has been previously employed in dogs and sheep to assess airway \( Q_{aw} \) (4, 6). LDF operates by lighting the tissue under observation with low-power laser illumination source. Laser light from one fiber is scattered within the tissue, and some of this light is scattered back to the sensor. Backscattered light from the tissue is produced by moving red blood cells and is sensed by another optical fiber. This signal returns to the monitor and is processed so that the Doppler shift (related to moving red blood cells) can be quantified.

Recently, we introduced this technique to further study OTTs for microvascular perfusion during the course of acute rejection (10). To assess \( Q_{aw} \), we use LDF probes in conjunction with the OxyLab LDF monitor. Perfusion data are collected at the same time that (anesthetized) mice are being assessed for tissue \( P_o_2 \) levels. The procedure entails the following steps: 1) for the grafted mouse under general anesthesia, tracheal grafts are carefully exposed using stay sutures to gently retract the strap muscles to reveal the anterior wall of the trachea. 2) The LDF probe is mounted on a micrometer in the same way that the \( P_o_2 \) probe is mounted, and measurements can be obtained at the same time that \( P_o_2 \) readings are performed. 3) BPU measurements are obtained by gently lowering the LDF probe onto the outer surface of tracheal grafts (Fig. 2, E and F). (The \( P_o_2 \) probe, by distinction, does not provide consistent measurements when used on the outside surface of OTTs apparently due to interference with the higher ambient \( P_o_2 \)). 4) The LDF monitor reports output signals on OTTs as BPUs by real-time measurements of red blood cells in flux that is proportional to the red blood cell perfusion. This measurement represents the transport of blood cells through the microvascularature and is the product of mean red blood cell velocity and mean red blood cell concentration in the volume of tissue under probe illumination.

**Bioprobe validation study 1: Vasodilator-vasoconstrictor study.** Transplanted and anesthetized mice were injected with either adeno-
sine (0.05 mg/kg, iv) or endothelin-1 (1 nm/kg, iv). Adenosine and endothelin-1 solutions were prepared in distilled water and injected in mice immediately before measurements.

Bioprobe validation study 2: Terminal anesthesia. For the terminal anesthesia procedure, mice were given an excess dose of ketamine/xylazine (240 mg/kg and 20 mg/kg) intraperitoneally. Next, trachea was exposed and examined for tissue Po2 by fluoresence oximetry and BPUs by LDF. In each experiment, tissue Po2 and BPUs were traced every 15 s until it drops to zero/minimum, and that reflects the death of mouse.

Human lung transplantation. All human studies are approved by the Stanford Human Subjects Institutional Review Board. The methods described below were developed in single lung transplant recipients at Stanford University Hospital. The rationale for using single lung transplant recipients is so that transplant airways can be directly compared with contralateral (diseased) native airways. Studies are performed on transplant patients during surveillance bronchoscopies.

Human airway tissue monitoring. Visible light spectroscopy is a clinically available, Food and Drug Administration-approved technology that can noninvasively measure tissue hemoglobin O2 saturation (StO2%) at capillary level. This technology is commercially available from Spectros and utilizes the T-stat 303 Microvascular Tissue Oximeter. The T-stat VLS monitor displays values for tissue hemoglobin saturation (StO2%), relative hemoglobin (rHgb), signal intensity, and an optional trend graph. The microprocessor uses a 1-GHz P3 CPU and a minimum of 64 MB internal memory. The operating system is Windows XP SP1, and it uses proprietary T-stat software for data collection and analysis. The input voltage is 100–240 V, and it requires 100 VA of power. Multiple alarms (low and high StO2, low and high rHgb, too bright, too dim, or unstable signal, and good data indicator) are available to ensure the quality of data. The sterile, single-use sensor probes connect to the monitor via a proprietary female type 1 Light-Jack connector. The sensor probe input is through 150 μM fiber return line, and the output source is a current-limited 5-V source. The currently available sensor probes include a rectal, a buccal, and two endoscopic probes of different lengths. A specially designed endoscopic probe to measure StO2 during gastrointestinal endoscopic procedures was used for our study. It is 1.5 mm thick and 2.3 m long and can be passed through the instrument channel of an endoscope. The available research options include ability to collect data on an internal flash or to export to external disks via USB port.

The range of measurement of StO2 is from 0–99%, with a resolution of 1%. The reproducibility and accuracy of these measurements are ±2%. Although we only used spot digital readings for StO2 at different anatomic sites in the bronchial tree, it also allows recording of a trend graph of StO2 over time. The range of measurements for rHgb is 0–0.99 mM and resolution of 0.01 mM. The reproducibility, accuracy, and stability of rHgb measurements are ±5 μM. The operation of the oximeter is based on the principle that different forms of hemoglobin have distinct spectroscopic properties that allow StO2% measurements based on spectral characteristics of the reflectance of light from tissue. The oximeter emits white light from a probe placed near the tissue and collects any light returning to the probe. The collected light is separated by wavelength into 2,048 bins, which are measured simultaneously. The reflectance of wavelengths between 475 and 600 nm is used to calculate the concentrations of the major forms of hemoglobin. StO2% is estimated as [oxyhemoglobin] + [deoxyhemoglobin + oxyhemoglobin] (2). The T-stat reports capillary-weighted oxygen saturation due to the selection of the range of light used. This oximeter uses visible light wavelength between 475 and 600 nm because in this range absorbance by hemoglobin is the strongest. Due to this strong absorbance, the transmitted light is almost completely absorbed by the larger hemoglobin containing structures such as arteries and veins. The light passing through the smallest structures is reflected back to the sensor for analysis. The T-stat light source is adjusted so that the reflected light is more than 95% capillary weighted and thus represents tissue O2 saturations. A specially designed endoscopic probe to measure StO2, during gastrointestinal endoscopic procedures, is commercially available. It is designed to measure StO2% in conjunction with T-stat tissue oximeter. It is 1.5 mm thick and 2.3 m long and can be passed through the instrument channel of an endoscope.

We use the following approach to measure airway tissue O2 saturations in human airways (5). The StO2 measurements are recorded using the following steps: 1) The study subjects are sedated and monitored using local conscious sedation or general anesthesia protocols. 2) Inspired oxygen concentrations are titrated to maintain the subjects’ arterial oxygen saturations of 94–96% as measured by pulse oximetry. 3) In nonintubated subjects, upper airway anesthesia is achieved by local application of 4% lidocaine solution. To minimize coughing, the lower airways are anesthetized using 2% lidocaine solution in all study subjects. 4) After insertion of bronchoscope into subject trachea, the sterile and single-use T-Stat Endoscope Sensor probe is passed through the bronchoscope working channel until the tip of the probe extended 1–4 cm past the distal end of bronchoscope. Under direct visual guidance and with bronchoscope manipulation, the probe is placed in the optimal position at different measurement sites. 5) The measurements are recorded by positioning the probe parallel to and 1–5 mm above the mucosa. The probe is held steady until a steady value (numerically and graphically) is achieved. Each measurement requires less than 5 s to achieve steady state and is recorded in the T-stat 303 Microvascular Tissue Oximeter. At each predefined site in the bronchial tree, three measurements are taken, and an average of these measurements is recorded. 6) To minimize error secondary to mucosal hyperemia, inflammation, or extravasated blood, all measurements are taken before any other endoscopic interventions. Also areas of apparent mucosal inflammation or hyperemia are avoided. 7) The anatomic sites of StO2% measurements included midtrachea, carina, bilateral secondary carina, bronchi to upper lobes, bronchi to middle (or lingula), and bronchi to lower lobes. After all the measurements are recorded, the endoscope is removed, and remaining bronchoscopic procedures can be completed.

Statistical analysis. GraphPad Prism version 5.0c was used for statistical analysis. Results are reported as means ± SE. Differences between values at multiple times were compared using repeated-measures ANOVA and test for linear trend for post hoc analyses. All data are represented as means ± SE, and P < 0.05 is considered significant.

RESULTS

In the OTT model, the vascular systems of the donor and recipients normally fuse (by secondary intention) at ≈4 days posttransplantation (Fig. 3A). In nonrejecting syngrafts (MHC-compatible transplants) and in immunosuppressed allografts (MHC-incompatible transplants), once the vascular connection is forged, the airways remain perfused over time. We discovered that nonimmunosuppressed allografts become completely ischemic after 10 days of rejection (Fig. 3B) and that the cessation of blood flow continues for several days before being reinvested with recipient-derived microvessels by 28 days following transplantation (Fig. 2C) (1, 9).

Pooling data from both published (9, 10) and unpublished results from the last 4 yr, we can show with remarkable interoperator consistency the kinetics of grafted airway tissue Po2 and Qaw using two methodologies described above (i.e., serial tissue Po2 monitoring by the fluorescence quenching technique and Qaw monitoring by LDF) (4). Tissue oxygenation and Qaw is consistently robust at 1 wk posttransplant.
In the absence of immunosuppression for allografts, there is a steady decline in tissue P O2 and Q aw in rejecting airway allografts until the P O2 nadirs ~10–12 days following transplantation. This process is further described in DISCUSSION. Our knowledge about whether tissues are perfused most directly relies on the FITC-lectin perfusion technique performed just before the animal being killed. Thus, currently, the FITC-lectin technique represents our gold standard for microvascular perfusion of airways. As noted, this process is not ideal in a kinetic study requiring in vivo evaluations at multiple time points. We have found that airway tissue P O2s of less than ≈16 mmHg are highly correlated with the absence of perfusion demonstrated by FITC-lectin technique (indicated by the gray field in Fig. 4).

To validate the new technique of assessing airway P O2 and new application of gauging airway Q aw, we measured serial airway oxygenation and perfusion values in OTTs immediately after challenges with adenosine and endothelin. As expected the vasodilator, adenosine, led to higher tissue P O2 values (Fig. 5A), whereas the vasoconstrictor, endothelin, resulted in relative hypoxia (Fig. 5B). Matching these findings, Q aw similarly improved following adenosine administration (Fig. 5C) and fell after endothelin injection (Fig. 5D). To further validate these techniques, we performed serial assessments on OTT recipients immediately following euthanizing doses of ketamine and xylazine (Fig. 6). Again, as expected, both P O2 (Fig. 6A) and Q aw (Fig. 6B) readings steadily decline during circulatory collapse of the expiring animal. In summary, these results validate the described techniques of assessing airway oxygenation and perfusion in OTT recipients.

Finally, we previously reported that airway oximetry can be successfully performed, using single-lung transplant recipients as a test population (5). Consistent airway StO2 readings can be obtained with this technique in patients maintained at a fixed level of supplemental oxygenation. Figure 7 illustrates that the simultaneous wave form tracings of both airway tissue (StO2) and pulse oximetry (SaO2) from a representative patient track together in a predictable pattern.

**DISCUSSION**

We describe in this study techniques for assessing airway tissue oxygenation and Q aw with special attention to rejecting allografts. The fall in tissue P O2 noted in rejection is followed by a slow but steady improvement in oxygenation and Q aw due
to a hypoxia inducible factor-1α (HIF-1α)-driven neovascularization of the airway (9). Furthermore, these nascent vessels are largely composed of recipient-derived cells (9). By contrast, syngeneic or immunosuppressed allogeneic grafts (not shown; Ref. 1) inosculate and retain PO2 values in the mid-30–atm range, syngeneic or immunosuppressed allogeneic grafts (not shown; Ref. 1) inosculate and retain PO2 values in the mid-30–

Fig. 5. Changes in tissue oxygenation and blood perfusion after adenosine and endothelin treatment. Tissue PO2 (A) and blood perfusion values (B) were plotted every 15 s after 10–15 min of adenosine injection in OTTs. OTT shows an increase in both tissue PO2 and BPUs after adenosine injection. Tissue PO2 (C) and blood perfusion (D) were plotted every 15 s after 5–10 min of endothelin-1 injection in OTTs. OTTs show a decrease in both tissue PO2 and BPUs after endothelin-1 injection. Data are shown as means with SE and each point representative of 2 different experiments (P < 0.0001 for all groups by repeated-measures ANOVA; n = 5 animals/group).

The ability to reverse acute rejection with various immunotherapies is highly correlated with the presence of a functional microcirculation (1). As a corollary notion, therapies that specifically preserved the microcirculation should extend the time in which an allograft could undergo rejection and be rescued from subsequent fibrotic remodeling (3). Accordingly, we tested this idea and subsequently identified HIF-1α as a key mediator in promoting microvascular repair in these hypoxic and ischemic airways (9). As predicted, upregulating HIF-1α extended the period of time that microvessels were perfused and prolonged the period of time that immunotherapy could be given to prevent fibroproliferative remodeling. Using the above-described techniques, we sought to determine which immune factors were specifically responsible for airway hypoxia and ischemia. We demonstrated that both CD4+ T cells and antibody-dependent complement activity were independently sufficient to mediate graft ischemia (10). At the same time, a surprising role for CD8+ T cells was uncovered; these lymphocytes are required for effective recipient-derived neo-

### Table 1

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of Subjects</th>
<th>Sex (Men/Women)</th>
<th>Age (yr)</th>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper extremity tumor resection</td>
<td>5</td>
<td>4/1</td>
<td>47–53</td>
<td>68%</td>
</tr>
<tr>
<td>Mastectomy</td>
<td>5</td>
<td>4/1</td>
<td>47–53</td>
<td>68%</td>
</tr>
<tr>
<td>Laparotomy</td>
<td>5</td>
<td>4/1</td>
<td>47–53</td>
<td>68%</td>
</tr>
<tr>
<td>Upper lobe carina</td>
<td>2</td>
<td>1/1</td>
<td>57–69</td>
<td>64%</td>
</tr>
<tr>
<td>Middle lobe carina</td>
<td>2</td>
<td>1/1</td>
<td>57–69</td>
<td>64%</td>
</tr>
<tr>
<td>Lower lobe carina</td>
<td>2</td>
<td>1/1</td>
<td>57–69</td>
<td>64%</td>
</tr>
</tbody>
</table>

The lung-transplant recipients were enrolled at the time of their routine surveillance flexible bronchoscopies. The subjects who were scheduled to undergo airway intubations for elective, noncardiothoracic surgical procedures served as normal controls. The median age of lung transplant recipients was 61 yr (range 57–69 yr), 64% were women, 68% were transplanted for idiopathic pulmonary fibrosis, and 68% had received right-lung transplant. The median time from transplant to the study was 264 days (range 19–418 days). The median age of normal controls was 50.5 yr (range 47–53 yr), and 50% were women. Their scheduled surgical procedures included upper extremity tumor resection, prostatetomy, mastectomy, and laparotomy. Table 1 shows the results of this previously published study (5).
significantly lower in the transplanted (healthy) lungs compared with the contralateral nontransplanted (diseased lung). These results are consistent with reduced oxygenation in transplanted lungs likely due to the lack of a restored bronchial artery circulation. Although the results demonstrate that transplant airways are relatively hypoxic, a 5% O₂ saturation difference is of unclear biological relevance. It is important to consider that the T-Stat oximeter assesses tissue oxygenation rather than arterial O₂ saturation. As such, these values represent a mixed venous oxygen (capillary) saturation. This type of estimation likely underestimates the true local arteriolar O₂ saturation difference and, rather, represents tissue oxygen deficit. Additionally, our animal studies demonstrate that inflammation itself induces airway tissue hypoxia (6) and could potentially reveal a larger difference during rejection episodes than the differences we published in healthy nonrejecting lungs. This new technique revealed for the first time in humans that there is a measurable physiological consequence that can be attributed to the omission of bronchial artery revascularization step at the time of lung transplantation.

Although most of the above-described techniques are most immediately germane to lung transplant studies, assessing decreased airway oxygenation in individuals with compromised bronchial artery circulation could be impactful for other pulmonary conditions. For example, patients with pulmonary atresia and ventricular septal defects likely develop collateral circulation originating from the bronchial arteries. Surgical repair of these congenital defects (called unifocalization) can involve sacrificing these circulations, and, interestingly, the procedures have been associated with the development of severe airway obstruction (20). The underlying pathology of

### Table 1. Human airway tissue oximetry study

<table>
<thead>
<tr>
<th>Airway Site</th>
<th>Transplant Lung StO₂% (N = 16)</th>
<th>Native Lung StO₂% (N = 16)</th>
<th>Normal Control StO₂% (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midtrachea</td>
<td>n/a</td>
<td>63.3</td>
<td>65.5</td>
</tr>
<tr>
<td>Carina</td>
<td>n/a</td>
<td>61.1</td>
<td>66.2</td>
</tr>
<tr>
<td>2° Carina</td>
<td>60.3</td>
<td>62.6</td>
<td>63.0</td>
</tr>
<tr>
<td>UL</td>
<td>59.8†</td>
<td>66.1</td>
<td>63.6</td>
</tr>
<tr>
<td>ML/Lingula</td>
<td>60.5*</td>
<td>66.7</td>
<td>63.6</td>
</tr>
<tr>
<td>LL</td>
<td>59.2†</td>
<td>65.4</td>
<td>64.7</td>
</tr>
</tbody>
</table>

For this study, significant differences between transplant, native, and normal lungs were identified by one-way analysis of variance with post hoc analysis using the least-significant difference test. Midtrachea and carina measurements were all native airway tissues because all single-lung transplant anastomoses were distal to the carina. These results were previously published, and this Table is modified from Ref. 7. LL, lower lobe; ML, middle lobe; StO₂, tissue oxygen saturation; UL, upper lobe. *Significantly lower than native airway StO₂ (P value < 0.05). †Significantly lower than native and normal control airway StO₂ (P value < 0.05).
Innovative Methodology

MONITORING AIRWAY TISSUE OXYGENATION AND PERFUSION

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the airways in these patients is not presently available and could be informative. The loss of bronchiolar circulation can also occur in sleeve lobectomy operations, which are performed as a treatment for bronchial carcinomas. Following sleeve lobectomy, surgically related bronchiolar pathology and infectious risk have been attributed, in part, to diminished $Q_{aw}$ (11, 24). By distinction, an increase in the bronchial circulation is associated with inflammatory conditions such as asthma, cystic fibrosis, noncystic fibrosis bronchiectasis, and eosinophilic bronchitis (8, 15, 22, 23, 26). Our understanding of these latter pulmonary conditions could also potentially benefit from the use of technologies described in this article.

In addition to the techniques described above, soluble inert gas uptake has also been developed as a noninvasive measuring $Q_{aw}$ in conducting airway mucosa (18). The methodology is based on determining the disappearance of a water-soluble gas, dimethylether (21). The method involves inhaling a gas mixture containing dimethylether, helium, and O$_2$ and using Fick’s principle to calculate $Q_{aw}$. This methodology has been used in humans and appears to be highly reproducible (25). This technique has value in assessing airway inflammation, estimating vascular remodeling, and evaluating postlung surgery airway blood flow and has been posited as a way to monitor the effectiveness of airway-relevant therapies. For example, the effectiveness of anti-inflammatory antibiotic therapy in cystic fibrosis, noncystic fibrosis bronchiectasis, and eosinophilic bronchitis (8, 15, 22, 23, 26).

In summary, the assessments with the soluble inert gas uptake technique, LDF, and FITC-lectin binding have proven valuable for understanding $Q_{aw}$. To complement these assessments, we have recently described new methods to directly monitor tissue PO$_2$ through the fluorescence-quenching technique and through tissue oximetry. The dynamic changes in airway oxygenation and $Q_{aw}$ observed in both developing and resolving airway conditions is a relatively unstudied area of pulmonary physiology. Heightened attention to these parameters will likely yield important clues about disease pathogenesis and treatment.

ACKNOWLEDGMENTS

We acknowledge Dr. Donald McDonald (UCSF) who provided the original detailed methods for the FITC-lectin technique.

GRANTS

This study was supported by NIH Grants HL095686, HL108797 and VA Merit BX000509 (M. Nicholls).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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