‘Oxygen Level in a Tissue’ – What Do Available Measurements Really Report?

H. M. Swartz, P. Vaupel, B. B. Williams, P. E. Schaner, B. Gallez, W. Schreiber, A. Ali, and A. B. Flood

Abstract The aim of the paper is to discuss what currently is feasible clinically to measure the level of oxygen and how that measurement can be clinically useful. Because oxygen in tissues is quite heterogeneous and all methods of measurement can only provide an average across heterogeneities at some spatial and temporal resolution, the values that are obtained may have limitations on their clinical utility. However, even if such limitations are significant, if one utilizes repeated measurements and focuses on changes in the measured levels, rather than ‘absolute levels’, it may be possible to obtain very useful clinical information. While these considerations are especially pertinent in cancer, they also pertain to most other types of pathology.

Keywords Oxygen heterogeneity · Imaging · |EPR · Radiation therapy · Immunotherapy

H. M. Swartz (✉)
Department Radiology, Dartmouth Medical School, Hanover, NH, USA

Section Radiation Oncology, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA
e-mail: Harold.M.Swartz@dartmouth.edu

P. Vaupel
Department Radiation Oncology, University Medical Center, Mainz, Germany

B. B. Williams
Department Radiology, Dartmouth Medical School, Hanover, NH, USA

Section Radiation Oncology, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA

P. E. Schaner
Section Radiation Oncology, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA

B. Gallez
Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium

W. Schreiber · A. B. Flood
Department Radiology, Dartmouth Medical School, Hanover, NH, USA

A. Ali
Department Radiation Oncology, Emory School of Medicine, Atlanta, GA, USA

P.-D. Ryu et al. (eds.), *Oxygen Transport to Tissue XLI*, Advances in Experimental Medicine and Biology 1232, https://doi.org/10.1007/978-3-030-34461-0_19

1 Introduction

The penultimate purpose of a clinical measurement is to obtain information that can be used to advance the clinical care of the patient. The ultimate test of the usefulness of the information is whether it impacts clinical outcomes. The outcomes can be survival or other clinically desirable parameters such as quality of life, cost-effectiveness, reduction of side effects, etc. The mechanisms by which the outcomes may be impacted can include enhancing diagnosis or improving individualized radiotherapy plans and/or combined treatments, as well as direct impacts on outcomes. But the bottom line is that when we make a clinical measurement, the ultimate criterion for its usefulness should be: does it improve clinical efficacy to an extent that offsets the costs (monetary, time, discomfort etc.) for getting that result and/or provide information that in the future could enhance clinical outcomes. Therefore, in evaluating the usefulness of a clinical measurement such as the value of oxygen in a tissue, we should take into account the purpose of making that measurement. When we focus on these considerations, we may find that, while it is not very valuable to state that there is a particular level of oxygen in a tissue, measurements of oxygen if repeated can reveal changes, and even if the amount is not rigorously quantified, these may be very useful indeed.

2 Oxygen Levels in Tissues

Heterogeneity of oxygen in the tissues of interest exists over many dimensions, including time, and over a wide range of scales [1]. Any practical method of measurement to characterize the tissue oxygenation and the heterogeneity present will necessarily provide data that represent some average over space and time; hence, careful considerations of the impacts of the averaging must be made to generate clinically relevant information. This average may include complex weighting factors with spatial, temporal, and oxygen dependencies. In some cases, additional processing of these data may provide other statistical measures, such as median, mean, max/min, etc. These practical considerations hold true for historic polarographic electrode measurements, modern PET and MRI approaches, optical methods, and all EPR techniques either in development or clinical implementation, as well as all other techniques. Consequently, there may be high value in using multiple techniques that together can provide complementary information.

There are spatial variances in oxygen levels in normal tissues due to the longitudinal gradient in oxygen as the blood passes through the microcirculatory bed (decreasing from the arterial inlet to the outlet microvessels) [2]. After the oxygen leaves the microvascular networks the oxygen partial pressures decrease from the vessels into the tissue as it diffuses through the tissues, due to oxygen consumption by the cells (radial gradients). Within the cells the oxygen decreases in a microspatially complex manner as it is intracellularly consumed, with most of the consumption occurring in the mitochondria. There also is growing evidence that the diffusion

of oxygen into the cell may be constrained, i.e., that oxygen does not freely and rapidly flow into cells across the membrane [3, 4]. As a result there are variations in oxygen levels from cell to cell (according to their distance from the microvessel) and then also within the cell. These variations of oxygen from micro vessels to dimensions that are smaller than cells currently cannot be measured; note as detailed below in discussing temporal variations, even if we could make such measurements of spatial heterogeneity, they would be inadequate to understand the full heterogeneity of oxygen. Also, in some normal tissues there is additional significant macroscopic heterogeneity of oxygen over space because of their physiology, e.g., macroscopic heterogeneity between grey and white matter of the brain, and between renal cortex and renal medulla as consequence of substantial differences in blood flow. There also are more minor heterogeneities in resting skeletal muscle or in the subcutis, due to low O_2 extraction rates.

There also are temporal changes in oxygen levels in normal tissues [5]. The supply of oxygen by arteries and arterioles can vary periodically due to rhythmic changes in microcirculatory blood flow, which is reflected at all levels from the inflow arteries to the microcirculation. Within the microcirculation there are variations in microvascular flow due to regional regulation in response to varying metabolic demands.

The presence of pathology often significantly increases the amount and extent of oxygen heterogeneity both spatially and temporally [6]. The presence of pathology often impacts the structure/morphology of the vessels. In tumors there often is a significant amount of neoangiogenesis which results in much less ordered and less functional blood vessels. These vessels are much less efficient in delivering blood and also tend to be much more prone to leak. The leakage from these vessels can cause variations in the interstitial pressure, which can reduce the effectiveness of the microcirculation due to reduction of the perfusion pressure in the tumor capillaries [7]. The pathological changes also can result in altered consumption of oxygen, potentially decreasing oxygen consumption due to poor oxygen delivery and because of a switch to glycolysis due to metabolic re-programming (i.e., the Warburg effect) as a consequence of HIF-overexpression, upregulation of oncogenes, downregulation of suppressor genes, activation of certain signaling pathways etc. The pathology can also impact the integrity of the blood vessels. For example, tumor growth may physically impinge on the integrity of the blood vessels, and the metabolic abnormalities in diabetes can impact the structure of blood vessels (microangiopathy, macroangiopathy). The results of these processes can produce very significant local variations in the availability of fully functional vascular structures, resulting in local hypoxic regions.

Pathology also can impact temporal changes of oxygen and the response to treatment, which can result in significantly greater variability in oxygen levels in the presence of pathology (especially shown in cancer and peripheral vascular disease). These include short term changes, especially associated with the structural abnormalities of the microcirculation resulting in increased local variability in flow and long-term changes over time, due to disease progression and responses to therapy [8]. There also is a potential for anemic hypoxia to be present in tumors due to the underlying anemia of the patient [9].

3 Use of EPR Oximetry to Illustrate the Thesis of the Paper

We first consider the measurement of oxygen levels in tumors by EPR, because it is one of the few (maybe only) specific techniques that is currently being used clinically to make direct measurements of oxygen in tissues [10]. We then consider how these conclusions apply to other pathophysiological processes and other available measurement techniques.

Using appropriate oximetric paramagnetic materials, EPR oximetry can provide direct measurements of oxygen, i.e., the EPR signal it is directly proportionate to the amount of oxygen. Most other clinically available techniques measure parameters that often are only indirectly related to oxygen levels in tissues, e.g., they assess oxygen in nearby microcirculatory units or measure blood flow. In contrast, EPR measurements are made in the tissues of interest, not in the vascular compartment; for example, compare EPR with NIRS of hemoglobin or BOLD MRI.

The measured parameter of an EPR spectrum that indicates the amount of oxygen present is the line width of the observed resonance peak. There usually is a fixed relationship between the line width and the amount of oxygen, with the relationship being specific for each type of paramagnetic material. Using particulate oximetric materials, the measurements can be continuous over any span of time and can be repeated indefinitely. The method does require that the sensing material be injected or implanted in one or more regions of interest, but thereafter all measurements can be made entirely non-invasively. Importantly the measurements can be carried out in a clinical setting and can fit into the workflow needed for patient care, etc.

The initial clinical EPR measurements of oxygen in tissues have used India Ink as the oxygen sensor [10]. The carbon particles are the components that respond to oxygen. After injection of 30–50 μl of the suspension through a small needle, the carbon particles disperse non-uniformly through the local region as small extracellular aggregates. They are often engulfed by macrophages. The resulting EPR spectra in the region probed by the resonator (i.e., the surface coil used for signal detection) are a composite of the oxygen-dependent line widths from each of the particles. In reality, because of the relatively broad lines from the India Ink particles, the range of ‘oxygen levels’ that are likely to be present in the tissue, and the limited number of particles in each subregion, it is a challenge to resolve directly even the major groups of similar line widths. So using the observed line width to provide a quantitative measure of oxygen would seem to have modest utility in itself.

The other method of clinical EPR oximetry is based on the use of micro-crystalline probes (e.g., LiPc, LiNc-BuO), encapsulated in biocompatible polymers [10]. Clinical measurements currently are being performed using the ‘OxyChip’ which consists of oxygen sensitive microcrystals of lithium octa-n-butoxynaphthalocyanine (LiNc-BuO) embedded in polydimethylsiloxane (PDMS) [11–13]. The dimensions currently used in humans are cylinders that are 5 mm long with a diameter of

0.6 mm. The EPR signal from the sensor (OxyChip) reflects the partial pressure of oxygen within the PDMS, which itself reflects an average of the partial pressure of oxygen in contact with the external surface of the cylinder. The dimensions of the OxyChip are much greater than those of a microcirculatory unit and therefore reflect many different such units. The microcirculatory networks sampled by the OxyChip are likely to include regions with quite different oxygen levels. The 'values' of oxygen that are obtained therefore do not, in themselves, provide any information on the heterogeneity of the sampled capillary networks.

Given the impacts of unavoidable averaging by any real method of oxygen measurements in tissues, one may question the value beyond that of more qualitative measures of absolute measurements (e.g., pO_2 in mm Hg). See Flood et al. [14] for details on statistical issues in this regard.

4 Examples of Circumstances in which Current Methods to Measure Oxygen Can Be Clinically Very Useful

4.1 Screening for Responsiveness to Hyperoxic Interventions

One of the clinical paradoxes in radiation oncology is that, although we have known for more than 100 years that the response of tumors to ionizing radiation would probably be very significantly enhanced if we could irradiate them at times of more favorable oxygenation status, this knowledge has not been able to be used directly in ways that are applicable to the usual clinical uses of radiation therapy. Although many techniques have been tried that were expected to increase the amount of oxygen present at the time of radiation therapy, their apparent ineffectiveness has not resulted in modifying clinical practice [15]. We and others have hypothesized that these clinical failures were due to the fact that a significant number of tumors do not respond to methods to increase oxygenation of the tumors; consequently, unless these intrinsic non-responders are identified and removed from the pool of patients in which the hyperoxic interventions are tested, their presence would make it very difficult to obtain statistically significantly improved outcomes in the sampled population [10, 16]. Therefore, if measurements of oxygen in tumors could provide information as to which tumors could respond to a particular hyperoxic intervention, it would allow targeting that subpopulation with attempts to change the tumor oxygenation during radiotherapy.

Now it is feasible to carry out proper tests of the efficacy of hyperoxic interventions in human subjects to change oxygen in individual tumors and determine if such interventions result in clinically significant improvements in outcomes. For example, EPR oximetry has been used in preclinical models to measure tumor pO_2 over time for strategies changing oxygen delivery [17], oxygen consumption [18], both oxygen delivery and oxygen consumption [19], or an allosteric modifier of

hemoglobin [20]. Ideally such a set of measurements would be carried out only on the hypoxic cells whose oxygenation needs to be improved, but this seems impractical at this time because of the lack of clinically applicable methods to measure oxygen levels selectively in the hypoxic cells. But it may be reasonable to assume that repeated measurements in representative parts of the tumor before and during the hyperoxic treatment, could be a workable method to determine the effectiveness of the treatment to increase oxygen throughout the tumor including the hypoxic cells. It would be desirable to test this directly by using such screening for responsiveness and then observing the impact on outcomes. It may be reasonable, given unavoidable impacts of spatial and temporal averaging and/or the use of a limited number of discrete measurements, to use data that only indicate whether or not the treatment resulted in a repeatable increase in oxygen, and not to need to focus on the quantitative aspects of the increase.

4.2 Impact of Hypoxia on the Effectiveness of Immunotherapy

There is increasing evidence indicating that the level of oxygen is an important determinant of the efficacy of immunotherapy [21–23]. Understanding these relationships and then exploiting them could have a very significant impact on the efficacy of immunotherapy, enabling physicians to determine which patients are likely to benefit from immunotherapy, providing early indications of the likelihood of response, and potentially providing a mechanism to enhance the effectiveness by altering the oxygen levels. These developments would be greatly aided by the use of repeated measures to determine changes in oxygen levels.

4.3 Role of Radiation-Induced Hypoxia on Fibrosis

Following large doses of radiation (as used in therapy), some individuals have significant fibrotic responses which can be uncomfortable and sometimes disabling. Hypoxia is considered potentially to be an important factor in the pathogenesis of fibrosis, but data to test this hypothesis are limited. By carrying out serial measurements of oxygen, it should be feasible to obtain more definitive data on the role of radiation-induced hypoxia in the pathogenesis of fibrosis. Serial measurements also might be useful for identifying subjects at risk before the changes become clinically obvious. Repeated measurements in the same individual, perhaps augmented by observing changes associated with breathing enriched oxygen mixtures, are key to gaining insights into the adaptive capacity of the vasculature. Measurements of relative changes should be appropriate for this purpose.

4.4 Quantitative Delineation of the Hypoxic Fraction

In principle such measurements would be ideal for use in guiding radiation therapy. Certainly, even relatively crude, one-time estimates using the Eppendorf electrode demonstrated the predictive capability of such measurements [24]. The problem is the technical difficulty in now making such measurements. The Eppendorf and related types of devices are no longer available for clinical use. Attempts to provide equivalent information by other methods have not been successful. This remains an area of opportunity for future developments of imaging. But it also should be noted that, especially in preclinical models as well as clinical studies using the oxygen electrode, some clinically useful correlations have been found between a limited number of measurements and outcomes, even if the measurements could not provide absolute measures of pO_2 [25].

4.5 Use of EPR Oximetry for Other Types of Pathology

The applicability and challenges of EPR oximetry are similar regardless of the types of pathology. Two instances where repeated measurements of oxygen would be especially valuable for guiding therapy are peripheral vascular disease and wound healing [10]. Although absolute measurements remain challenging, serial measurements of *relative* changes should be quite sufficient to help guide therapy, providing indications of the success of interventions to improve local oxygenation.

5 Conclusions

The previous discussion of what types of EPR-generated information about tissue oxygen have practical utility for medical decision-making extends to all types of such measurements [26, 27]. Some techniques have the advantage of directly measuring oxygen, but even they cannot fully resolve spatial and temporal heterogeneities. Others have the additional limitation of not being able to make repeated measurements. It would appear that repetition is likely to be particularly important for solving these problems, since it allows characterization of both spatial and temporal *changes,* which may be the most valuable clinical information.

Although it does not seem practical for any clinically available technique to provide a meaningful single (or 'absolute') number for oxygen levels in tissues, that does not need to be a fatal flaw if these limitations are recognized and adaptations are made to use the information in conjunction with other techniques that, when combined, provide the information needed to solve the clinical problem and to obtain the goal of using oxygen measurements to improve patient outcomes.

Acknowledgments Major funding is from the National Cancer Institute, PPG Grant P01CA190193. We gratefully acknowledge all the other scientists, clinicians, engineers and coordinators on the PPG. Disclaimer: ABF and HMS are owners of Clin-EPR, LLC which manufacturers clinical EPR instruments for investigational use only.

References

1. Harrison D, Vaupel P (2014) Heterogeneity in tissue oxygenation: from physiological variability in normal tissues to pathophysiological chaos in malignant tumors. Adv Exp Med Biol 812:25–31
2. Erickson K, Braun RD, Yu D et al (2003) Effect of longitudinal oxygen gradients on effectiveness of manipulation of tumor oxygenation. Can Res 63(15):4705–4712
3. Khan N, Shen J, Chang TY et al (2003) Plasma membrane cholesterol: a possible barrier to intracellular oxygen in normal and mutant CHO cells defective in cholesterol metabolism. Biochemist 42:23–29
4. Kurokawa H, Ito H, Inoue M et al (2015) High resolution imaging of intracellular oxygen concentration by phosphorescence lifetime. Sci Rep 5:10657. https://doi.org/10.1038/srep10657
5. Griffith TM (1996) Temporal chaos in the microcirculation. Cardiovasc Res 3:342–358
6. Vaupel P, Mayer A (2016) Tumor hypoxia: causative mechanisms, microregional heterogeneities, and the role of tissue-based hypoxia markers. Adv Exp Med Biol 923:77–86
7. Fukumura D, Duda DG, Munn LL et al (2010) Tumor microvasculature and microenvironment: novel insights through intravital imaging in pre-clinical models. Microcirculation 17(3):206–225
8. Konerding MA, Fait E, Gaumann A (2001) 3D microvascular architecture of pre-cancerous lesions and invasive carcinomas of the colon. Brit J Cancer 84:1354–1362. https://doi.org/10.1054/bjoc.2001.1809
9. Vaupel P, Mayer A (2014) Hypoxia in tumors: pathogenesis-related classification, characterization of hypoxia subtypes, and associated biological and clinical implications. Adv Exp Med Biol 812:19–24
10. Swartz HM, Williams BB, Zaki BI et al (2014) Clinical EPR: unique opportunities and some challenges. Acad Rad 21(2):197–206
11. Hou H, Khan N, Gohain S et al (2018) Pre-clinical evaluation of OxyChip for long-term EPR oximetry. Biomed Microdevices 20(2):29
12. Jarvis LA, Williams BB, Schaner PE et al (2016) Phase 1 clinical trial of OxyChip, an implantable absolute pO_2 sensor for tumor oximetry. Int J Rad Onc Biol Phys 96(2):S109–S110
13. Hou H, Khan N, Nagane M et al (2016) Skeletal muscle oxygenation measured by EPR oximetry using a highly sensitive polymer-encapsulated paramagnetic sensor. Adv Exp Med Bio 923:351–357. https://doi.org/10.1007/978-3-319-38810-6_46
14. Flood AB, Schaner PE, Vaupel P et al (2019) Clinical and statistical considerations when assessing oxygen levels in tumors: illustrative results from clinical EPR oximetry studies. Adv Exp Med Bio. (In Press)
15. Overgaard J (2007) Hypoxic radiosensitization: adored and ignored. J Clin Oncol 10;25(26):4066–4074
16. Swartz HM, Williams BB, Hou H et al (2016) Direct and repeated clinical measurements of pO_2 for enhancing cancer therapy and other applications. Adv Exp Med Biol 923:95–104. https://doi.org/10.1007/978-3-319-38810-6_13
17. Ansiaux R, Baudelet C, Jordan BF et al (2005) Thalidomide radiosensitizes tumors through early changes in the tumor microenvironment. Clin Cancer Res 11:743–750
18. Gallez B, Neveu MA, Danhier P et al (2017) Manipulation of tumor oxygenation and radiosensitivity through modification of cell respiration: a critical review of approaches and imaging biomarkers for therapeutic guidance. Biochim Biophys Acta 1858(8):700–711

19. Jordan BF, Sonveaux P, Feron O et al (2004) Nitric oxide as a radiosensitizer: evidence for an intrinsic role in addition to its effect on oxygen delivery and consumption. Int J Cancer 109(5):768–773
20. Hou H, Khan N, O'Hara JA et al (2004) Effect of RSR13, an allosteric hemoglobin modifier, on oxygenation in murine tumors: an *in vivo* electron paramagnetic resonance oximetry and bold MRI study. Int J Radiat Oncol Biol Phys 59(3):834–843
21. Vaupel P, Multhoff G (2019) Fatal alliance of hypoxia-/HIF-1α-driven microenvironmental traits promoting cancer progression and resistance to therapy. Adv Exp Med Biol. (In press)
22. O'Hara JA, Blumenthal RD, Grinberg OY et al (2001) Response to radioimmunotherapy correlates with tumor pO_2 measured by EPR oximetry in human tumor xenografts. Radiat Res 155:466–473
23. Noman MZ (2015) Hypoxia: a key player in antitumor immune response. A review in the theme: cellular responses to hypoxia. Am J Physiol Cell Physiol 309(9):C569–C579
24. Vaupel P, Höckel M, Mayer A (2007) Detection and characterization of tumor hypoxia using pO_2 histography. Antioxid Redox Signal 9:1221–1235
25. Colliez F, Gallez B, Jordan BF (2017) Assessing tumor oxygenation for predicting outcome in radiation oncology: a review of studies correlating tumor hypoxic status and outcome in the preclinical and clinical settings. Front Oncol 25(7):10
26. Springett R, Swartz HM (2007) Measurements of oxygen *in vivo*: overview and perspectives on methods to measure oxygen within cells and tissues. Antioxid Redox Signal 9(8):1295–1301
27. Flood AB, Satinsky VA, Swartz HM (2016) Comparing the effectiveness of methods to measure oxygen in tissues for prognosis and treatment of cancer. Adv Exp Med Biol 923:113–112. https://doi.org/10.1007/978-3-319-38810-6_15.