Intravital microscopy methods in microvascular blood flow and tissue oxygenation

David M. Eckmann, Ph.D., M.D.

Horatio C. Wood Professor of Anesthesiology and Critical Care, University of Pennsylvania, Philadelphia, Pennsylvania USA

CORRESPONDENCE EMAIL: eckmanndm@uphs.upenn.edu

Gas embolism bubbles enter the vasculature both accidentally and deliberately in medical procedures and during decompression events, posing a health threat regardless of the gas source. For instance, arterial gas embolism remains a fact in cardiac surgery both on-pump from various cardiopulmonary bypass circuit sources and during off-pump procedures.

A PubMed search of “gas embolism” limited to humans in the past three years reveals more than 400 articles. This body of literature indicates that gas embolic phenomena remain pervasive in many branches of medicine, having been documented during endoscopies, gynecological procedures, laparoscopy, tissue biopsy, neurosurgery, central venous line placement and removal, radiological procedures, urological surgery, pacemaker placement, ophthalmological surgery, positive-pressure ventilation, and as iatrogenic embolization with injector equipment use. Of course, the diving and hyperbaric medicine communities realize it occurs in commercial and recreational divers and recognize vascular gas embolism to be an occupational hazard for hyperbaric chamber care providers [1].

Intra-arterial gas bubbles are transported with the blood flow and can deposit into the microcirculation of any end organ. Cerebral embolization is probably the most relevant due to its relation to cardiac surgery [2]. Following coronary bypass surgery, 50% to 70% of patients have a measurable decrement in brain function. The pathophysiology is little studied at a tissue, cellular or molecular level, largely due to our lack of experimental methods available to capture the important coupling of blood flow and oxygen transport mechanics occurring at the tissue level and at even smaller-length scales. Historically, major innovations in this realm have included the introduction and implementation of significant physiology tools such as the pulmonary artery catheter to assess cardiac output [3] and blood gas analyzer technologies spawned by development of oxygen-sensing devices [4]. The benefits to clinical medicine resulting from utilization of such devices have come through applications aimed at understanding and treating derangements of global physiological indices, while local abnormalities occurring at the tissue or even down to the cellular level may not be detected.

As an example, global blood flow and oxygenation measures can be completely normal in cardiac surgical patients, yet these patients demonstrate clinical manifestations of brain injury compatible with multifocal cerebrovascular gas embolism [5-7]. The neurological deficits include stroke, impaired consciousness, seizures and cognitive impairment limiting function, just as those that occur in other forms of cerebral arterial gas embolism. Beyond hyperbaric therapy, no alternative treatment strategy has yet emerged, despite there being a large population who risk clinically significant gas embolic events. No doubt a better understanding of the specific tissue and cellular phenomena occurring in terms of blood flow and oxygen transport during gas embolic events could reveal possible strategies to mitigate central nervous system injury or tissue damage occurring within other organ systems.

In this issue of UHM, Torres and colleagues [8] (Pages 537-548) report their success using a combination of intravital microscopy methods, including bright field and phosphorescence microscopy, to examine simultaneously microvascular blood flow and tissue oxygen tension in vivo in an experimental model of arterial gas embolization. Using a well-established rat cremaster muscle microcirculation platform with ipsilateral femoral artery cannulation and microbubble injection (9-12) to induce intra-arterial gas embolization, these authors have enhanced our understanding of the coupling of alterations of local microcirculatory blood flow dynamics and tissue oxygenation following gas embolization. They have employed a laser-based phosphorescence quenching method to determine microvascular and interstitial partial pressures of oxygen (PO₂), and they have probed in and around the microcirculation
of tissue that has been directly affected by arterial gas embolization. Notably, they have assessed red blood cell velocity as well as oxygen partial pressure in arterioles, venules and interstitium while tracking vessel occlusion by bubbles. The significant results of this research were that both microvascular blood flow and local (i.e., intravascular and interstitial) PO2 decreased following vessel occlusion by bubbles. A distinguishing feature of their findings was that blood flow redistribution occurred in nearby microvessels, enabling oxygen transport to be partially maintained so that the locally embolized tissue bed did not manifest complete hypoxia.

Essentially this work represents the microvascular equivalent of having a pulmonary artery catheter and a blood gas analyzer – tools for global assessment of blood flow and mass transport – at one’s disposal for making measurements within microcirculatory environments in which important derangements of physiology are at play. Why is this important when it is already known that intravascular bubbles occlude vessels, diminish perfusion and initiate thrombotic and inflammatory pathways? [13]. The main answer is that minimizing activation of pathophysiological responses evoked by intravascular bubbles may prevent injury from developing in the first place, and studying resultant abnormalities of oxygenation in tissue micro-environments otherwise undetectable by global measures (e.g., pulse oximetry, arterial blood gas analysis) is an ideal starting point to guide us to effective treatments. What we do for arterial gas embolism, for instance, at present, is provide hyperbaric oxygen therapy (HBO2T), but often this is not feasible for hours after embolism occurrence. Moreover, HBO2T does not address the root cause of injury resulting from microvascular and cellular responses to bubbles. Torres et al. [8] have demonstrated in this first study that it is both possible and practical to assess relevant microvascular physiology in gas embolism and even, by extension, in other vaso-occlusive conditions, and this opens wide the doors to pursuit of novel therapeutic interventions.

What lies within the realm of possibilities? The marriage of these intravitreal microscopy techniques lets us be imaginative in studying chemical agents that decrease the biological responses to gas embolism in pursuit of pharmacologic (i.e., non-hyperbaric therapy) options to reduce organ injury that otherwise may ensue. Considering that the overall population exposure risk for gas embolism is significant, the health sequelae for different end organs involved remain somewhat elusive and that no easily-implemented treatment or prophylaxis currently exists, having a toolset to investigate the effectiveness of experimental treatments at the tissue level is of great value.

To understand some of the pathophysiological issues that can be addressed more fully as a result of Torres et al.’s innovation, consider that intravascular bubbles provide an interface for adsorption of circulating blood macromolecules such as proteins. Proteins can unfold as a result, exposing domains that can activate biochemical pathways involved in blood clotting, inflammatory responses and promotion of bubble adherence to the endothelium. Each of these mechanistically can be related to changes in local blood flow and tissue oxygenation. There exist numerous drugs and compounds having anti-thrombotic or anti-inflammatory properties which may have utility in preventing embolism-related tissue injury. Even the most obvious of interventions, use of oxygen-carrying compounds that may enhance local O2 delivery, can be evaluated in a new reference frame, and this has already been recognized by Torres et al. [8].

Another potential method of treatment or injury prevention in gas embolism that be pursued with greater physiological detail is the use of surfactants. Coating the interface with inert surfactants is likely to shield biological moieties from encountering an adsorptive surface (14). This prevents initiation of pathophysiological processes resulting from adverse molecular interactions between the bubble surface, an adsorbed surface layer, and the luminal endothelial surface or blood components. Surfactants such as perfluorocarbons, polydimethylsiloxanes and polyols are well characterized for physical characteristics (e.g., aqueous solubility, vapor pressure, boiling point, viscosity), are stable, chemically inert and essentially non-toxic. The literature, including our work, demonstrates their biocompatibility and effects on gas embolism [10,11,15], inflammation [16] and interactions with blood components [17,18]. While surfactant competition for interfacial occupancy is an obvious therapeutic strategy, there is now an important advance in experimental capabilities for investigating bubble interactions with blood and cells in terms of perfusion and mass transfer.

The optical methods Torres et al. [8] have combined in producing their manuscript are also amenable to further enhancement by inclusion of fluorescence probes to assess alterations in endothelial cellular function regulating vascular responses to gas embolism. Many of these functions depend on chemo- and mechanotransduction beginning with the endothelium sensing the luminal environment or responding to a surface receptor binding
event. Functional change can be reflected in a change in intracellular Ca\(^{2+}\) concentration, indicating endothelial cell activation. This occurs with mechanical shear [19], normal stress [20] and hypoxia [21]. As intracellular Ca\(^{2+}\) levels rise, calmodulin binding increases. The Ca\(^{2+}\)-calmodulin complex activates calmodulin kinase, which phosphorylates proteins [22] into their active forms. For example, phosphorylation activates endothelial nitric oxide synthase (eNOS) [23]. Increased nitric oxide (NO) synthesis produces vasodilation. We regularly observed a prolonged hyperemic phase of vasodilation and loss of vessel tone regulation following bubble passage through the arteriolar microcirculation [9-12]. This indicates an embolism-induced event. This may result from activation of Ca\(^{2+}\) and NO-dependent pathways. This may contribute to neural injury and impairment of endothelial function reported in other gas embolism studies [24,25]. Given that there are fluorophores available to study these pathways, it may be possible to build in this direction, too, on the valuable contributions Torres and colleagues are making to the field of microvascular physiology in gas embolism.

Ultimately, if we are going to be able to implement new clinical therapies for gas embolism, we will need a better understanding of the underlying physiology and injury development at the tissue and cell level. Fortunately, in Torres et al. [8] we have been provided an experimental platform for advancing such work in the microcirculation in vivo.

**REFERENCES**


