Abstract: There is strong clinical evidence that controlling cerebral venous oxygenation (oxyhemoglobin saturation) is critically important for patients with severe traumatic brain injury as well as for patients undergoing cardiac surgery. However, the only available method for cerebral venous blood oxygenation monitoring is invasive and requires catheterization of the internal jugular vein. We designed and built a novel optoacoustic monitor of cerebral venous oxygenation as measured in the superior sagittal sinus (SSS), the large midline cerebral vein. To the best of our knowledge, optical monitoring of cerebral venous blood oxygenation through overlying extracerebral blood is reported for the first time in this paper. The system was capable of detecting SSS signals in vivo at 700, 800, and 1064 nm through the thick (5–6 mm) sheep skull containing the circulating blood. The high (submillimeter) in-depth resolution of the system provided identification of the SSS peaks in the optoacoustic signals. The SSS peak amplitude closely followed the actual SSS blood oxygenation measured invasively using catheterization, blood sampling, and “gold standard” CO-Oximetry. Our data indicate the system may provide accurate measurement of the SSS blood oxygenation in patients with extracerebral blood over the SSS.

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1. Introduction

There is strong clinical evidence that monitoring cerebral venous oxygenation (oxyhemoglobin saturation) can be useful in patients with severe traumatic brain injury and in patients undergoing cardiac surgery because cerebral venous oxygenation reflects cerebral ischemia early and can be used to guide therapeutic interventions [1,2]. Cerebral venous oxygenation below 50% is associated with death or severe neurologic complications (normal range 55–75%) [3]. However, the only clinically useful technique for cerebral venous blood oxygenation monitoring is invasive, requiring catheterization of the internal jugular vein and measurement of blood oxygenation in the jugular bulb [4]. Another invasive method measures regional brain tissue pO₂ (partial oxygen tension) with an intracranial probe inserted directly into the brain parenchyma. Both methods can cause complications due to the invasiveness of the procedures and have technical limitations associated with frequent recalibration. The intraparenchymal probe can measure only local brain tissue pO₂, while the catheter in the jugular bulb often provides false values associated with contact with the vessel wall. Near-infrared (NIR) spectroscopy, a non-invasive optical technique, can monitor tissue oxygenation based on detection of light diffusively scattered from a target tissue [5,6]; however, NIR spectroscopy measures only volume-averaged oxygenation of brain tissue and cannot distinguish between venous, capillary, and arterial blood.

We proposed to use an optoacoustic technique for monitoring of blood oxygenation including cerebral venous blood oxygenation [7,8]. This technique detects optoacoustic (ultrasound) waves generated in tissue due to absorption of NIR pulses followed by thermo-elastic expansion of the irradiated volume. In vivo experiments conducted by our group [9–11] confirmed the clinically useful potential of this technique. Recently, we demonstrated high accuracy of optoacoustically measured oxygenation in the superior sagittal sinus (SSS), a large central cerebral vein [12]. Although photoacoustic tomography was studied for quantitative imaging of oxyhemoglobin saturation in the cerebral vasculature of small animals [13], the imaging depth in these experiments was not sufficient for human applications. Moreover, the small vessel size in these animals did not allow for validation of cerebral blood vessel oxygenation with “gold standard” invasive methods requiring blood samples.

To the best of our knowledge, accurate monitoring of cerebral venous blood oxygenation through overlying extracerebral blood is reported for the first time in this paper. The in vivo measurements were performed through the thick (5–6 mm) sheep skull using our novel multi-wavelength, optoacoustic system by probing the SSS which is located on top of the brain and collects blood from both hemispheres. In this study we tested the capability of the optoacoustic technique to probe the SSS through extracerebral blood and evaluated accuracy of the SSS blood oxygenation measurements through extracerebral blood. We validated the optoacoustic monitoring of the SSS blood oxygenation through extracerebral blood using the “gold standard” CO-Oximetry. This situation simulated much more challenging and clinically relevant conditions than those studied without diploic and other veins in [12] because sometimes diploic and other veins can overlie cerebral blood vessels and complicate cerebral blood oxygenation monitoring.

2. Materials and methods

A recently developed multi-wavelength optoacoustic system was used in the study (Fig. 1). For a source of pulsed tunable NIR radiation, the system uses a compact optical parametric oscillator (OPO; Opolette 532 II, Optron Inc., Carlsbad, CA) with the following parameters: range of available wavelengths 680–2440 nm; pulse duration, 10 ns; repetition rate, 20 Hz. We developed a highly sensitive optoacoustic probe, which incorporated a broadband (3 MHz) piezoelectric transducer and a 4-fiber light-delivery system. The fibers had a core diameter of 1 mm and were mounted surrounding the transducer. The signals from the probe
were amplified and digitized with a 100-MHz digitizer (National Instruments Corp., Austin, TX). Using a specially developed software package and a laptop, the OPO was controlled and the digitized signals were acquired and processed in real time.

The system was calibrated with as a “gold standard” CO-Oximeter (IL 682, Instrumentation Laboratories, Lexington, MA) using a procedure described in detail in [12]. The calibration was performed using model blood vessels in a tissue phantom with optical properties close to that of tissue in the NIR spectral range at three different wavelengths (700, 800/805, and 1064 nm) in a wide range of blood oxygenation (17–94%). Simultaneously, actual blood oxygenation was measured using blood sampling and the CO-Oximeter. The calibration curve obtained in [12] was used in this study to predict the SSS blood oxygenation.

The three wavelengths were used because they provide accurate measurement of blood oxygenation due to the strong hemoglobin absorption coefficient dependence on oxygenation at 1064 and 700 nm [14–16]. At 1064 nm the hemoglobin absorption increases with oxygenation, while at 700 nm it decreases. Moreover, at 1064 nm, both melanin and water have low absorption and scattering in tissues [17–20]. In contrast, 800 nm and 805 nm were used for reference measurements and normalization of signals because they are in the middle of the typical range of 795 nm to 810 nm reported in the literature for the isosbestic point [15,16,21], where optical absorption of blood does not depend on oxygenation. The difference in hemoglobin absorption at these two wavelengths is very small that allows for using them interchangeably in oxygenation studies.

After performing these calibration studies, we conducted in vivo experiments in 6 adult merino sheep. Optoacoustic signals were detected from the SSS through the intact skull (thickness: 5-6 mm). Although the adult sheep skull is thinner than the adult human skull (8–10 mm), the bone over the SSS is thick enough to demonstrate the capability of optoacoustic technique to measure cerebral blood oxygenation. In addition, the structure of the sheep skull is anatomically similar to that of the human skull and both have areas with increased blood content consisting of diploic veins and/or increased capillary network.

The Institutional Animal Care and Use Committee at the University of Texas Medical Branch (UTMB) approved the protocol for the study. The animals were housed at the UTMB’s Animal Resources Center under daily supervision of full-time veterinarians. During the experiments, the sheep were anesthetized with a 1.5% to 2.0% isoflurane and kept in a prone position. Oral-tracheal intubation was used for the delivery of both isoflurane and a gas mixture of oxygen and nitrogen to the animal.
We were able to change venous blood oxygenation throughout a wide range of 10%–100% by varying the fraction of oxygen (FiO\textsubscript{2}) in the inhaled gas mixture in the range from 10% to 100%. To generate the gas mixtures varying from 10% to 100% inspired oxygen, we combined medical grade oxygen and nitrogen using precision flow meters to produce a total gas flow rate of 2L per minute that provides an adequate flow for anesthesia. We performed at least 2-3 complete cycles of decreasing SSS blood oxygenation and increasing it in each animal. Some sheep were subjected to up to 5 complete cycles.

The scalp was cut along the midline, reflected to the sides, and the skin and other soft tissues over the skull at the site of measurement were removed before attaching the optoacoustic probe. A small craniotomy was made close to the site of optoacoustic measurements to insert a blood-sampling catheter into the SSS to draw samples immediately after each optoacoustic measurement. A small (5 x 10 mm) burr hole was placed in the skull over the SSS and the SSS was cannulated by direct visualization using PE-50 tubing. The cannula was secured with bone wax and sutures in the scalp. Samples were drawn and blood oxygenation was measured with the CO-Oximeter. Although the diploic and other extracerebral veins are small, sometimes they are visible as darker spots in the skull. When the probe was placed on these areas, the optoacoustic signal had an additional (second) peak after the first peak, which was generated on the skull surface.

During the experiment we continuously monitored the animal’s vital signs. Blood pressure was measured using a catheter inserted into femoral artery, while a pulse oximeter attached to lip, tongue, or ear continuously monitored arterial blood oxygenation. Heart rate and cardiac rhythm were monitored by electrocardiography. At the end of the experiment the sheep was given saturated KCl solution intravenously (about 1 cc/kg) under deep (>4%) isoflurane anesthesia.

A thin layer of ultrasound gel ensured acoustic coupling of the optoacoustic probe with tissue. To obtain SSS signals with the highest amplitude, we scanned the probe using a 3D translation stage. Laser pulse energy was attenuated to provide incident laser fluence of about 4 mJ/cm\textsuperscript{2}, which is well below the maximum permissible exposure for skin (20–100 mJ/cm\textsuperscript{2}) in this spectral range [22].

At each oxygenation level we consecutively acquired optoacoustic signals at 700 nm, 805 nm, and 1064 nm. Every recorded signal was an average of 400 acquired signals to increase signal-to-noise ratio. A blood sample was drawn from the SSS after each set of the three-wavelength measurements that took approximately 1.5–2 min.

3. Results and discussion

Figure 2 shows typical optoacoustic signals recorded at the wavelengths of 700 nm (a), 805 nm (b), and 1064 nm (c) from the SSS and the sheep skull with low blood content. The signals were measured at three different oxygenation levels: 30%, 75%, and 95% (green, blue, and red lines, respectively). The leftmost peak in each signal was produced by the absorption of light in the upper layers of the skull (major absorbers within the skull are lipids, water, hemoglobin, and collagen [23]). The next prominent peak was delayed from the first one by 2-2.35 μs in different sheep. This time delay can be converted into the skull thickness by multiplying by the speed of sound in the cranial bone, \(c_s\). The reported data for the speed of sound in cranial bone differ. Fry et al. [24] reported that \(c_s = 2.5 \text{ mm/μs}\) for the diploe (porous internal layer of the bone) and \(2.9 \text{ mm/μs}\) for both inner and outer tables. In skull bone a mean \(c_s = 3.36 \text{ mm/μs}\) was reported by Enderle [25]. Using these estimates and our optoacoustic signals, one can calculate the minimal and maximal possible bone thickness as \(\Delta_{\text{min}} = 2 \text{ μs} \times 2.7 \text{ mm/μs} = 5.4 \text{ mm}\) and \(\Delta_{\text{max}} = 2.35 \text{ μs} \times 3.36 \text{ mm/μs} = 8 \text{ mm}\), respectively. These numbers are in good agreement with the range of the cranial bone thicknesses from 5 to 8 mm that we measured in the sheep after the experiments. The SSS is located directly beneath the skull bone, separated from it by only a thin layer of connective tissue with very
low blood content \((\text{dura mater})\). Since hemoglobin is the prevailing absorber at these wavelengths, one can conclude that the second peak in each signal is generated in SSS blood.

All the signals in Fig. 2 were normalized to the amplitude of the surface peak to minimize influence on the SSS oxygenation measurements of instability in acoustic contact and in the OPO pulse energy. Although, the optoacoustic signal amplitude depends on the light fluence on the skull surface and absorption coefficient at the employed wavelengths, the blood content of the upper dense layer of the skull bone (the outer table) is extremely low. It implies that the changes in the amplitude of the surface peak were not produced by the variations of blood oxygenation, but rather by the instability of acoustic contact and incident light fluence. Hence, the normalization of the signals by this amplitude removes this dependence and increases the accuracy of the SSS oxygenation measurements. The optical absorption of the skull is wavelength-dependent, but it does not influence substantially the accuracy of the SSS blood oxygenation measurements, because the bone absorption has a weak dependence at these wavelengths [23]. The peak-to-peak SSS signal amplitude measured at 700 nm decreased with the SSS oxygenation. At 1064 nm, the opposite dependence was valid: the amplitude increased with the SSS oxygenation. Since 805 nm is in the isosbestic range in the near-infrared spectrum of whole blood absorption [15,16,21], the optical absorption of blood does not depend on oxygenation and the amplitude of the SSS peak at 805 nm was changing due to hemodynamic phenomena described below.

The optoacoustic signal from the exposed skull over the SSS had an additional peak adjacent to the bone surface peak in the presence of the extracerebral blood vessels (Fig. 3). The time delay between the two peaks was about 0.4 \(\mu\)s, which translates into 1.2 mm
distance in bone. The outer table thickness in sheep is about 1.0-1.5 mm. Therefore, the peak was most probably generated in a diploic vein located under the probe. This conclusion is further confirmed by the fact that the amplitude of this “subsurface” peak changed similarly to the amplitude of the SSS peak, without any delay (Fig. 4 shows both trends at 700 nm). Therefore, the changes in the cerebral blood oxygenation resulted in the synchronous response from these two optoacoustic sources.

Despite the presence of the diploic vein that absorbed some amount of light, the signal from the SSS was strong and easily identifiable. The fact that the SSS signal was detectable

![Optoacoustic Signal](a)

![Optoacoustic Signal](b)

![Optoacoustic Signal](c)

Fig. 3. Optoacoustic signals from the sheep skull with a diploic vein and the SSS at 700 nm (a), 800 nm (b), and 1064 nm (c) for different SSS blood oxygenations: 89%, 17.5%, and 94%.

![Amplitude of the SSS and diploic vein peaks](d)

Fig. 4. Amplitudes of the SSS and diploic vein peaks (blue line with triangles and pink line with open dots, respectively) in normalized optoacoustic signals at the wavelength of 700 nm.
through the blood above the SSS is very encouraging from clinical point of view. In patients with head trauma, the brain may be inadequately perfused, while extracerebral blood oxygenation can be normal. Therefore it is important to detect these two blood vessels separately. Figure 3 demonstrates that the optoacoustic technique is capable of separating the signals generated in extracerebral and cerebral blood.

It is worth noting that this specific experiment continued for 5 hours and we were able to perform four cycles of oxygenation change. Figure 5 shows the SSS blood oxygenation measured invasively during this experiment (black line with dots, left Y-axis) along with the peak-to-peak SSS signal amplitude in normalized optoacoustic signals (colored lines with triangles, right Y-axis). As in the sheep measured in a region without a diploic vein, the SSS signal amplitude at 700 nm changes in the direction opposite to that of actual blood oxygenation. In contrast, both of these parameters change concurrently at 1064 nm. These trends are in good agreement with the optical absorption spectrum of blood [14–16,20,21].

The SSS signal amplitude measured at 800 nm correlated with the actual SSS blood oxygenation as well. However, these changes are not related to the absorption properties of blood, as the extinction coefficient of hemoglobin at 800 nm is not dependent on its oxygenation level. However, there are other factors that can influence the amplitude of the optoacoustic signal from the SSS. The decrease of the fractional oxygen (FiO₂) in the inspired gas mixture causes hypoxemia in sheep (decreased partial pressure of oxygen in arterial blood, PaO₂), which, in its turn, leads to hypoxia (lack of oxygen in the body). There are physiologic compensatory mechanisms to ensure adequate oxygen supply to vital organs during hypoxia. The first response is redistribution of the blood flow so that regional blood flow increases in vital organs (including brain). The major mechanism that serves this purpose is vasodilation of the brain vessels. As the diameter of the sheep SSS (about 1 mm) is
smaller than the active size of the optoacoustic probe (10 mm$^2$), the increase in the SSS diameter due to vasodilation means a larger effective size of the acoustic source, and, hence, a higher SSS signal amplitude. Respectively, when cerebral blood vessels constrict, the SSS signal amplitude decreases.

At the same time, hypoxia triggers splenic contraction. This process is much slower than vasodilation and results in an additional red blood cell (RBC) release into the circulation [26–28]. After splenic contraction, the oxygen delivery to organs is maintained by the increase of the blood hemoglobin concentration, and the regional blood flow re-distribution returns to normal. As the absorption coefficient of blood is linearly dependent on the concentration of hemoglobin, this transient elevation of blood hemoglobin concentration manifests itself in the higher SSS signal amplitude. When the hypoxic state ends, the spleen again sequesters excessive RBCs, and the SSS signal decreases.

Figure 6(a) shows the time trend of both total hemoglobin concentration [THb] (red line with dots, left Y-axis) and blood oxygenation (black line with dots, right Y-axis) during the 5-hour experiment. When venous oxygenation (measured as hemoglobin saturation) dropped below a threshold (around 50% in most experiments), the [THb] started to increase, most likely due to splenic contraction. When blood oxygenation returns to higher levels, [THb] decreases (spleen sequesters the RBCs). The negative correlation in the graph is not perfect, though, due to occasional adjustments of the flow rate of intravenous Ringer’s lactate solution given during the study. When the animal’s vital signs were getting unstable (normally occurred during the acute hypoxic state), the infusion rate of the Ringer’s solution was increased which temporarily lowered the [THb], and vice versa. This might explain the presence of the minima on the rising slopes of the [THb] trend, when blood oxygenation still was going down and [THb] was supposed to keep increasing. For comparison, Fig. 6(b) shows similar trends obtained in the experiment with another sheep: the negative correlation of these two parameters is more obvious in this graph.

Figure 7(a) shows the same [THb] trend as in Fig. 6(a), superimposed now with the peak-to-peak SSS signal amplitude measured at 800 nm. The correlation of these two parameters is easily seen, although the [THb] trend was somewhat distorted due to the reasons of infused Ringer’s lactate as discussed above. However, the correlation was sometimes almost ideal as shown in Fig. 7(b) (it shows the data obtained during one cycle of FiO$_2$ change in another sheep).

Another reason for changes of the SSS peak amplitude at 800 nm can be gradual distortion of the alignment of the optoacoustic probe with the SSS. Although the sheep’s head was fixed in a custom-made head frame, some motion due to cranial muscle contraction could lead to a small displacement of the probe from the best-aligned position and distort the signal.
The optoacoustic signals were acquired at the three wavelengths as rapidly as possible within one set of measurements to keep them close in time and thus, to provide similar measurement conditions. In this case, the three confounding factors discussed above had similar influence on the signals measured at all the three wavelengths. This allowed a significant increase in measurement accuracy by normalizing the SSS signal amplitudes at 700 nm and 1064 nm using the amplitude at 800 nm. The normalized peak-to-peak SSS signal amplitudes were calculated as the ratios of the amplitudes measured at 700 nm or 1064 nm to those measured at 800 nm (Fig. 8). The correlation between the amplitude ratio and actual SSS blood oxygenation measured invasively is substantially higher compared to that for the original amplitudes (Fig. 5 ((a) and (c)), colored lines). Figure 9 demonstrates that the amplitude ratio is linearly dependent on blood oxygenation (data for the fourth cycle from Fig. 8 are presented). The lines of the corresponding color fit the data point sets linearly. The correlation coefficients are much higher, $R^2 = 0.71$ and 0.91 at 700 nm and 1064 nm, respectively, while before the normalization they were 0.569 and 0.776, respectively (data not shown).

We predicted the SSS blood oxygenation values and assessed accuracy of the optoacoustic measurements. Figure 10(a) shows high correlation between the optoacoustically predicted and actual SSS blood oxygenation measured with the CO-Oximeter ($R^2 = 0.91$). To predict the SSS blood oxygenation, we calculated the ratio of the SSS peak amplitude measured at 1064 nm to that measured at 700 nm and then used the calibration curve reported in [12].
calibration curve is the dependence of the ratio of the signal amplitude measured from sheep blood in the phantom at 1064 nm to that measured at 700 nm vs. blood oxygenation.

To assess accuracy of the optoacoustic measurements, we calculated the bias and standard deviation using the difference between the optoacoustically predicted and actual oxygenation measured with the CO-Oximeter (Fig. 10(b)). These data demonstrate that, despite the presence of the extracerebral blood, the accuracy of the optoacoustically predicted SSS blood oxygenation approaches that of measured without extracerebral blood that was reported in [12] ($R^2 = 0.91$ vs. 0.965, bias = 2.0% vs. −9.3%, and SD = 8.5% vs. 4.2%, respectively).

Although, no tissue phantom can provide perfect calibration for in vivo measurements, the calibration obtained from blood in vitro in the tissue phantom in [12] allowed for accurate prediction of SSS blood oxygenation. This is because: 1) the phantom had optical properties (the effective attenuation coefficient and the spectral dependence of the attenuation coefficient) close to that of tissue; 2) the blood signal amplitude was measured in vivo at 1064 nm and 700 nm within a short time to minimize changes in the tissue optical properties during the measurement; and 3) although the diploic and other extracerebral veins produce a noticeable signal, they are small and their effect on the fluence attenuation is not strong. The
veins produce a noticeable signal because they are close to the irradiated surface and fluence at these depths is not strongly attenuated. The data presented in Figs. 10(a) and 10(b) on accuracy of the optoacoustic measurement of SSS blood oxygenation through the extracerebral blood confirm that the diploic and other extracerebral veins are not optically thick and do not substantially reduce accuracy of the SSS blood oxygenation measurements.

Since we use the ratio measurements for predicting the SSS blood oxygenation, the acoustic wave aberration and attenuation effects on the accuracy of the SSS blood oxygenation measurements are minimal. Acoustic reverberations do not influence the accuracy of the SSS oxygenation measurements because they arrive at the optoacoustic probe later than the SSS signal due to longer acoustic path. Moreover, the reverberation signal amplitude is low due to stronger acoustic attenuation in the bone over the longer path.

As noted above, it is important to differentiate between extracerebral and cerebral blood. Our results show that the optoacoustic technique allows for it due to high resolution. Besides, it can accurately quantify the SSS blood oxygenation. In this study we did not simultaneously produce different states of oxygenation in the diploic and sagittal sinus veins. It is a difficult technical and physiologic task that was beyond the scope of this study. We plan to address in our next studies the accuracy of the SSS blood oxygenation measurement when oxygenation is different in the two veins.

To avoid the lateral scanning of the optoacoustic probe, one can use optoacoustic arrays for fast detection of the SSS signals. Recently developed optoacoustic arrays that provide optoacoustic images deeply in highly scattering media and tissues in vivo [29–32] can be adapted for the SSS signal detection. This will further improve system performance and minimize influence of motion artifacts.

4. Conclusions

This study demonstrated that the multi-wavelength optoacoustic system provides accurate monitoring of cerebral venous blood oxygenation through overlying extracerebral blood. The system is capable of detecting SSS signals in vivo through the thick sheep skull containing circulating blood. The high in-depth resolution of the system provided easy identification of the SSS peaks in the optoacoustic signals. The SSS peak amplitude closely followed the actual SSS blood oxygenation measured invasively using catheterization, blood sampling, and “gold standard” CO-Oximetry. Our data indicate that this system may provide accurate measurement of the SSS blood oxygenation in patients.

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