Tissue oxygenation index is a useful monitor of histologic and neurologic outcome after cardiopulmonary bypass in piglets

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Objective: Tissue oxygenation index is a novel monitoring indicator derived by near-infrared spectroscopy. We hypothesized that tissue oxygenation index could predict a minimum safe flow rate for specific bypass conditions.

Methods: Thirty-six piglets (age, 43 ± 5 days; weight, 9.0 ± 1.1 kg) underwent cardiopulmonary bypass with cerebral near-infrared spectroscopy (NIRO-300; Hamamatsu Photonics K.K., Hamamatsu City, Japan). Animals were cooled for 40 minutes to 15°C, 25°C, or 34°C (pH-stat, hematocrit value of 20% or 30%, and pump flow of 100 mL · kg^{-1} · min^{-1}), followed by low-flow perfusion (10, 25, or 50 mL · kg^{-1} · min^{-1}) for 2 hours. Neurologic and behavioral evaluations were determined for 4 days. The brain was then fixed for histologic assessment. Tissue oxygenation index was defined as the average signal during low-flow bypass.

Results: Animals with an average tissue oxygenation index of less than 55% showed cerebral injury, whereas animals with an index of greater than 55% showed minimal or no evidence of injury. Correlations were found between average tissue oxygenation index and histologic score (Spearman rho = −0.65, $P < .001$) and neurologic deficit score (Pearson $r = -0.50$, $P = .002$) on the first postoperative day. Temperature ($P < .001$), flow rate ($P < .001$), and hematocrit value ($P = .002$) were multivariable predictors of tissue oxygenation index, as determined by means of multivariable analysis of variance.

Conclusion: Tissue oxygenation index is a useful monitor for defining the minimum safe flow rate during cardiopulmonary bypass. An index value of less than 55% is a strong predictor of neurologic injury.

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Neurologic injury continues to be an important problem after cardiopulmonary bypass (CPB).[1-9] Previous work in this area has focused on deep hypothermic circulatory arrest (DHCA), which is now used infrequently in pediatric heart surgery.[3-5] As an alternative to DHCA, reduced-flow hypothermic CPB has been used.[6-9] However, there are no guidelines for a minimal safe flow rate or its duration under specific CPB conditions, including hematocrit value and temperature.[6-15]

Near-infrared spectroscopy (NIRS) is a relatively new technique for assessment of cerebral oxygenation.[16,17] We have previously reported development of a piglet model with NIRS, allowing real-time monitoring during DHCA.[18-21] Although an important finding was that the safe duration of DHCA could be monitored by using the oxyhemoglobin nadir time, nevertheless, NIRS was of limited value because it could only report a change from baseline rather than an absolute value. This is a consequence of lack of information regarding the path length of the infrared beam between the transmitting and receiving optode. However, the limitation has been partially addressed with the development of the NIRO-300 instrument (Hamamatsu

Photonics K.K., Hamamatsu City, Japan), which incorporates 3 receiving optodes with different path lengths. This allows calculation of a tissue oxygenation index (TOI) in addition to changes in oxyhemoglobin (Hbo_2) and deoxyhemoglobin (HHb) concentrations and redox state of cytochrome oxidase.[22-24] TOI is an absolute value and allows comparison among different conditions.

The present study was designed to evaluate the risk of neurologic injury at various levels of reduction of CPB flow rates at different temperatures and hematocrit values during hypothermic low-flow CPB. We hypothesized that TOI could predict a minimum safe flow rate and that this level would vary according to the specific bypass conditions.

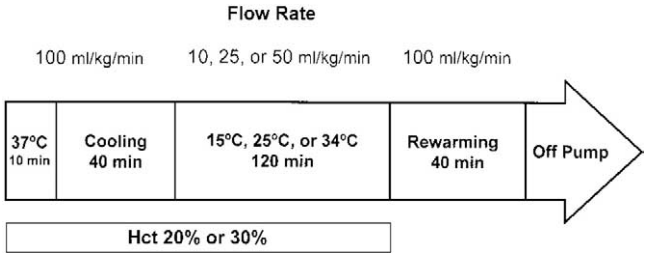

Figure 1. Experimental protocol depicting bypass conditions. All animals underwent the pH-stat strategy. NIRS signals were recorded every 10 seconds after induction of anesthesia and for 3 hours after weaning from CPB. *Hct,* Hematocrit.

Materials and Methods

Surgical Preparation

All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised in 1996.

Thirty-six 5- or 6-week-old Yorkshire piglets weighing 9.0 ± 1.1 kg were sedated with intramuscular ketamine (20 mg/kg) and xylazine (4 mg/kg) and intubated with 5-mm cuffed endotracheal tubes. Each animal was ventilated with an inspired oxygen fraction of 0.21 at a respiratory rate of 15 to 18 breaths/min to achieve a normal pH and $Paco_2$. Optodes for NIRS were placed over the frontal lobes, with an interoptode distance of 4.0 cm. The receiving optode incorporates 3 detectors. After an intravenous bolus injection of fentanyl (50 μg/kg) and pancuronium (0.5 mg/kg), anesthesia was maintained by continuous infusion of fentanyl (25 $\mu g \cdot kg^{-1} \cdot h^{-1}$), midazolam (0.2 $mg \cdot kg^{-1} \cdot h^{-1}$) and pancuronium (0.2 $mg \cdot kg^{-1} \cdot h^{-1}$) through the entire experiment.

All surgical procedures were performed under sterile conditions. For intraoperative monitoring and blood sampling, arterial and venous lines were placed in the left superficial femoral artery and right femoral vein, respectively. The right femoral artery was exposed for the CPB arterial cannula, and a right anterolateral thoracotomy was performed in the third intercostal space to expose the right atrium for venous cannulation. After systemic heparinization (300 IU/kg), an 8F arterial cannula (Medtronic BioMedicus, Eden Prairie, Minn) and a 28F venous cannula (Research Medical, Inc, Midvale, Utah) were inserted into the right femoral artery and right atrial appendage, respectively.

Experimental Protocol

Thirty-six piglets were randomized according to the experimental protocol. The study protocol is depicted in Figure 1. Arterial pressure was monitored continuously throughout each experiment and was recorded every 10 minutes. Arterial blood gas values, including pH, Po_2, Pco_2, hemoglobin, hematocrit, electrolyte, glucose, and lactate concentrations, were measured at baseline, every 10 minutes during cooling and rewarming, every 15 minutes during low-flow bypass, and after the procedure as needed (NOVA 900; Nova Biomedical, Waltham, Mass).

Experimental Groups

Hematocrit value. During the cooling phase, a hematocrit value of either 20% or 30% was maintained.

Flow rates during the low-flow period. Flow rates of 10, 25, or 50 $mL \cdot kg^{-1} \cdot min^{-1}$ were used.

Temperature during the low-flow period. A pharyngeal temperature of either 15°C, 25°C, or 34°C was used for 2 hours.

The experimental design included these 3 parameters, with 2 or 3 possible values resulting in 18 ($2 \times 3 \times 3$) experimental settings. Each setting was performed in 2 piglets. In the group with a hematocrit value of 20%, the CPB prime consisted of 400 mL of blood and 800 mL of crystalloid solution. The other group with a hematocrit value of 30% had a setting of 1200 mL of whole-blood prime.

CPB Technique

The CPB circuit consisted of a roller pump (Cardiovascular Instrument Corp, Wakefield, Mass), membrane oxygenator (Minimax; Medtronic Inc, Anaheim, Calif), and sterile tubing with a 40-μm arterial filter (Olson Medical Sales, Inc, Ashland, Mass). Fresh whole blood from a donor pig, drawn on the operative day, was transfused into the prime as required to adjust the hematocrit value to either 20% or 30%. Methylprednisolone (30 mg/kg), furosemide (0.25 mg/kg), sodium bicarbonate 7.4% (10 mL), cefazolin sodium (25 mg/kg), fentanyl (50 μg/kg), and pancuronium (0.5 mg/kg) were added to the prime. Full bypass flow was set at 100 $mL \cdot kg^{-1} \cdot min^{-1}$, and pH-stat management was selected. CPB was started, and the animals were perfused for 10 minutes at normothermia (37°C). Animals were then cooled to a pharyngeal temperature of 15°C, 25°C, and 34°C over 40 minutes according to the experimental protocol. Ventilation was stopped after the establishment of CPB. After cooling, low-flow perfusion at a flow rate of 10, 25 or 50 $mL \cdot kg^{-1} \cdot min^{-1}$ was initiated for 120 minutes. Before rewarming, methylprednisolone (30 mg/kg), furosemide (0.25 mg/kg), sodium bicarbonate (10 mL), and mannitol (0.5 g/kg) were administered into the pump. Rewarming was begun at a rate of 100 $mL \cdot kg^{-1} \cdot min^{-1}$, and each animal was warmed to 37°C. The heart was defibrillated as necessary at a pharyngeal temperature of 30°C. Fresh whole blood from a donor pig was transfused into the prime as required to increase the hematocrit value to at least 25% in all groups during rewarming. Ventilation (fraction of inspired oxygen, 1.0) was started 10 min-

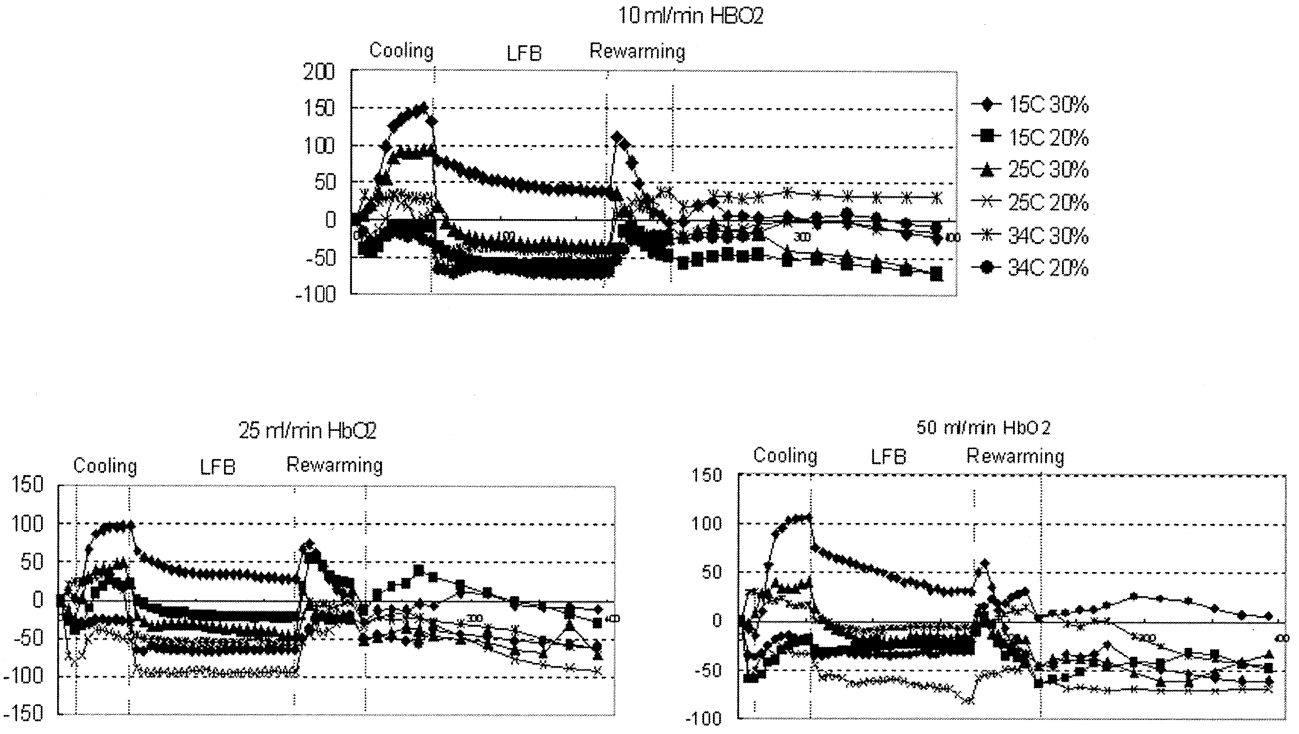

Figure 2. Changes in Hbo_2 determined by means of NIRS. *LFB,* Low-flow bypass.

utes before weaning from CPB. After 40 minutes of rewarming, animals were weaned from CPB, and the arterial and atrial cannulas were removed. Protamine (5 mg/kg) was administered intravenously after the animals were in a hemodynamically stable condition. The wound was closed in a sterile fashion.

Near-Infrared Spectroscopy

A pair of fiberoptic optodes was attached to the head of the animal with a probe holder after induction of anesthesia. The spacing of optodes was 4.0 cm in the coronal plane. These 2 optodes, a transmitter and a receiver of laser light of near-infrared wavelength, were connected to the NIRS device (NIRO-300). Data were recorded every 10 seconds after the induction of anesthesia and for 3 hours after weaning from CPB. This device calculated the relative concentration changes in Hbo_2, HHb, oxidized cytochrome a,a3 ($Cyto_2$), and TOI.

Postoperative Management

Animals remained sedated and paralyzed and were mechanically ventilated and monitored continuously for 12 hours postoperatively. The chest tubes were removed, and animals were extubated.

Postoperative Evaluation

Neurologic and behavioral evaluations were performed at 24-hour intervals by a veterinarian blinded to the experimental protocol beginning on postoperative day (POD) 1, as described previously.[25] Neurologic scoring data were adapted from the neurologic deficit score (NDS; 500 = brain death, 0 = normal) and overall performance category (5 = brain death, 1 = normal). On POD 4, the brain was fixed with 4 L of 4% formaldehyde solution, and the histologic assessment was done by a single neuropathologist in a blinded fashion (5 = cavitated lesions with necrosis, 4 = significant damage to neurons, 3 = large clusters of injured neurons, 2 = small clusters of damaged neurons, 1 = isolated neuronal damage, and 0 = normal).[26]

Statistical Analysis

A power analysis indicated that a sample size of 36 animals would provide 80% power to detect an effect size of 1.0 (mean difference/common standard deviation) with respect to outcome scores between the bypass conditions (version 5.0, nQuery Advisor; Statistical Solutions, Boston, Mass). In addition, the total N would provide 80% power to evaluate the correlation between TOI and the neurologic and histologic scores. All continuous variables were checked for normality by using the Shapiro-Wilk test, and significant skewness was detected for total histologic score.[27] Therefore means and standard deviations are used for TOI and NDS, and medians and ranges are used for histologic scores. The Pearson product-moment correlation coefficient *(r)* was used to measure the association between TOI and NDS and the Spearman rho correlation for TOI and histologic scores. Multivariable analysis of variance was applied to test the effects of hematocrit value, temperature, and flow rate on outcome scores.[28] Temperature and flow rate conditions were compared with the nonparametric Kruskal-Wallis test and Mann-Whitney *U* test for comparing histologic scores and analysis of variance for assessing differences in NDS.[29]

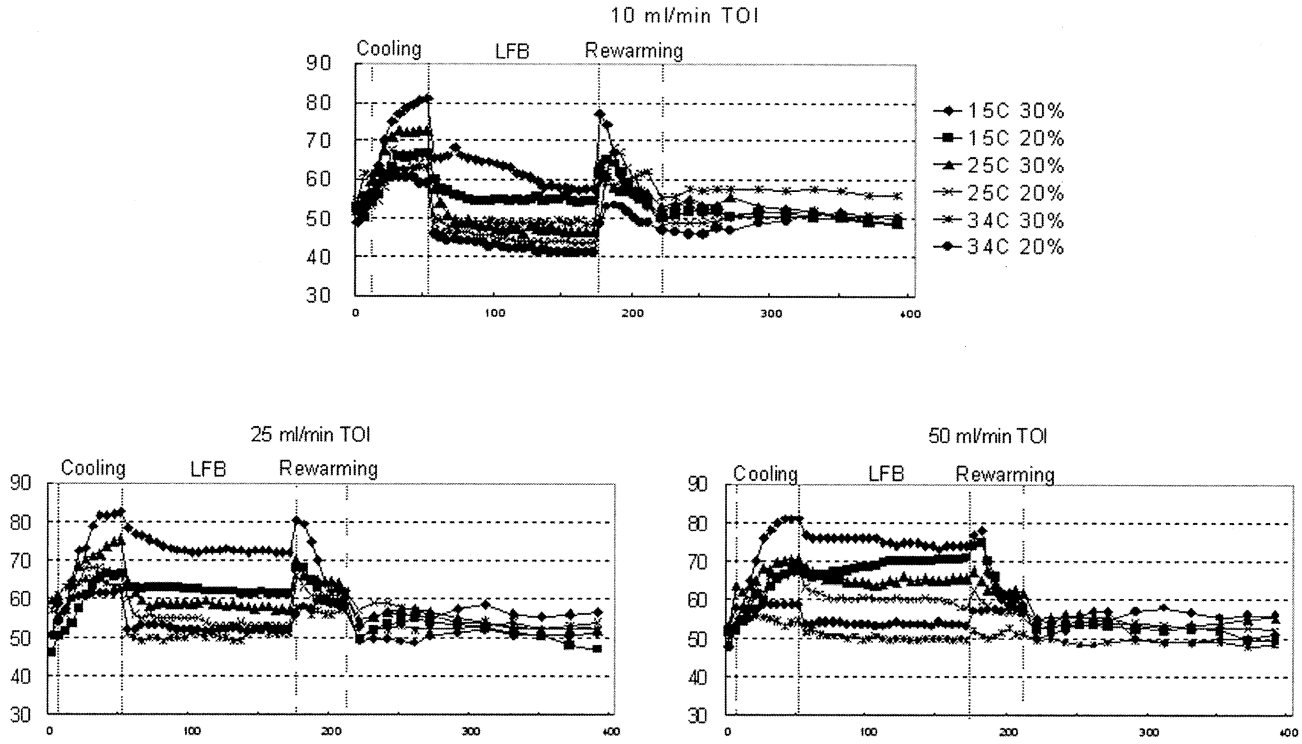

Figure 3. Changes in TOI at 3 different low-flow rates (10, 25, 50 mL · kg^{-1} · min^{-1}). *LFB,* Low-flow bypass.

Statistical significance was defined by a Bonferroni-corrected P value of less than .05. Analysis of the data was performed with the SPSS software package (version 12.0; SPSS, Inc, Chicago, Ill). All reported P values are 2 tailed.

Results

Baseline measurements (before CPB) showed no significant differences between experimental conditions for body weight, hematocrit values, blood gases, and parameters derived by NIRS.

Hbo_2 Signal, HHb Signal, and $Cyto_2$ Signal

The Hbo_2 signal increased during the cooling phase in all treatment groups (Figure 2). In the groups with hematocrit values of 30%, the Hbo_2 signal continued to increase during the entire cooling phase, whereas the Hbo_2 signal reached a plateau after about 20 minutes with a hematocrit value of 20%. The HHb signal showed an inverse decrease compared with the Hbo_2 signal during the cooling phase. From the onset of low-flow bypass, there was a decrease in Hbo_2 and $Cyto_2$ signals and a reciprocal increase in HHb signal. Some groups showed a gradual decrease in Hbo_2 signal, whereas other groups showed a rapid decrease. Groups that maintained a fixed value during low-flow bypass were 25°C and 34°C at a flow rate of 10 mL · kg^{-1} · min^{-1}, 25°C with hematocrit values of 20%, and 34°C at flow rates of 25 and 50 mL · kg^{-1} · min^{-1}.

Tissue Oxygenation Index

TOI increased during the cooling phase in all groups (Figure 3). Lower temperature and higher hematocrit values were associated with a higher index for the same flow rate. From the onset of low-flow bypass, TOI demonstrated a rapid decrease and then maintained a fixed value throughout low-flow perfusion, except for 2 groups (10 mL/15°C/30% hematocrit and 25 mL/15°C/30% hematocrit). During low-flow bypass, the 15°C group with a hematocrit value of 30% had the highest index, and the groups at 34°C had the lowest index. Average TOI was defined as the average signal during low-flow bypass. Average TOI was highly correlated with minimum TOI and TOI at 15 minutes after the onset of low-flow bypass (Pearson $r \geq 0.98$).

Neurologic and Behavioral Evaluations

Three animals assigned to 34°C and a flow rate of 10 mL · kg^{-1} · min^{-1} could not be weaned from mechanical ventilation because of a lack of spontaneous breathing. Scoring showed brain death on POD 1 (NDS = 500, overall performance category = 5). Another animal in the same group died on POD 2 because of respiratory abnormality

TABLE 1. TOI and total histologic and neurologic deficit scores for low-flow bypass conditions

Condition*	Temperature, flow rate (mL · kg^{-1} · min^{-1})	TOI (%)	Total histologic score, median (IQR)	NDS on POD 1, mean ± SD
1	15°C, 10	59 ± 6	0 (0-0)	128 ± 75
2	15°C, 25	68 ± 7	0 (0-0)	78 ± 47
3	15°C, 50	71 ± 4	0 (0-0)	52 ± 32
4	25°C, 10	49 ± 3	5 (0-14)	134 ± 118
5	25°C, 25	55 ± 3	0 (0-2)	75 ± 44
6	25°C, 50	63 ± 5	0 (0-0)	120 ± 102
7	34°C, 10	44 ± 5†	16 (12-22)†	480 ± 28†
8	34°C, 25	53 ± 3	1 (0-9)	125 ± 45
9	34°C, 50	52 ± 3	1 (0-3)	111 ± 8

TOI, Tissue oxygenation index; *IQR*, interquartile range; *NDS*, neurologic deficit score; *POD 1*, postoperative day 1; *SD*, standard deviation. *Each condition, n = 4 animals. †Statistically significant compared with the other conditions.

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with persistent seizures. In other groups NDS and overall performance category showed daily recovery. Higher flow rate and lower temperature were associated with lower scores (less injury) and a rapid return to normal (NDS = 0, overall performance category = 1).

Histologic Assessment

Neuropathologic injury was evaluated in the same manner as in previous studies. Evidence of hypoxic-ischemic injury was indicated by the presence of hypereosinophilic shrunken neurons with karyorrhectic nuclei. Damage scores for all regions were summed and shown as a total score. Histologic damage was found predominantly in the neocortex and hippocampus. All groups perfused at 15°C showed no histologic damage, whereas all groups perfused at 34°C showed damage. At 25°C, the groups perfused at a flow rate of 50 mL · kg^{-1} · min^{-1} demonstrated no brain injury, whereas the groups perfused at 10 mL · kg^{-1} · min^{-1} had injury.

There was a tendency for severe scores when temperature was higher, flow rate was lower, and hematocrit value was lower. Table 1 summarizes the total histologic scores, NDS on POD 1, and average TOI stratified according to 9 different conditions on the basis of temperature and flow rate. Hematocrit value was not included in this table because it was not found to be predictive of histologic ($P = .66$) or neurologic ($P = .61$) scores on the basis of multivariable analysis, whereas temperature and flow rate were highly predictive of these outcomes (all $P < .001$). This table clearly shows that animals subjected to a temperature of 34°C and a flow rate of 10 mL · kg^{-1} · min^{-1} had very low average TOIs and significantly worse histologic scores and NDSs than the other conditions.

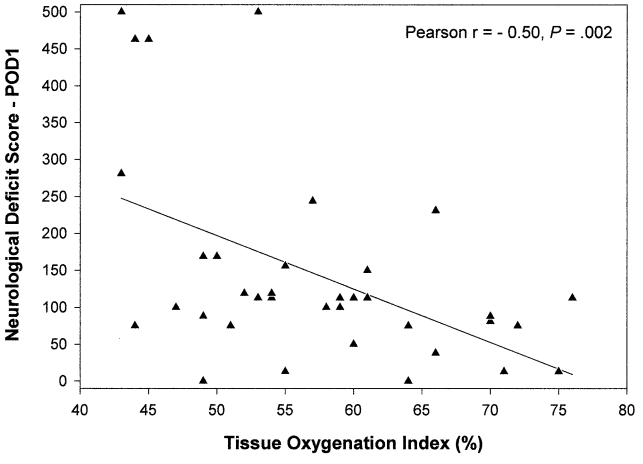

Figure 4. A significant inverse correlation was identified between average TOI and NDS on the first postoperative day (Pearson $r = -0.50$, $P = .002$).

Correlation Between Average TOI and Neurologic and Histologic Outcome

Figure 4 depicts a significant inverse correlation between average TOI and NDS on POD 1 (Pearson $r = -0.50$, $P = .002$). There were 18 animals that had TOIs of less than 55% and 18 animals with TOIs of greater than 55%. In comparing these 2 groups, the mean NDS was significantly higher in the former group (208 ± 160 vs 82 ± 57; $P = .004$, unpaired Student t test). The mean difference is 126 points. Significant correlation was found between average TOI and histologic score ($r = -0.65$, $P < .001$, Figure 5).

Multivariable analysis of variance with repeated measures revealed significant effects of temperature, flow rate, and hematocrit value on TOI during low-flow bypass (temperature, F = 37.4, $P < .0001$; flow rate, F = 18.6, $P < .0001$; hematocrit, F = 11.1, $P = .002$). All 3 variables were independently predictive of TOI, and the F tests indicate the strength of each predictor in terms of its effect on TOI. This can be best discerned by looking at the average TOIs for the 18 combinations of temperature, flow rate, and hematocrit

value, as shown in Table 2. Among the 18 experimental conditions, 9 had an average TOIs of less than 55% (shown in bold) and reflect unsafe CPB conditions in terms of the potential for cerebral injury.

Discussion

This study has confirmed that the TOI derived from NIRS is a useful monitor for determining bypass conditions that are likely to result in functional and structural neurologic injury. Animals that maintained an average tissue oxygen index of less than 55% during the 2-hour low-flow bypass period exhibited both structural and functional neurologic injury in every case. On the other hand, animals in which the average tissue oxygen index remained greater than 55% during the low-flow period had little or no evidence of injury.

TOI was found to increase during cooling, as did the Hbo_2 signal. Presumably, tissue oxygen increases during this time because of the decreasing metabolic rate in the setting of normal cerebral oxygen delivery. When the flow rate is decreased, however, the tissue oxygen index decreases, eventually achieving a plateau level. The level of the plateau varies with the perfusion conditions (ie, it is higher at a higher flow rate, lower at a higher temperature, and higher with a higher hematocrit value).

Identification of perfusion conditions that minimize the risk of brain injury during pediatric cardiovascular surgery has focused primarily on DHCA.[3-5] The current study builds on previous studies from our laboratory in which we examined the influence of hematocrit value, temperature, duration of arrest, and pH strategy in DHCA models.[30-33] NIRS in a piglet model during DHCA demonstrated that higher temperature, lower hematocrit value, more alkaline pH, and longer hypothermic circulatory arrest duration are predictive of more severe damage to the brain.[32,33] These previous experiments used the same survival model as this study, with daily neurologic observation by a blinded veterinarian, as well as histologic assessment by a blinded neuropathologist. Hbo_2 nadir time was calculated as the duration from reaching the nadir until reperfusion of the Hbo_2 signal during DHCA. Hbo_2 nadir time normalized according to the perfusion conditions was positively correlated with neurologic recovery on the fourth postoperative day and with total histologic injury score. Intravital microscopy in a piglet model demonstrated that a higher hematocrit value during cooling did not impair cerebral microcirculation and reduced white cell–endothelial activation after DHCA.[31] pH-Stat management increased both microvascular diameter and tissue oxygenation at the end of cooling.[30] Leukocyte activation is increased with higher temperatures and lower flow rates in the same model after low-flow CPB.[34]

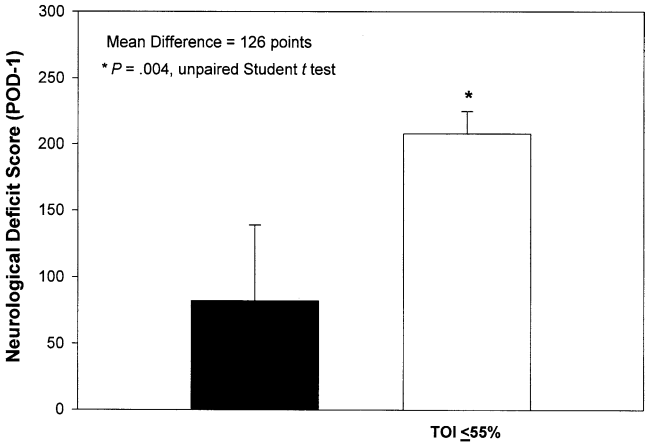

Figure 5. Significant difference in mean NDS according to TOI level. *POD-1*, Postoperative day 1.

In the present study animals were assigned to 2 different hematocrit values and 3 different temperatures and were perfused at 3 different flow rates to evaluate the minimal safe flow rate of low-flow hypothermic CPB. We have concluded from both laboratory and clinical studies that pH-stat strategy is preferable to alpha-stat strategy,[35,36] and therefore we used the pH-stat strategy for all groups. Several reports have suggested that low-flow bypass is a more advantageous strategy in terms of brain protection than DHCA[6-8,10-12,14,15]; however, the optimal flow rate and minimal safe flow rate for specific conditions during low-flow bypass have not previously been defined. In this current report neurologic outcome, including both histologic assessment and behavioral evaluation, have been assessed to determine minimal safe conditions for low-flow hypothermic CPB. All groups maintained at a temperature of 34°C during low-flow bypass had evidence of injury histologically or behaviorally, whereas a minimum flow rate of 10

TABLE 2. Average percent TOI during 2-hour low-flow bypass period

Flow rate	15°C Hct 30%	15°C Hct 20%	25°C Hct 30%	25°C Hct 20%	34°C Hct 30%	34°C Hct 20%
10 mL · kg^{-1} · min^{-1}	62.7	55.7	**49.3**	**45.7**	**49.4**	**43.2**
25 mL · kg^{-1} · min^{-1}	73.6	62.4	59.4	**54.8**	**50.9**	**52.6**
50 mL · kg^{-1} · min^{-1}	75.4	68.8	65.5	60.5	**50.3**	**53.7**

Hct, Hematocrit; *TOI,* tissue oxygenation index.

$mL \cdot kg^{-1} \cdot min^{-1}$ at a temperature of 15°C provided adequate oxygen supply and resulted in no cerebral injury. At a temperature of 25°C, all groups with a flow rate of 10 $mL \cdot kg^{-1} \cdot min^{-1}$ and some groups at a flow rate of 25 $mL \cdot kg^{-1} \cdot min^{-1}$ sustained cerebral injury, suggesting that a flow rate of 50 $mL \cdot kg^{-1} \cdot min^{-1}$ is needed to ensure that sufficient oxygen is delivered to the brain to minimize the risk of cerebral injury. The results are in agreement with those of animal studies by Swain,[37] Watanabe,[38] and their associates. Swain and colleagues[37] found that a flow rate of 10 $mL \cdot kg^{-1} \cdot min^{-1}$ was safe with a temperature of 15°C for 2 hours without pH-stat management. Watanabe and associates[38] demonstrated that a flow rate of 40 $mL \cdot kg^{-1} \cdot min^{-1}$ was needed to allow aerobic metabolism in the brain at a temperature of 15°C for 2 hours (pH-stat strategy).

Analysis of the NIRS data has clarified the role of hematocrit value and temperature at different flow rates by demonstrating their effect on cerebral oxygenation. The NIRO 300 is based on technology used in the earlier NIRO 500 and a prototype spatially resolved spectrometer.[22,23,39] Four wavelengths of light (775, 810, 850, and 910 nm) are delivered by 4 pulsed laser diodes, and scattered light is detected by 3 closely placed photodiodes. The concentration changes of the chromophores Hbo_2, HHb, total hemoglobin, and cytochrome oxidase are measured by the middle photodiode with the use of a modified Beer-Lambert law, whereas TOI is measured by all 3 photodiodes and application of the principle of spatially resolved spectrometry. TOI provides an absolute value, and average TOI was termed as the average signal for TOI during low-flow bypass in this study. The average TOI was highly correlated with minimum TOI and TOI at 15 minutes after the beginning of low-flow bypass. All animals with TOIs of greater than 60% had no histologic or behavioral injury, whereas all animals with TOIs of less than 55% had evidence of injury.

There are several reports evaluating the efficacy of TOI derived from the NIRO 300. Al-Rawi and coworkers[24] applied the NIRO 300 to patients undergoing carotid endarterectomy and demonstrated that a change in TOI was predominantly associated with internal carotid artery clamping and that TOI reflects cerebral tissue oxygenation with a high degree of sensitivity and specificity. McLeod and colleagues[40] demonstrated with severely brain-injured patients that TOIs followed similar patterns to jugular venous oxygen saturation and brain tissue oxygen tension during normobaric hyperoxia.

A limitation of NIRS is the fact that only a superficial volume to a depth of approximately 1 to 2 cm below the skin can be assessed. Deeper cerebral sites cannot be studied with this noninvasive continuous monitoring method. However, the most vulnerable regions are the neocortex, hippocampus, and caudate nucleus from our laboratory studies of hypothermic circulatory arrest, and those sites are similarly injured in terms of histopathologic assessment.[22,32,33] Thus in this piglet model recognition of injury by observation of the neocortex and cortex predicts whole brain damage.

Another limitation of this study is that all animals were exposed to 2 hours of low-flow bypass. Nevertheless, the average TOI during this period correlated well with the minimum TOI that animals experienced. Further work is needed to assess the effect of a shorter exposure from a TOI of less than 55%, the cutoff level defined in this study as predictive of injury.

The final limitation of this study is the difference in structure between piglet and human hemoglobin. This difference might lead to misinterpretation of the NIRS signal, especially in the setting of cyanosis. Furthermore, fetal hemoglobin has different absorption spectra relative to mature hemoglobin for both oxygenated and deoxygenated hemoglobin so that NIRS signals might require careful interpretation in neonates.

In summary, the TOI has been shown in this study to be a useful monitor of safe minimum flow rate. TOI is appropriately influenced by the specific bypass conditions of temperature and hematocrit value and represents an absolute value rather than a percentage change from baseline. Therefore, this index can be used and interpreted with good reliability to confirm the adequacy of perfusion, irrespective of variations in bypass conditions.

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Discussion

Dr Randall B. Griepp *(New York, NY).* This is a very interesting article, and the TOI looks like a useful method of monitoring. I am curious as to whether you measured sagittal sinus saturations, which are a very simple way of looking at the same sort of thing, and what the correlation was between sagittal sinus saturations and your TOI.

Dr Hagino. Thank you very much for your question. We did not sample blood directly from the sagittal sinus. We did obtain jugular venous samples for venous saturation, though in the setting of very low-flow bypass the volume of venous return was low.

Dr Frank W. Sellke *(Boston, Mass).* You are placing a lot of emphasis on oxygenation, which is appropriate. Have you considered administering adjuvants, such as deferoxamine or superoxide dismutase, to animals to see whether that correlation still exists as much as it does in your current study. I assume that a lot of the injury is due to increased oxidative stress. If so, can you do something other than increase the oxygen-carrying capacity to alleviate that using your model?

Dr Hagino. You mean oxygenation of the brain, the usual model?

Dr Sellke. Yes. If you compare a hematocrit value of 20% versus 30%, you will see improved outcomes clinically and experimentally using this model. Have you considered giving something as an adjuvant to increasing the hematocrit value to improve neurologic outcomes and other indices of recovery?

Dr Hagino. We think that a higher hematocrit and higher flow rate increased oxygen delivery to the brain. Cerebral temperature is important in determining cerebral oxygen demand. Lower temperature, higher hematocrit, and higher flow rate provide greater safety for the brain, but under certain surgical conditions a reduced flow rate improves exposure. We found that a TOI level of greater than 55% is safe and allows determination of minimum safe flow rate.

Dr Sellke. Those findings are very good. My question is this: Can you give something else in addition to that, something to reduce the number of free radicals, like superoxide anion and hydroxyl radical, that presumably will cause some of the injury in the brain?

Dr Hagino. We have previously demonstrated in our laboratory that a higher temperature exacerbates the inflammatory response including leukocyte activation. Therefore it is possible that adjunctive methods will reduce brain injury.

Dr W. Randall Chitwood, Jr *(Greenville, NC)*. I think what he was asking regarded the question of what adjunctive drugs can be given to help.

Dr Hagino. Dr Jonas' laboratory studied many adjunctive drugs in the past including N-methyl-D-aspartate (NMDA) antagonists as well as manipulation of nitric oxide. However, we did not study adjunctive drugs in this current study.

Dr Paul Kurlansky *(Miami Beach, Fla)*. This might be part of the same question in a way, but I notice that there was actually a reversal of some of the lines, that you might not be dealing with a simple phenomena here, and that some of the lowest readings that you were getting actually turned into some of the highest readings after perfusion, and therefore you might be dealing with an ischemic problem and a reperfusion problem. The phenomena that you are measuring might be more complex. That is why the correlation coefficient was only in the 0.5 range, which is about 50/50, and the scatter was very great across the line that you drew.

Therefore, I was wondering what specific histologic things you had looked at to measure neurologic injury and whether you had attempted to correlate it. There are actually, believe it or not, clinical grading systems for pigs as to their neurologic status. If you had used any of these scales, the pigs were alive for 4 days afterward, and you could certainly tell whether they were up, whether they were feeding, et cetera, et cetera. There are various simple neurologic scales, 1 to 5, that can be used to assess the clinical neurologic status, if you will, of pigs, if you had attempted to correlate it with the histologic findings and with the flow findings.

Dr Hagino. Thank you very much for your comments and questions. The neurologic deficit score and overall performance category as described in our study are methods for grading the functional outcome in piglets. These grading systems have been adapted from the grading systems used to assess dogs as described by the Pittsburgh group. Both in this study and in previous studies we have demonstrated a correlation between the histologic findings and functional results.

Dr Bradley S. Allen *(Houston, Tex)*. As I understand it, what you are trying to show is that this method measures actual tissue oxygen levels, as opposed to a percentage change from baseline. The study seems to support this assertion, and there is a nice consolation with tissue injury. From a clinical standpoint, however, does your study tell us how long you can be below a certain oxygen level, as this is what is important in the clinical setting? One hundred twenty minutes of low flow is a long time at 34°C and not something most surgeons would do clinically. Are you planning follow-up studies, or do you have ideas on how you might be able to determine the safe duration at a particular oxygen level?

Dr Hagino. Thank you very much for your question. It is not possible from this study to specify a TOI level that would indicate a safe perfusion flow rate for specific bypass conditions in the clinical setting. However, we do believe that the study supports investigation of TOI in the clinical setting.