Is There a Relationship between Pressure Gradients through Contemporary Oxygenators and Immune Cell Proliferation during Cardiopulmonary Bypass? A Pilot Study

Roger D.P. Stanzel, PhD, MSc, BScH, CPC; Mark Henderson, CCP CPC

Cardiovascular Perfusion, Nova Scotia Health Authority, Halifax, Nova Scotia, Canada

Abstract: There have been many advances in the perfusion equipment used for cardiopulmonary bypass (CPB) surgery. A key component, the membrane oxygenator, has had a number of modifications in recent years and a recent clinical evaluation demonstrated disparity in various aspects of device performance. One difference among oxygenators, which to-date has received little attention, was the impact on the patient’s immune cells, with some oxygenators producing a significantly greater increase in immune cell numbers after cross clamp. Such increases in immune cell proliferation may contribute to the development of a systemic inflammatory response (SIR), which has been demonstrated to have a negative impact on patient outcomes. Although factors contributing to immune cell proliferation during CPB are recognized to be multi-factorial, the goal of the current communication was to perform an ad hoc analysis of these raw data for evidence that pressure gradients through an oxygenator contributes to this outcome. Despite the observation that higher-pressure gradient oxygenators appeared to associate with increased immune cell proliferation, no correlation was detected in this analysis. This finding, however, provides further evidence for the complex nature of inflammation during CPB, which deserves ongoing discussion and investigation.

Keywords: cardiopulmonary bypass, perfusion, heart-lung machine, oxygenator, cardiac surgery, inflammation, transmembrane pressure, shear stress, neutrophils.

Recently, our perfusion team had the opportunity to conduct a limited quality assurance audit of our current oxygenator and new oxygenators available in Canada with the goal of identifying the optimal replacement product for the care of our cardiopulmonary bypass (CPB) patients. In this evaluation, variation in product performance was observed including differences in oxygen transfer and carbon dioxide removal (1). Of interest, variation in pressure drop (pressure gradient) through the oxygenators was also observed. From the highest to lowest pressure gradient, there was nearly a 4-fold difference. It is reasonable to postulate that such a variation could translate into differences in shear stress exerted on the blood components by the oxygenator. Shear stress is affected by, but not limited to, a number of variables including blood viscosity, blood flow rate, prime volume, and pressure drop as seen in the following formula:

\[ \tau_{oxy} = \frac{\sqrt{\eta \times Q \times \Delta P}}{V_{priming}} \]

where, \( \tau_{oxy} \) is shear stress in the oxygenator; \( \eta \) is the absolute viscosity of the blood, \( Q \) is the blood flow rate, \( \Delta P \) is the pressure gradient across the oxygenator, and \( V_{priming} \) is the prime volume of the oxygenator (2).

De Somer discussed the impact of shear stress on blood traveling through an oxygenator in a recent review (3). In terms of cellular impact, there was no evidence presented to demonstrate that oxygenators with greater levels of shear stress induced greater damage to red blood cells (RBCs) as evidenced by increased plasma-free hemoglobin. This was recently corroborated in vitro. Using normalized index of hemolysis as a surrogate of RBC damage, Venema et al. (2) compared oxygenators with varying pressure gradients and reported no evidence for high-pressure gradients inducing more hemolysis than low-pressure gradient oxygenators. However, the impact of pressure gradient on the other blood cellular components was not examined.
Whereas the impact of oxygenators on RBC hemolysis has historically garnered the majority of attention, the effect on platelets and white blood cells (WBCs) may be more relevant because of the fact that they are more sensitive to shear stress than RBC. In fact, both platelets and immune cells are approximately 10-fold more sensitive to shear stress (4–6).

In the aforementioned recent clinical evaluation of contemporary oxygenators, the impact on blood cell counts was also evaluated to determine if variation existed among oxygenators. Briefly, a sample of blood from the post-heparin sample taken by anesthesia (baseline sample) and a sample taken 5–10 minutes after cross clamp removal were analyzed. Blood cell counts from the post-cross clamp sample were normalized to the post-heparin sample and expressed as “percent baseline.” Whereas no differences were observed in the level of RBC or platelets after cross clamp removal among the oxygenators examined, variation was observed with immune cell levels and specifically the neutrophils. Stanzel and Henderson did not attempt to establish a causative relationship between pressure gradient and WBC proliferation; however, a trend was apparent: with one exception, higher-pressure gradient oxygenators had the largest mean increase in immune cell number after cross clamp removal (1).

Although other assessments, such as platelet function and quantification of inflammatory mediators were identified as being of interest in this evaluation, the ethics acquired in this “quality assurance audit” did not permit these evaluations and the window of opportunity to evaluate these oxygenators did not permit the necessary time to acquire the ethics clearance required. Furthermore, patient outcomes were also not permitted to be examined.

The goal of the current brief communication was to conduct an ad hoc analysis of these raw data for evidence of a causative relationship between pressure gradient through an oxygenator and immune cell proliferation with the hypothesis that there is a direct relationship between pressure gradient and immune cell proliferation during CPB.

**METHODS**

Ethics approval for this evaluation was obtained by the hospital’s Research Ethics Board as a “quality assurance initiative.” In the recent clinical evaluation, six large adult oxygenators (Sorin Synthesis, Sorin Inspire 6F, Sorin Inspire 8F, Medtronic Fusion, Maquet Quadrox-i, and Terumo FX25) and one small oxygenator (Terumo FX15) were evaluated (1).

CPB was carried out according to the established departmental guidelines. Equipment included Sorin S5 heart-lung machine with a roller pump as the arterial pump (Sorin Group Italia, Mirandola, Italy), GEM Premier 4000 (Instrumental Laboratory, Bedford, MA), and Actalyte XL Max-ACT (Helena Laboratories, Beaumont, TX). Cerebral perfusion was monitored by cerebral near infrared spectroscopy placed over the patient’s left and right frontal lobes (Covidien, Mansfield, MA). Perfusion data were captured on a hand-written perfusion record. In addition to the oxygenator being evaluated, the CPB circuit included a CSC14 cardioplegia heat exchanger (Sorin Group Italia) and an external Medtronic EL 404 (Medtronic Inc., Minneapolis, MN) cardiotomy reservoir to allow for sequestration of shed mediastinal blood. If required, a 4F76R2 hemocoagulator from Medtronic (Medtronic Inc.) was used. The tubing used for all cases was supplied by Sorin (P.h.y.s.i.o.-coated), as per usual practice. CPB prime solution for patients included 2 L of Normosol (Hospira Healthcare Corporation, Montreal, QC), 10,000 U heparin (PPC Pharmaceutical partners of Canada, Richmond Hill, ON), .5 g/kg mannitol (PPC Pharmaceutical partners of Canada), 4 g of Cefazolin (Hospira Healthcare Corporation), if not contraindicated. The cardioplegia solution was cold 4:1 blood cardioplegia (88 mmol KCl in 1,000 mL 5% dextrose and .225% NaCl) (Baxter Corporation, Mississauga, ON), with 100 mEq sodium bicarbonate added (Hospira Healthcare Corporation). Temperatures monitored during the case included nasopharyngeal, arterial, and venous blood temperatures. Blood samples were collected on CPB every 20–30 minutes for activated clotting times assessment (maintained greater than 480 seconds) and paired arterial and venous blood gases.

For the current analysis, only the large adult oxygenators (flows up to at least 6 L/min) were included. Thirty of each oxygenator were evaluated, excluding the Inspire 6F, with which 10 were evaluated. Evidence for a relationship between pressure gradients through an oxygenator and immune cell activation was observed with oxygenators with larger pressure gradients had the largest mean increase in immune cells after cross clamp removal, with the exception of the Inspire 6F (1). The raw data from this manuscript were re-examined to test for evidence of such a relationship, specifically, the raw data that were used to produce Figures 4 (mean pressure gradient through the oxygenator) and 8 (immune cell levels after cross clamp removal (1). The linear regression analysis tool in Excel® was used to evaluate the strength of the relationship between pressure gradient and immune cell proliferation.

**RESULTS**

To identify for the possibility of bias in the original analysis, case and patient demographic data were compared between oxygenators. There were no differences in
patient age, gender, weight, body surface area or case type (coronary bypass grafts, valves, etc.), pump times, or clamp times among these six oxygenators (1). Hemoconcentrators were rarely used (twice) and data from cases in which they were failed to demonstrate a difference in immune cell proliferation to cases in which they were not.

Variation in Pressure Gradients and Immune Cell Numbers after Cross Clamp Removal

The evidence that prompted the current analysis for a potential relationship between pressure gradient through an oxygenator and immune cell proliferation is derived from Figures 4 and 8 from Stanzel and Henderson, respectively, which contain data from the six large adult oxygenators are presented in Figure 1 (1). With the exception of Inspire 6F, of which only 10 oxygenators were evaluated, there appears to be a positive relationship between pressure gradient and immune numbers after cross clamp removal with the higher-pressure gradient oxygenators exhibiting the greatest increase in immune cell numbers.

Variation in WBC Levels after Cross Clamp Removal

As a first step in investigating the potential relationship between pressure gradient and immune cell proliferation, the raw data were displayed as the frequency of cases for each oxygenator in which WBC levels were above baseline after cross clamp (Figure 2). For all oxygenators except the Terumo FX25 and Sorin Inspire 6F, the majority of cases resulted in WBC levels after cross clamp removal above baseline values and ranged from 60% to 88% of cases for the other oxygenators (Figure 2A). The frequency of cases with WBC levels of 1.5-fold baseline decreased (Figure 2B) with Sorin Inspire 8F, Sorin Synthesis, and Medtronic Fusion having the greatest frequency followed by Maquet Quadrox-I, Terumo FX25, and Sorin Inspire 6F. The frequency of cases with WBC above 2-fold baseline decreased further; however, there again was variation between oxygenators with Sorin Inspire 8F and Medtronic Fusion having the greatest frequency followed by Sorin Synthesis, Terumo FX25, Maquet Quadrox-i, and Sorin Inspire 6F (Figure 2C). The overall range for normalized WBC levels for the oxygenators were Sorin Synthesis: 63–250% baseline, Sorin Inspire 8F: 68–250% baseline, Sorin Inspire 6F: 52–150% baseline, Medtronic Fusion: 73–280% baseline, Maquet Quadrox-i: 43–280% baseline, and Terumo FX25: 50–200% baseline.

The raw data pertaining to neutrophil levels were also displayed as the frequency of cases for each oxygenator in which levels were above baseline after cross clamp (Figure 3). Unlike WBC, the majority of cases produced neutrophil proliferation after cross clamp removal for all oxygenators, except the Sorin Inspire 6F (38%), although variation was observed and ranged from 62 to 96% of cases (Figure 3A). As was the case with WBC, the frequency of cases with neutrophil levels above 1.5-fold baseline decreased (Figure 3B) with Sorin Inspire 8F, Sorin Inspire 6F, and Medtronic Fusion having the greatest frequency...
followed by Sorin Synthesis, Terumo FX25 and Maquet Quadrox-i. The frequency of cases with neutrophil levels above 2-fold baseline decreased further; however, there again was variation between oxygenators with Sorin Inspire 8 having the greatest frequency followed by Sorin Synthesis, Medtronic Fusion, Sorin Inspire 6F, Terumo FX25, and Maquet Quadrox-i (Figure 3C). The overall range for normalized WBC levels for the oxygenators were Sorin Synthesis: 69–400% baseline, Sorin Inspire 8F: 78–330% baseline, Sorin Inspire 6F: 50–220% baseline, Medtronic Fusion: 82–290% baseline, Maquet Quadrox-i: 40–300% baseline, and Terumo FX25: 66–270% baseline.

Although these data are suggestive of a potential relationship between pressure gradient and immune cell proliferation, further analysis of the individual cases are required. To test for evidence of such a relationship, the raw data for all cases were used to generate scatter plot graphs with immune cell numbers plotted as a function of pressure gradients for each individual case regardless of oxygenator.

When the WBC proliferation (percent baseline) was plotted as a function of mean pressure gradient for each case (regardless of oxygenator) no relationship was observed between WBC proliferation and mean pressure gradient, \( r^2 = .026 \) (Figure 4A). However, it is possible that the maximum pressure gradient through an oxygenator may also contribute to WBC proliferation. When these WBC proliferation data were plotted against maximum observed pressure, there was also no relationship observed, \( r^2 = .069 \) (Figure 4B). These same steps were then taken to examine the possibility of a relationship between neutrophil proliferation and pressure gradients through an oxygenator. Again, no relationship was observed between neutrophil proliferation and mean pressure gradient, \( r^2 = .0485 \) (Figure 4C). However, it is again possible that the maximum pressure gradient through an oxygenator may also influence neutrophil proliferation. When these neutrophil proliferation data were plotted against maximum observed pressure, there was also no relationship observed, \( r^2 = .0879 \) (Figure 4D).
Another factor that may contribute to inflammatory cell proliferation is the duration of CPB and/or cross clamp. In the initial study, no differences were observed in these times across oxygenators. To more thoroughly investigate this possibility, scatterplots were produced by plotting WBC and neutrophil increases over baseline as functions of CPB and cross clamp times similar to the previously mentioned one. No relationships were observed to suggest a link between CPB or cross clamp time and the extent of immune cell proliferation (data not shown).

DISCUSSION

Cardiac surgery requiring CPB results in the development of a complex systemic inflammatory response (SIR) of varying degrees (7–9). Tissue damage, contact with foreign surfaces of the CPB circuit, and ischemia-reperfusion injury are all involved in the activation of the immune system. The inflammatory response involves activation of both cellular and humoral aspects of the immune system. Activated and damaged cells produce an array of inflammatory mediators. These mediators act as chemoattractants and activators for immune cells to permit migration of these leukocytes, largely neutrophils into the damaged tissues. Once in the damaged tissues, these activated neutrophils release an extensive repertoire of chemokines, cytokines, reactive oxygen species, and proteinases (10). Whereas this immune response is necessary for tissue repair after surgery, the uncontrolled activation of the immune system is associated with exaggerated immune activation and is associated with increased patient morbidity and mortality (7–10).

The recent clinical evaluation of contemporary oxygenators used during cardiac surgery suggested evidence for a relationship between pressure gradient through the oxygenator and the immune response as evidenced by oxygenators with the greatest pressure gradients largely producing the most robust increase in immune cells, as evidenced by Figure 1 (derived from 1). Despite the multifactorial nature of inflammation during cardiac surgery, this observation raised the question, “could pressure gradient
through an oxygenator contribute to the patient's immune response during CPB?"

Re-evaluating the raw data from Stanzel and Henderson (1), the higher-pressure gradient oxygenators appeared over-represented in the distribution of cases in which the immune cell number after cross clamp removal was above baseline (Figures 2 and 3), with the exception of Inspire 6F. However, when the immune cell number change over baseline values (WBC or specifically neutrophil) were plotted against the mean and maximum pressure gradients for all cases, no relationship was observed. This finding is in stark contrast to the current hypothesis and requires further investigation.

Clearly, the Inspire 6F appears to be an outlier in this evaluation given the high-pressure gradient and relatively low level of immune cell proliferation observed. It is difficult to understand why this high-pressure gradient oxygenator did not produce the same level of immune response as the Sorin Synthesis and especially Sorin Inspire 8F given that it is a smaller version of this product. One difference between the Sorin Inspire 6F and 8F is the surface area, 1.4 and 1.75 m², respectively (11). Although a smaller surface area may play a role, it is unlikely that a .35 m² difference in surface area could be responsible for a significant variation in immune cell proliferation. A further potential factor is hemodilution. The Inspire 6F had the lowest post-cross clamp Hgb, albeit not a statistically lower value. Currently, the role of hemodilution in immune cell proliferation is not well understood as evidenced by the conflicting findings of Siminelakis et al. (12) and Franke et al. (13). It is important to recall, however, that with only 10 products evaluated, it may not represent the true outcome with this oxygenator.

While there were differences noted between oxygenators in terms of immune response, what factors are responsible, if not the differences in pressure gradients? To answer this question, these differences between oxygenators must be identified and evaluated.

The first disparity is the coatings each company uses for its product. Whereas the majority of CPB circuit was provided by Sorin (Physio) in our evaluation, the oxygenators themselves were treated as per manufacturers' signature coatings, specifically, Sorin coated with PH.I.S.O. PC®, Medtronic Fusion with Balance® Bio-surface, Maquet Quadrox-i with Softline®, and Terumo...
FX25 with X Coating®. A comprehensive investigation on the differential impacts of these physiological coatings on immune cell proliferation has not, to the authors' knowledge, been published to-date.

Since pressure gradient data were captured by handwritten charting and not continuous electronic copying, it is possible that peak pressure gradients were not captured. The use of continuous electronic charting would have aided in this matter. Furthermore, these data may not adequately represent the true shear stress levels that have been shown to induce immune cell activation. Shear stress through an oxygenator is inherently difficult to measure and would require expert engineering assistance, which is why the pressure gradient values were used as a surrogate of shear stress.

Although clinical outcomes were beyond the scope of ethics approval of the recent clinical oxygenator evaluation, the observed variation in immune cell proliferation post cross clamp removal between oxygenators remains intriguing. It is tempting to associate these differences with the onset of an unregulated inflammation after CPB (SIRs) and the poor patient outcomes that follow despite the low n-value of the study. The clinician must, however, temper these by recalling the multi-factorial nature of cardiac surgery and the importance of multi-center, randomized controlled trials. Clearly, further work is required to examine the relationship between different oxygenators and the inflammatory response. As these products are relatively new to the market, should the differences observed in the recent study be representative, a careful surveillance, possibly through the use of perfusion data registries, may shed light into differential patient outcomes and generate interest in discovering the underlying mechanism(s) (1). This would benefit not only the patient, but also the industry in future oxygenator designs.

Limitations

As this communication is a post hoc analysis, there are significant limitations including the inability to control for a number of confounding variables. Such variables include, but are not limited to, the effects of ischemia-reperfusion on immune cell activation and the timing of the post-cross clamp samples. In addition, the original evaluation was a small (n = 30 cases per oxygenator, except Inspire 6F with only 10), single-center evaluation. A larger, multi-center, prospective evaluation would be invaluable in elucidating the impacts of pressure gradients or other factors on immune cell proliferation during contemporary CPB.

Although no differences were observed in patient or case demographics, it is important to note that the quality assurance audit level of ethics permitted limited acquisition of patient data. That is, it was limited to patient age, gender, height, weight, and body surface area. A number of pre-existing factors could influence the inflammatory reaction including diabetes and organ function that were not captured. Furthermore, responses to CPB such as hyperlactatemia, hyperglycemia, hemolysis, creatinine, and organ function could also play a role in inflammation independently of the oxygenator (7,9). Such data, if they had been collected would require sophisticated multi-variant analysis which was beyond the capacity of the investigators. It is possible that such analysis could have yielded intriguing findings.

There were also 12 perfusionists involved, which conceivably could play a role in affecting outcomes. An examination of individual perfusionists’ immune cell numbers failed to yield evidence of perfusionist-specific bias. That is, all perfusionists had cases with high and low immune cell proliferation independent of oxygenator used.

Another possible concern is the use of immune cell numbers alone as a surrogate of inflammation. Ideally, quantification of key inflammatory cytokines, such as IL-1β or TNF-α, would have been performed to corroborate the involvement of immune cell proliferation with inflammation. The quantification of inflammatory mediators was, as mentioned, beyond the capacity of the quality assurance audit but could provide valuable information for any future investigations into this matter. In fact, the current technology permits quantification of up to 30 relevant mediators at a time in a multiplex assay, which would be of considerable utility in such investigations (14). It is important to understand the distinction between increases in WBC and neutrophils. Whereas some WBC, such as macrophages can play an anti-inflammatory role or post-inflammatory role; neutrophils are neither anti-inflammatory nor “pedestrian” and if present in increasing numbers is an accurate indication of an inflammatory state (7,9).

A further concern may be our understanding of the relationship between pressure gradients through an oxygenator and the subsequent contribution to shear stress. For the purposes of this manuscript, we assumed a direct relationship, when in fact the calculation of shear stress through an oxygenator is complex and factors other than blood viscosity, flow rate, priming volume, and pressure gradient may be more influential. In fact, a publication by Girdhar and Bluestein investigating the impact of shear stress through prosthetic devices such as artificial heart valves highlighted this point (15). These authors pointed to the importance of factors, such as flow distributions, peak velocity, time of peak velocity and flow transitions of blood through the oxygenator bundle fiber in calculating shear stress using computational flow dynamics analysis (15). Although this was beyond the scope of the current communication, which used pressure gradient as the lone surrogate of shear stress, it is conceivable that overall oxygenator design leading to higher shear stress may play a role in immune cell physiology during CPB.
Ultimately, the analysis presented in the current manuscript did not find evidence for a relationship between pressure gradients through an oxygenator and immune cell activation. Given the poor outcomes attributed to SIRs after CPB, this article should promote discussion for the perfusion community. Clearly, this article has raised more questions than it has answered and could serve as a catalyst for a larger-scale investigation into the underlying mechanisms of inflammation during CPB. This would require a clear protocol with multiple centers using different products, detailed data collection, and sophisticated statistical analysis. Although difficult to conduct, the results may answer a number of critical questions. It is important that the mechanisms of inflammation during cardiac surgery be re-evaluated periodically because of the influence of changing technology (e.g., oxygenators and circuit coatings) and techniques in cardiac surgery (e.g., minimally invasive) (8). At the very least, it is the hope of the authors that this article promotes vigilance in product performance and generates discussion among clinical staff and between clinical staff and product representatives regarding potential relationships between perfusion products and patient care.

Summary

Despite the observation that higher-pressure gradient oxygenators appeared to associate with increased immune cell proliferation after cross clamp removal in Stanzel and Henderson (1), no correlation was detected in the analysis presented in the current manuscript. However, this outcome can be attributed to the complex nature of inflammation during CPB, which deserves ongoing discussion and investigation.

Future Studies

To further evaluate the potential causal relationship between pressure drop and immune cell proliferation, more data are required, as mentioned earlier. We will be in a position to pursue this matter further as our center will be using a high-and a low-pressure gradient oxygenator from the initial evaluation for our practice. This will allow us to address a short coming of the current manuscript, sample size. In this future evaluation, we propose a sample size greater than 200 of each oxygenator and will be consulting our divisional statistician for assistance in study design. We will expand upon data collection to incorporate more patient demographical details and details of CPB volume balances. The additional patient data will include pre-existing factors that could influence the inflammatory reaction (diabetes renal failure, etc.) and capture CPB data pertaining to hyperlactatemia, hyperglycemia, hemolysis, and creatinine. To properly examine these data, a multi-variant analysis will be conducted by our statistician. Depending on the strength of these data, the next step would be to initiate a multi-center evaluation using this study protocol as a template.

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