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Volatile anesthetics in oxygenators during cardiopulmonary bypass

Anestésicos volátiles en oxigenadores durante la circulación extracorpórea

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What do we know about this topic?

Volatile anesthetics are widely used during cardiopulmonary bypass (CPB) due to their myocardial protective effects. However, their exact concentrations in oxygenator sweep and exhaust gases remain unclear, potentially affecting efficacy as well as environmental exposure. Previous studies have evaluated anesthetic retention in different oxygenators, but there is limited data comparing volatile anesthetic concentrations across various oxygenator types. A better understanding of these differences is crucial for optimizing anesthesia management during CPB and minimizing unnecessary anesthetic waste.

What is the contribution of this study?

This study quantifies volatile anesthetic concentrations in the sweep and exhaust gases of three oxygenator types during CPB. By comparing these concentrations, it provides insights into anesthetic retention and potential exposure risks. The findings help refine anesthetic dosing strategies, optimize CPB management, and contribute to environmental safety by reducing anesthetic waste. This study fills a critical knowledge gap, supporting evidence-based decision-making in perfusion and anesthesia practices.

How to cite this article

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Abstract

Introduction

Continuous monitoring of exhaled concentrations and CO2 levels is often lacking during the administration of inhaled anesthetics in cardiopulmonary bypass (CPB), these levels often being adjusted intermittently based on blood gas values. This approach disregards variations in fresh gas and circulatory flows between blood samples.

Objective

To assess gas behavior during bypass circulation, evaluate data reliability, and analyze gradients through the membrane oxygenator.

Methods

Real-time monitoring of inhaled and exhaled volatile anesthetics, CO2, and oxygen was conducted at the oxygenator inlet and outlet ports during CPB. Seventy adult patients undergoing cardiac surgery on CPB were included in order to analyze the impact of circulatory flow across different oxygenators.

Results

A strong correlation was found between end-tidal CO2 and arterial blood gas CO2 (Spearman's Rho = 0.74, p = 0.00). Isoflurane gradients differed significantly among the Affinity, Fusion, and Terumo oxygenators (p = 0.015). Equilibrium for Isoflurane was reached in 493.9 ± 164.98 seconds (95% CI: 454–532 seconds). When circulatory flow was reduced to 0.5 L/min, exhaled concentrations increased significantly (Fisher's T, p = 0.07). Sevoflurane washout varied significantly across oxygenators at CPB initiation (mean: 117.5 s).

Conclusions

Continuous monitoring of inhaled and exhaled gases during CPB should be mandatory to optimize anesthetic delivery and achieve targeted plasma concentrations.

Keywords

Cardiopulmonary bypass monitoring; Anesthesia during bypass; Membrane oxygenator; Volatile anesthetics, Real-time monitoring.

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Resumen

Introducción: La monitorización continua de las concentraciones exhaladas y los niveles de CO2 a menudo falta durante la administración de anestésicos inhalados en la circulación extracorpórea (CEC), niveles que se ajustan de manera intermitente basándose en los valores de gases en sangre. Este enfoque no considera las variaciones en los flujos de gas fresco y circulatorio entre las muestras de sangre.

Objetivo: Evaluar el comportamiento de los gases durante la circulación extracorpórea, evaluar la fiabilidad de los datos y analizar los gradientes a través del oxigenador de membrana.

Métodos: Se realizó una monitorización en tiempo real de los anestésicos volátiles inhalados y exhalados, CO2 y oxígeno en las entradas y salidas del oxigenador durante la CEC. Se incluyeron 70 pacientes adultos sometidos a cirugía cardiaca con CEC para analizar el impacto del flujo circulatorio en diferentes oxigenadores.

Resultados: Se encontró una fuerte correlación entre el CO2 al final de la espiración y el CO2 en los gases arteriales (Rho de Spearman = 0,74, p = 0,00). Los gradientes de isoflurano difirieron significativamente entre los oxigenadores Affinity, Fusion y Terumo (p = 0,015). El equilibrio para el isoflurano se alcanzó en 493,90 ± 164,98 segundos (IC 95 %: 454-532 segundos). Cuando el flujo circulatorio se redujo a 0,5 L/min, las concentraciones exhaladas aumentaron significativamente (T de Fisher, p = 0,07). El lavado de sevoflurano varió significativamente entre los oxigenadores al inicio de la CEC (media: 117,5 s).

Conclusiones: La monitorización continua de los gases inhalados y exhalados durante la CEC debería ser obligatoria para optimizar la administración de anestésicos y alcanzar las concentraciones plasmáticas deseadas.

Palabras clave: Monitorización de la circulación extracorpórea; Anestesia durante la circulación extracorpórea; Oxigenador de membrana; Anestésicos volátiles; Monitorización en tiempo real.

INTRODUCTION

Volatile anesthetics are widely used in medical centers globally to maintain anesthetic concentrations during cardiopulmonary bypass procedures. In addition to providing anesthesia, they also have anti-inflammatory properties. They can reduce the release of proinflammatory cytokines, affect alveolar macrophage function, protect against free radical-induced myocardial injury, and reduce neutrophil and platelet uptake in the coronary circulation. These effects contribute to the preservation of cardiac function during ischemia and reperfusion, and may provide a valuable tool for the prevention and treatment of intraoperative cardiac ischemic dysfunction. (1-3)

The pharmacokinetics of volatile drugs during bypass is influenced by three primary factors: alterations in the blood/gas partition coefficient, changes in tissue solubility, and oxygenator design. (4) The design of the oxygenator has a substantial impact on the absorption and elimination of these gases during anesthesia. Acting as a reservoir for volatile anesthetics, the oxygenator impedes their passage, with different brands giving rise to varying gas diffusion behaviors. This effect is created by gas exchange through PMP membranes, which mimics the natural process of gas diffusion across solid gas-permeable cell membranes. In this process, gas transfer within the solid layer depends on partial pressure gradients and the specific permeability properties of the membrane (5). Additionally, variations in fresh gas flow and temperature affect the uptake and elimination of anesthetics (6).

During cardiopulmonary bypass and cardiovascular anesthesia, hypothermia not only reduces the need for anesthetics but also affects the accuracy of anesthesia depth monitoring methods, such as the bispectral index (BIS), which has shown significant variability. In some cases, the electroencephalographic (EEG) pattern may indicate mild sedation, while clinical indicators of anesthesia depth may suggest a deeper unconsciousness level. (7)

Continuous monitoring of inhaled and exhaled gases during extracorporeal circulation is needed to maintain adequate concentrations and avoid insufficient levels of volatile anesthetics that could increase the risk of intraoperative awareness. Installing a gas scavenging system in the oxygenator outlet for monitoring all incoming and outgoing gases is recommended when using a volatile anesthetic supply system. This allows tracking and adjustment of carbon dioxide levels in accordance with anesthesia safety standards. (4,8,9)

Several in vivo and in vitro studies have been conducted to measure sequential plasma concentrations of inhaled anesthetics. However, routine performance of these measurements during surgery is not feasible due to reproducibility issues. Proper sampling under controlled temperature conditions and immediate access to laboratory facilities are required for these measurements. (4,5)

Understanding the behavior of inhaled anesthetics during cardiopulmonary bypass is important in the determination of the correct inhaled anesthetic concentrations for assessing their impact on the transfer of other gases. Some manufacturers recommend adjusting the inspired oxygen fraction and fresh gas flow for 1.3% and 2.6% isoflurane and sevoflurane concentrations, respectively, in order to achieve the desired gas transfer performance (10).

Therefore, in this study, we conducted in vivo measurements during extracorporeal circulation to determine the behavior

of inhaled and exhaled gases across the oxygenator membrane. We employed sampling lines at both the inlet and outlet of the oxygenator. This was done to ensure the reliability and consistency of the data, rendering it apt for routine monitoring of inhaled and exhaled gases such as oxygen, carbon dioxide, sevoflurane, and isoflurane throughout the course of cardiopulmonary bypass. This monitoring technique is particularly relevant during this surgical phase, as it aids in assessing anesthetic depth in accordance with clinical indicators.

METHODS

Study design

This prospective observational study was conducted at Fundación Cardioinfantil in Bogotá, Colombia. The study enrolled adult patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) using either Medtronic Fusion, Affinity or Terumo membrane oxygenators. The study was approved by the Ethics and Research Committee (approval number N. 15-2017, May 3, 2017) and classified as "minimal risk research" according to Article 11, Resolution 008430 of 1993 of the General Health Law on Health Research. Written informed consent was not required due to the study's observational nature and absence of modifications to anesthetic, perfusion, or surgical procedures.

The protocol was submitted to the Ethics Committee of Fundación Cardioinfantil for evaluation and approval. Based on the minimal risk nature of the research project and the absence of changes in patient behavior, written informed consent was not required according to Paragraph 1 of Title II, Chapter 1 of the resolution mentioned above.

Participants

A total of 70 adult patients were included in the study. Patients were excluded if they had a contraindication to volatile anesthetics or if the membrane oxygenator was not used during surgery.

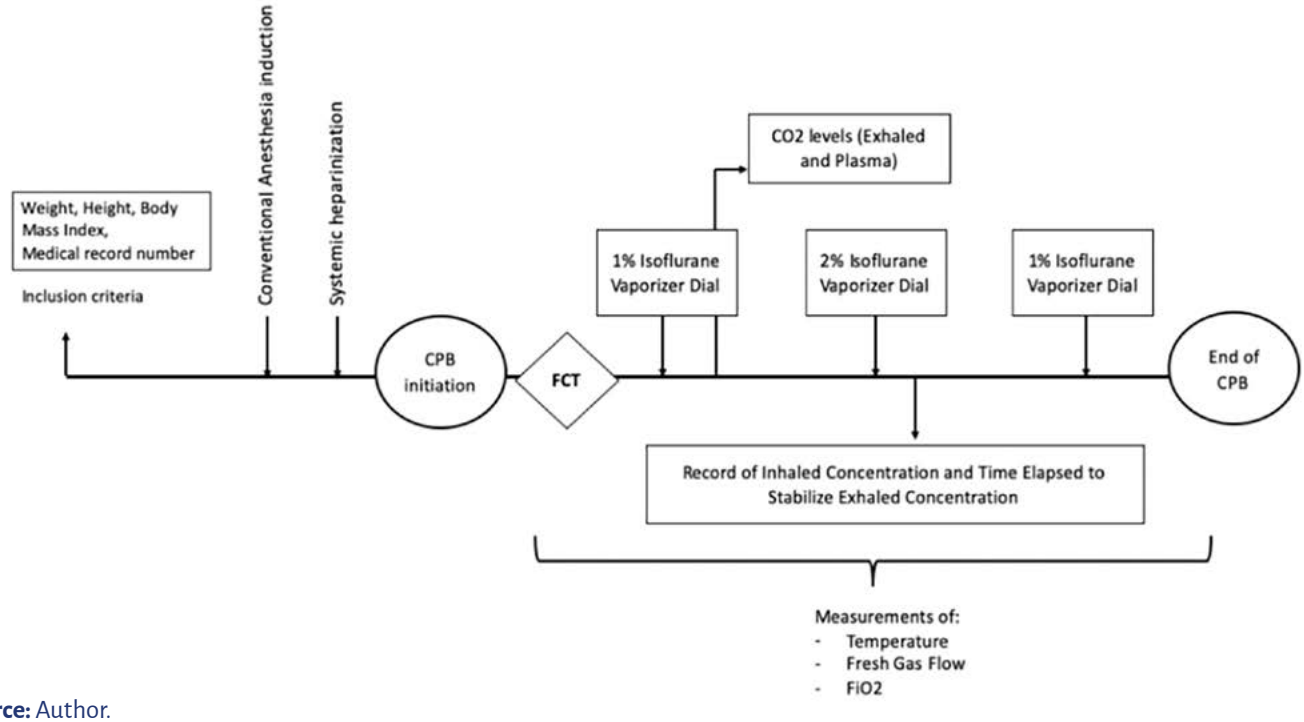
The sample size was calculated for a 5% margin of error, a 95% confidence interval, and a variance of 400, based on a standard deviation of +/- 20% for the isoflurane captured by the membrane oxygenators.

Efforts were made to handle missing data, including a structured protocol for real-time gas monitoring and recording. In those cases in which data points were missing due to equipment malfunction or recording errors, missing values were not imputed, and only complete cases were analyzed.

Anesthetic management

All patients received a conventional balanced anesthetic induction with sevoflurane, maintaining an exhaled concentration of 2.5% until CPB initiation. Upon starting CPB, 1% isoflurane was administered, with continuous real-time monitoring of concentrations in both the inspiratory and expiratory branches of the oxygenator. The TEC7 isoflurane vaporizers were calibrated prior to the study and they were monitored using a General Electric Carescape Monitor B650 gas analyzer. The timeline of events can be seen in Figure 1.

Figure 1. Timeline of the events.



Source: Author.

The following membrane oxygenators were used during cardiopulmonary bypass: 1) Terumo Capiox RX25 (microporous polypropylene hollow fibers, 2.5 m^2 membrane surface area, 0.5-7.0 L/min recommended flow rate, 4,000 mL reservoir capacity); 2) AFFINITY NT™ (microporous polypropylene hollow fibers, 2.5 m^2 membrane surface area, 1-7 L/min recommended blood flow rate, 4,000 mL reservoir capacity); 3) Affinity Fusion (microporous polypropylene hollow fiber, 2.5 m^2 membrane surface area, 1-7 L/min recommended flow rate, 4,500 mL reservoir capacity).

Measured outcomes

The primary outcome variables included were: 1) real-time inhaled and exhaled concentrations of volatile anesthetics; 2) CO2 and oxygen levels at the oxygenator inlet and outlet ports; 3) circulatory flow, temperature, and arterial blood gas samples to assess gas transfer dynamics

The study recorded the decrease in sevoflurane exhaled concentrations from the start of CPB until 0.5% was reached, along with the time required for these changes. Isoflurane concentration adjustments (increasing to 2% and then reducing to 1%) were also monitored, with time recordings for each transition. In-line measurements of inspired oxygen fraction and carbon dioxide were compared with arterial gas sample results. Values were analyzed in relation to temperature, time on extracorporeal circulation, and machine flow effects on the measurements.

Statistical analysis

Continuous variables were summarized as means (SD) or medians (IQR) based on their distribution. Differences between oxygenator types were assessed using the Kruskal-Wallis H test for nor-parametric comparisons and one-way ANOVA for normally distributed variables. Post hoc pairwise comparisons were performed using Dunn´s test or Tukey´s HSD test. Spearman correlation coefficients were used for assessing relationships between exhaled CO2 and arterial blood gas CO2 levels. Figures were created using ggplot2 and exported for publication.

RESULTS

A total of 70 patients were included in the study; 75.7% (n=53) were males, and 24.3% (n=17) were females, with an age range between 18 and 82 years (mean: 62.5). The majority of patients (68.9%) underwent a single procedure, while 22.9% and 71.1% had two and three combined procedures, respectively, 71.1% had three combined, and lastly 1.4% underwent more than 3 procedures.

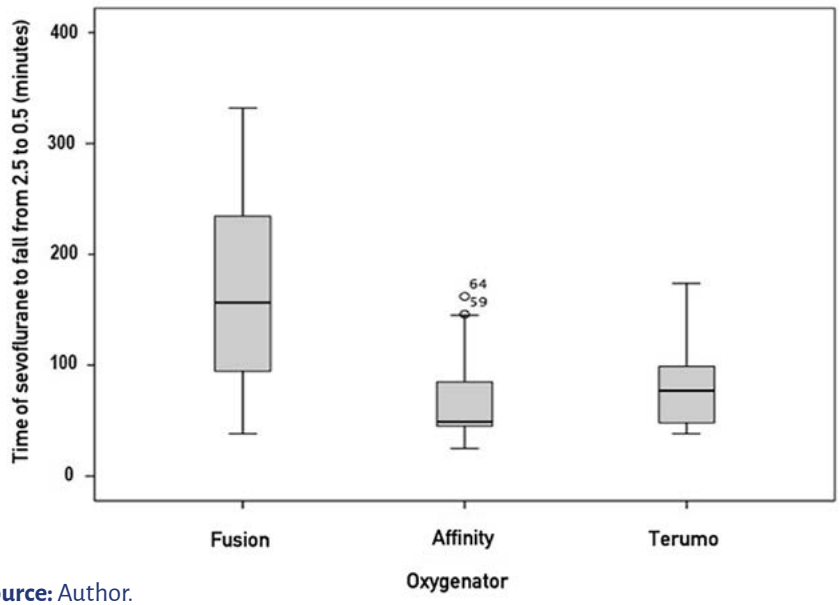
The procedures performed included both combined and single surgeries, with myocardial revascularization being the most frequent, followed by aortic valve replacement and mitral valve replacement. The oxygenator brand most commonly used during cardiopulmonary bypass was the Medtronic Fusion (n=48 patients, 68.6%), followed by Affinity (n=17 patients, 24.3%) and Terumo (n=5 patients, 7.1%).

The sevoflurane exposure time from the beginning of the anesthetic procedure until the start of bypass showed a non-normal distribution due to the different procedures involved, with a median time of 150 minutes (range: 210 minutes; 75-285 minutes).

When sevoflurane was discontinued at the start of bypass, the mean time required for the exhaled concentration of the same medication to decrease to 0.5% while maintaining a stable flow in the extracorporeal circulation was 117.5 minutes (range: 307, 25-332 minutes). There were no significant differences between the duration of sevoflurane exposure and the time to reach an exhaled concentration of 0.5% (Spearman´s Rho=0.064, p=0.59).

A difference was observed among the oxygenators regarding the time between vaporizer closure and reaching a 0.5% exhaled sevoflurane concentration (Kruskal–Wallis H, p=0.01, Figure 2). A post hoc analysis revealed that the time needed for the exhaled concentration of sevoflurane to decrease when using the Fusion oxygenator was greater than that needed for the Affinity oxygenator (p=0.01) and the Terumo oxygenator (p=0.041). However, there was no difference in the time necessary for the exhaled concentration of sevoflurane to reach

Figure 2 Sevoflurane elimination time by oxygenator.



Source: Author.

0.5% between the Affinity and Terumo oxygenators (p=0.48), and no correlation was found between patient body surface area and the time needed for sevoflurane levels to fall (p=0.97).

The average time required for the exhaled concentration to stabilize when 1% isoflurane was administered at the start of bypass was 493.3 seconds (SD +/-164.98 sec, 95% CI 454 sec - 532 sec). Comparisons across oxygenators did not reveal major differences when comparing the mean oxygenator times using one-way ANOVA and normal variable distributions (p=0.12).

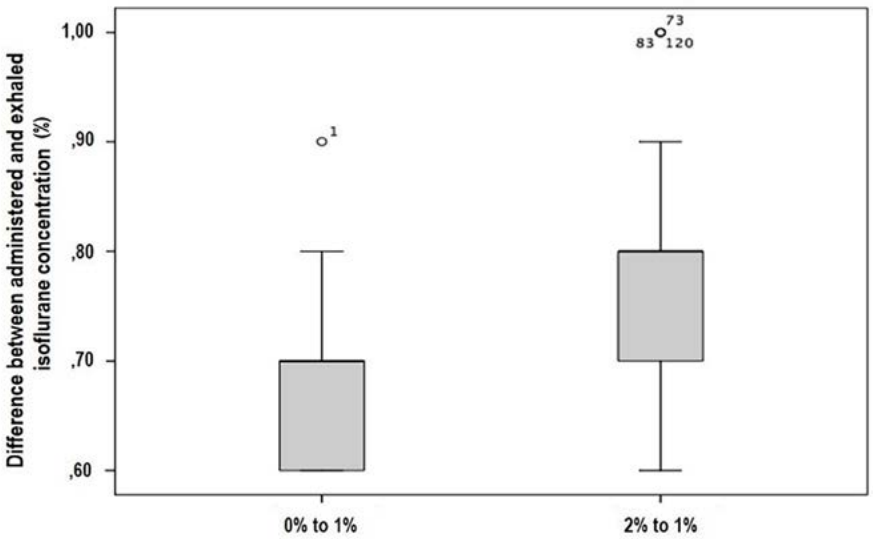
The time required to balance the isoflurane concentration when changing the vaporizer dial from 1% to 2% was, on average, 448.5 seconds (SD +/- 18.6; 95% CI 411.33-485.67 sec), with no differences between the oxygenators (p=0.106). The mean time to balance the isoflurane concentration when turning the vaporizer dial back from 2% to 1% was 431.2 seconds, with differences found among the oxygenators (p=0.139). No difference was found in the time required to balance the isoflurane concentration between 0% and 1% or between 1% and 2% (p=0.083).

The Kruskal–Wallis test was used to determine whether there was a difference between administered and exhaled isoflurane concentrations with the vaporizer dial at 1% at the start of bypass according to the type of oxygenator used, revealing a significant difference among the groups (p=0.015).

There was a greater difference between the administered concentration minus the exhaled concentration when using the Fusion oxygenator than when using the Affinity oxygenator (p=0.035). A similar result was found for the Terumo oxygenator compared to the Affinity, (p=0.013). However, there was no significant difference between the Terumo and Fusion oxygenators (p=0.082). The difference between administered and exhaled concentrations was also significant when changing the dial from 0% to 1%, as compared to changing it from 2% to 1% (p=0.00, see Figure 3) and between the oxygenators when changing the vaporizer dial from 1% to 2% (p=0.007) and from 2% to 1% (p=0.029) (see Figures 3 and 4).

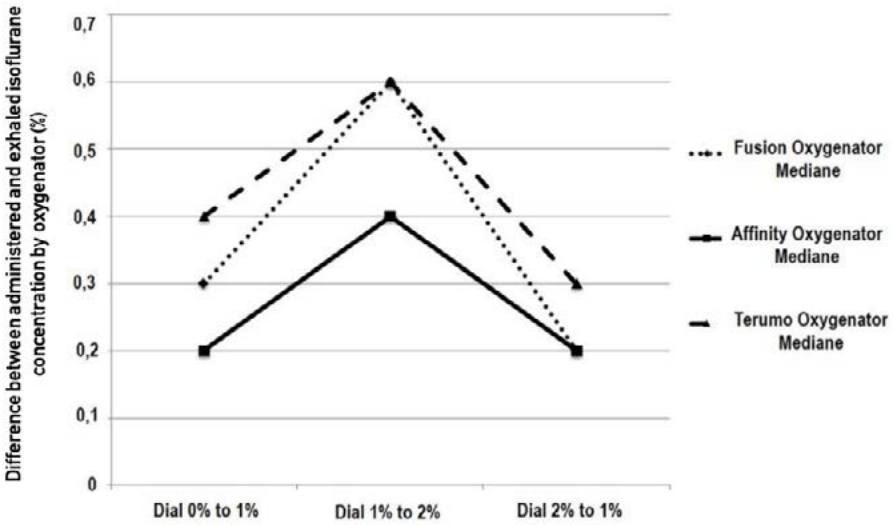
Regarding CO_2 behavior, a strong correlation was found between exhaled CO_2 and arterial blood gas CO_2 levels (Spearman´s Rho 0.74, p<0.0001). When analyzed by oxygenator type, this correlation remained strong for 3 of the 4 oxygenators, both the Medtronic and Fusion (Spearman´s Rho 0.85, p<0.0001), and the Affinity (Spearman´s Rho 0.81, p<0.0001). However, for the Terumo oxygenator, the correlation was weaker and not statistically

Figure 3. Difference between administered and exhaled isoflurane concentrations.



Source: Author.

Figure 4. Difference between the administered and exhaled isoflurane concentrations when changing the oxygenator dial from 2% to 1%.



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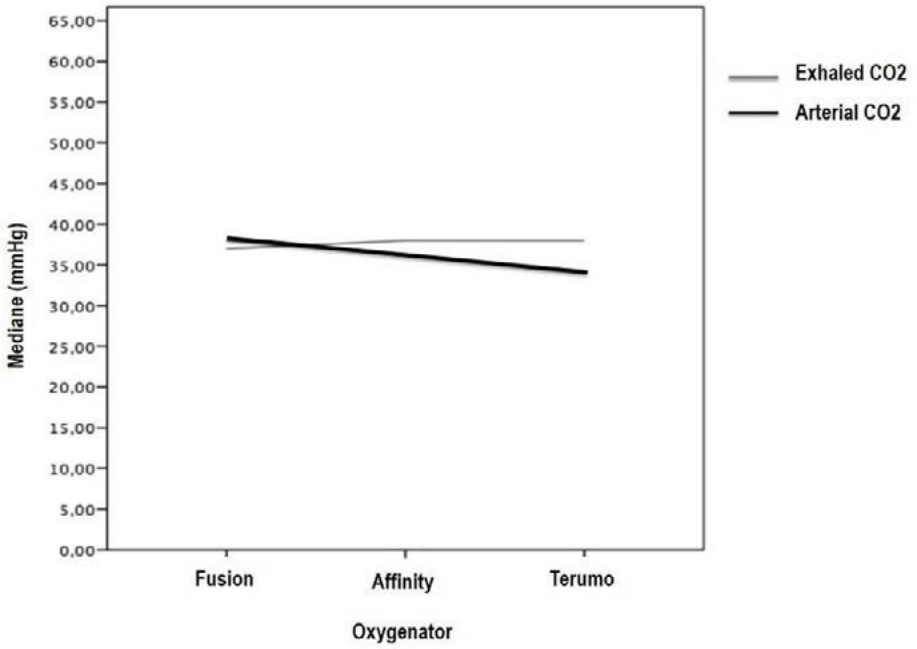
significant (Spearman´s Rho 0.61, p= 0.27). Subsequent stratification by gas sample group showed a stronger correlation for the first sample group (Figure 5).

The median inspired oxygen fraction during bypass was 88% (range: 73-99%), while the median expired oxygen fraction was 79% (range: 64-92%). The difference between the inspired and exhaled oxygen fractions was analyzed separately, revealing a mean difference of 7% (Figure 6). This difference was found to change between the first and second arterial gas samples (p=0.014), prompting further exploration of differences over time. When comparing the median difference in inspired minus exhaled oxygen fractions over time, greater discrepancies were observed during the first 40 minutes of CPB (Mann–Whitney U, p=0.005) (Figure 7).

Figure 5. Correlation between exhaled CO2 and arterial blood gas CO2 levels by oxygenator type.



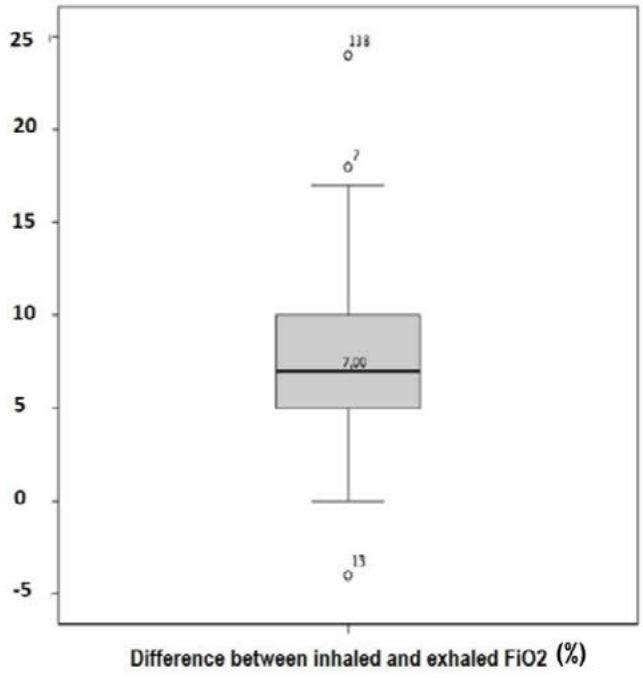
Source: Author.

DISCUSSION

Over time, intraoperative anesthesia monitoring has increased to ensure optimal care during surgery. However, in cardiovascular surgery, when cardiopulmonary bypass is initiated, monitoring of halogenated gases is suspended, while monitoring of oxygen and CO2 continues intermittently at regular intervals.Consideringthatcardiopulmonary bypass is an integral part of the anesthetic procedure, ensuring therapeutic levels for appropriate anesthetic depth, analgesia, and neuromuscular relaxation becomes essential. Therefore, it is crucial to maintain monitoring during the extracorporeal circulation process. In fact, in the event of leaks in the inspiratory branch, it would be the earliest and most acute method to identify any oxygenation issues.

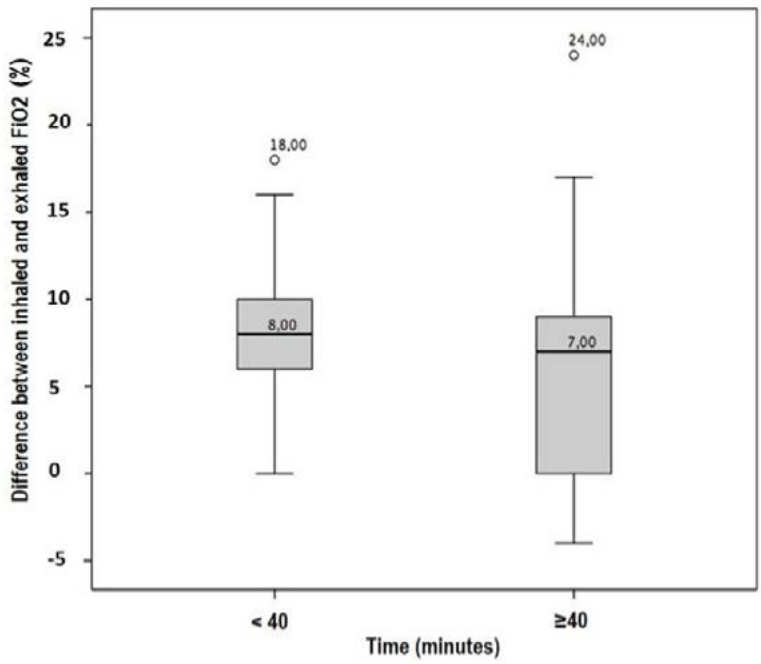
According to the Standards and Guidelines for Perfusion Practice, it is recommended to use an in-line oxygen analyzer at the junction between the oxygen source inlet line and the connection to the oxygenator. However, a limitation of this setup is the placement of the oxygen analyzer upstream of the oxygenator, (8)

Figure 6. Average difference between the fractions of inspired and exhaled oxygen over time.



Source: Author.

Figure 7. Discrepancies in inspired minus exhaled oxygen fractions during the first 40 minutes of extracorporeal circulation.



Source: Author.

meaning that a deficit of oxygen in the downstream region may go undetected until the patient's arterial saturation starts to decline. To address this issue, authors such as Srey et al. (12) and Caruso et al. (13) suggest monitoring the fraction of expired oxygen (FEO2) that exits through the oxygenator exhaust port using a connector attached to the port; this allows early and acute detection of oxygenation problems and leaks in the inspiratory branch. However, monitoring FEO2 alone is insufficient for validating the configuration of the inspired oxygen fraction (FiO2) in gas blenders. To ensure accurate FiO2 regulation and verification, the use of an independent O2 analyzer is necessary as a means to confirm that the oxygenated gas has passed through the oxygenator and that the FiO2 is proportional to the blender setting. On the other hand, in-line CO2 measurements allow for early adjustment of inspired gas flows to achieve the desired values before taking the first blood gas samples.

The efficiency of gas transfer across the oxygenator membrane is highly sensitive to changes in machine flow. This rapid transfer of gases across the membrane leads to an immediate drop in halogenated gas concentrations after closing the vaporizer at the start of cardiopulmonary bypass, exposing the patient to levels below the minimum alveolar concentration (MAC) and increasing the risk of intraoperative recall. Therefore, early administration of halogenated gases is crucial for maintaining therapeutic efficacy. The time required to achieve halogenated gas equilibrium in the patient can vary significantly due to variations in distribution compartments. This unpredictability necessitates constant monitoring to avoid exposing the patient to intraoperative recall events.

Our study revealed a significant difference between inspired and expired concentrations of halogenated gases. This difference can be attributed not only to entrapment by the tissues once the body becomes saturated, but also to the influence of the oxygenator membrane material. These findings are supported by previous research by Wiesenack (5), who demonstrated that isoflurane uptake and clearance are significantly reduced by the new plasma-tight PMP membrane compared to conventional PPL membranes. According to this model, a possible explanation for the extremely reduced uptake of isoflurane by diffusion membrane oxygenators is a very low diffusion coefficient of isoflurane in the solid layer of the new membrane due to its molecular size.

When the dial of the vaporizer was returned to 1%, exhaled concentrations were higher than the initial concentrations. This is because the previous increase in concentration to 2% saturates the tissues, leading to a delay in removal time and slower equilibration.

The differences between the selected fraction of inspired oxygen and the actual inspired value can be attributed to the lack of dial precision, where slight variations can result in a change of 1% or 2%. Therefore, in-line monitoring is necessary to establish the exact concentration.

Our study revealed a strong positive correlation between exhaled CO2 and arterial CO2 measured in arterial gas samples during cardiopulmonary bypass (CPB). Our findings agree with those of Zia et al., (14) who also identified a significant relationship between arterial CO2 measured by a blood gas analyzer and arterial CO2 determined by capnography of the oxygenator exhaust. We also observed a weak but statistically significant and positive correlation between the temperature measured at the time the gases were taken and the CO2 measured in the expiratory branch of the system. This result supports the study by Potger et al. (15), who found a reasonable correlation between PECO2 and temperature-corrected CO2 throughout all phases of CPB.

Additionally, our results showed a moderate negative correlation between the temperature at the time of arterial gas sampling and the CO2 delta (arterial

CO_2 concentration minus exhaled CO_2 concentration). These findings are also supported by the literature, as is the case of the studies by Baraka et al. (16) and Graham et al. (17), where the latter identified good agreement between arterial and exhaust CO_2 tensions during CPB, particularly under normothermic conditions and stable hypothermia. Our findings coincide with those of previous studies and support the use of exhaled CO_2 to reliably indicate arterial CO_2 during CPB. Furthermore, our study highlights the importance of considering temperature when interpreting oxygenator exhaust capnography results, in order to improve arterial CO_2 control and optimize clinical outcomes.

During bypass, condensation released by the oxygenator through the heat exchanger leads to saturation of the exhaled gas measurement filter, resulting in decreased sensitivity during exhaled measurements and gradient increases. To maintain reliable measurements, it is advisable to place a water trap in the expiratory branch filter or exchange the filter after the first hour of bypass.

The increase in exhaled halogenated gases when reducing the machine flow to 50% is physiologically explained by the decrease in blood flow speed through the membrane. This decreases the gradient between the inspired halogenated gases and those in the blood, allowing the exhaled branch to register a higher concentration, closer to the inspired value. Conversely, high flows cause fast blood passage, thus increasing the gradient and resulting in lower measured values in the exhaled branch. Therefore, all measurements were performed at total blood flow for each patient.

The current investigation has noteworthy limitations that warrant consideration. One of these constraints is associated with the measurement mechanism employed. This apparatus was custom-designed and manufactured by the researchers themselves, and was originally not intended for highly precise concentration measurements. Moreover, the dial utilized for setting the desired concentration lacks a high degree of accuracy. As previously noted, even minor fluctuations can yield changes of approximately 1% to 2%. Consequently, to achieve precise concentrations, ongoing real-time monitoring is imperative. It is also imperative to underscore the challenge posed by the disparity in oxygenator types utilized and the intricate task of comparing such disparate mechanisms. In this study, the diverse range of patient body weights potentially affected the absorption of the anesthetic during CPB. However, we addressed this issue by employing membrane oxygenators of varying sizes tailored to individual patient weight class. This limitation underscores the necessity of implementing supplementary measures — such as continuous monitoring — to guarantee the precision and dependability of the findings derived from this study.

CONCLUSION

In summary, this research highlights the importance of continuous monitoring due to the variability in the time required for halogenated gas stabilization and the significant differences between inspired and exhaled halogenate gas concentrations. Although the pharmacokinetics and pharmacodynamics of inhaled anesthetics are well understood, it is important to consider that several factors may affect these behaviors, such as the retention of the anesthetic in tissues and the diffusion capacity of anesthetics based on the material and composition of each membrane. This variability could be due to retention in the oxygenator membrane, which may be influenced by gas transfer efficiency and the sensitivity to changes in machine flow. Consequently, these variations increase the risk of encountering subtherapeutic halogenated gas concentrations, thereby increasing the potential for intraoperative recall events.

ETHICAL DISCLOSURES

Ethics committee approval

The study was approved by the Ethics and Research Committee (approval number N. 15-2017, May 3, 2017).

The protocol was submitted to the Ethics Committee of Fundación Cardioinfantil for evaluation and approval.

Protection of human and animal subjects

The authors declare that no experiments were performed on humans or animals for this study. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Data confidentiality

The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent

The authors declare that no patient data appear in this article.

The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of said document.

ACKNOWLEDGEMENTS

Authors' contributions

LG: Conceptualization; Idea and hypothesis development; formulation of the research goals. Investigation; research and experiments.

JK: Methodology; design of the methodology; development of the process which was followed. Verification of study results, experiment and data; supervision; leading the research, mentoring and project oversight.
EB: Data curation; research data management and keeping, including data cleaning. Writing; writing the initial version of the manuscript. Review & editing; review, revision and approval of the final version.
OQ: Conceptualization; idea and hypothesis development.
NM: Writing the initial version of the manuscript.
LP: Visualization; creation of graphs, charts and figures.

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Conflicts of interest

None declared.

·Presentations

None declared.

Appreciation

None declared.

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