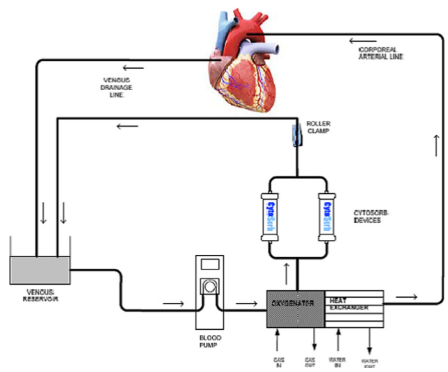
# Hemoadsorption to Reduce Plasma-Free Hemoglobin During Cardiac Surgery: Results of REFRESH I Pilot Study

*Thomas G. Gleason, MD,[*] Michael Argenziano, MD,[†] Joseph E. Bavaria, MD,[‡] Lauren C. Kane, MD,[§] Joseph S. Coselli, MD,[¶] Richard M. Engelman, MD,[‖] Kenichi A. Tanaka, MD,[**] Ahmed Awad, MD,[††] Michael E. Sekela, MD,[‡‡] and Joseph B. Zwischenberger, MD[‡‡]*

Generation of plasma-free hemoglobin (pfHb) and activated complement during complex cardiac surgery contributes to end-organ dysfunction. This prospective, multicenter REFRESH I (REduction in FREe Hemoglobin) randomized controlled trial evaluated the safety and feasibility of CytoSorb hemoadsorption therapy to reduce these factors during prolonged cardiopulmonary bypass (CPB). Eligible patients underwent elective, nonemergent complex cardiac surgery with expected CPB duration ≥3 hours. Exclusions included single procedures including primary coronary artery bypass graft, single valves, transplant, and left ventricular assist device extraction. TREATMENT used 2 parallel 300 mL CytoSorb hemoadsorption cartridges in a side circuit during CPB. CONTROL was standard of care. Of 52 enrolled patients, 46 underwent surgery (Safety group, $n = 23$ vs Control, $n = 23$), and




The CytoSorb® device.

*Abbreviations:* AE, adverse event; AKI, acute kidney injury; AKIN, acute kidney injury network; CABG, coronary artery bypass graft; CPB, cardiopulmonary bypass; ICU, intensive care unit; IGFBP-7, insulin-like growth factor-binding protein; LVAD, left ventricular assist device; MODS, multiple organ dysfunction syndrome; MOF, multiple organ failure; pfHb, plasma-free hemoglobin; pRBC, packed red blood cells; RBC, red blood cell; RCT, randomized controlled trial; SAE, serious adverse event; SIRS, systemic inflammatory response syndrome; TIMP-2, tissue inhibitors of metalloproteinases

*University of Pittsburgh Medical Center, Department of Cardiothoracic Surgery, Pittsburgh, Pennsylvania
†Columbia University Medical Center-New York Presbyterian Hospital, Department of Surgery, New York, New York
‡Hospital of the University of Pennsylvania, Department of Surgery, Philadelphia, Pennsylvania
§Texas Children's Hospital, Department of Surgery, Houston, Texas
¶Texas Heart Institute, Department of Surgery, Houston, Texas
‖Baystate Medical Center, Department of Surgery, Springfield, Massachusetts
**University of Maryland School of Medicine, Department of Anesthesiology, Baltimore, Maryland
††Cooper University Hospital, Camden, New Jersey
‡‡University of Kentucky College of Medicine, Department of Surgery, Lexington, Kentucky

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Address reprint requests to Joseph B. Zwischenberger, MD, Department of Surgery, University of Kentucky College of Medicine, 800 Rose Street, MN264, Lexington, KY 40536-0298. E-mail: jzwis2@uky.edu


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38 were evaluated for pfHb reduction (EFFICACY group, $n = 18$ vs CONTROL, $n = 20$). Type and number of serious adverse events (44 vs 43 CONTROL) were similar, as was 30-day mortality. Transient reduction in platelets during CPB was observed in both groups, especially TREATMENT, but returned to pretreatment levels after CPB without bleeding. Peak pfHb was positively correlated with CPB length ($P$ = 0.01) but the high variability of pfHb, due to the broad surgical procedure mix, prevented detection of changes in pfHb in the overall EFFICACY population. However, the valve replacement surgery subgroup (8 vs 10 CONTROL) had the highest peak pfHb levels, and TREATMENT demonstrated significant pfHb reductions vs CONTROL ($P \leq 0.05$) in CPB ≥3 hours. In the EFFICACY group, C3a and C5a were significantly reduced by treatment throughout surgery. Intraoperative hemoadsorption with CytoSorb was safe and feasible in this randomized, controlled pilot study during complex cardiac surgery. Treatment with CytoSorb resulted in significant reductions in pfHb during valve replacement surgery and reductions in C3a and C5a in the overall EFFICACY group. Future studies will target complex cardiac surgery patients with prolonged CPB to assess hemoadsorption effect on end-organ dysfunction and outcomes.

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**Keywords:** Plasma-free hemoglobin, Cardiopulmonary bypass, Hemoadsorption

**Central Message**

REFRESH (REduction in FREe Hemoglobin) Pilot Study was the first FDA clinical trial assessing safety and feasibility of intraoperative CytoSorb hemoadsorption in a complex cardiac surgery population.

**Perspective Statement**

The REFRESH Pilot Study evaluated the safety and feasibility of blood hemoadsorption technology based on biocompatible, highly porous polymer beads and showed reductions in major inflammatory mediators including plasma-free hemoglobin and activated complement C3a and C5a during cardiopulmonary bypass in elective, nonemergent, complex cardiac surgery in a multicenter randomized controlled trial.

## INTRODUCTION

Cardiac surgery requiring cardiopulmonary bypass (CPB) has been routine in clinical practice for over 50 years. However, there is still the risk of postoperative morbidity and mortality, particularly in complex cardiac surgery patients. During CPB, hemolysis, release of plasma-free hemoglobin (pfHb), activation of complement, and the generation of inflammatory mediators are directly correlated with acute kidney injury (AKI),[1−3] pulmonary and systemic hypertension,[4] and intestinal mucosal injury.[5] Specific mechanisms of pfHb-induced injury include oxygen radical formation, direct toxicity to renal tubules,[6] scavenging of nitric oxide,[7] and myocardial ischemia/reperfusion injury.[8,9]

Normally during CPB, pfHb concentrations are nominal. Red blood cells (RBCs) can hemolyze due to many factors including negative pressure cardiotomy suction, blood material interactions, increases in the ratio of foreign surfaces to blood volume, and shear stress due to high flow rates through artificial blood circuits.[10] Mechanical forces associated with blood pumps generate shear forces, further contributing to RBC injury and hemolysis.[11] The degree of hemolysis during cardiac surgery, therefore, is directly correlated with length and complexity of the cardiac surgery.[12] The resulting hemolysis and release of cell-free hemoglobin can overwhelm the body's natural hemoglobin scavenging system, resulting in increased circulating levels of pfHb.

In order to reduce pfHb and other inflammatory mediators during complex cardiac surgery, we evaluated a blood hemoadsorption technology based on biocompatible, highly porous polymer beads, CytoSorb (CytoSorbents Corporation, New Jersey, USA), a 300 mL extracorporeal blood purification cartridge that is approved as a cytokine adsorber (European Union) (Video 1). For cardiac surgery, CytoSorb connects to a parallel side circuit (postoxygenator to venous reservoir) during CPB without the need for an additional external pump. Each cartridge is validated for use up to 24 hours. This closed system does not result in plasma loss or hemolysis. CytoSorb is filled with biocompatible, porous polydivinylbenzene copolymer sorbent beads approximately 0.5 mm in diameter that can reduce a broad range of mid-molecular weight substances from blood via pore capture and surface adsorption of hydrophobic substances. By narrowing the pore size range to exclude large substances such as immunoglobulin and cells, decreasing the adsorption of hydrophilic substances, and utilizing concentration gradients, CytoSorb primarily reduces substances at high concentration. CytoSorb does not use affinity agents such as antibodies, biologics, or other ligands. CytoSorb utilization for reduction of albumin, platelets, and certain drugs has been reported, but the reductions have not been clinically significant and have been medically manageable.[13−17] The benefit of acutely reducing dangerous levels of toxins has been reported in life-threatening conditions such as sepsis, trauma, and complications of cardiac surgery.[18−22]

A single CytoSorb device used intraoperatively during CPB was deemed safe and feasible in several studies.[21−25] In a retrospective study involving 40 cardiac surgery patients undergoing hypothermic cardiac arrest and anterograde cerebral perfusion (20 Control vs 20 CytoSorb treated intraoperatively), CytoSorb was safe with statistically significant postoperative reductions of inflammatory mediators including IL-6, pro-calcitonin, fibrinogen, and CRP.[22] An interim analysis of approximately 180 patients (out of a target 300) undergoing elective myocardial revascularization in a prospective, randomized controlled, single center 3-arm study (off pump, on-pump, on-pump with CytoSorb), CytoSorb was safe and associated with reduced IL-6 levels and decreased postoperative infections with lower antibiotic usage.[24] In a randomized, controlled single-center study ($n = 37$) evaluating CytoSorb use in low-to-moderate risk cardiac surgery patients, CytoSorb use was safe and associated with significantly reduced immune reactivity, as measured by LPS-induced TNF-$\alpha$ expression.[21]

Unlike these previously published studies, our study is the first randomized, controlled pilot trial to evaluate the safety and performance of CytoSorb in reducing pfHb when used intraoperatively during CPB in subjects undergoing complex cardiac surgery and prolonged CPB. Unlike all other published studies that only used one CytoSorb cartridge, this pilot study evaluated the safety of using dual cartridges, in a parallel configuration, during surgery.

## METHODS

Eight academic medical centers participated based on investigator experience and site resources. Investigators could not deviate from the protocol, except with prior written sponsor approval or concerns for patient safety. Each participating site obtained institutional review board approval including informed consent documents and recruitment materials. The protocol was a 2-arm, multicenter pilot study designed by the investigators and CytoSorbents Corporation, New Jersey). Eligible patients were undergoing elective, nonemergent, complex cardiac surgery with expected CPB duration ≥3 hours. Patients excluded had planned primary coronary artery bypass graft, single-valve procedures, cardiac transplant, or left ventricular assist device extraction. Of 297 patients screened, 52 met criteria (Fig. 1). Following informed consent, patients were randomized prior to study entry. Of the 52 patients, 6 withdrew consent. The 46 remaining subjects randomized in a 1:1 ratio to either standard of care (CONTROL) or CytoSorb as an adjunct to standard of care (TREATMENT). The SAFETY group consisted of all 46 subjects who underwent surgery. The pfHb EFFICACY group (EFFICACY) consisted of 38 patients with nonmissing, validated pfHb sampling (18 TREATMENT and 20 CONTROL patients). PfHb levels were assessed at baseline (after induction of anesthesia, but just prior to sternotomy) and then hourly during CPB, at the end of CPB, and then once daily postoperatively while in the intensive care unit (ICU).

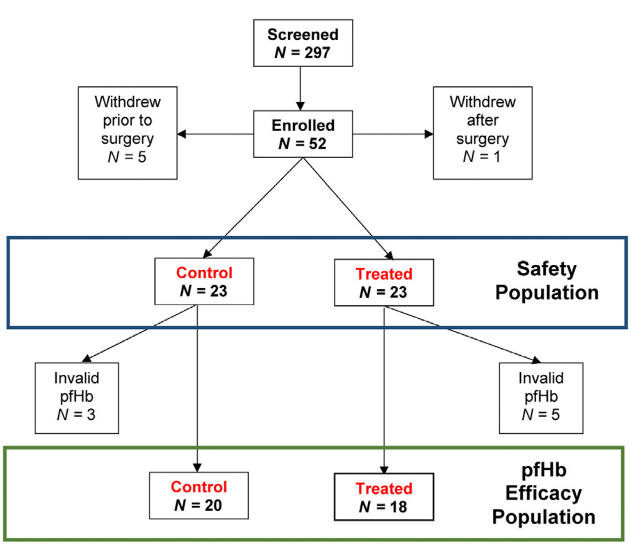


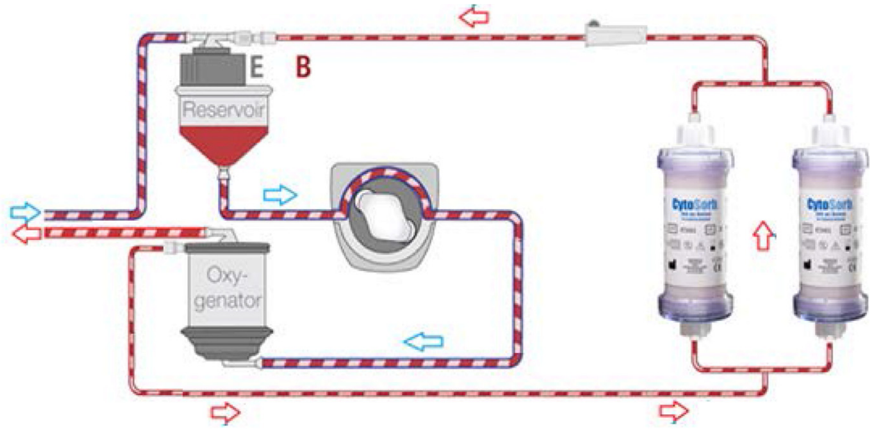

**Figure 1.** Flow diagram of patients.

Two CytoSorb cartridges (CytoSorbents Corporation, New Jersey; 300 mL hemoadsorption cartridges; porous divinylbenzene copolymer sorbent; 10−60 kDa target) were placed in a parallel configuration in a side circuit connected between the oxygenator and venous reservoir prior to the start of the CPB procedure as shown in Figure 2. An adjustable roller clamp distal to the cartridges controls blood flow through the CytoSorb circuit to maintain a total blood flow ~600 mL/min (minimum flow 350 mL/min). Because the flow into the 2 CytoSorb cartridges returns to the venous reservoir rather than to the patient, total flow from the CPB pump is adjusted to achieve the desired CPB flow to the patient. Blood flow through the CytoSorb devices was initiated 1 hour after the patient went on CPB (when pfHb levels elevate) and stopped at the end of the CPB procedure. CONTROL had no CytoSorb circuit. Approximately 300 mL/min, or approximately 4 total blood volumes in 1 hour, flows through each CytoSorb device. All sites used their standard CPB circuits with full heparin anticoagulation and no clotting was observed.

The primary EFFICACY endpoint was the change in pfHb from presternotomy baseline to the end of CPB. Secondary EFFICACY endpoints were ventilator time (h) through ICU discharge, days in the ICU, days in the hospital post-CPB through discharge, incidence and progression of postoperative AKI as defined by serum creatinine criteria and present if any of the 3 defined acute kidney injury network (AKIN) stages were identified through ICU discharge,[26] and postprocedure 30-day all-cause mortality. Exploratory EFFICACY endpoints included complement C3a, C5a, and IL-6 collected until ICU discharge. The primary safety endpoint was the assessment of device-related serious adverse events (SAEs) until ICU discharge. Secondary safety endpoints were adverse events (AEs; device related or not) through 30-day postprocedure; vital signs (blood pressure, pulse, and temperature); and safety laboratory assessments (hematology, comprehensive metabolic panel, and arterial blood gas) until ICU discharge.

### Statistical Analysis

Descriptive statistics for continuous variables include the mean, median, standard deviation, and range. Confidence intervals were included when applicable. Categorical variables used frequency counts and percentages. The primary efficacy endpoint was the change of pfHb from preprocedure to the end of CPB. Because the baseline pfHb values were below the




Configured in a parallel circuit between oxygenator and venous reservoir

- Target 600 mL/min total blood flow
- Initiated 1 hour after start of CPB and stopped at end of CPB
- Intended for patients with ≥3 hours CPB (i.e. ≥2 hours treatment)

**Figure 2.** CytoSorb device in CPB circuit.

limit of detection, and because of the variability in the timing of the blood sampling at the end of CPB, the peak pfHb value during CPB was used as the endpoint in many of the analyses in this pilot study. Data were summarized by arm (CONTROL or TREATMENT). Unless otherwise stated, continuous variables were compared using either *t* tests or Wilcoxon sign rank tests. Categorical data were summarized using Fisher's exact test. All testing was 2-sided. Because longer duration of CPB has been associated with elevated pfHb, analysis of covariance was used to assess peak pfHb after correcting for duration of CPB as a continuous covariate. Similarly, logistic regression was applied to determine the odds of experiencing AKI after correcting for duration of CPB and peak pfHb.

## RESULTS

The demographics including age, gender, height, weight, and BMI of the SAFETY and EFFICACY populations are shown in Table 1. Demographics were similar. The number of comorbid conditions at baseline determined the preprocedure severity of illness. Twenty-one comorbid conditions were recorded, ranging from cardiac-related diseases (eg, coronary artery disease, atrial fibrillation) and metabolic conditions such as diabetes and renal disease, to hematology-related disorders. Within-patient totals ranged from 1 to 10. No significant differences in the mean or median baseline number of conditions were noted between TREATMENT and CONTROL.

The length of surgical procedures was highly variable in the SAFETY population (Table 2). Despite intending to enroll patients with CPB duration greater than 3 hours, CPB duration was less than 3 hours in 37% (17/46), due to either an intraoperative change in the surgical plan or an unexpectedly shorter procedure. Since CytoSorb is initiated 1 hour after start of CPB, shorter CPB duration results in shorter treatment duration. The mean duration of CytoSorb treatment was 2.5 ± 1.2 hours (range: 0.8−5.0 hours). Following surgery, mean length of stay in the ICU was more variable in the CONTROL (6.8 ± 12.7 days; range: 1−54 days) vs TREATMENT (4.3 ± 3.3 days; range: 1−14 days) as was hospitalization postsurgery in CONTROL (14.0 ± 14.1 days; range 4−57 days) vs

**Table 1.** Demographics of the Safety and Efficacy Populations

| | Safety Population | | | Efficacy Population | | |
|---|---|---|---|---|---|---|
| | Control *N* = 23 | CytoSorb *N* = 23 | *P* Value | Control *N* = 20 | CytoSorb *N* = 18 | *P* Value |
| Age (mean ± std) | 61 ± 17 | 66 ± 8 | 0.20 | 60 ± 17 | 67 ± 7 | 0.11 |
| BMI (mean ± std) | 29 ± 7 | 28 ± 4 | 0.29 | 30 ± 7 | 28 ± 4 | 0.51 |
| Gender (male) | 78% | 56% | 0.21* | 75% | 56% | 0.31* |
| Hispanic | 4% | 0% | 1.00* | 5% | 0% | 1.00* |
| Current smoker | 9% | 30% | 0.13* | 5% | 22% | 0.17* |

All values expressed as (mean ± std).
*P* values: *t* test.
*Fisher's exact test.

**Table 2.** Surgery and ICU Statistics of Safety Population

| | Control *N* = 23 | CytoSorb *N* = 23 | *P* Value |
|---|---|---|---|
| Surgery length (h) | 8.1 ± 2.0 (5.3–12.2) | 8.4 ± 2.7 (4.9–13.7) | 0.66 |
| CPB time (h) | 3.3 ± 1.1 (1.7–5.6) | 3.8 ± 1.3 (1.9–6.6) | 0.18 |
| CPB time >5 h | 1 (4%) | 4 (17%) | 0.35* |
| Length of CytoSorb Treatment (h) | n/a | 2.5 ± 1.2 (0.8–5.0) | n/a |
| Length of ICU stay (d) | 6.8 ± 12.7 (1–54) | 4.3 ± 3.3 (1–14) | 0.38 |
| Hospitalization post-CPB (d) | 14.0 ± 14.1 (4–57) | 11.8 ± 5.9 (6–28) | 0.49 |

All values expressed as (mean ± std [min–max]).
*P* values: *t* test.
*Fisher's exact test.

TREATMENT (11.8 ± 5.9 days; 6–28 days), but the differences were not statistically significant.

The majority of patients underwent multiple procedures that included some combination of valve replacement, aortic reconstruction, coronary artery bypass graft surgery, valve repair, or congenital defect repair. Despite the intention to perform multiple procedures, approximately 22% (5/23) of CONTROL and 13% (3/23) of TREATMENT patients underwent a single procedure due to an operative change in plan. The most common surgical procedures performed in the SAFETY population were valve replacement surgery (48% [11/23] CONTROL; 56% [13/23] TREATMENT) and aortic reconstruction (65% [15/23] CONTROL; 56% [13/23] TREATMENT). Table 3 shows CPB duration by type of surgical procedure.

Higher peak pfHb levels were correlated with longer duration of CPB ($P = 0.06$) in the EFFICACY population and were typically observed at or near the end of CPB. However, pfHb levels varied by the surgical procedure. In CONTROL patients in the EFFICACY group (no pfHb removal), high pfHb levels were most pronounced in patients undergoing multiple valve replacement alone or valve replacement with other procedures (Table 4). Procedures involving valve replacement trended toward higher median pfHb levels compared to nonvalve replacement procedures (123 mg/dL vs 61 mg/dL, $P = 0.13$). CPB times tended to be shorter in the valve replacement subgroup (Table 3) compared to other CONTROL patients. The high variability in pfHb levels caused by different procedures, CPB duration, and small study population led to the inability to observe overall pfHb reductions from preprocedure to end of CPB ($P = 0.59$).

In order to assess the performance of the device in a more homogenous patient cohort with highest peak pfHb levels, we analyzed the subpopulation of patients undergoing valve replacement surgery. In this subgroup, we observed lower peak pfHb levels over all CPB durations in TREATMENT ($N = 11$) vs CONTROL ($N = 10$). For patients with CPB ≤5 hours ($N = 8$ TREATMENT, $N = 10$ CONTROL), a significant reduction in pfHb was achieved at CPB of 3.5 and 4.0 hours (48 and 61 mg/dL reduction, $P \leq 0.05$; Table 5 and Fig. 3).

**Table 3.** Length of Cardiopulmonary Bypass (H) by Type of Surgical Procedure; Safety Population

| | | Control | CytoSorb | *P* Value |
|---|---|---|---|---|
| All Surgeries | *n* | 23 | 23 | 0.25 |
| | Median | 3.2 | 3.7 | |
| | (min–max) | (1.7–5.6) | (1.9–6.5) | |
| Valve replacement | *n* (%) | 11 (48%) | 13 (56%) | 0.13 |
| | Median | 2.8 | 3.7 | |
| | (min–max) | (1.7–4.4) | (1.9–6.2) | |
| Nonvalve replacement | *n* (%) | 12 (52%) | 10 (43%) | 0.92 |
| | Median | 4.0 | 3.8 | |
| | (min–max) | (2.2, 5.6) | (2.7–6.5) | |
| Valve repair | *n* (%) | 5 (22%) | 5 (22%) | 0.69 |
| | Median | 3.5 | 4.0 | |
| | (min–max) | (1.8, 4.8) | (2.2, 5.0) | |
| Aortic reconstruction | *n* (%) | 15 (65%) | 13 (6%) | 0.44 |
| | Median | 3.3 | 3.9 | |
| | (min–max) | (2.2,5.6) | (2.2, 6.5) | |
| CABG | *n* (%) | 6 (26%) | 8 (35%) | 0.13 |
| | Median | 3.2 | 4.4 | |
| | (min–max) | (1.7–4.9) | (2.9–6.2) | |
| Congenital defect repair | *n* (%) | 2 (9%) | 0 | n/a |
| | Median | 3.4 | | |
| | (min–max) | (2.4–4.4) | | |

*P* values: Wilcoxon test comparing median values. Patients may be included in more than 1 category if multiple procedures were performed during the same surgery.

**Table 4.** Peak PfHb in Different Surgical Procedures (mg/dL); Efficacy Population, Control Group

| Procedure | *N* | Mean | Std | Median | Min | Max |
|---|---|---|---|---|---|---|
| All | 20 | 104.0 | 58.54 | 101 | 23 | 245 |
| Multiple valve replacement | 9 | 121.0 | 46.57 | 123 | 57 | 204 |
| Single or multiple valve replacement and/or aortic reconstruction | 17 | 103.9 | 46.92 | 116 | 41 | 204 |
| Nonvalve replacement | 11 | 90.1 | 65.60 | 61 | 23 | 246 |

Patients may be included in more than 1 category if multiple procedures were performed during the same surgery.

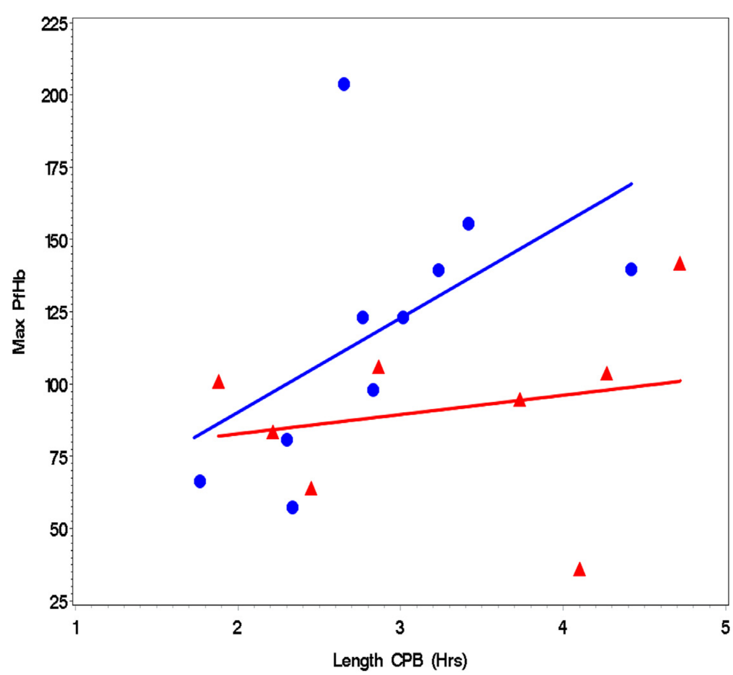
**Table 5.** Predicted Mean Difference in Peak pfHb Between Control and Treatment in Valve Replacement Subgroup During CPB ≤ 5 Hours

| CPB Length (h) | Control−Treatment Mean Peak pfHb (mg/dL)* | *P* Value |
|---|---|---|
| 3.0 | 35 | 0.09 |
| 3.5 | 48 | 0.04 |
| 4.0 | 61 | 0.05 |
| 4.5 | 74 | 0.07 |

Treatment = 11, Control = 10.
*Least-square mean differences based on analysis of covariance (ANCOVA) of mean peak pfHb between treatment groups adjusted for CPB length as a continuous covariate.

To assess the decrease of inflammatory mediators, we measured treatment effects on activated complement. The TREATMENT patients showed significant reductions in C3a and C5a during surgery. In addition, sustained reductions in levels of C5a in the TREATMENT group compared to CONTROL were observed throughout the duration of ICU stay (Fig. 4).

Based on AKIN criteria using creatinine as a primary measure, the incidence of AKI in the overall safety population was not significantly different between TREATMENT (30% or 7/23) and CONTROL (26% or 6/23; $P = 1.0$), but is in agreement with previously published rates of AKI observed during cardiac surgery.[27,28] In some previous studies, the risk of postprocedure AKI was directly correlated with length of CPB and pfHb levels.[28,29]




Control —— Treatment ——
Points represent observed peak pfHb (mg/dL) values by length of CPB (hrs) among patients with valve replacement surgery. Linear predicted LS-mean values determined by ANCOVA with fixed effect treatment and length CPB as continuous covariate. A greater increase in pfHb associated with length of CPB is predicted among the control patients.

**Figure 3.** Observed and predicted plasma-free hemoglobin (mg/dL) values by length of CPB in valve replacement subgroup.

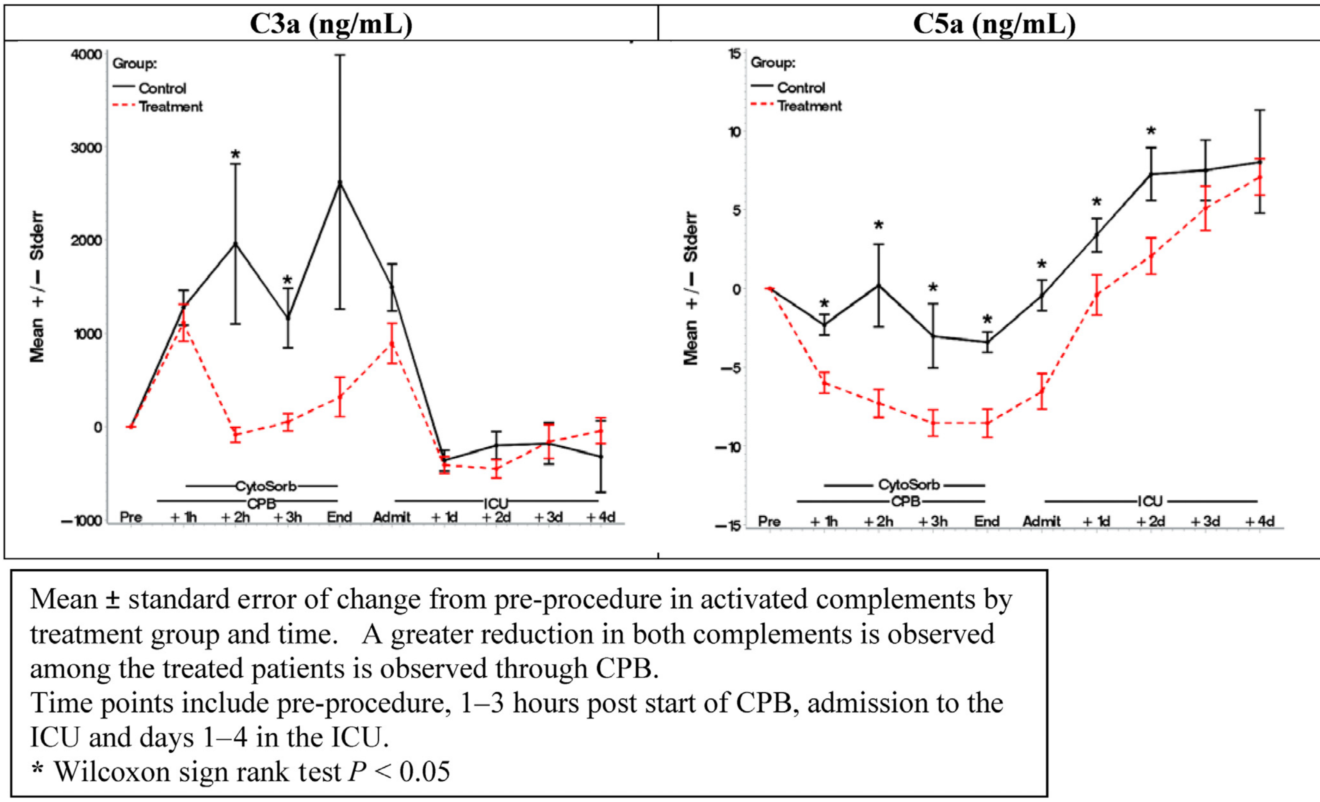



**Figure 4.** Effect of CytoSorb on activated complement C3a and C5a.

A logistic regression performed with TREATMENT and length of CPB suggests a potential reduction in risk of AKI with an odds ratio of 0.43 at the average length of CPB for the overall safety population ($P = 0.34$), and 0.26 for the valve replacement population (alone or in conjunction with other procedures, $P = 0.54$). Neither is statistically significant, and a larger study would be required to evaluate the impact of treatment on the incidence of AKI. Other secondary efficacy endpoints are summarized in Table 2.

There was no significant difference between AEs or SAEs between the CONTROL and TREATMENT groups. Type and number of SAEs (44 vs 43 CONTROL) were similar, with 2 device-related SAEs (thrombocytopenia) that resolved without sequelae. The frequency of AEs and SAEs was similar in both CONTROL and TREATMENT groups (Tables 6 and 7). There was no difference in 30-day mortality.

Prior to surgery, CONTROL patients in the SAFETY population had higher baseline platelet levels compared to TREATMENT patients. The initiation of CPB led to significant mean reductions in platelet counts in both the CONTROL (38%) and TREATMENT (50%) groups at 1 hour post-CPB start, in the absence of CytoSorb treatment. After the initiation of CytoSorb treatment, an additional mean drop in platelets was observed in TREATMENT (56%) vs CONTROL (4%) that remained stable throughout CPB, with only 4% (1/23) of CONTROL patients and 13% (3/23) of TREATMENT patients receiving platelets during the procedure ($P = 0.61$). In most cases, platelet levels returned to 1 hour prior to CytoSorb levels for both groups by ICU admission (Fig. 5). There were no significant differences in postoperative coagulation parameters (PT or PTT), AEs, or SAEs related to perioperative bleeding complications in CONTROL or TREATMENT groups. There was no significant difference in the median administration of

**Table 6.** Adverse and Serious Adverse Events, Safety Population

| | Control $N = 23$ | CytoSorb $N = 23$ |
|---|---|---|
| Total number AEs | 137 | 121 |
| Postprocedure, prior to hospital discharge | 119 | 86 |
| Patients with at least 1 AE | 21 (91.3%) | 23 (100%) |
| Total number SAEs | 43 | 44 |
| Number patients with at least 1 SAE | 11 (47.8%) | 16 (69.6%) |
| Patients with at least 1 AE leading to death | 1 (4.3%) | 2 (8.7%) |
| Patients with at least 1 surgical related AE (probable or definite) | 15 (69.5%) | 19 (82.5%) |
| Total device-related AEs | n/a | 2 |
| Patients with at least 1 device-related AE* | n/a | 2 (8.7%) |
| Unanticipated device-related AEs | n/a | 0 |

*One mild and 1 severe thrombocytopenia, resolved without sequelae.

**Table 7.** Serious Adverse Events (SAEs) Reported in Safety Population

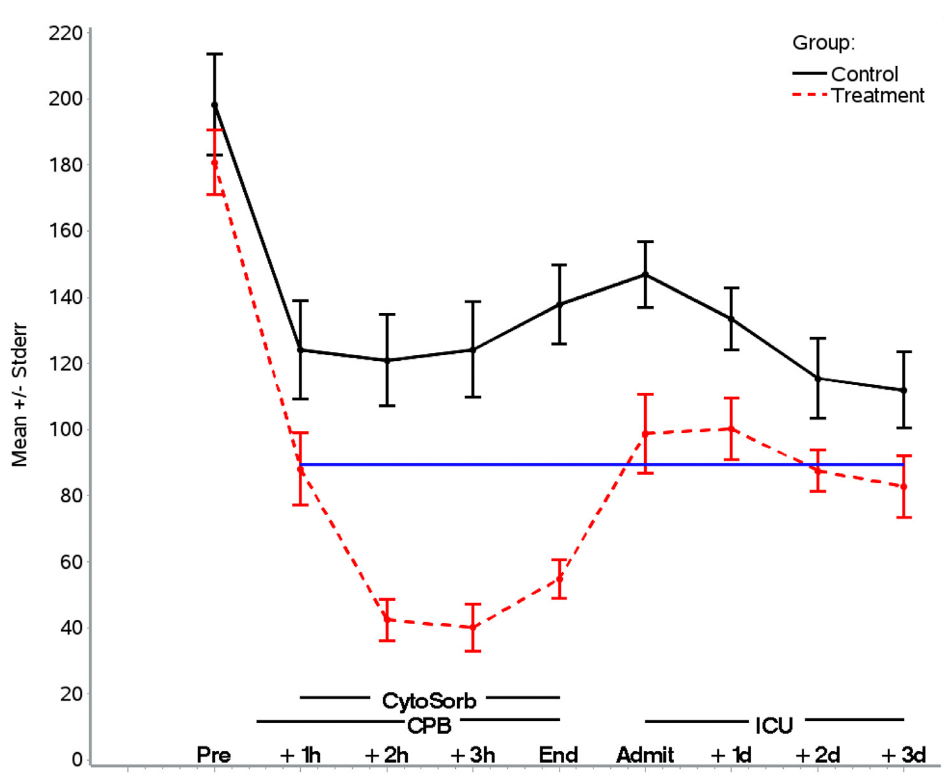
| Type of SAE | Patients Experiencing SAEs | |
|---|---|---|
| | Control *N* = 23 | CytoSorb *N* = 23 |
| Cardiac disorders | 6 (26%) | 10 (43%) |
| Atrial fibrillation | 2 (9%) | 2 (9%) |
| Respiratory, thoracic, and mediastinal disorders | 5 (22%) | 3 (13%) |
| Pleural effusion | 3 (13%) | 2 (9%) |
| Procedural complication | 4 (17%) | 3 (13%) |
| Anemia – postoperative | 2 (9%) | 2 (9%) |
| Renal and urinary disorders | 6 (26%) | 6 (26%) |
| Vascular disorders | 3 (13%) | 3 (13%) |
| Hypotension | 2 (9%) | 2 (9%) |
| Blood/lymphatic system disorders | 1 (4%) | 4 (17%) |
| Thrombocytopenia | 1 (4%) | 2 (9%) |
| Metabolism/nutrition disorders | 3 (13%) | 2 (9%) |
| Nervous system disorders | 3 (13%) | 0 (0%) |
| Stroke or CVA | 2 (9%) | 0 (0%) |

platelets, pRBCs, or plasma from surgery until ICU discharge among all patients in the SAFETY population (Table 8).

Blood flow rates, as measured by transonic blood flow sensors, were analyzed at each trial site. Certain trial sites reported flow limitations in their CPB circuits and could not achieve targeted blood flow rates of 300 mL/min per cartridge (600 mL/min through both cartridges) in all cases. These sites had "low" mean blood flow rates of approximately 206 mL/min per device (range 148–286 mL/min), while other sites had "high" mean blood flow rates of approximately 313 mL/min per device (range: 269–339 mL/min).

## DISCUSSION

This REFRESH I trial is the first randomized, controlled, multicenter pilot trial characterizing the safety and efficacy of pfHb removal by dual parallel CytoSorb cartridges when used intraoperatively during complex cardiac surgery. The study demonstrated that the use of dual CytoSorb cartridges within the CPB circuit is feasible with no major reported technical issues. The CytoSorb hemoadsorption cartridge represents a




Mean ± standard error of platelet levels by treatment group and time. The blue line represents mean pre-procedure platelet level within the treated group. Time points include pre-procedure, 1–3 hours post start of CPB, admission to the ICU and days 1–3 in the ICU.

**Figure 5.** Perioperative platelet levels over time.

**Table 8.** Transfusions Administered to Safety Population – Surgery Through ICU Stay

| Type of Transfusion | | *N* | Mean | Std | Median | *P* Value |
|---|---|---|---|---|---|---|
| Platelets | Control | 23 | 1.2 | 1.6 | 1 | 0.10 |
| | Treated | 23 | 2.4 | 2.6 | 2 | |
| pRBC and autologous blood | Control | 23 | 2.6 | 4.4 | 2 | 0.26 |
| | Treated | 23 | 4.1 | 5.8 | 2 | |
| Plasma | Control | 23 | 1.0 | 1.8 | 0 | 0.23 |
| | Treated | 23 | 2.3 | 3.5 | 1 | |
| At least 1 transfusion | Control | 17 (74%) | | | | 0.7222* |
| | Treated | 19 (83%) | | | | |

*P* values: Wilcoxon rank sum test.
*Fisher's exact test.

novel strategy to directly reduce excessive pfHb, activated complement, cytokines, and other circulating inflammatory mediators during cardiac surgery in real-time. The CytoSorb system has been approved in the European Union, India, Russia, South Africa, Saudi Arabia, and other countries (though it is not yet approved in the United States), and it has been reported to be safe and well tolerated when used as a single cartridge during cardiac surgery in low-to-moderate risk patients and in patients with acute infective endocarditis. Reported benefits were improved intra- and postoperative hemodynamic stabilization, reduced infections and need for antibiotics, and reductions in postoperative inflammatory mediators.[21−25]

PfHb is generated by blood shear forces during cardiac surgery. High levels of pfHb can lead to oxygen radical generation and oxidative injury,[30,31] nitric oxide scavenging leading to vasoconstriction, systemic and regional hypertension,[4,32,33] as well as postoperative systemic inflammatory response syndrome.[2,34,35] These changes can cause postoperative cardiac strain, direct tissue injury, and inflammation that can potentially lead to major postoperative complications such as multiple organ dysfunction syndrome and multiple organ failure.[36]

In this pilot study, we observed that pfHb levels are dependent on both the length of CPB and the type of surgical procedure. In general, the longer the procedure, the higher the peak pfHb levels. Multiple valve replacements or valve replacements with another procedure, such as aortic reconstruction, were associated with rapid pfHb generation and the highest peak pfHb levels, while nonvalve replacement surgeries were not. The surgical procedure determines the length of CPB, amount of pericardial shed blood, and the negative pressure cardiotomy suction needed, which correlates with hemolysis and the release of pfHb. The combination of a small study size, variable peak pfHb levels among patients undergoing different procedures, different CPB times, and preferential ability of CytoSorb to reduce substances at high concentrations, impacted changes of pfHb or organ injury in the broader SAFETY population. However, in the valve replacement subgroup where peak levels of pfHb were greater, CytoSorb treatment in patients undergoing CPB ≤5 hours led to an absolute reduction of pfHb between 35 and 74 mg/dL through 4.5 hours of CPB, representing an approximately 35−45% reduction in peak pfHb levels relative to CONTROL.

Exploratory endpoints evaluating other inflammatory mediators in the SAFETY population showed significant reductions of activated complement C3a and C5a with CytoSorb treatment. In contrast to pfHb, complement appears to activate very rapidly across all patients undergoing CPB and implies an immediate risk of inflammation and potential organ injury. Ideally, CytoSorb treatment should be initiated at the start of CPB in order to have the maximum protective impact, rather than the delayed start of 1 hour after CPB that we used in this trial. The study was neither designed nor powered to prospectively or retrospectively evaluate clinical benefit of CytoSorb in the entire population or subgroups.

To our knowledge, CytoSorb is the first blood purification technology to demonstrate the ability to reduce intraoperative levels of pfHb and activated complement C3a and C5a. Likewise, CytoSorb may have additional benefits by reducing other inflammatory mediators. A larger study will be needed to evaluate the effect of CytoSorb treatment on clinical outcomes and postoperative complications.[22] AKI is directly associated with peak pfHb levels and is one of the most common adverse events following complex cardiac surgery. We observed an incidence of approximately 30%, consistent with the reported incidence of AKI after CPB.[37,38] This population would be the logical target of a larger CytoSorb trial.[39−43]

In our trial, CytoSorb was safe with no major differences in the type or number of adverse events compared to CONTROL. One anticipated risk factor was a reduction in platelets, as CPB and CytoSorb activate platelets. Although no decrease in platelets was reported in a recently published RCT in patients undergoing cardiac surgery with a single CytoSorb cartridge treatment,[21] the effect of dual CytoSorb cartridges on platelets was not significant. We observed a drop in platelet levels with the initiation of CPB in both CONTROL and TREATMENT, primarily attributable to the known dilution effect of the crystalloid priming volume in the CPB circuit, organ sequestration, and adherence to the CPB circuit. Initiation of CytoSorb treatment 1 hour after CPB start led to a further reduction in platelets. Part of this decrease is predictable due to 8−10% dilution from ~400 mL of crystalloid in the CytoSorb cartridge priming circuit. Part of the decrease is attributable to platelet adherence to the CytoSorb beads, which is partially mitigated by maintaining blood flow rates of 300 mL/cartridge or more. The clinical relevance of intraoperative thrombocytopenia during CPB is unclear. Patients are already anticoagulated with systemic heparin during CPB while platelet levels rebound to pre-CytoSorb treatment levels within hours. Importantly, the number of blood transfusions (eg, platelets, pRBCs, and plasma) administered from the beginning of surgery through the ICU stay was not significantly different.

There are several limitations of this study. First, the trial was too small to account for the site-specific variability in the standard of care and case mix. Second, we used "expected CPB time ≥3 hours" as the primary inclusion criteria. However, we found that this variable was difficult to estimate a priori, with 37% of patients ultimately having CPB times of less than 3 hours, resulting in reduced opportunity for pfHb generation, decreased length of CytoSorb treatment, and lower risk of organ injury. For those patients with CPB ≥3 hours, there was a wide variation in peak pfHb levels. The type of surgical procedure and CPB length was a better predictor of peak pfHb levels than CPB length alone, with valve replacement surgery resulting in the highest values, compared to nonvalve replacement surgeries.

A future study will evaluate the safety and efficacy of CytoSorb on clinical outcomes such as a reduction in the severity of AKI. In addition, the use of preoperative risk assessment criteria such as the Cleveland Clinic Score may identify patients with risk factors for developing postsurgical AKI. Based on power calculations and a 1:1 randomized controlled trial, such a trial would require about 400 patients to see a 10−15% absolute reduction in AKI risk. The cost of the device, if approved in the United States, will be determined by clinical benefit and cost effectiveness.

## CONCLUSIONS

This was the first randomized, clinical pilot trial demonstrating safety and feasibility of intraoperative use of dual, parallel CytoSorb hemoadsorption cartridges in an elective, nonemergent complex cardiac surgery population. We demonstrated that CytoSorb use was safe and capable of reducing concentrations of pfHb and activated complement, especially in patients undergoing valve replacements surgery (eg, multiple valve replacements or single valve with other procedures). Future clinical studies will focus on the complex cardiac surgery population to evaluate benefit of removing pfHb and other inflammatory mediators by CytoSorb to decrease the risk of postoperative inflammation.

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## SUPPLEMENTARY MATERIAL

The following is the supplementary data to this article:

**Video 1.** CytoSorb in cardiopulmonary bypass.

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