

# Evaluation of MP4OX for Prevention of Perioperative Hypotension in Patients Undergoing Primary Hip Arthroplasty with Spinal Anesthesia

## *A Randomized, Double-blind, Multicenter Study*

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### ABSTRACT

**Background:** MP4OX (oxygenated polyethylene glycol-modified hemoglobin) is an oxygen therapeutic agent with potential applications in clinical settings where targeted delivery of oxygen to ischemic tissues is required. The primary goal of this study was to investigate MP4OX for preventing

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### What We Already Know about This Topic

- Oxygenated polyethylene glycol–modified hemoglobin is useful in preventing hypotension and ischemia in animals subjected to hemorrhage and may reduce hypotension in surgical patients.

### What This Article Tells Us That Is New

- In more than 350 patients undergoing primary hip arthroplasty under spinal anesthesia, oxygenated polyethylene glycol–modified hemoglobin reduced hypotensive episodes significantly more than hydroxyethyl starch but was associated with a higher risk of adverse events, including elevations of liver enzyme and troponin concentrations and nausea.

hypotensive episodes. An additional goal was to establish the safety profile of MP4OX in a large surgical population.

**Methods:** Patients ( $n = 367$ ) from 18 active study sites in six countries, undergoing elective primary hip arthroplasty with spinal anesthesia, were randomized to receive MP4OX or hydroxyethyl starch 130/0.4. Patients received a 250-ml dose at induction of spinal anesthesia and a second 250-ml dose if the protocol-specified trigger (predefined decrease in systolic blood pressure) was reached. The primary end point was the proportion of patients who developed one or more hypotensive episodes.

**Results:** The proportion of patients with one or more hypotensive episodes was significantly lower ( $P < 0.0001$ ) in the MP4OX group (66.1%) versus controls receiving hydroxyethyl starch 130/0.4 (90.2%). More MP4OX-treated patients experienced adverse events compared with controls (72.7% vs. 61.4%;  $P = 0.026$ ). Transient elevations in laboratory values (e.g., alanine aminotransferase, aspartate aminotransferase, lipase, and troponin concentrations) occurred more frequently in the MP4OX group. There were no sig-

- ◆ This article is accompanied by an Editorial View. Please see: Levy JH: Hemoglobin-based oxygen carriers for reversing hypotension and shock: NO way, NO how? ANESTHESIOLOGY 2011; 114:1016–8.

nificant differences in the incidence of serious adverse events or in the composite morbidity and ischemia outcome end points, but nausea and hypertension were reported more often in MP4OX-treated patients.

**Conclusion:** MP4OX significantly reduced the incidence of hypotensive episodes in patients undergoing hip arthroplasty, but the adverse event profile does not support use in routine low-risk surgical patients for the indication evaluated in this study.

**S** PINAL anesthesia frequently causes hypotension in surgical patients.<sup>1,2</sup> This is because of a gravitational redistribution of blood volume caused by the anesthesia-induced sympatholytic reduction in vascular tone, leading to functional hypovolemia, with resultant reduction in venous return, cardiac filling, and cardiac output and consequent hypotension. Elderly patients with advanced cardiovascular disease receiving spinal anesthesia are, therefore, potentially at risk for organ ischemia if insufficient perfusion occurs.<sup>3,4</sup>

MP4OX, oxygenated polyethylene glycol–modified hemoglobin, formerly referred to as Hemospan® (Sangart Inc., San Diego, CA) is a novel oxygen therapeutic agent that may have potential applications in several therapeutic indications for which targeted delivery of oxygen to ischemic tissues is required.<sup>5,6</sup> Preclinical studies of hemorrhage resuscitation in swine<sup>7</sup> and rats<sup>8</sup> have demonstrated greater efficacy of MP4OX in preventing hypotension and ischemia compared with crystalloid and colloid and have shown that MP4OX is capable of preserving capillary perfusion *in vivo*<sup>9</sup> without any evidence of vasoconstriction in awake hamsters instrumented to allow direct visualization of the vasculature.<sup>10</sup>

The clinical development of MP4OX has been performed in accordance with guidance and scientific advice from regulatory authorities regarding investigation of oxygen therapeutic agents. The draft guidance issued in 2004 by the US Food and Drug Administration\*\*\* recommended that oxygen therapeutic agents establish their tolerability profiles “initially in controlled settings such as elective surgery before embarking on studies in unstable surgical patients or unstable trauma patients.” In addition, surrogate markers of efficacy are acceptable if they are reasonably likely to predict clinical benefit. Therefore, clinical evaluation of MP4OX has been primarily performed in stable patients who undergo elective orthopedic surgery.<sup>11,12</sup> In a previous phase 2 study<sup>11</sup> in 90 patients undergoing hip replacement or repair with spinal anesthesia, the incidence of hypotension was significantly reduced ( $P < 0.001$ ) by half with MP4OX (250 or 500 ml) compared with crystalloid (Ringer’s acetate) infu-

sion and standard of care. More patients in the 500-ml MP4OX group reported adverse events (AEs; mostly bradycardia, nausea, and hypertension), and there were transient elevations in lipase, amylase, and aspartate aminotransferase concentrations in some patients, although the mean values were not significantly different from those of the control group. Further studies involving more patients are needed to clarify the relevance of these laboratory findings, with respect to future clinical use of MP4OX in high-risk patient populations exposed to ischemia and oxygen debt, in whom the oxygen therapeutic effect of MP4OX is expected to be more beneficial.

Because patients who undergo hip arthroplasty represent the largest orthopedic surgery population routinely receiving spinal anesthesia, the current multicenter study focused on evaluating MP4OX in these patients, relative to controls treated with colloid. The objectives of this study were 2-fold: (1) to investigate the ability of MP4OX to prevent hypotensive episodes (during surgery and up to 6 h after skin closure) and (2) to establish the safety profile of MP4OX in a large homogeneous population of low-risk patients undergoing major elective surgery.

## Materials and Methods

### Patient Population

This study was approved by the regulatory authorities in each participating country and by the centralized and local ethics committee for each participating site. The study was conducted in accordance with good clinical practice and with country-specific laws and regulations governing clinical studies of investigational products. Compliance with these requirements also constitutes conformity with the Declaration of Helsinki. The study was registered.†††

Before any study-related procedure was performed, each patient or an authorized legal representative gave written informed consent to participate. Patients or legal representatives were free to withdraw the patients from the study at any time. Patients from 18 study sites in six countries (Belgium, Czech Republic, The Netherlands, Poland, Sweden, and the United Kingdom) were included in the study. Adult female (surgically sterile or postmenopausal) or male patients were eligible to participate if they were 50 yr or older, classified as American Society of Anesthesiology physical status class II or III, and scheduled to undergo elective primary hip arthroplasty (based on osteoarthritis diagnosis) under spinal anesthesia.

Major exclusion criteria included the following: hip fracture or nail/pin extraction procedures; evidence of uncontrolled cardiovascular, infectious, psychiatric, metabolic, or systemic disorders, including diabetes mellitus and rheumatoid arthritis; evidence of hypertension (systolic blood pressure [SBP] > 180 mm Hg or a difference in SBP of  $\geq 15$  mm Hg between arms, measured in the supine position); recent history or evidence of myocardial infarction (MI) or stroke (within 6 months); known alcohol or drug dependency; currently taking oral anticoagulant therapy, except for low-dose

\*\*\* Food and Drug Administration, Center for Biologics Evaluation and Research, October 2004. Guidance for Industry: Criteria for Safety and Efficacy Evaluation of Oxygen Therapeutics as Red Blood Cell Substitutes. Available at: <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm074920.htm>. Accessed November 19, 2010.

††† NCT00421200. Available at: <http://www.clinicaltrials.gov>. Accessed January 9, 2007.

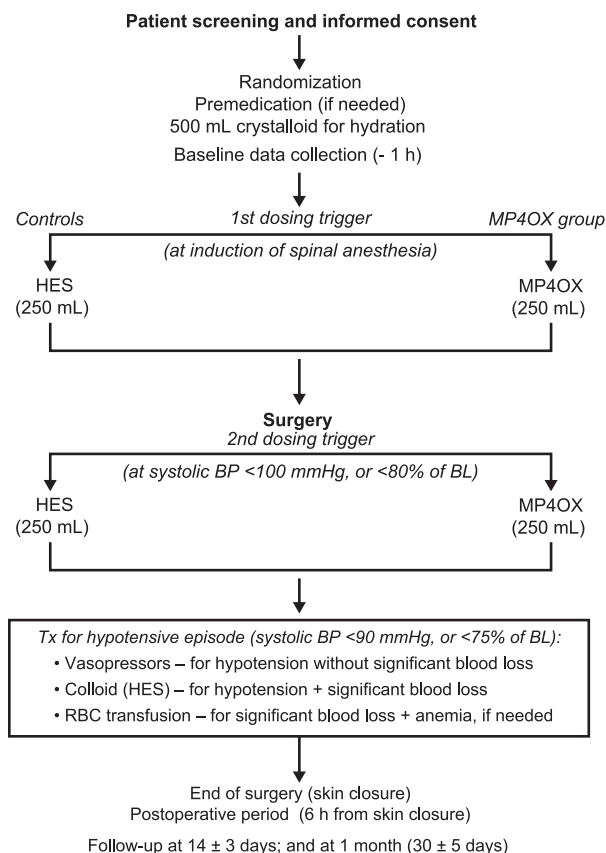
acetylsalicylic acid; history of coagulopathy; and involvement in any investigational drug or device trial within 30 days before this study.

### Investigational Products and Study Design

This was a multicenter, randomized, double-blind, comparator-controlled study. MP4OX is manufactured and supplied by Sangart, Inc., and is prepared from purified stroma-free hemoglobin that is chemically modified by attachment of seven to eight monofunctional maleimide-activated polyethylene glycol molecules.<sup>13</sup> MP4OX is formulated at 4.3 g/dl under oxygenated conditions in isotonic lactated electrolyte solution (280 mOsm/l), has a high oxygen affinity with a P50 (50% saturation level) of approximately 5 mm Hg, and is hyperoncotic, with a colloid osmotic pressure of approximately 70 mm Hg. Voluven® (hydroxyethyl starch [HES] 130/0.4, 6%, in NaCl solution, 0.9%), purchased from Fresenius Kabi (Bad Homburg, Germany), is a commercially available colloid (308 mOsm/l with a colloid osmotic pressure of approximately 36 mm Hg) for volume replacement<sup>†††</sup> and was used in the control group as the active comparator because it is a commonly used colloid in Europe.<sup>14</sup>

All patients received a 500-ml infusion of isotonic crystalloid for hydration within 30 min before induction of spinal anesthesia as an initial volume load to improve hydration status and reduce the risk of hypovolemia. Patients could also receive benzodiazepine (approximately 0.1 mg/kg) for anxiety, if needed, 1 h before induction. Baseline measurements were recorded within 1 h before induction of spinal anesthesia. Per protocol, spinal anesthesia consisted of a standard dose (approximately 12–20 mg) of plain isobaric bupivacaine, 0.5%. If required, propofol (up to 2 mg · kg<sup>-1</sup> · h<sup>-1</sup>) was permitted after induction of anesthesia if required for additional sedation when surgical procedures took longer than planned. A schematic illustration of the sequence of events in the study protocol is shown in figure 1.

Both treatment groups received the first 250-ml dose of investigational product (*i.e.*, either MP4OX or HES, according to randomization) at the induction of spinal anesthesia. A second 250-ml dose (MP4OX or HES) was administered only if the protocol-defined dosing trigger (SBP either lower than 100 mm Hg or lower than 80% of baseline, whichever was the greater value) occurred during surgery. Investigational product was administered using a standard intravenous infusion set with a drip chamber, but all lines were shrouded to prevent unblinding. Gravity infusion was recommended (10–15 min per 250-ml dose, corresponding to an infusion rate of 17–25 ml/min). After administration of the second dose, if patients reached the protocol-defined level of hypotension (*i.e.*, SBP either lower than 90 mm Hg or lower than 75% of baseline, whichever was the greater



**Fig. 1.** Schematic flow chart of the study protocol procedures. BL = baseline; BP = blood pressure; HES = hydroxyethyl starch solution 130/0.4; MP4OX = oxygenated pegylated hemoglobin; RBC = red blood cell; Tx = treatment.

value), they could receive additional fluids (isotonic crystalloids or colloids) or vasopressor support at the discretion of the investigator and as clinically indicated to maintain hemodynamic stability. Similarly, if there was a need for erythrocyte transfusion during surgery, patients in either group could receive packed erythrocytes (or autologous predonated blood, if available). Treatment of these hypotensive episodes was handled according to the anesthetic, surgical, and medical practices in effect at each site for determining whether patients needed any additional interventions with intravenous fluids, vasopressor agents, or blood products.

Blood pressures were measured using the hospital's calibrated automatic blood pressure monitoring equipment. Baseline blood pressure was measured in the supine position (in the same arm to be used during surgery for blood pressure monitoring) before starting infusion of the 500-ml crystalloid for hydration and before insertion of the spinal needle. Intraoperatively (from induction of anesthesia until skin closure), blood pressure was measured automatically every 5 min and then every 15 min for the first 2 h of the postoperative period, immediately after skin closure. During the remainder of the postoperative period (2.5–6 h after skin closure), blood pressure was measured every 30 min using the same (or similar) automated blood pressure equipment.

††† Voluven (6% HydroxyEthylStarch 130/0.4) Product Monograph. Fresenius Kabi Deutschland GmbH, Bad Homburg. Available at: <http://www.fresenius-kabi.ca/pdfs/Voluven%20Product%20Monograph%20Eng%20Oct07.pdf>. Accessed November 19, 2010.



### Efficacy Variables

The primary efficacy end point was the proportion of patients who develop at least one hypotensive episode (prospectively defined as an SBP lower than 90 mm Hg or lower than 75% of the baseline concentration, whichever was the greater value) during anesthesia/surgery and the first 6 h after skin closure. Successful prevention of hypotension was defined as the absence of any hypotensive episode during this period.

Secondary variables to further compare hemodynamic stability and volume status included the following: (1) time to the first hypotensive episode (SBP lower than 90 mm Hg or lower than 75% of baseline) after administration of the second dose, (2) time to the administration of a second dose (measured from induction of anesthesia until the dosing trigger was reached) and proportion of patients requiring only one dose and avoiding any hypotensive episode until 6 h after skin closure, (3) total duration and duration of the longest period of hypotension until 6 h after skin closure, (4) intervention with a vasopressor agent to treat a hypotensive episode until 6 h after skin closure, and (5) postoperative intervention with a diuretic for volume overload or inadequate urine output until postoperative day (POD) 3.

To evaluate clinical benefit, the incidence of serious complications (using a composite morbidity outcome that included acute heart failure, MI, ischemic stroke, or renal failure) until follow-up at POD 30 ( $\pm 5$  days) was compared, as was the incidence of operative and postoperative organ dysfunction relating to ischemia and/or tissue hypoxia (using a composite ischemia outcome that included evidence of cerebral ischemia, myocardial ischemia, or renal dysfunction) until the POD 30 follow-up. The protocol-defined components for each of the two composite end points are provided in appendix 2.

### Safety Evaluation

An AE was defined as any unwarranted reaction, symptom, or illness that occurred or worsened from randomization until safety follow-up at POD 30 ( $\pm 5$  days), whether related to infusion of investigational product or not. Criteria to define serious AEs (SAEs) were based on current International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines.<sup>§§§</sup> AEs were assessed by the blinded investigator based on spontaneous patient reports and observations by clinical staff and regular physical examination. AEs were rated as mild, moderate, or severe. All AEs and SAEs were recorded, and the relationship to investigational product was judged by the blinded principal investigator at each site to be not related, unlikely to be related, possibly related, probably related, or definitely related. Details of any death in the study

population during the entire study period were reported within 24 h. Patients were monitored *via* clinical evaluations (vital signs and physical examination), laboratory assessments performed locally by each hospital's certified laboratory (including clinical chemistry, hematology, coagulation, and urinalysis), 12-lead electrocardiographic recordings at selected points (baseline, 2 h after skin closure, and PODs 1, 2, and 14), and continuous Holter monitoring for the first 24 h starting at 1 h before spinal anesthesia.

An independent Data Safety Monitoring Board, composed of two anesthesiologists, a cardiologist, and a biostatistician, performed two scheduled interim safety reviews of blinded data after enrollment of approximately one third and two thirds of the patients; and a final review of the unblinded data after the database was locked and unblinded. A separate Clinical Events Committee, composed of a neurologist, a cardiologist, and a nephrologist, was contracted at the end of the study to independently adjudicate all relevant AEs and related laboratory data to determine which patients met the protocol-defined criteria (described in appendix 2) for inclusion in the two secondary composite outcome end points.

### Sample Size, Allocation, and Blinding

The sample size was determined based on previous phase 2 study data showing an 87% incidence of hypotensive episodes in the crystalloid-treated control group.<sup>11</sup> For the current study, an incidence of 70% was assumed because a colloid was used in the control group and a reduction from 70% to 50% was considered clinically relevant. At 95% power, using a two-sided significance level of 5% and assuming a dropout rate of up to 10%, 370 patients were required; these patients were randomized in a 1:1 ratio, with 185 in each treatment arm. Patients were randomized by site to minimize the potential effect of variation in protocols for standard of care between sites (*e.g.*, additional fluids and vasopressor administration). Patient enrollment per site was limited to a maximum of 40 treated patients to ensure balance across multiple sites and to prevent undue influence from a few high-enrolling sites.

The randomization list to assign study treatment allocation was prepared by the biostatistics group at the clinical research organization and generated using a block size of four. The list was provided to the unblinded pharmacist or designee at each site. The sponsor, the clinical research organization's data management group, and all investigators remained blinded until after the final database was locked. One designated nurse/investigator remained unblinded at each site to prepare and administer the investigational product, which was kept shrouded at all times to ensure blinding of patients and all other investigators and support personnel.

### Statistical Analyses

Statistical analyses were performed using computer software (SAS<sup>®</sup> version 8.2 or higher; SAS Institute Inc., Cary, NC). The *intent-to-treat population* was defined as

§§§ International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline E2A: Clinical safety data management: Definitions and standards for expedited reporting. October 27, 1994. Available at: <http://www.ich.org/LOB/media/MEDIA436.pdf>. Accessed October 28, 2010.

all patients randomized and was the population analyzed for safety. The *modified intent-to-treat (MITT)* population was defined as those who received any amount of investigational product and was the population analyzed for efficacy and safety.

For the primary efficacy analysis, the following variables were calculated: proportion of patients with at least one hypotensive episode in each group, group difference, 95% CIs, and odds ratio. A Cochran–Mantel–Haenszel test, stratified by center with a one-sided significance level of 2.5% (to allow “extreme cases” to one side of the treatment effect), was used to test the null hypothesis that the number of patients with at least one hypotensive episode in the MP4OX group was the number in the HES group or greater.

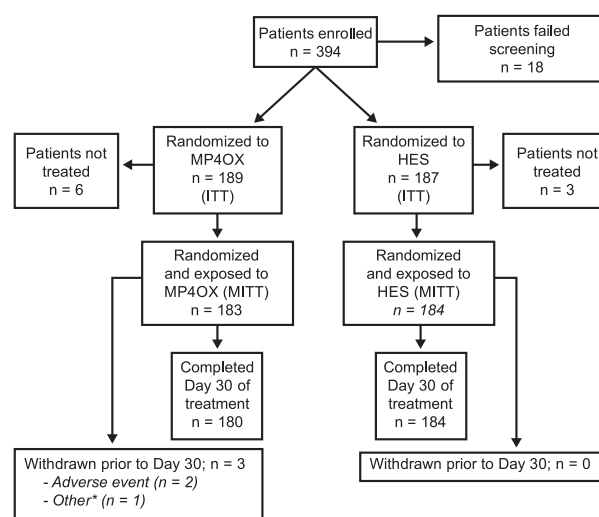
All other analyses used two-sided significance at the 5% level. Where applicable, the incidence, the difference in incidence between treatment groups (with 95% CIs), and odds ratios (with 95% CIs) were calculated. The Cochran–Mantel–Haenszel test (adjusting for centers) was used to test the null hypothesis that the number of events (*e.g.*, morbidity, ischemia, mortality, one dose, avoidance of hypotension, and incidence of intervention with a vasopressor or diuretic agent) in the MP4OX group was not different from the number of events in the control group; it was used to test the alternative hypothesis that the number of events in the MP4OX group was different from that in the control group.

The Kaplan–Meier method was used to compare time to first hypotensive episode and time to second dose; the difference for these measures was tested using the two-sided log-rank test, stratified by center. ANOVA, with treatment and center as variables, was used to test for a group difference in the duration of all hypotensive episodes and duration of longest period of hypotension. The Fisher exact test was used for comparing categorical variables and incidence rates for safety assessments, including laboratory parameters. The Wilcoxon rank–sum test was used for comparing continuous variables (*e.g.*, baseline characteristics, surgical and laboratory parameters, and vital signs), and this nonparametric method was used to compare between-group differences in clinical chemistry data that were not normally distributed (based on the results of the Shapiro–Wilk test). These data are presented as medians with 25<sup>th</sup>–75<sup>th</sup> interquartile ranges. Multiple comparisons for repeated measurements were used to prevent the risk of type I error (false-positive statistical inference), and *P* values were adjusted using the step-down Bonferroni method.

## Results

### Patient Disposition

Patient flow through the study is shown in figure 2. A total of 376 patients who underwent primary hip arthroplasty were randomized (intent-to-treat population), of whom 367 were exposed to treatment and, therefore, compose the MITT population. This study was executed with excellent protocol compliance and low patient dropout. Block randomization



\*Other: site lost contact with the patient before Day 14

**Fig. 2.** Patient consort diagram showing patient disposition for all patients screened and randomized. HES = hydroxyethyl starch solution 130/0.4; ITT = intent to treat; MITT = modified intent to treat; MP4OX = oxygenated pegylated hemoglobin.

by site and high enrollment at most sites helped to balance between-site differences in clinical practice. Of 18 sites, 13 treated approximately 95% of all patients (minimum of 14 patients each), with the top six sites each contributing from 32 to 40 patients (maximum limit allowed by the protocol); only five sites enrolled fewer than 10 patients each. Only eight patients deviated from the protocol, and because all efficacy analyses supported the findings in the MITT population, these were not presented separately and were included in the MITT analyses. Patient screening began on February 7, 2007; and the first patient received a dose on February 12, 2007. The last patient completed the study follow-up on June 11, 2008.

### Surgical Parameters

Baseline demographics for the MITT population and selected surgical parameters are shown in table 1. Patients ranged in age from 50 to 86 yr, 58% were female, and all were white. There were no differences in baseline characteristics between the MP4OX and HES groups or the patients who were not treated. For most treated patients (approximately 72%), the level of block achieved by the spinal anesthesia ranged from T12 to T8, whereas 16% had a level of T7 or T6 and only 4% reached a level of T5 to T3. The incidence of propofol administration was similar between groups (44.3% *vs.* 40.2%; *P* = 0.460), with total doses per patient averaging 198 mg in the MP4OX group *versus* 190 mg (*P* = 0.235) in the HES group.

As shown in table 1, both groups were well balanced on median values for duration of surgery (93 *vs.* 96 min; *P* = 0.954), estimated intraoperative blood loss (350 *vs.* 340 ml; *P* = 0.894), estimated total day 0 blood loss (700 *vs.*

**Table 1.** Patient Baseline Characteristics and Selected Surgical Parameters

Characteristics	MP4OX	HES	P Value*
MITT Population, No. of Patients	183	184	
Treated			
Age, Median, yr	67 (59–74) [50–86]	66 (60–73) [50–86]	0.707
Male/Female Ratio	81/102	73/111	0.398
Weight, kg	79 (67–92)	77 (68–88)	0.323
Surgery Duration, min	93 (76–121) [51–221]	96 (76–121) [41–206]	0.954
Patients Receiving Cemented Cups, %	49.7	47.3	0.677
Intraoperative Blood Loss until Skin Closure, mL	350 (200–600) [50–2,150]	340 (200–600) [100–1,800]	0.894
Surgical Blood Loss Total for Day 0, mL	700 (500–1,062) [0–2,640]	655 (450–1,100) [100–2,080]	0.482
Day 0 Total IV Fluids, mL	2,700 (1,900–3,600) [500–6,500]	3,000 (2,075–3,795) [750–7,700]	0.063
Cumulative IV Fluids through POD 3, mL	3,950 (3,080–5,300) [500–13,250]	3,838 (3,000–5,100) [750–10,100]	0.605

Data are given as the median (25th–75th interquartile range) [minimum–maximum] unless otherwise indicated.

\* Statistical significance tests: *P* values based on the Fisher exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables.

HES = hydroxyethyl starch solution 130/0.4; MITT = modified intent to treat; MP4OX = oxygenated pegylated hemoglobin; POD = postoperative day.

655 ml; *P* = 0.482), and total volume of intravenous fluids (crystalloid and colloid) administered (2,700 *vs.* 3,000 ml [*P* = 0.063 on day 0] and 3,950 *vs.* 3,838 ml [*P* = 0.605 through POD 3]) for MP4OX *versus* HES, respectively.

The most commonly reported preexisting condition (other than a diagnosis of osteoarthritis required in all patients for inclusion) from the patient's medical histories was hypertension (114 MP4OX-treated patients [62.3%] *vs.* 108 patients [58.7%] in the HES group; *P* = 0.522). There were no notable between-group differences in preoperative use of  $\beta$ -blockers, acetylcholine esterase inhibitors, angiotensin II blockers, or calcium channel blockers; the percentage of patients taking any one of these medications before surgery was low, ranging from 0.5 to 2.2%. The most commonly reported concomitant medications were enoxaparin sodium (low-molecular-weight heparin to prevent thrombosis: 144 [78.7%] *vs.* 149 [81.0%]; *P* = 0.605) and paracetamol (acetaminophen) for pain (141 [77.0%] *vs.* 146 [79.3%]; *P* = 0.615) for the MP4OX group *versus* the HES group, respectively. Additional use of other nonsteroidal antiinflammatory drugs, including ketoprofen and ibuprofen, was less common and was also similar between groups (24.6% *vs.* 28.8% in the MP4OX *vs.* HES group; *P* = 0.409).

There were no clinically relevant treatment group differences noted in the mean changes from baseline for any hematologic parameters (including hemoglobin concentration, hematocrit, erythrocyte count, leukocyte count, and platelet count), and there were no changes in coagulation (including international normalized ratio of prothrombin time, fibrinogen concentration, and fibrin split products). Mean ( $\pm$  SD) hemoglobin concentrations

started at  $137 \pm 13$  g/L at baseline in both groups, decreased to  $114 \pm 16$  g/L *versus*  $115 \pm 14$  g/L (*P* = 0.795) at 2 h postoperatively, and reached a low of  $101 \pm 13$  g/L *versus*  $103 \pm 13$  g/L (*P* = 0.148) at POD 3 (MP4OX *vs.* HES group). The incidence of transfusion of allogeneic erythrocytes on day 0 was similar (14.8% *vs.* 15.8%; *P* = 0.885) for patients in the MP4OX and HES groups. However, by hospital discharge, significantly more patients in the MP4OX *versus* the HES group received an allogeneic erythrocyte transfusion (37.7% *vs.* 27.2%; *P* = 0.034), although the average total number of erythrocyte units ( $2.4 \pm 1.4$  *vs.*  $2.5 \pm 1.2$  units for the MP4OX *vs.* the HES group; *P* = 0.442) was similar between groups. There were no differences in the incidence of autologous blood transfusion (16.9% *vs.* 18.5%; *P* = 0.785) and the number of autologous units given ( $1.4 \pm 1.1$  *vs.*  $1.1 \pm 0.8$  units; *P* = 0.345) for the MP4OX group *versus* HES controls, respectively.

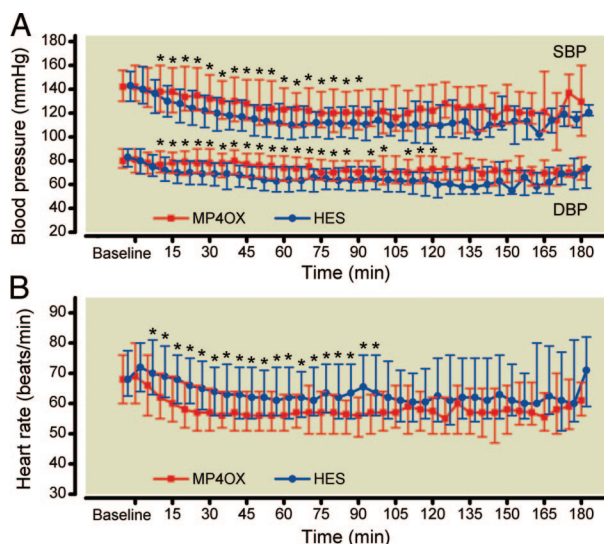
### Vital Signs

The results for heart rate and blood pressure (systolic and diastolic) monitoring during the intraoperative period are shown in figure 3. Administration of MP4OX maintained higher blood pressures and lower heart rates compared with the HES group, with differences being statistically significant for much of the intraoperative period.

### Primary Efficacy Analysis

The proportion of patients with at least one hypotensive episode during surgery or the postoperative period (to 6 h after skin closure) was significantly lower (*P* < 0.0001) in the MP4OX group (66.1%) *versus* the HES group (90.2%). The group difference (MP4OX-HES) was  $-0.24$  (95% CI,





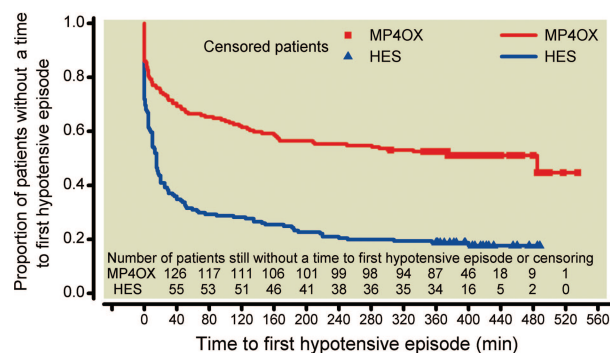
**Fig. 3.** Vital signs during the intraoperative period showing systolic and diastolic blood pressure (A) and heart rates (B). Data are shown as median with 25th–75th interquartile range. \*Significant between-group differences are based on adjusted  $P < 0.0013$ . DBP = diastolic blood pressure; HES = hydroxyethyl starch solution 130/0.4; MP4OX = oxygenated pegylated hemoglobin; SBP = systolic blood pressure.

–0.32 to –0.15), with an odds ratio of 0.19 (95% CI, 0.11–0.35).

### Secondary Efficacy Variables

**Time to the First Hypotensive Episode.** From the Kaplan–Meier curve shown in figure 4, the median time to the first hypotensive episode was significantly longer in patients in the MP4OX group (485 min [95% CI, 166; upper limit not presented because of the many patients censored at the upper quartile]) than in the HES group (15 min [95% CI, 10–20];  $P < 0.0001$ ).

**Time to the Administration of a Second Dose.** The median time to the administration of the second dose during surgery was significantly longer in the MP4OX group than in the HES group (75 min [95% CI, 65–95 min] *vs.* 40 min [95% CI, 34–45 min];  $P < 0.0001$ ). More patients were treated



**Fig. 4.** Time to the first hypotensive episode. HES = hydroxyethyl starch solution 130/0.4 [Voluven]; MP4OX = oxygenated pegylated hemoglobin.

successfully with a single dose and avoided hypotension during surgery in the 6-h postoperative period in the MP4OX group (51 patients [27.9%]) compared with the HES group (11 patients [6.0%]). The difference between groups was significant (0.22 [95% CI, 0.15–0.30];  $P < 0.0001$ ), with an odds ratio of 0.14 (95% CI, 0.07–0.29).

**Duration of Hypotensive Episodes.** The mean total duration of all hypotensive episodes was significantly shorter in the MP4OX group (64.9 min; SD, 73.64 min; range, 0–400 min) compared with the HES group (139.2 min; SD, 127.90 min; range, 0–475 min). ANOVA with treatment center and group as variables showed that the effect of treatment was highly significant ( $P < 0.0001$ ). The mean duration of the longest period of hypotension was significantly shorter in the MP4OX group (48.4 min; SD, 61.60 min; range, 0–400 min) compared with the HES group (98.8 min; SD, 107.36 min; range, 0–470 min). ANOVA showed that the effect of treatment was again significant ( $P < 0.0001$ ).

**Interventions Required.** Fewer patients required intervention with a vasopressor agent in the MP4OX group (40 patients [21.9%]) compared with the HES group (91 patients [49.5%]). The difference was significant (–0.28; 95% CI, –0.36 to –0.18;  $P < 0.0001$ ), with an odds ratio of 3.83 (95% CI, 2.36–6.23). The average per-patient dose of vasopressor therapy (predominantly ephedrine) was similar between groups (16 *vs.* 18 mg in the MP4OX *vs.* HES group;  $P = 0.066$ ). There was no significant difference in patients treated with a diuretic in the MP4OX group (34 patients [18.6%]) compared with the HES group (23 patients [12.5%]) ( $P = 0.097$ ).

**Morbidity and Ischemia Composite Outcomes.** There was no statistically significant difference between the MP4OX and HES groups for the composite morbidity or ischemia outcomes. However, there were few patients with AEs that qualified for inclusion (as shown in table 2, only 6 in the composite morbidity and 22 in the composite ischemia outcome groups).

### Safety Analysis

**Mortality.** There were two deaths in the MP4OX group. The first occurred in an 83-yr-old woman with a history of hypertension and chronic venous insufficiency, who received 500 ml MP4OX. She had postoperative gastrointestinal bleeding, hematemesis on POD 2, and paralytic ileus from POD 3 to POD 6; she also received 9 units of transfused erythrocytes. Aspiration with shortness of breath and cardiac arrest occurred on POD 7; the patient was resuscitated successfully. She continued to experience hemodynamic insufficiency and died on POD 8, with the primary cause of death listed as pulmonary edema. The investigator classified the event as unlikely to be related to the investigational product.

The second death occurred in a 70-yr-old woman with a history of cholelithiasis and cholecystectomy, who received 250 ml MP4OX. She developed lymphatic leg edema on POD 5, with circulatory insufficiency; and bacterial infec-

**Table 2.** Frequency of Adverse Events Composing the Secondary Composite End Points

End Points	MP4OX (n = 183)	HES (n = 184)	P Value
Composite Morbidity Outcome	5 (2.7)	1 (0.5)	0.124
Ischemic Stroke	0	0	NA
Acute Heart Failure	0	0	NA
Myocardial Infarction	4 (2.2)*	1 (0.5)	0.221
Renal Failure	1 (0.5)	0	0.305
Composite Ischemia Outcome	12 (6.6)	10 (5.4)	0.598
Cerebral Ischemia	0	0	NA
Myocardial Ischemia	0	0	NA
Renal Dysfunction	12 (6.6)	10 (5.4)	0.598

Data are given as number (percentage) of patients. Details for the clinical definitions that were provided in the protocol for each component of the two secondary composite end points are given in appendix 2.

\* Two MP4OX-treated patients were symptomatic and had myocardial infarctions reported on POD 3 and POD 14 as serious adverse events, and the other two patients were asymptomatic but exhibited significant troponin elevations from postoperative day 1 to 3 and were, therefore, included in the composite as myocardial infarctions by the Clinical Events Committee.

HES = hydroxyethyl starch solution 130/0.4; MP4OX = oxygenated pegylated hemoglobin; NA = not applicable.

tion was suspected on POD 9. Despite attempts at antibiotic therapy, the patient died on POD 11 from circulatory insufficiency and cardiac arrest. The primary cause of death was bacterial infection. Blood culture results later determined that the organism cultured was not susceptible to the antibiotics administered. The investigator (T.M.) classified the event as unrelated to the investigational product.

**SAEs and AEs Classified as Severe.** There were 29 SAEs reported in 28 patients (17 patients [9.3%] in the MP4OX group *vs.* 11 patients [6.0%] in the HES group;  $P = 0.245$ ). There were no significant between-group differences for the more frequently reported medical SAEs, including wound secretion and wound infection (3 patients [1.6%] each in MP4OX group only), MI (2 patients [1.1%] in MP4OX group *vs.* 1 patient [0.5%] in the HES group), and cardiac arrest (2 patients [1.1%] in the HES group only). Other AEs that were reported as severe were reported more frequently in the MP4OX than in the HES group (16 patients [8.7%] *vs.* 5 patients [2.7%];  $P = 0.014$ ). All severe AEs were reported by no more than one patient in either group, except for wound secretion (2 patients [1.1%] in the MP4OX group).

**Overall AEs.** In the intent-to-treat population, 134 patients (73.2%) in the MP4OX group and 118 patients (64.1%) in the HES group ( $P = 0.072$ ) experienced at least one AE (including AEs that occurred before infusion of the investigational product). The number of patients reporting at least one AE in the MITT population was significantly higher in the MP4OX than in the HES group: 133 patients (72.7%) *versus* 113 patients (61.4%) ( $P = 0.026$ ). Most AEs were mild or moderate in severity. The overall incidence of AEs

**Table 3.** Patients with Frequently Occurring ( $\geq 3\%$ ) Adverse Events

Adverse Events	MP4OX (n = 183)	HES (n = 184)	P Value
Nausea	38 (20.8)	22 (12.0)	0.024
Anemia*	33 (18.0)	25 (13.6)	0.256
Vomiting	24 (13.1)	21 (11.4)	0.637
Hypotension	17 (9.3)	23 (12.5)	0.403
Bradycardia	17 (9.3)	12 (6.5)	0.341
Peripheral Edema	17 (9.3)	15 (8.2)	0.716
Procedural Pain	12 (6.6)	14 (7.6)	0.839
Fever†	15 (8.2)	10 (5.4)	0.309
Wound Secretion	12 (6.6)	5 (2.7)	0.088
Hypertension	8 (4.4)	1 (0.5)	0.020
Constipation	7 (3.8)	7 (3.8)	1.000
Abdominal Pain	7 (3.8)	2 (1.1)	0.105
Hematoma	6 (3.3)	6 (3.3)	1.000
Headache	6 (3.3)	1 (0.5)	0.067
Procedural Nausea	3 (1.6)	7 (3.8)	0.337

Data are given as number (percentage) of patients (MITT population).

\* Anemia also includes postoperative anemia and hemoglobin decreased. † Fever also includes pyrexia and body temperature increased.

HES = hydroxyethyl starch solution 130/0.4; MITT = modified intent to treat; MP4OX = oxygenated pegylated hemoglobin.

that was reported as possibly or probably related to the investigational product was low in both groups (16 patients [8.7%] in MP4OX group *vs.* 7 patients [3.8%] in the HES group;  $P = 0.055$ ). Significantly more MP4OX-treated patients reported AEs of nausea (20.8% *vs.* 12.0% in HES controls;  $P = 0.024$ ) and hypertension (4.4% *vs.* 0.5% in HES controls;  $P = 0.020$ ). Table 3 lists the frequently reported AEs (occurring in  $\geq 3\%$  of patients in either group).

**Cardiac AEs and Troponin Concentrations.** The heart is one of the organs of concern for potential hemoglobin-based toxicity based on the “class effects” reported for many earlier-generation hemoglobin-based oxygen carrier (HBOC) solutions.<sup>15</sup> A listing was compiled to include all cardiac-related AEs reported (table 4 shows the preferred terms for these events), regardless of severity or cause. For all cardiac AEs, there was no difference between groups (13.7% *vs.* 10.9%;  $P = 0.431$ ). The incidence of mild or moderate events was mostly because of bradycardia and was similar between the MP4OX and HES groups (9.3% *vs.* 6.5%;  $P = 0.341$ ). Similarly, the remaining more important cardiac AEs were also reported at a similar frequency (1.6% in the MP4OX group *vs.* 2.2% in the HES controls;  $P = 1.000$ ).

Troponin concentrations over time are shown in figure 5. The troponin concentrations for each patient are expressed as a multiple of the upper limit of normal (ULN) for that site’s reference range because there is considerable variability in sensitivity between different assays (which can differ by 10-fold). There was a higher incidence of transient postoperative elevations (more than  $2 \times$  ULN) in cardiac troponin (I or T) concentrations in the MP4OX-treated patients than in the



**Table 4.** Cardiac-related Adverse Events and Laboratory Findings

Variables	Treatment Groups (MITT Population)		P Value
	MP4OX (n = 183)	HES (n = 184)	
Total Patients with Any Cardiac-related AE	25 (13.7)	20 (10.9)	0.431
Patients with $\geq 1$ SAE	3 (1.6) (MI or acute coronary syndrome)	4 (2.2) (atrial fibrillation, cardiac arrest, or MI)	1.000
Patients with $\geq 1$ "Severe" AE	1 (0.5) (angina pectoris)	0	0.499
Patients with $\geq 1$ "Other" AE			
Mild	17 (9.3) (bradycardia)	12 (6.5) (bradycardia)	0.341
Moderate	3 (1.6) (angina pectoris, arrhythmia, or tachycardia)	4 (2.2) (angina pectoris or tachycardia)	1.000
Patients with $\geq 1$ Laboratory Abnormality Reported as an AE or Signs of Myocardial Ischemia from the Electrocardiogram	3 (1.6) (troponin increased, troponin T increased, or electrocardiographic signs of myocardial ischemia)	0	0.123

Data are given as number (percentage) of patients. Reported AEs are given by preferred terms.

AE = adverse event; HES = hydroxyethyl starch solution 130/0.4; MI = myocardial infarction; MITT = modified intent to treat; MP4OX = oxygenated pegylated hemoglobin; SAE = serious adverse event.

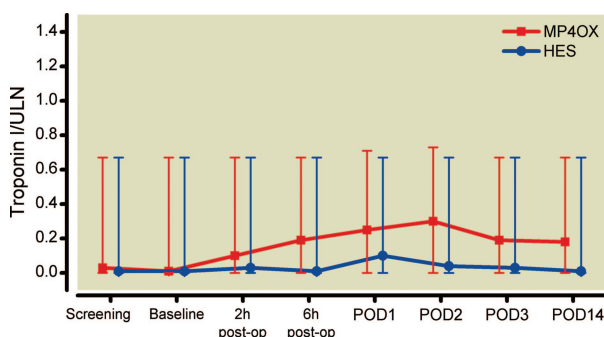
HES controls (6.6% *vs.* 1.1%;  $P = 0.006$ ). Because of the high sensitivity of some troponin I kits, and variability between assays and normal ranges, many clinicians regard an elevation to greater than 0.1  $\mu\text{g/L}$  (approximately  $5\times$  ULN range for many of the most sensitive troponin I assays) as being a better diagnostic indicator of an MI. By using a cutoff of more than  $5\times$  ULN, the incidence of any troponin elevation (I or T) was 3.8% in the MP4OX group *versus* 0% in the HES controls ( $P = 0.007$ ).

**Electrocardiographic Findings.** Blinded review of the 12-lead electrocardiographic recordings (QECG Services, Quintiles, Mumbai, India) found only sinus tachycardia as the treatment-emergent event that was both "abnormal" and "clinically significant." The incidence of sinus tachycardia after dosing, that was not present at baseline, was not significantly different (4.4% *vs.* 3.8% in the MP4OX *vs.* HES group;  $P = 0.799$ ); and there was no other evidence of myocardial ischemia (*i.e.*, no ST-segment changes).

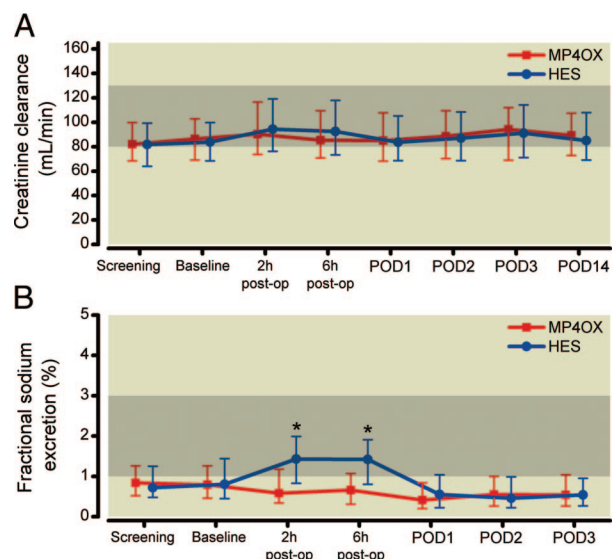
The 24-h continuous Holter recordings were also reviewed externally by blinded cardiologists at QECG Services (Quintiles). The most common finding was a higher incidence of sinus bradycardia (defined as  $\geq 4$  beats at  $\leq 45$  beats/min from the Holter data) observed for MP4OX-treated patients (49.2% *vs.* 26.1% in the MP4OX *vs.* HES group;  $P < 0.0001$ ). Conversely, supraventricular tachycardia tended to occur more frequently in the HES group (14.1% *vs.* 8.2%;  $P = 0.097$ ). Ventricular tachycardia ( $\geq 5$  beats at  $\geq 100$  beats/min) tended to be more frequent in the MP4OX patients (4.4% *vs.* 1.6%;  $P = 0.139$ ).

**Renal, Hepatic, and Pancreatic Findings.** For renal function assessments, there were no differences observed between groups in total serum creatinine concentrations, incidence of serum creatinine elevations of 0.5 mg/dl or greater or  $2\times$  baseline concentrations or greater, and calculated creatinine clearance (fig. 6A). For fractional excretion of sodium in urine (fig. 6B), there was a significant difference between groups observed at the 2- and 6-h points, with higher sodium excretion seen in the HES group, albeit still within the normal range. Urine output on day 0 was lower in the MP4OX than the HES group (median, 1,000 *vs.* 1,548 ml;  $P < 0.0001$ ), consistent with a trend for greater IV fluid volumes being administered on day 0 in the HES than the MP4OX group (median, 3,000 *vs.* 2,700 ml;  $P = 0.063$ ; table 1). Postoperatively, there were no differences in urine output between groups on POD 1 (median, 1,800 *vs.* 1,725 ml;  $P = 0.919$ ) and on POD 2 (median, 1,748 *vs.* 1,675 ml;  $P = 0.463$ ), resulting in similar total urine output through POD 3 (median, 6,200 *vs.* 6,805 ml;  $P = 0.118$ ) for the MP4OX *versus* the HES group.

According to the RIFLE (Risk, Injury, Failure, Loss, End-stage renal disease) criteria used to assess the risk of kidney injury,<sup>16</sup> a serum creatinine increase of 0.3 mg/dl or greater or  $1.5\times$  or greater baseline concentrations may be a more



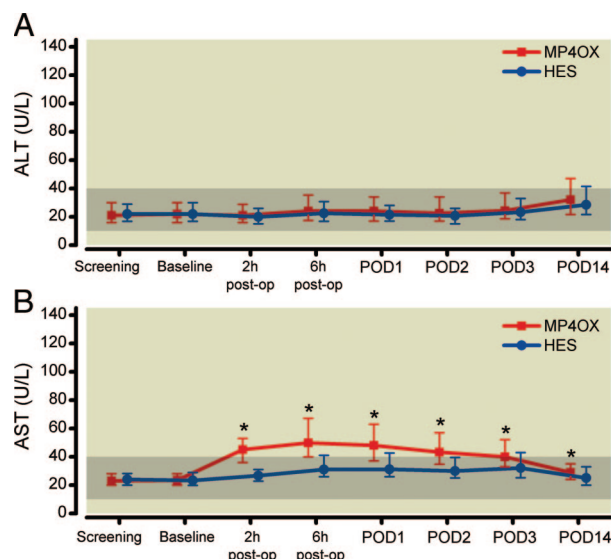
**Fig. 5.** Serum troponin I concentrations, normalized by expressing each value as a multiple of the upper limit of normal (ULN). Data are shown as median with 25th–75th interquartile range. HES = hydroxyethyl starch solution 130/0.4; MP4OX = oxygenated pegylated hemoglobin; POD = postoperative day.



**Fig. 6.** Calculated creatinine clearance (A) and fractional excretion of sodium in urine (B). The shaded area represents the approximate normal range. Data are shown as median with 25th–75th interquartile range. \*Significant between-group differences are based on adjusted  $P < 0.0071$ . HES = hydroxyethyl starch solution 130/0.4; MP4OX = oxygenated pegylated hemoglobin; POD = postoperative day.

appropriate and sensitive indicator of risk than an increase of 0.5 mg/dl or greater or 2× or greater baseline concentrations, which may indicate potential kidney injury. When using the more sensitive cutoff for a *post hoc* analysis, there was evidence of an imbalance in the MP4OX-treated patients, seen on POD 1 in the 500-ml dose group (12.7% vs. 3.7% for the MP4OX vs. HES group;  $P = 0.008$ ). Overall, for any postbaseline time points (table 5), a significant imbalance was only found in patients who received 500 ml investigational product (20.2% in the MP4OX group vs. 11.0% in the HES group;  $P = 0.039$ ).

MP4OX administration was also associated with transient elevations, typically peaking at 6 h or on POD 1, in



**Fig. 7.** Serum concentrations of alanine aminotransferase (A) and aspartate aminotransferase (B). The shaded area represents the approximate normal range. Data are shown as median, with 25th–75th interquartile range. \*Significant between-group differences are based on adjusted  $P < 0.0063$ . ALT = alanine aminotransferase; AST = aspartate aminotransferase; HES = hydroxyethyl starch solution 130/0.4; MP4OX = oxygenated pegylated hemoglobin; POD = postoperative day.

selected liver enzyme (*i.e.*, aspartate aminotransferase and alanine aminotransferase) and pancreatic enzyme (typically lipase and occasionally amylase) concentrations. As shown in figure 7A, there were no between-group differences in median alanine aminotransferase concentrations; however, median aspartate aminotransferase concentrations (fig. 7B) were significantly higher at all postoperative points in MP4OX-treated patients, despite having recovered to the normal range by POD 3. Amylase concentrations were significantly higher at 2 and 6 h and on POD 1 in the HES group (fig. 8A), whereas lipase concentrations were significantly higher

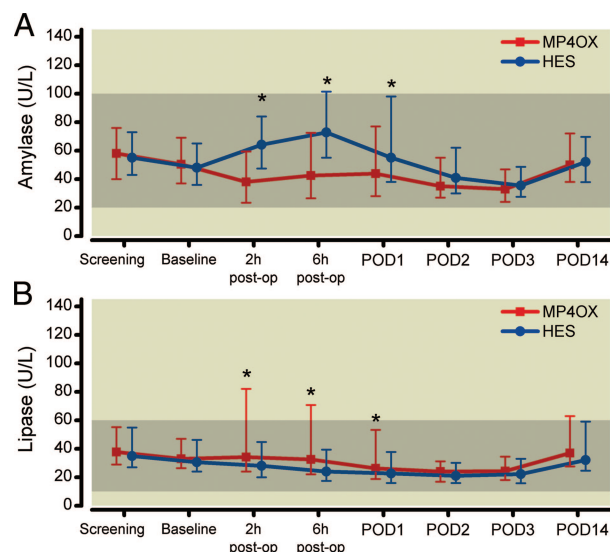
**Table 5.** Serum Creatinine Elevations ( $\geq 0.3$  mg/dl or  $\geq 1.5 \times$  Baseline Levels)

Time Point	MP4OX Group			HES Group			Total (N = 367)
	MP4OX, 250 ml (n = 69)	MP4OX, 500 ml (n = 114)	Overall MP4OX (n = 183)	HES, 250 ml (n = 20)	HES, 500 ml (n = 164)	Overall HES (n = 184)	
2 h Postoperatively	0	2 (1.8)	2 (1.1)	0	0	0	2 (0.6)
6 h Postoperatively	0	3 (2.7)	3 (1.7)	0	3 (1.9)	3 (1.6)	6 (1.7)
POD 1	3 (4.3)	14 (12.7)*	17 (9.5)	0	6 (3.7)	6 (3.3)	23 (6.4)
POD 2	4 (5.8)	8 (7.1)	12 (6.6)	1 (5.0)	8 (4.9)	9 (4.9)	21 (5.8)
POD 3	2 (2.9)	7 (6.3)	9 (5.0)	1 (5.3)	5 (3.1)	6 (3.4)	15 (4.2)
POD 14	2 (3.1)	6 (5.4)	8 (4.5)	1 (5.0)	7 (4.4)	8 (4.4)	16 (4.5)
At Any Post-BL Point	6 (8.7)	23 (20.2)*	29 (15.8)	2 (10.0)	18 (11.0)	20 (10.9)	49 (13.4)

Data are given as number (percentage) of patients. Percentages are based on the number of patients in the modified intent-to-treat population in each treatment group who also had laboratory test results at each visit. The table is based on a *post hoc* analysis using the RIFLE (Risk, Injury, Failure, Loss, End-stage kidney disease) criteria for assessing the risk of kidney injury.<sup>16</sup>

\* Significantly different from HES, 500-mL controls ( $P = 0.008$  at POD 1;  $P = 0.039$  at any post-BL point).

BL = baseline; HES = hydroxyethyl starch solution 130/0.4; MP4OX = oxygenated pegylated hemoglobin; POD = postoperative day.



**Fig. 8.** Serum concentrations of amylase (A) and lipase (B). The shaded area represents the approximate normal range. Data are shown as median, with 25th–75th interquartile range. \*Significant between-group differences are based on adjusted  $P < 0.0063$ . HES = hydroxyethyl starch solution 130/0.4; MP4OX = oxygenated pegylated hemoglobin; POD = postoperative day.

in the MP4OX group at the same points (fig. 8B). There were more MP4OX-treated patients (20 [11%]) with lipase elevations more than  $3 \times$  ULN compared with HES controls (4 [2.2%]) ( $P < 0.001$ ), but only one of the MP4OX-treated patients had an AE of pancreatitis reported; however, this AE was not treatment emergent because it began the day before baseline measurements and dosing occurred. Three MP4OX-treated patients had transient lipase concentrations more than  $10 \times$  ULN at either 2 or 6 h after dosing, and two of these patients also had other gastrointestinal-related AEs reported, including nausea and vomiting.

## Discussion

Our study demonstrated that MP4OX was superior to HES 130/0.4 in reducing the proportion of patients experiencing at least one hypotensive episode throughout anesthesia/surgery and the 6-h postoperative period. Additional efficacy measures showed that MP4OX was associated with a longer median time to the first hypotensive episode, and more MP4OX-treated patients were treated successfully with a single dose and had a shorter total duration of hypotensive episodes. Therefore, MP4OX was effective in preventing perioperative hypotension, and fewer patients required intervention with a vasopressor agent in the MP4OX group. There were no significant differences in clinical outcomes based on the composite morbidity and ischemia end points; however, the few patients with these outcomes rendered our study insufficiently powered to demonstrate any clinical benefit using these secondary end points or to establish any correlation between administration of MP4OX and the SAEs that were reported.

A general class of side effects that have frequently been described for many earlier-generation HBOCs includes hypertension; bradycardia; MI; increased troponin, pancreatic and liver enzyme (*i.e.*, lipase, amylase, aspartate aminotransferase, and alanine aminotransferase) concentrations; jaundice; renal dysfunction; platelet dysfunction; dysphagia; and nausea.<sup>17</sup> Several of these AEs were also observed in our study with MP4OX, but they appear to occur at a lower frequency and severity than with other HBOCs. This may be a consequence of the lower doses administered or the beneficial effects from pegylation, which are discussed later. Any direct comparison of the incidence of these AEs in previous studies with other HBOCs *versus* our study is difficult because various HBOCs have different properties and the patient populations vary considerably across studies (which have included trauma and cardiac, vascular, and orthopedic surgery).

In agreement with preclinical animal data, MP4OX does not appear to cause acute systemic hypertension; it appears to differ from earlier-generation HBOCs in this important respect. Earlier-generation HBOCs have been poorly tolerated because of induction of vasoconstriction and hypertension, which limits capillary perfusion and oxygen delivery<sup>18</sup> and possibly contributes to the increased incidence of MI and mortality described by Natanson *et al.*<sup>15</sup> This phenomenon has been attributed to nitric oxide scavenging by cell-free hemoglobin<sup>19</sup> and led to the premature dismissal of the therapeutic potential of the whole HBOC class by some investigators. For example, Natanson *et al.* performed a meta-analysis of HBOC safety data in which 16 studies were included, from which they concluded that HBOCs as a class carry a significantly increased risk of mortality and MI. However, this meta-analysis included incomplete data from only one small phase 2 study with MP4OX.<sup>20</sup> The current cardiac findings in our large phase 3 study suggest that, although MI events have been reported, the incidence rate appears to be low compared with the results compiled by Natanson *et al.* for other HBOC solutions.

Patients receiving MP4OX in our study exhibited lower heart rates, which appears to be consistent with an improvement in venous return and, in some cases, reflex vagally mediated bradycardia in response to short-term expansion of plasma volume and higher blood pressure based on a *post hoc* blinded evaluation of all Holter electrocardiographic tracings that contained clinically significant arrhythmias (mostly bradyarrhythmias) by an independent expert in electrocardiography and electrophysiology (Eric N. Prystowsky, M.D., F.A.C.C., Director, Clinical Electrophysiology, St. Vincent's Hospital and Health Care Center, Indianapolis, IN; oral communication; July 2010). The more frequent AE reports of hypertension (4.4% in MP4OX-treated patients *vs.* 0.5% in HES controls;  $P = 0.020$ ) may be partially associated with the volume effect and higher blood pressure levels after MP4OX administration, although most of the hypertension AEs occurred in the postoperative period. Although speculative, this may also explain the higher incidence of



erythrocyte transfusion in MP4OX-treated patients because higher blood pressures throughout the intraoperative and immediate postoperative period may have contributed to greater oozing of blood from cut bone surfaces.

The proportion of patients with AEs after receiving investigational product was higher in the MP4OX than the HES group (72.7% *vs.* 61.4%;  $P = 0.026$ ), but most AEs were mild or moderate and were deemed to be unlikely or not related to treatment. One severe AE of hypertension was considered to be possibly related to MP4OX administration. SAEs were reported in 9.3% of patients receiving MP4OX *versus* 6.0% of HES controls ( $P = 0.245$ ). There were no group differences for specific SAEs, and most medical SAEs occurred in single patients. The most common SAEs included joint dislocation ( $n = 3$  in the MP4OX group and  $n = 2$  in the HES controls), wound secretion and wound infection ( $n = 3$  each in the 500-ml MP4OX group and  $n = 0$  in HES controls), MI ( $n = 2$  in the 250-ml MP4OX group and  $n = 1$  in the 500-ml HES controls), and cardiac arrest ( $n = 0$  in the MP4OX group and  $n = 2$  in the 500-ml HES controls).

A few of the clinical chemistry parameters evaluated exhibited abnormal elevations that were observed in both groups. Compared with HES controls, MP4OX administration was more frequently associated with transient elevations in aspartate aminotransferase, alanine aminotransferase, lipase, and troponin concentrations, as reported in previous phase 2 studies.<sup>11,12</sup> Such elevations may be associated with mild transient organ dysfunction, although most patients with these abnormal laboratory findings remained asymptomatic and had no related clinical symptoms. A potential explanation for the early spike in lipase concentrations may be a transient constriction of the common bile duct, possibly related to a localized effect of nitric oxide binding by MP4OX. This short-lived spike in lipase concentrations is not typical of pancreatic injury because most patients had no increases in amylase and the duration of the lipase elevations was shorter than what is typically observed for inflammatory pancreatitis.<sup>21</sup> Reports from other clinical<sup>22</sup> and preclinical<sup>23</sup> studies have suggested that a transient constriction of the common bile duct or the sphincter of Oddi, due to scavenging of nitric oxide by HBOCs, might be causing the observed spike in lipase concentrations.

The exact mechanism behind the transient increase in liver transaminase and lipase concentrations and the cardiac troponin elevations has not been defined. A localized effect of MP4OX on nitric oxide concentrations in smooth muscle in some tissues and/or possible oxidative stress from heme iron oxidation and degradation cannot be excluded as a potential contributing factor. Additional nonclinical studies will be needed to better understand these transient laboratory findings. In addition, some degree of laboratory interference of colorimetric analytical assays has affected certain clinical chemistry results when HBOCs are present. Significant interference typically occurs only at higher plasma hemoglobin concentrations than those achieved with

the 250- or 500-ml dose of MP4OX in our study.<sup>24,25</sup> These doses result in low concentrations of MP4OX in plasma that are within the range of what is typically handled by the hemolysis index correction that many analyzers apply to plasma/serum samples that contain visible hemoglobin because of erythrocyte hemolysis.

Overall, renal function did not appear to be affected by MP4OX, based on creatinine clearance and fractional sodium excretion, both of which remained essentially unchanged from baseline and within the normal range (fig. 6). However, a more sensitive *post hoc* assessment of serum creatinine concentration detected a difference between groups; 20.2% of patients receiving two doses (*i.e.*, 500 ml) of MP4OX exhibited a 0.3 mg/dl or greater increase over baseline at any postdosing point, *versus* 11.0% of patients receiving 500 ml HES ( $P = 0.039$ ). Because MP4OX is a hyperoncotic colloid, the possibility exists that higher doses may have a transient impact on the kidney to affect filtration gradients and thereby decrease urine output, which would be consistent with the renal effects that have been described for other hyperoncotic colloid-based plasma volume expanders, such as HES 200/0.5 solution, 10%.<sup>26,27</sup> A *post hoc* analysis of the transient troponin and serum creatinine elevations in our study revealed that there was a higher incidence in patients older than 65 yr in the MP4OX group. There was no age effect observed in the HES controls for either serum creatinine or troponin (more than  $2 \times$  ULN) elevations.

Our study design had several limitations. (1) The patients who underwent primary hip arthroplasty and were selected for this study were not at high risk of oxygen debt; therefore, the study was underpowered to evaluate any oxygen therapeutic clinical benefit of MP4OX. (2) Invasive monitoring was not feasible in these patients for collection of arterial (or central venous) blood gases, lactate concentrations, or cardiac output; thus, base deficit, oxygen dynamics (*i.e.*, central venous oxygen saturation and oxygen delivery or consumption), or flow-related variables (*i.e.*, cardiac output and systemic vascular resistance) cannot be determined. (3) Baseline central venous pressures were not recorded in this study, making it impossible to document whether all patients were euvolemic at the start of the surgical procedure. (4) The commercially available plasma expander, Voluven® (HES 130/0.4), was the best choice as a colloid comparator in the control group (despite having a lower oncotic pressure and a shorter circulating half-life) because it is commonly used for volume replacement in surgery and because no other oxygen therapeutic agent is indicated for use in Europe.

The chemical modification of MP4OX by pegylation imparts multiple beneficial effects, including the following: (1) reduced toxicity normally associated with high concentrations of free hemoglobin in plasma; (2) higher oxygen affinity (P50, approximately 5 mm Hg), which targets oxygen delivery to hypoxic/ischemic tissues; (3) a higher colloid osmotic pressure (approximately 70 mm Hg) to enhance plasma expansion and improve capillary blood flow and tis-

sue perfusion; and (4) enhanced nitrite reductase reactivity (due to specific pegylation of the  $\beta$ -93 cysteine residues<sup>28</sup>) that potentially gives MP4OX the ability to regenerate bioavailable nitric oxide and thereby attenuate the vasoconstriction caused by nitric oxide scavenging that was commonly observed with earlier-generation HBOCs.

The superior efficacy of MP4OX for hemodynamic stability may be because MP4OX is a more potent colloid (*i.e.*, it has a higher oncotic pressure) compared with HES and has a longer intravascular half-life in circulation (the half-life for MP4OX was approximately 20–24 h in patients undergoing orthopedic surgery, depending on total dose and degree of surgical blood loss).<sup>11,12</sup> In contrast, HES 130/0.4 has a rapid initial elimination, with a half-life of approximately 1.5 h and a terminal elimination half-life of approximately 12 h. Although our study did not include any direct measurement of oxygen off loading from MP4OX, these results may still suggest a potential oxygen therapeutic effect of MP4OX in addition to its hyperoncotic volume expansion properties. This is also supported by preclinical studies in which MP4OX has reduced cardiovascular dysfunction compared with an oncologically matched colloid, HES 260/0.45, 10% (Pentastan®; B. Braun, Irvine, CA), used in the control group.<sup>7,8</sup>

Consistent with the results in our study, previous clinical and preclinical studies<sup>10,29,30</sup> of MP4OX have demonstrated a safety profile, without any significant hypertension or evidence of systemic vasoconstriction, which may support the potential utility of MP4OX for use in unstable clinical populations at higher risk of ischemia. Although animal studies<sup>10</sup> have shown the absence of vasoconstriction in the hamster, the hemodynamic data collected in our study cannot definitively distinguish between the possibility of a vasoconstrictive effect in some vascular beds due to MP4OX binding of nitric oxide and a hyperoncotic colloidal volume expansion benefit of MP4OX treatment.

In clinical settings with greater morbidity and/or mortality than the current orthopedic surgery population, the benefit–risk profile of MP4OX would likely be enhanced. For example, in hypovolemic shock with lactic acidosis, MP4OX may improve perfusion and oxygenation of ischemic organs. A recently completed exploratory phase 2a study<sup>31</sup> in 51 trauma patients demonstrated an immediate and sustained decrease of lactic acid in MP4OX-treated patients *versus* controls, with a trend toward more MP4OX-treated patients achieving normal lactate concentrations by 4 h after treatment and more patients being discharged from the hospital by day 28.

In summary, our study demonstrated that administration of MP4OX was able to prevent acute hypotensive episodes in patients undergoing hip arthroplasty with spinal anesthesia, but our study was not sufficiently powered to demonstrate any clinical benefit in morbidity that might result from localized tissue hypoxia due to ischemia or anemia. Nevertheless, this study has provided a large safety data set in elderly

surgical patients to establish the expected AE profile for MP4OX, which was needed to support new clinical studies to explore the potential utility of MP4OX as an oxygen therapeutic agent in other clinical settings. MP4OX is not being proposed for use in routine surgery in low-morbidity populations without an oxygen deficit. The AE profile for MP4OX doses up to 500 ml documented in our study demonstrates a satisfactory safety profile for severe trauma populations and warrants further clinical evaluation of MP4OX to reverse the oxygen deficit after severe hemorrhagic shock when administered in addition to standard of care, including transfusion of blood products.

## Appendix 1: Study 6084 Clinical Investigators (Number of Patients Randomized by Site)

Belgium: Mona Momeni, M.D. (Principal Investigator, national coordinating center); Barbara Brui, M.D., Valerie Collet (Department of Anesthesiology, Cliniques Universitaires Saint-Luc, Brussels; n = 4); Sven Van Poucke, M.D. (Principal Investigator), René J. Heylen, M.D., J.D., Ria Hens, R.N. (Department of Anesthesiology, Z.O.L. Campus Sint-Jan, Genk; n = 24); Piet J. Libbrecht, M.D. (Principal Investigator), Bruno De Turck, M.D., Lieve D'haenen, R.N., Jean-Claude De Mey, M.D., Ludwig Deruyck, M.D. (Department of Orthopedic Surgery, AZ St Lucas Hospital, Ghent; n = 18); Marcel Vercauteren, M.D., Ph.D. (Principal Investigator), Ellen Hendrickx, M.D., Luc Sermeus, M.D., Vera Saldien, M.D., Carine Smits, R.N., (Department of Anesthesiology, University Ziekenhuis Antwerp, Edegem; n = 5).

Czech Republic: Ivo Kofránek, M.D. (Principal Investigator, national coordinating center), Petr Hajný, M.D., Alan Konopásek, M.D., Radouan Kubes, M.D., Martin Kafunek, M.D., Lucie Kompeletova, M.D., Ivana Stastna, R.N., (Department of Orthopedic Surgery, Fakultni nemocnice Na Bulovce, Praha; n = 39); David Jahoda, M.D. (Principal Investigator), Lubos Vcela, M.D., Jan Tomajda, M.D., David Pokomy, M.D., Rastislav Hromadka, M.D., Antonin Sosna, R.N., Alena Hykesova, R.N., (Department of Orthopedic Surgery, Fakultni nemocnice Motol I, Praha; n = 32); David Pellar, M.D. (Principal Investigator), David Doležal, M.D., Magdalena Winklerova, R.N., (Department of Orthopedic Surgery, Fakultni Nemocnice Hradec Kralove, Hradec Kralove; n = 14); Thomas Novak, M.D. (Principal Investigator), Petr Nekvinda, M.D., Petra Valova, M.D. (Department of Orthopedic Surgery, Oblastni Nemocnice Kladno, Kladno; n = 3).

The Netherlands: Ris Dirksen, M.D. (Principal Investigator, national coordinating center), Cornelius Gort, M.D., Ineke Bergshoeff, R.N., Florent Luijkx, R.N. (Department of Anesthesiology, Sint Maartenskliniek, Nijmegen; n = 39); Gert Jan Scheffer (Principal Investigator), Gert-Jan van Geffen, M.D., Judith Beurskens-Meijerink, R.N. (Department of Anesthesiology, Universitair Medisch Centrum St Radboud, Nijmegen; n = 7).

Poland: Jacek A. Majewski, M.D. (Principal Investigator, national coordinating center), Rafal Szczygiel, M.D., Jacek Hermanon, M.D., Jan Pajak, M.D. (Department of Anesthesiology, SP Wojewódzki Szpital Chirurgii Urazowej, Piekary Śląskie; n = 39);

Andrzej Z. Górecki, M.D. (Principal Investigator), Piotr Nowakowski, M.D., Paweł Skowronek, M.D., Radosław Gorski, M.D., Paweł Olszewski, M.D. (Department of Orthopedic Surgery, SK Dzieciątka Jezus, Warszawa; n = 40); Tomasz Mazurkiewicz, M.D. (Principal Investigator), Agnieszka Koman-Izko, M.D., Jan Blacha, M.D., Robert Wętkowski, R.N., Maciej Janosz Patyra, R.N., (Department of Orthopedics, SPSK nr 4, Klinika Ortopedii, Lublin; n = 33); Tadeusz Gaździk, M.D. (Principal Investigator), Marek Bozek, M.D., Tomasz Bielecki, M.D., Alberski Ewa Kuk, M.D. (Department of Orthopedic Surgery, Wojewódzki Szpital Specjalistyczny nr 5, Sosnowiec; n = 14).

Sweden: Christina I. Olofsson, M.D., Ph.D. (Principal Investigator, study 6084 and national coordinating center), Eva-Britt Nygårds, M.D., Sinnika Omaniemi Olsson, R.N. (Department of Anesthesiology, Karolinska University Hospital, Solna, Stockholm; n = 16); Sixten Bredbacka, M.D. (Principal Investigator), Caroline Hällsjö-Sander, M.D., Anders Ekelund, M.D., Ritva Thule, R.N. (Department of Anesthesiology, St Göran Hospital, Stockholm; n = 22); Anders Winterfeldt, M.D. (Principal Investigator), Eva Nordenskjöld, M.D., Carl Erik Högman, R.N. (Department of Anesthesiology, Skaraborg Hospitals, Skövde; n = 17).

United Kingdom: Alan Aitkenhead, M.D., Ph.D. (Principal Investigator, national coordinating center), Jon Hardman, M.D. (Department of Anesthesiology, University Hospital, Queen's Medical Centre, Nottingham; n = 1).

## Appendix 2: Criteria for the Morbidity and Ischemia Composite Outcome End Points

If a patient exhibited any one of the perioperative complications defined for the two composite end points, the patient was counted as one “event” and assigned to the appropriate composite end point according to the seriousness of the event. If a particular organ dysfunction that initially qualified for inclusion in the ischemia composite (table 6) worsened and later also met the more serious definitions for the morbidity outcome (table 7), this patient would be counted as one event in each of the two composite outcomes. Conversely, if a patient experienced an SAE that met the definition for the serious morbidity composite (e.g., myocardial infarction), this patient would only be counted once in the morbidity composite and would not also be included in the ischemia composite.

**Table 6.** Composite Ischemia End Point for Ischemic Organ Dysfunction: Definitions

Cerebral Ischemia	Occurrence of a transient ischemic attack that resolves within 24 h and a reversible ischemic neurologic deficit lasting >24 h, with resolution within 7 days of onset.
Myocardial Ischemia	Electrocardiographic signs of myocardial ischemia (e.g., new ST-segment depression $\geq 0.1$ mV or new ST-segment elevation $\geq 0.2$ mV).
Renal Dysfunction	Postoperative serum creatinine level $>177 \mu\text{mol/L}$ (approximately 2.0 mg/dL), with an increase over preoperative baseline level of $>62 \mu\text{mol/L}$ ; or a $>25\%$ decrease in $C_{Cr}^*$ with evidence of tubular damage by an increase in fractional excretion of sodium $>1\%$ . <sup>32</sup>

\* Estimated using the Cockcroft–Gault equation.  $C_{Cr}$  is creatinine clearance;  $S_{Cr}$  is serum creatinine in mg/dl; age is in years, and weight is in kg.

$$C_{Cr}(\text{mL min}^{-1}) = \frac{(140 - \text{age}) \times \text{weight}}{72 \times S_{Cr}} \times (0.85 \text{ if female})$$

**Table 7.** Composite Morbidity End Point for Serious Complications: Definitions

Ischemic Stroke	<i>Stroke</i> is defined as an acute new disturbance of focal neurologic function lasting >24 h or resulting in death. Ischemic stroke will be confirmed by a computed tomographic or magnetic resonance imaging scan within 3 weeks after onset that either is normal or shows an infarct in the expected area on the basis of the clinical findings or demonstrates evidence of cerebral infarction at autopsy. <sup>33</sup>
Acute Heart Failure	Acute heart failure is characterized by dysfunction of the myocardium that leads to reduced cardiac output, tissue hypoperfusion, pulmonary edema, and tissue congestion. Diagnosis should be made according to published guidelines of the European Society of Cardiology that define the various clinical stages of acute heart failure. <sup>34</sup>
Myocardial Infarction	Myocardial infarction will be diagnosed when there is a typical increase and gradual decrease of laboratory markers specific to myocardial necrosis (troponin I or T), preferably in combination with ischemic symptoms and/or typical electrocardiographic signs (e.g., development of new pathologic Q waves or new persistent ST-segment or T-wave changes). <sup>35</sup>
Renal Failure	<i>Acute renal failure</i> is defined by an increased serum creatinine level that is $3\times$ over baseline or $>75\%$ decrease in calculated $C_{Cr}^*$ or dysfunction requiring dialysis. <sup>36,37</sup>

\* Estimated using the Cockcroft–Gault equation, see table 6 footnote for details.



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## ANESTHESIOLOGY REFLECTIONS

### Ombrédanne Inhalers in the “Falklands War”



During the 74-day Falklands Conflict in 1982, Argentina and the United Kingdom battled for control of the Falkland Islands. Two photographs (*left*) depicting Argentine losses from the “Falklands War” reflect the British military’s burning of a Vietnam War helicopter and their sinking of a World War II-era light cruiser, the ARA *General Belgrano*. Remarkably, the ill-prepared junta that ordered the invasion supplied its Argentine Medical Corps with Ombrédanne Inhalers (*right*) for battlefield anesthetics. These ether inhalers were designed prior to World War I by Dr. Louis Ombrédanne (1871–1956), a Parisian pediatric and plastic surgeon. (Copyright © the American Society of Anesthesiologists, Inc. This image also appears in the *Anesthesiology Reflections* online collection available at [www.anesthesiology.org](http://www.anesthesiology.org).)

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