Calcium Chloride for prevention of blood loss from uterine atony during intrapartum cesarean delivery

Study Protocol and Statistical Analysis Plan

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Calcium Chloride for prevention of blood loss from uterine atony during intrapartum cesarean delivery

A placebo controlled, double blind randomized controlled trial and pharmacokinetic pharmacodynamic study

PI: Jessica Ansari, MD
Senior Faculty Advisors: Dr. [redacted], Dr. [redacted]
Data Safety Monitoring Committee: Dr. [redacted], Dr. [redacted]

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SPECIFIC AIMS:

We **propose to perform a randomized controlled trial (RCT) assessing calcium chloride ($CaCl_2$) as a novel intervention to prevent hemorrhage from uterine atony with nested PK/PD analysis.**

PPH is the leading cause of maternal morbidity and mortality worldwide[1,2]. Concerningly, the incidence of PPH has been rising[3]. Uterine atony, defined as failure of the uterus to adequately contract after placental delivery, causes approximately 80% of PPH[2]. Current medications used to prevent or treat uterine atony are limited by poor efficacy, adverse side effects, expense, and contraindications to use[2]. As such, there is a critical need for effective, safe, and inexpensive pharmacologic measures for uterine atony.

Intravenous $CaCl_2$ is an inexpensive, shelf-stable drug with good biologic and epidemiologic plausibility to treat and/or prevent uterine atony. Calcium is already frequently utilized in obstetric anesthesia for indications such as hypocalcemia from massive transfusion and overcoming magnesium toxicity. Multiple *in vitro* studies have demonstrated that adequate extracellular calcium levels are necessary for forceful myometrial contraction in response to oxytocin[5-7]. Epidemiologic studies show that low ionized calcium levels predict the development of severe postpartum hemorrhage in a dose-response fashion[8].

We recently conducted a 40-patient placebo-controlled, double-blind pilot RCT assessing a one-gram dose of intravenous $CaCl_2$ as a novel agent to prevent uterine atony during cesarean delivery. This pilot demonstrated the feasibility of enrollment and good patient tolerance of the intervention. Moreover, we found a trend toward efficacy in prevention of uterine atony in women who received calcium as compared to placebo infusion (p=0.07, relative risk 0.38, 95% CI 0.15-1.07, number needed to treat 3.3).

Pharmaceutical development and pharmacokinetic characterization in pregnancy has become a National Institute of Health priority[9]. The NICHD has established an Obstetric and Pediatric Pharmacology and Therapeutics Branch dedicated to advancing knowledge of drug PK/PD in pregnancy and childhood given critical knowledge gaps in these areas[9]. We will collect and analyze PK/PD for intravenous $CaCl_2$ within our trial. Building upon the promising results from our pilot study, we aim to:

AIM 1: Establish the effect of a $CaCl_2$ infusion on blood loss and uterine tone during cesarean delivery in parturients at heightened risk for uterine atony in a double-blind, randomized, placebo-controlled trial. We hypothesize that, as suggested by our pilot study, $CaCl_2$ infusion will reduce quantitative blood loss (QBL, primary outcome) and improve a numerical rating score for uterine tone (NRS_{UT}, secondary outcome) compared to placebo without significant safety or tolerability concerns.

AIM 2: Measure PK/PD of intravenous $CaCl_2$ in pregnant patients. Establishing the PK of intravenous $CaCl_2$ in parturients is of great relevance whether or not $CaCl_2$ is shown to be effective in preventing atony in Aim 1, given the multiple indications for calcium administration in obstetric patients. We will generate a PK/PD model for calcium in pregnancy that includes estimates of covariate effects and intra-and interindividual variability using non-linear mixed effects modeling. Meanwhile, PD modeling of calcium effect upon uterine tone will allow us to quantify calcium's effect on myometrial function.

Significance: If $CaCl_2$, an inexpensive, widely available drug, has efficacy in the prevention or treatment of uterine atony, it has the potential to change the maternal standard of care and decrease the leading cause of maternal morbidity and mortality. We will leverage the intervention tested in the RCT, the infusion of $CaCl_2$, to define the PK/PD of calcium in pregnant women at term. This has potential relevance in defining approaches for administering calcium in various obstetrical contexts and will facilitate dose optimization.

BACKROUND AND SIGNIFICANCE

Uterine atony and postpartum hemorrhage: PPH, defined by the American College of Obstetricians and Gynecologists as a cumulative blood loss of >1000mL within 24 hours of birth, is the leading cause of maternal major morbidity and mortality worldwide.[1,2] Uterine atony, defined as failure of the uterus to adequately contract after placental delivery, causes about 80% of PPH.[3] Rates of PPH requiring blood transfusion in the United States increased 5-fold from 1993 to 2014, an increase largely attributed to rising incidence of uterine atony[4].

Current clinical management of uterine atony involves prophylaxis with oxytocin and treatment with second-line uterotonics including methylergonovine, carboprost, and misoprostol[2]. However, the latter medications all carry significant limitations of poor efficacy, adverse side effect profiles, expense, or contraindications to use.[2] For example, methylergonovine is contraindicated in women with preeclampsia or hypertensive diseases. Carboprost, which may cost up to $1000 per dose, is contraindicated in women with asthma and requires refrigeration, significantly limiting its utility in poor resourced settings. Misoprostol causes fever and rigors and its efficacy for treating uterine atony has been called into question.[2] As such, additional prevention or treatment modalities for uterine atony--especially inexpensive, shelf-stable, and well-tolerated drugs—are essential.

Calcium: Intravenous $CaCl_2$ is an inexpensive, widely-available, shelf-stable drug which is familiar to anesthesiologists in a variety of contexts. On obstetric units, calcium is utilized for indications including hypocalcemia from rapid transfusion of citrate-containing blood products and overcoming magnesium toxicity.

Biological plausibility: calcium and uterine contractility: *In vitro* studies have established calcium's important physiological role in uterine contractility. Uterine myometrial contractility is dependent upon an influx of calcium from intracellular stores in the sarcoplasmic reticulum as well as extracellular calcium.[5-7] Myometrial contraction amplitude diminishes in the setting of low extracellular calcium.[5-7] Oxytocin's efficacy in inducing myometrial contraction diminishes by 60-75% in the setting of low or absent extracellular calcium levels in *in vitro* studies with human uterine strips.[7]

Clinical and epidemiologic evidence: serum calcium levels impact myometrial function: In a large retrospective cohort study, low ionized calcium levels predicted severity of PPH in a dose-response relationship, with an odds ratio of 1.97 for each 0.1mmoL/L decrease in ionized calcium level[8]. A prospective observational study also linked serum calcium levels with spontaneous labor onset, finding higher serum calcium in laboring than matched non-laboring patients at the same gestational age[10]. Finally, calcium channel blockers are regularly utilized to prevent uterine contractility in the setting of preterm labor[11].

In one prior small RCT, Farber and colleagues examined the effect of low dose $CaCl_2$ administered during scheduled cesarean delivery in 60 patients upon hemodynamics (primary outcome). No differences in development of uterine atony (secondary outcome) were noted among the study groups who received 0, 200mg, or 400mg of $CaCl_2$; however, there were only 4 total cases of uterine atony and none in the 400mg group, such that the study was grossly underpowered with respect to this outcome[11].

Maternal hemorrhage and pharmacokinetic drug studies and maternal hemorrhage are National Institutes of Health (NIH) priorities: The National Institute of Child Health and Human Development (NICHD) specifically calls for investigations dedicated to "improv[ing] the prevention of and response to labor and delivery complications that lead to maternal morbidity and mortality and extend[ing] studies of key adverse events to the postpartum period ("the fourth trimester") to include hemorrhage"[9]. In addition, pregnant women are historically excluded from drug trials, and despite physiologic changes including larger plasma and adipose volumes, increased glomerular filtration rate, and bidirectional changes in hepatic enzyme function, PK/PD drug studies in this population are often lacking[13]. The NICHD 2020 strategic plan includes themes centered upon funding PK/PD studies in pregnant women and children and improving pregnancy outcomes[9]. The proposed work for this FAER award meets both of these NICHD strategic goals by addressing maternal hemorrhage and PK/PD.

PRELIMINARY STUDIES

We performed a 40-patient double-blind pilot RCT examining the impact of 1 gram of $CaCl_2$ or saline placebo in addition to standard oxytocin during cesarean delivery in parturients with heightened risk of uterine atony. In the pilot study, we demonstrated feasibility by successfully enrolling all 40 patients in a one-year timeframe despite a lack of dedicated funding or research staff. The drug infusion was well-tolerated, with no adverse hemodynamic consequences. An equal number of patients in the calcium and placebo groups reported possible drug side effects including flushing, nausea, or vomiting (30% in each arm). Obtaining and measuring venous blood specimens for PK analysis also proved feasible.

Although inferring intervention efficacy is not the aim of a pilot trial, there were several trends noted within our pilot trial data that suggest $CaCl_2$ warrants a larger, definitive study as a potential intervention for uterine atony (**Table 1**). Only 20% of patients who received calcium had uterine atony, pre-defined as requirement for a second line uterotonic agent, placement of an intrauterine balloon for tamponade, or hemorrhage with blood loss >1000mL, compared to 50% of patients who received the saline placebo infusion (relative risk 0.38, *P*=0.07, 95% CI 0.15-1.07, NNT 3.3). Blood loss, uterine tone score, total oxytocin dose, and intraoperative fluid requirements all showed similar promising trends (**Table 1**).

Table 1: Outcomes related to atony and hemorrhage, pilot RCT. Values are provided as median [interquartile range]

	Calcium (n=20)	Placebo (n=20)	*P* value
Uterine atony incidence	20% (n=4)	50% (n=10)	0.07
Blood Loss *(mL)*	750 [600-800]	850 [650-1000]	0.15
Uterine tone NRS *(0-10)*	9 [8-9]	8 [7-9]	0.13
Oxytocin			
Total bolus dose (units)	2 [2-2]	2 [2-4]	0.14
Maximal infusion rate (units/hr)	7.5 [7.5-15]	15 [7.5-22.5]	0.41
Fluids and transfusion			
Crystalloid (mL)	1200 [1000-2000]	1750 [1500-2000]	0.03
Colloid (mL)	0 [0-0] range [0-500]	0 [0-0] range [0-1000]	0.08

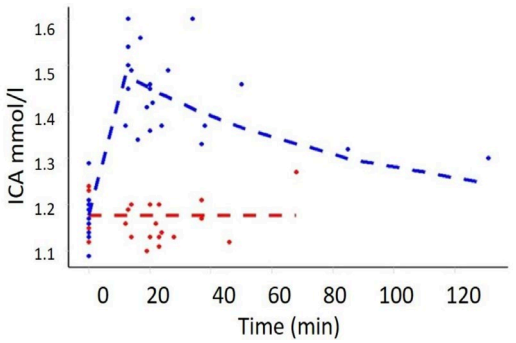

Figure 1: pilot data: ionized calcium concentrations over time. Blue data points represent ionized calcium venous blood concentrations from 13 patients who received calcium, and red data points represent ionized calcium in 11 patients who received a saline placebo infusion.

As part of the pilot RCT, we also gathered serial serum venous blood samples from a subset of participants. This was done for two main purposes: 1) to ensure a reliable but safe rise in ionized calcium from the 1-gram dose of $CaCl_2$, and 2) to generate sample size calculations for a definitive study of $CaCl_2$ PK in a similar pregnant patient cohort. Data and a 1-compartment PK model are shown in **Figure 1**. As anticipated, a rapid and reliable and safe-level peak in ionized calcium was obtained in subjects who received $CaCl_2$.

EXPERIMENTAL DESIGN AND METHODS

SPECIFIC AIM 1 OVERVIEW: Based on our preliminary data, we propose a double-blind, placebo-controlled, 120-patient RCT to assess the effect of prophylactic $CaCl_2$ infusion upon blood loss and uterine tone at the time of intrapartum cesarean delivery. Patients will be randomly assigned to receive a prophylactic infusion containing one gram of dilute $CaCl_2$ or saline placebo in addition to standard of care oxytocin after fetal delivery. We will assess quantitative blood loss and serial measurements of uterine tone.

Study population: The study will enroll adult pregnant patients age 18-45 years of age at >34 weeks gestation who require intrapartum cesarean delivery and have received an exogenous oxytocin infusion for labor induction or augmentation, as these are known risk factors for uterine atony[1,2]. Exclusions include emergent surgery, known cardiac dysfunction, renal dysfunction with serum Cr >1.0, hypertension necessitating intravenous hydralazine or labetalol during labor, and treatment with calcium channel blockers or digoxin.

Subject enrollment: All laboring patients on the unit receive a brief informational handout about this study upon admission (**Appendix 1**). Research staff visit all laboring patients twice daily after permission to approach and introduction by the bedside nurse, once in the morning and once in the afternoon, to obtain informed consent from all willing laboring patients if they require cesarean delivery. Consent and study inclusions/exclusions are confirmed by the clinical anesthesiologist at the time of decision by the obstetrics team to proceed with an intrapartum cesarean delivery.

Subject exposures: Sequential subject study ID numbers will be randomized in 1:1 ratio to one of two experimental groups. Study drugs will be prepared daily by the research pharmacy and labeled only with the subject ID # and "study drug". The patient, anesthesiologist, obstetricians, and research staff will remain blinded to assignment. All study subjects will receive standard care with prophylactic oxytocin bolus and infusion, as well as second-line uterotonics on an as-needed basis per institutional protocol. (**Appendix 2**)

- *Calcium:* 1 gram $CaCl_2$ in total volume 60mL delivered over 10 minutes beginning 2 minutes after fetal delivery.
- *Placebo:* 60mL sterile saline delivered over 10 minutes beginning 2 minutes after fetal delivery

Outcomes and Measurement: The primary outcome will be quantitative blood loss (QBL) in mL, which is calculated by standardized methodology including volumetric (suction cannister contents after subtracting amniotic fluid volume) and gravimetric (weighing soiled surgical sponges, drapes, and pads) assessment. Secondary outcomes will include the following: uterine tone assessments by the obstetric attending on a validated 10-point numerical rating scale[14] (NRS_{UT}) at study drug initiation 2 minutes after fetal delivery and reassessed every 5 minutes thereafter until fascial closure. Additionally, total oxytocin bolus and infusion doses, second line uterotonics, change in hematocrit from preoperative to postoperative day one values, total intravenous fluids, and a composite outcome indicating hemorrhage morbidity including transfusion during the hospital stay, hysterectomy, uterine artery embolization, intensive care admission, and death will be recorded.
Please see Appendix 3 for forms to be filled out by research staff.

Data Analysis: Analyses will be conducted using R and supervised by a biostatistical consultant from the Stanford Quantitative Sciences unit. Continuous data will be represented using mean (± SD) or median (interquartile range) for variables not normally distributed and compared using parametric or non-parametric t-tests as appropriate. Categorical data will be presented using proportions and compared using a chi-square (or Fishers Exact) test. Two-sided p-values less than 0.05 will be considered statistically significant.

Analysis of Primary Aim*:* The primary outcome of the study is the difference in quantitative blood loss between the two groups on an intention-to-treat basis. Patients who are found to have non-atonic reasons for blood loss including morbidly adherent placenta, hysterotomy extension, cervical, or vaginal lacerations will be included in primary outcome assessment. The trial is also powered for planned subgroup analysis excluding these cases. In addition, subgroup analyses based upon risk factors for atony including chorioamnionitis, total oxytocin exposure, indication for cesarean, magnesium infusion, and preeclampsia will be performed.

Sample size: Power analysis of the data from our pilot RCT revealed that a sample size of 31 patients per arm will have 90% power to detect a difference of 200mL in quantitative blood loss between groups at the $p<0.05$ significance level. The sample size was increased to 60 patients per arm to allow sufficient power for the planned subgroup analysis excluding patients with non-atonic etiologies of blood loss which may occur in up to 40% of cases. This same sample size will also generate 86% power to detect a rank test difference of 2 points in the ordinal secondary outcome measure of NRS_{UT}. We anticipate signing consent documents with approximately 1200 laboring patients to obtain the 120 subjects who meet enrollment criteria, as most of these patients will have normal spontaneous vaginal delivery, some may later decline to participate, some may develop exclusions such as renal dysfunction, and some may require stat delivery without time to safely administer the study medications in addition to standard care.

Patient safety: All potential side effects of calcium will be recorded as part of this study, including flushing, arm discomfort, nausea, vomiting, bradycardia, tachycardia, arrhythmia, and hypertension. A single dose of dilute $CaCl_2$, infused slowly through an intravenous (IV) line in the sensate upper extremity of an un-sedated patient, is unlikely to cause severe adverse effects. The major concern with $CaCl_2$ is tissue necrosis from extravasation. Any discomfort in the IV site will warrant immediate study drug infusion discontinuation with careful assessment of the extremity. The clinical anesthesiologist caring for the patient will also monitor for potential hemodynamic consequences of calcium infusion including bradycardia, hypotension, or hypertension. The anesthesiologist providing care to the patient will be instructed to assume the study drug contains 1 gram of $CaCl_2$ for all cases, and to immediately discontinue the infusion if they believe any the patient may be having a severe adverse effect. A data safety monitoring committee of two attending anesthesiologists with no involvement in the trial and with a track record of data safety monitoring has been created for this trial. Any potential adverse events including patient hemodynamic compromise or concern for drug extravasation will be reviewed immediately ad hoc, and they will determine whether unblinding is required and when the study may resume.

Management of hypercalcemia: Acute hypercalcemia may manifest as altered mental status, bradycardia, or hypotension. We do not anticipate clinical hypercalcemia given the patient population (young, healthy, renal dysfunction and digoxin use are contraindications), the dilute and slow infusion, the single dose of calcium, and the excellent patient tolerance and peak ionized calcium levels obtained during our pilot study. However, management of acute hypercalcemia would include immediately pausing the infusion if still running, administering a fluid bolus of 500mL to 1000mL isotonic fluid, and obtaining a serum calcium level at the discretion of the clinical anesthesia team. If necessary, the team could consider additional administration of 10-20mg intravenous furosemide to lower serum calcium further if needed.

Feasibility: Stanford Children's Hospital performs 4600-4700 deliveries per year, of which 1600-1950 are cesarean. More than 400 cesarean deliveries per year meet study inclusion criteria. Without dedicated funding or research staff, we were easily able to enroll 40 patients in the pilot study in a 1-year time period. Plans for the study have been accepted by the group of 15 fellowship trained obstetric anesthesiologists who cover labor and delivery, all of whom participate in monthly division research meetings and are well-versed in study protocol for any actively recruiting studies. In addition, we have the support and collaboration of an excellent obstetric division. There are no concurrent competing studies in this study cohort.

Potential difficulties: Given our successful pilot, we believe this study will proceed smoothly but are ready for unanticipated problems. We have received support from obstetricians, the quantitative sciences unit (statistics), and labor and delivery nursing for the study. The greatest source of potential difficulties involves enrollment of 120 patients at a single site during a pandemic. We have infrastructure in place to allow research assistants obtain consent remotely utilizing iPads if necessary. In addition, we have obtained a letter of commitment from a second site at Brigham and Women's Hospital if needed to facilitate enrollment.

Weaknesses: The primary outcome (QBL) and secondary outcome of NRS_{UT} are surrogate outcomes for clinical endpoints of major morbidity or mortality but allow for a more reasonable study sample size for a single center.

SPECIFIC AIM 2 OVERVIEW: Serial venous blood sampling for ionized calcium level in willing study participants will allow us to also establish the PK/PD of $CaCl_2$ in term parturients. PK findings have applicability to a variety of clinical indications for calcium in pregnant patients, while PD will enhance mechanistic understanding of the impact of serum ionized calcium level upon uterine contractility.

Venous blood samples for pharmacokinetic analysis: 0.5 mL venous blood will be collected at up to 6 timepoints for willing study participants at each of the following time intervals relative to study drug infusion: baseline (prior to fetal delivery), 0-5 minutes, 5-10 minutes, 10-30 minutes, 30-60 minutes, and 60-240 minutes. Ionized calcium level and pH of blood specimens will immediately be analyzed using an iSTAT machine with a EG7+ cartridge (Abbott Core Laboratory Systems, Lake Forest, IL) in an adjacent operating room by research staff or obstetric anesthesia fellows uninvolved in patient care or data analysis. Venous blood pH and ionized calcium values will be recorded directly into the secure research database but not entered into the patient's medical record as this would compromise blinding. Blood specimens will not be stored.

As a second peripheral IV is frequently placed in the opposite arm from the primary peripheral IV for patients undergoing intrapartum cesarean delivery as part of standard care in case of hemorrhage, this IV will be used for blood collection whenever possible. If not possible to draw blood or this IV is not placed, the patient will be asked for permission to obtain blood specimens using a 22g winged blood collection needle. If possible, these specimens will be collected from the lower extremity (saphenous vein or foot), as these veins are dilated and insensate in patients undergoing cesarean delivery under neuraxial anesthesia. Sterile technique will be utilized for all blood collections.

Pharmacokinetic and pharmacodynamic analysis: We will assess the data with graphical validation to assure data validity. General analyses will be conducted with R statistical programming language (R Foundation for Statistical Computing, Vienna, Austria). PK and PD models will be characterized using nonlinear mixed effects modeling with the program NONMEM 7.4[16].

We anticipate a two-compartment mammillary model with an effect site. Calcium is administered into the central compartment, also the site of drug sampling. Calcium is also eliminated from the plasma compartment. Calcium may distribute from the plasma compartment to one or more compartments. Drug effect is mediated by the concentration in a hypothetical "effect site", in this case the uterus[17]. The effect site permits modeling of hysteresis between the time course of plasma calcium concentration increase and increase in uterine tone measured in Aim 1. PK modeling will be done in the volume and clearance domain and include the influence (if any) of age, weight, gestational age, pH, starting heamtocrit, and blood loss.

Equation 1 is an equation for the sigmoidal PD model. Because the measure of drug effect is the NRS_{UT} is an ordinal measure, it will be dichotomized into 4 categories, 0-3, 4-10, 7-10, and 9-10, and modeled as the probability of being in each category as shown in **Figure 2**. PD models will be estimate p_0, p_{MAX}, Ce_{50}, and γ (the baseline probability of uterine tone X, the maximum probability of uterine tone X, the concentration associated with 50% of the change in probability, and the steepness of the concentration versus probability relationship). The effect of pH and uterotonics including oxytocin, methylergonovine, and carboprost on the PD parameters will be explored.

$$p(NRS_{UT} \leq X) = p_0 + (p_{MAX} - p_0)\frac{Ce^{\lambda}}{Ce_{50}^{\gamma} + Ce^{\lambda}}$$

Equation 1: Pharmacodynamic model for the probability of uterine NRS and calcium concentration

Inter-subject random effects (e.g., subject to subject differences) on model parameters will be estimated and applied using additive, proportional, and exponential models as appropriate. Residual intrasubject variability (e.g., noise) will typically require an additive and multiplicative error model. The influence of covariates effects (e.g., age, weight, gestational age, pH, uterotonics including oxytocin, methylergonovine, and carboprost) on model parameters will be analyzed by serial inclusion/exclusion with significance determined by the likelihood ratio test. Covariates on the PK and PD parameters deemed significant by likelihood ratio test will be validated using jackknife cross-validation as described by Sheiner[17] and only included in the final model if validated.

N	Parameter	Mean	95% Confidence Bound		Range	Correction	Corrected Range
			Lower	Upper			
24	Cl_1	0.93	0.78	1.70	98%	1.76	173%
	V_1	75.6	52.8	88.6	47%		83%
	Baseline	1.18	1.16	1.19	3%		4%
60	Cl_1	0.93	0.80	1.43	68%	1.39	**94%**
	V_1	75.6	62.9	84.0	28%		**39%**
	Baseline	1.18	1.17	1.19	2%		**2%**
120	Cl_1	0.93	0.81	1.24	46%	1.26	57%
	V_1	75.6	68.5	80.6	16%		20%
	Baseline	1.18	1.17	1.18	1%		1%

Table 2: Bootstrap analysis using pilot ionized calcium values. The mean and 95% confidence intervals are shown for each parameter.

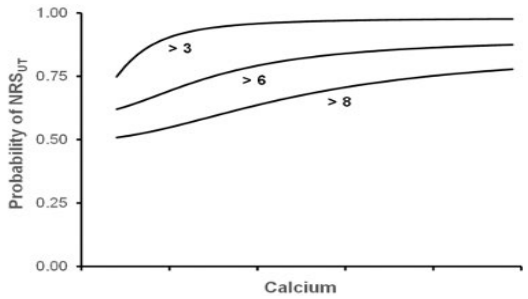

Figure 2: Ordinal model of uterine NRS showing hypothetical probability of NRS>3, >6, and >8 as a function of calcium

Table 2 shows the results of 1000 bootstrap replications of data from the original 24 individuals in the pilot PK study, resampled with N=60 (half of the proposed study population), and N=120 (the entire proposed study population)[18]. As expected, the estimates are more precise with increasing study size. As seen in the table, the parameters estimated for the initial 24 subjects had considerable uncertainty for clearance and the volume of distribution. If the study gathers PK samples in half of the patients (N=60), V_1 will be estimated within less than a 40% range (**Table 2**, numbers in red). This should be sufficient to predict the dose of calcium required to reach a target calcium concentration in parturients. Our study will have greater power than predicted by the bootstrap as we will collect 5-6 samples per patient rather than the 2-3 samples obtained in the pilot study.

Rigor and Transparency: Prior research that serves as the key support for the proposed study is rigorous as there is basic science and epidemiologic plausibility for this intervention. Sample size and statistical power for both aims of this research proposal build upon our own pilot clinical trial data. Weaknesses of the only prior clinical trial to investigate calcium's relationship to uterine atony included low calcium dose, small sample size, and a study population with a very low baseline incidence of uterine atony[12]. We have addressed all of these weaknesses with our clinical trial design by using a higher dose of calcium that we have shown in our pilot study accomplishes a measurable peak in ionized calcium levels shortly after infusion, a larger sample size based on rigorous estimation techniques, and a study population with very high baseline incidence of uterine atony. Our blinding and randomization should ensure a robust and unbiased approach. Finally, the proposed study takes into account sex as a biological variable by studying a condition unique to pregnant women. It also promises to bridge gaps in pharmacokinetic and pharmacodynamic studies in pregnant women.

Implications: If $CaCl_2$ shows efficacy in reducing blood loss from atony, it has potential to change the maternal standard of care worldwide and directly impact the leading cause of morbidity and mortality in pregnant women.

Future Directions: The likely next step will include obtaining NIH funding to execute a larger, multicenter RCT with expanded inclusion criteria.

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Appendix 1: Patient informational handout

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Stanford OB Anesthesia Study Information Sheet for Patients

Trial Investigating Calcium to Prevent Bleeding during Cesarean

This information sheet is provided to laboring patients to provide some information in case you might later qualify for one of our studies. **At some point, an anesthesiologist or research assistant might approach you about this study.** They would at that point go through a much more detailed explanation and consent with your permission. However, you are welcome to ask questions any time!

What is the study? In this study, Stanford OB Anesthesiologists are investigating whether giving some **extra calcium** through a patient's IV after the baby is delivered during a **cesarean** can help the uterus to contract and prevent blood loss.

I am in labor, not having a Cesarean. Why am I receiving this information? Until babies are born, we never know for sure that cesarean delivery will not be required. In this study, we are particularly trying to enroll patients who require **cesarean delivery after laboring**, as these patients have a higher risk of blood loss from poor uterine contraction after delivery.

How would this study change my care? If you were to qualify and consent for this study, you would receive all of the same medications and doses that any other patient receives. If you took part in this study, after your baby was delivered, an anesthesiologist would ALSO give a dose of extra calcium or some plain saline (known as a placebo) through your IV. Your anesthesiologist and you would not know whether you received calcium or the saline placebo. With your permission, the anesthesiologist may also collect several small blood samples from your IV during your cesarean to measure your calcium levels. You would still be welcome to participate without giving blood samples.

Why calcium? Why is this being studied? Calcium is present in all of our bodies and is essential for normal functions like blood clotting, heart, bone, and muscle function. Research shows that it is possible that extra calcium may also help the uterus muscle contract, which is important to help prevent bleeding after delivery. We are investigating whether women who require Cesarean have less blood loss and better uterine contraction after delivery if they receive some extra calcium. This study will help us to answer that important question.

For Participant's rights questions, contact 1-866-680-2906.

Appendix 2: Stanford Labor and Delivery protocol for intrapartum cesarean delivery

Monitoring: All patients undergoing intrapartum cesarean delivery are monitored by continuous pulse oximetry, 3-lead EKG, and noninvasive blood pressure oscillometry. Blood pressures are obtained every 1 minutes prior to fetal delivery. For clinically stable patients at the discretion of the anesthesia team, blood pressure frequency may be decreased to every 2.5-5 minutes after fetal delivery.

Antibiotics:

- Patients without antibiotic allergy or chorioamnionitis receive 2 grams intravenous cefazolin (3 grams if >120 kg) prior to incision and 500 mg of intravenous azithromycin over a one-hour infusion for endometritis prophylaxis.
- Patients with a clinical diagnosis of chorioamnionitis instead receive intravenous ampicillin 2g, gentamycin 5 mg/kg over a one-hour infusion, and clindamycin 900 mg intravenously

Neuraxial anesthesia:

- Patients with a labor epidural in situ receive epidural top-up with divided doses of 2% lidocaine with epinephrine 1:200,000 to achieve a T4 dermatomal level to pinprick bilaterally (generally 15-25 mL total volume). 100 mcg epidural fentanyl is added at the discretion of the attending anesthesiologist. All patients receive 3 mg epidural morphine prior to case conclusion for postoperative analgesia.
- Patients without a labor epidural in situ receive a single-shot spinal or combined spinal-epidural anesthetic, with a standard intrathecal medication cocktail of 1.6 mL 0.75% hyperbaric bupivacaine, 15 mcg fentanyl, and 100 mcg morphine.

Uterine tone assessment and uterotonic medications:

- At the time of fetal delivery, 2 units of intravenous oxytocin are administered, and an oxytocin infusion is initiated at 7.5 units/hr
- 2 minutes after fetal delivery, the uterine tone is assessed by palpation of the uterine fundus and rated on a scale from 0-10, with 0 representing a completely atonic uterus and 10 representing a firm and completely contracted uterus. This score is recorded. For uterine tone score <8/10, an additional 2 units oxytocin are administered, and oxytocin infusion increases to 15 units per hour
- 7 minutes after fetal delivery, uterine tone is again assessed by palpation and score recorded. For scores <8/10, oxytocin 2 units are administered for a 3rd time, oxytocin infusion increases to 30 units/hr, and the anesthesia and obstetric teams discuss whether a second line uterotonic may be warranted. If so, the anesthesia team begins preparation of the second line uterotonic agent. This agent is administered if uterine tone is still deemed inadequate at the discretion of the anesthesia and obstetric teams.
- 12 minutes after delivery, uterine tone is again assessed by palpation and score recorded. For scores <8/10, the obstetric and anesthesia teams will discuss whether a second line uterotonic may be clinically indicated.
- The uterotonic protocol outlined can be changed based on clinical picture or obstetrician request.

Quantitative blood loss:

Quantitative blood loss (QBL) is measured for all cesarean deliveries by a standardized protocol. The protocol follows nationally recommended methodology that combines gravimetric (measurement of suction cannister contents after subtraction of the amniotic fluid volume) and volumetric (weighing all surgical sponge and drape and subtracting dry weights) QBL.[1,2] This process is performed by the clinical labor and delivery nurses, all of whom receive standardized training in performing the technique.

1. *Quantitative Blood Loss in Obstetric Hemorrhage: ACOG COMMITTEE OPINION, Number 794. Obstetrics and gynecology 2019;134(6):e150-e156.*
2. *Lyndon A, Lagrew D, Shields L, Main E, Cape V: Improving Health Care Response to Obstetric Hemorrhage.(California Maternal Quality Care Collaborative Toolkit to Transform Maternity Care). Published by the California Maternal Quality Care Collaborative. 2015.*

Appendix 3 Protocol Sheet: CALCIUM CHLORIDE FOR PREVENTION AND TREATMENT OF BLOOD LOSS FROM UTERINE ATONY DURING INTRAPARTUM CESAREAN DELIVERY

STUDY ID NUMBER (on study drug syringe): _____

Reminder: This study investigates whether one gram of calcium chloride IV, administered over 10 minutes starting 2 minutes after fetal delivery, decreases blood loss from uterine atony during intrapartum cesarean.

- Primary outcome: Quantitative Blood Loss
- Secondary outcomes: Uterine tone scores, uterotonics, side effects

Please confirm inclusions with a checkmark or X:

☐ Patient is 18-55 years old

☐ Patient is having an intrapartum cesarean delivery

☐ Patient received oxytocin infusion for labor induction or augmentation.

☐ Patient still wants to participate and has signed consents

Please confirm this patient has NO EXCLUSIONS:

☐ Patient does not have renal dysfunction with serum Cr>1.0 mg/dL

☐ Patient does not have any known cardiac conditions

☐ Patient is not receiving a calcium channel blocker (none in last 24 hours)

☐ Patient is not receiving digoxin for a maternal or fetal indication

☐ This is not an emergency in which participation in the study could hinder or delay care

Study drug:

- The syringe contains **60mL saline with 1-gram Calcium Chloride** OR **60mL saline placebo**

- Administer via syringe pump and microbore tubing over 10 minutes
  - Program a basic infusion on Alaris pump for basic infusion, **360mL/hr**
  - Attach microbore tubing to injection port closest to the patient
  - Start infusion **2 minutes after fetal delivery (**1 minute after cord clamp)

- Assume that every infusion contains 1 gram of calcium and **discontinue** if concerns for extravasation (arm pain and redness or signs of infiltration), hemodynamic compromise (bradycardia and hypotension most common). Contact Dr. Ansari via email or cell (760) 845-0328 for any concerns and document on next page.

PLEASE FILL OUT ALL INFORMATION ON THE NEXT PAGE.

STUDY ID NUMBER (on study drug syringe): _____

USE EPIC COMPUTER CLOCK FOR ALL TIMES
Fetal Delivery and oxytocin 2 unit bolus : Time: _____

2 minutes after fetal delivery:

- Ask obstetrician to rate uterine tone 0-10: Tone: _____
- Start study drug infusion: Time Started: _____
- *If infusion was paused or stopped* *Time paused:* _____

  Time restarted: _____

7 minutes after fetal delivery:

- Ask obstetrician to rate uterine tone 0-10: Tone: _____

12 minutes after fetal delivery:

- Ask obstetrician to rate uterine tone 0-10: Tone: _____

19 minutes after fetal delivery:

- Ask obstetrician to rate uterine tone 0-10: Tone: _____

Side effects (did the patient experience any of the following possibly related to the study drug? Circle Yes/No)

- Arm or IV site discomfort? YES / NO
- Concern for extravasation? YES / NO
- Heart rate change or arrhythmia? YES / NO Describe: _____
- Hypertension or hypotension YES / NO Describe: _____
- Flushing YES / NO
- Abnormal sensations or tastes YES / NO Describe: _____
- Nausea YES / NO
- Vomiting YES / NO
- Other YES / NO Describe: _____

Was the infusion started exactly 2 minutes after fetal delivery? YES / NO

If not, please explain why: _____

Was the whole infusion given? If not or if paused, please explain why below

_____

STUDY ID NUMBER (on study drug syringe): _____

If patient consents to up to 6 lab draws of 0.5mL venous blood:

- Research staff (fellow or research assistant) to analyze samples in a **different operating room**.
- **DO NOT** participate in patient care or converse with staff or the patient in the delivery room other than to obtain permission to obtain blood samples, as your knowledge of these lab values could compromise blinding of the patient, anesthesiologist, or obstetrician.

Times to target for blood samples relative to the start of study drug infusion:

- Baseline (prior to study drug start)
- 0-5 minutes
- 5-10 minutes
- 10-30 minutes
- 30-60 minutes
- 60-240 minutes

Carefully document the time that the blood was drawn using **EPIC computer clock** for each sample.
Use the **research iSTAT machine** and research EG7+ cartridges.
For patient ID on the iSTAT, please enter the study ID number and not the MRN

Time study drug started: _____

Baseline: Time: _____ pH: _____

iCa: _____

Sample 2: Time: _____ pH: _____

iCa: _____

Sample 3: Time: _____ pH: _____

iCa: _____

Sample 4: Time: _____ pH: _____

iCa: _____

Sample 5: Time: _____ pH: _____

iCa: _____

Sample 6: Time: _____ pH: _____

iCa: _____