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Original contribution

Minimum effective fluid volume of colloid to prevent hypotension during caesarean section under spinal anesthesia using a prophylactic phenylephrine infusion: An up-down sequential allocation study☆

Christian Loubert, FRCPC (Consultant anesthesiologist, Clinical Assistant Professor) [a,*],
Pierre-Olivier Gagnon, FRCPC (Consultant anesthesiologist) [a],
Roshan Fernando, FRCA (Consultant anaesthetist, honorary clinical senior lecturer in anaesthesia) [b]

[a] Maisonneuve-Rosemont Hospital affiliated to the University of Montreal, 5415 boul. L'Assomption, Montreal, (QC), H1T 2M4, Canada
[b] University College London Hospitals NHS Foundation Trust, 235 Euston Road, London, NW1 2BU, United Kingdom

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ABSTRACT

Study objective: The aim of this study was to de termine the minimum effective fluid volume (MEFV) of hydroxyethyl starch 130/0.4 (HES) infused in a preload fashion which would prevent hypotension in 50% of parturients undergoing caesarean section. A secondary objective was to measure the hemodynamic effect of fluid loading on the subjects.
Design: This is a prospective, double-blinded, dose-finding study using an up-down sequential allocation design.
Setting: In the operating room.
Patients: Thirty healthy parturients undergoing caesarean section under spinal anesthesia using a prophylactic phenylephrine infusion were included in this study.
Intervention: The initial HES volume infused in the first patient was 500 mL. A failure of treatment to HES preload was defined as a single episode of systolic hypotension below 20% of baseline value. The next patient in the sequence was given a volume of HES adjusted by either an increment or a decrement of 100 mL according to the previous subject response to fluid preload.
Measurements: Stroke volume and cardiac output were measured with a bioreactance-based non-invasive cardiac output monitor (NICOM).
Main results: The MEFV of HES was 733 mL (95% CI: 388-917 mL). Fluid loading before the administration of the spinal anesthesia resulted in an increase in stroke volume and cardiac output. The combined effect of spinal anesthesia and a phenylephrine infusion reduced the maternal heart rate and cardiac output, but not the stroke volume.
Conclusion: Our study is the first to investigate variable fluid loading volumes in this population. A HES preload of approximatively 700 mL prevented maternal hypotension in 50% of the parturients under the conditions of this study. We suggest that up-down sequential allocation design is a useful tool to compare different fluid loading regimens in this setting.

1. Introduction

Spinal anesthesia-induced hypotension, which is pronounced in term parturients undergoing elective caesarean section, may be deleterious to both the mother and the fetus. Obstetric anesthetists are increasingly opting for prophylatic phenylephrine infusions as first line therapy to prevent hypotension in pregnant women undergoing caesarean section [1]. Phenylephrine infusions significantly reduce the incidence of hypotension, nausea and vomiting and increases patient comfort while being safe for both the mother and the baby [2,3].

Some experts have questioned the usefulness of prophylactic fluid boluses for prevention of spinal anesthesia-induced maternal hypotension [4]. Several studies have shown that volumes of crystalloid up to 30mL kg^{-1}, given before the administration of spinal anesthesia (preloading) only moderately prevented hypotension [5-7]. Various fluid loading strategies including the use of fluid coloading (infusion of fluid at the time of the administration of spinal anesthesia) have

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* Corresponding author: Department of Anesthesiology, Maisonneuve-Rosemont Hospital, Montreal, (QC), H1T 2M4, Canada. Tel.: +1 514 567 7340.
E-mail addresses: loubertch@yahoo.fr (C. Loubert), pogagnon@gmail.com (P.-O. Gagnon), roshanagfernando@gmail.com (R. Fernando).

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proven superior to crystalloid preloading, although their clinical effectiveness is moderate [8-10]. Very few studies have evaluated the additional benefit of fluid loading in terms of hypotension prevention in patients already receiving a prophylactic infusion of an alpha-receptor agonist such as phenylephrine [11,12]. The studies which aimed to find an ideal fluid volume which would prevent such hypotension are limited by their design which compared fixed volumes. To our knowledge, there is no study investigating variable fluid volumes in women receiving a phenylephrine infusion to prevent spinal anesthesia-induced hypotension. The primary aim of this study was to determine the minimum effective fluid volume (MEFV) of colloid infused as a preload which would prevent hypotension in 50% of healthy term pregnant women undergoing caesarean section with spinal anesthesia used in conjunction with a phenylephrine infusion. We measured the effect of fluid loading several maternal hemodynamic parameters as a secondary objective.

2. Methods

After obtaining institutional review board approval (IRB: #11 051) and written informed consent, 30 pregnant women were enrolled in this prospective, double-blind, dose-finding study between October 2011 and September 2012 (ClinicalTrial.gov: #NCT01415284). Healthy parturients (ASA score I-II) at term gestation (37 weeks and above) with a singleton pregnancy undergoing elective caesarean section under spinal anesthesia were considered eligible for recruitment. Exclusion criteria included hypertensive disorders (chronic or gestational hypertension, pre-eclampsia and eclampsia), cardiac pathologies, a body mass index above 35at the time of delivery, abnormal placentation, known allergy to hydroxyethyl starch, refusal to participate or urgent caesarean section.

Subjects were instructed to fast from midnight on the day of the surgery and were premedicated with 30 mL of sodium citrate 0.3 mol/L on the preoperative unit before leaving for the operating theater. In the waiting area vital signs were taken every 5 minutes over 10 minutes (3 measurements) by a research nurse. All measurements were averaged to determine baseline vital signs. Abio-reactanceNon-Invasive Cardiac Output Monitor (NICOM) (Cheetah Medical, Boston, MA) was used to evaluate the ejection volume and cardiac output. The complete mechanisms underlying the use of this technology are extensively detailed by Keren etal [13] and briefly described in Appendix A. Once baseline vital signs were recorded, a total of 4 sensors were applied on the back of the patients by the research nurse in accordance with the manufacturer's instructions.

In the operating room, the subjects were placed in the left-wedged supine position and a large-bore (18G) intravenous line was inserted in the left forearm. The NICOM sensors were connected to the NICOM device and baseline ejection volume and cardiac output were determined. The studied volume of hydroxyethyl starch (HES) 130/0.4 in NaCl 0.9% (Voluven, Fresenius-Kabi, Germany) was then infused under pressure using an inflatable fluid pump as rapidly as possible while vital signs and hemodynamic parameters were recorded every minute over 10 minutes by the research nurse. The study HES volume was prepared preoperatively by the same research nurse who then covered the HES bags with an opaque plastic bag in order to ensure that both the attending anesthetist and the patient were blinded to the infusion volume. A lactated ringer infusion was administered at a rate of 100 mL h^{-1} with a Baxter infusion pump from the end of the HES infusion until the end of the study period, that is, the delivery of the baby.

After the initial 10 minute period for fluid preloading, the subjects were placed into the sitting position. A standardized «needle-through-needle» combined spinal-epidural anesthesia technique was performed by the anesthetist attending the patient's care at the L2-L3 or L3-L4 vertebral interspace level using a 17G Tuohy epidural needle and a 27G Whitacre spinal needle. The anesthetist remained in the operating room to ensure routine anesthetic care throughout the caesarean section. A dose of 10.5mg hyperbaric bupivacaine with fentanyl 15 μg and morphine 150 μg was injected in the intrathecal space after which an epidural catheter was inserted 4 to 5cm into the epidural space. At the start of the spinal injection, a phenylephrine infusion at a rate of 0.5 μg kg^{-1} min^{-1} (based on the body weight at first antenatal clinic visit) was initiated. The patient was then placed in the left-wedged supine position. Vital signs and hemodynamic parameters were recorded every minute until the delivery of the baby. The obstetricians were instructed to prepare the patient for the caesarean section in order to perform the skin incision exactly 20 minutes after the spinal injection. A loss of sensation to cold at the T4 level, measured by the attending anesthetist, at the time of skin incision was considered adequate to start surgery. This evaluation was performed at 15 minutes after the spinal injection and repeated at 20 minutes. In cases where the sensory block had not reached the T4 level at 15 minutes, a 5 mL bolus of lidocaine 2% with epinephrine 5 μg/mL was injected in the epidural space through the indwelling epidural catheter. If the sensory block was considered inadequate for surgery at 20 minutes, the patient was withdrawn from the study and left to the care of the attending anesthetist. The individual study period ended at delivery of the baby.

The up-down sequential allocation method described by Pace and Stylianou was used to determine the HES study volume [14]. In this approach, the volume injected to the next patient is determined by the clinical response of the previous patient. In our study, the initial HES volume infused to the first patient was 500 mL. This volume was arbitrarily chosen as the best estimation of the MEFV based on 2 investigations which showed that a preloading of HES of 0.5 liter reduced the incidence of hypotension between 35 and 40% [8,15]. A failure of treatment was defined as a single episode of systolic hypotension below 20% of baseline value. In such a case, the next patient was given a higher volume by an increment of 100 mL. A successful response was defined as an absence of hypotension, with, the next patient in the sequence receiving a lower volume by an decrement of 100 mL, and so on, until the last subject was enrolled. The treatment results as well as all trial data were collected on a paper case record file which was secured in a key-locked drawer within the anesthesia department. Only the research nurse involved in this trial had access to the trial CRFs and could use them in order to prepare the infused volumes of HES for each subject. Unblinding was made possible in agreement with the *International Conference for Harmonization of Good Clinical Practice E6*-based institutional protocol. For clinical safety and ethical considerations, we kept the volume infused within a range of 200 mL to 1500 mL.

When needed, vasopresors were administered by the attending anesthetist according to the study protocol. Episodes of systolic hypotension (defined as systolic blood pressure below 20% of baseline value) without bradycardia (HR >55 bpm) were treated with an intravenous bolus of phenylephrine 100 μg, whereas an episode of hypotension associated with bradycardia (HR< 55 bpm) was treated with an IV bolus of ephedrine 5 mg. An isolated episode of bradycardia with the systolic blood pressure within 20% of the baseline SBP was treated with a bolus of IV glycopyrrolate 0.2 mg. An episode of hypertension above 120% of baseline SBP was treated by reducing the phenylephrine infusion rate to 25% of its initial rate. At any time, the attending anesthetist was allowed to deviate from the study protocol if patient safety was thought to be at risk. In this instance, the patient was excluded from the trial.

The primary outcome of this study was the volume of hydroxyethyl starch infused as a preload before spinal anesthesia which would prevent an episode of systolic hypotension below 80% of baseline in 50% of the subjects (EV50). Secondary outcomes included the incidence of hypotension and of hypertension, the total dose of vasopressors administered, nausea and vomiting episodes, sensory block level, 1 and 5 minute Apgar scores as well as umbilical artery and venous cord blood gases.

2.1. Statistics

We used an up-down sequential allocation design previously described by Pace and Stylianou to estimate the MEFV of HES under the conditions of this trial [14]. In this trial design, the response of the previous patient to a treatment determined the volume administered to the next patient. Isotonic regression, which is described in Appendix B, was used to estimate the MEFV of HES and calculate the 95% confidence intervals. We selected the μ_3 isotonic estimator of MEFV as it has been shown that this has the narrowest confidence intervals. The MEFV estimator and 95% confidence intervals were computerized with a parametric bootstrap routine developed by Stylianou. The algorithms were written in GAUSS by Stylianou and translated into the public domain R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria) by Pace. These functions call for the boot function of Canty's Bootstrap package. We used R version 3.0.2 to computerize the bootstrap functions for this trial.

Theoretically rigourous rules to calculate the necessary sample size for a prespecified precision of the estimation of MEFV cannot be developed owing to the non-independence and unknown distribution of data of an up-down sequential allocation method. Simulation studies suggest however that enrolling between 20 and 40 patients will provide stable estimates of the MEFV for most scenarios [14]. Therefore, we decided to include 30 subjects in our trial.

Non-parametric statistical tests accounting for the non-independence of the data were used to analyze our results when applicable. Association between variables were assessed with Spearman's r test for correlations. Hemodynamic variable trends were evaluated with analysis of variance for repeated measures (RM-ANOVA). A $P < .05$ was considered statistically significant. Statistical analysis were performed with Prism 6.0 for Mac OS X (GraphPad Software Inc., San Diego, CA, USA).

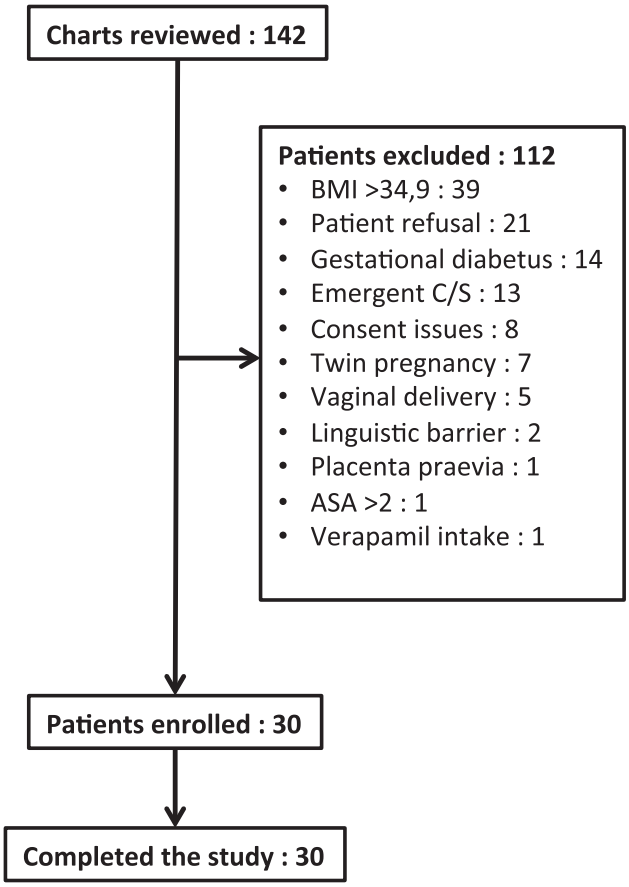

Fig. 1. CONSORT flow diagram.

3. Results

All 30 subjects included in this trial completed the study (Fig. 1). Demographic data and indications for caesarean section are presented in Table 1. Baseline hemodynamic parameters are presented in Table 2. The median [interquartile range] sensory block height at skin incision was T3 [T2 – T4]. No subject required supplemental epidural local anesthetic. The median effective volume which prevented hypotension in 50% of the subjects, calculated by isotonic regression, was found to be 733 mL (95% confidence intervals: 388-917 mL) (Fig. 2).

Trends in hemodynamic variables during the 10 minutes following the onset of infusion of the HES bolus are shown in Fig. 3. There was a statistically significant increase in cardiac output ($P < 0{,}001$), heart rate ($P = .02$), and stroke volume ($P < .001$) (RM-ANOVA) over time. There was no correlation between the volume of HES infused and the magnitude of the increase (expressed as the highest value minus the lowest value) in these variables (Spearman's r; $P > .05$).

Fig. 4 shows trends in hemodynamic variables over the 20 minutes following the onset of spinal anesthesia and starting the phenylephrine infusion. There was a statistically significant decrease in cardiac output and heart rate over time (RM ANOVA; $P < .001$) while the stroke volume remained constant (RM ANOVA; $P = .27$). The systolic blood pressure reach its lowest values at the seventh minute following the onset of spinal anesthesia, then increased and plateaued until the end of the trial period. The heart rate reached a nadir after 11 minutes and remained lower than baseline for the following 20 minutes. There was no correlation between the volume of HES infused and the magnitude of changes in these variables (Spearman's r; $P > .05$).

The median (interquartile range) total dose of phenylephrine administered during the trial period was 1486 (1110–1660) μg. There was a weak inverse correlation between the total dose of phenylephrine administered and the volume of infused HES (Spearman's $r = -0.367$; $P = .046$).

The overall incidence of hypotension was 46.7% (14 patients). Of these 14 patients, 7 presented with only one hypotensive episode which was resolved with a single 100 μg bolus of phenylephrine while the phenylephrine infusion was still running at a fixed rate. Three patients presented with bradycardia below 55 beats per minute. Of these, two patients had an associated episode of hypotension. The abnormal hemodynamic parameters of these patients were promptly treated with a glycopyrrolate 0.2mg IV bolus or an ephedrine 5mg IV bolus. There was a single episode of systolic hypertension (systolic above 120% baseline) in 4 patients. In 3 of these patients, the hypertensive episode occurred only at the first measurement after the spinal. In the fourth patient, boluses of phenylephrine and ephedrine were administered by the attending anesthetist to treat a hypotensive episode associated with bradycardia. This lead to an overshoot hypertensive episode of one to two minutes duration.

There was no correlation between the timing of spinal anesthesia (performed before versus after 12:00 pm) and the incidence of hypotension, requirements for vasopressors, or nausea and vomiting. The correlation between the incidence of hypotension and nausea or

Table 1
Demographic data and indications for caesarean delivery.

Primiparity (n)		6
Multiparity (n)		24
BMI (kg. m^{-2} ± SD)		30,1 ± 2,9
Indications for C/D	Contraindication to TOLAC	23
	Breech or transverse presentation	5
	Cervical cerclage	1
	Failure of induction	1
Timing of C/D (n)	AM	20
	PM	10

C/D: Caesarean delivery.
TOLAC: Trial of labor after caesarean.

Table 2
Baseline haemodynamic parameters.

Systolic blood pressure (mmHg ± SD)	116 ± 12
Heart rate (bpm ± SD)	89 ± 11
Ejection volume (mL ± SD)	47 ± 30
Indexed ejection volume (mL m^{-2} ± SD)	38 ± 15
Cardiac output (L min^{-1} ± SD)	6,1 ± 1,2
Indexed cardiac output (L min^{-1} m^{-2} ± SD)	3,6 ± 1,0

SD: standard deviation.

vomiting (33.3% - 10 patients) was statistically significant (Spearman's $r = 0.472$; $P = .008$).

Neonatal outcomes are shown in Table 3. There was no correlation between the amount of fluid infused, the incidence of hypotension, the total dose of phenylephrine infused, and Apgar scores. There were weak negative correlations between the occurrence of maternal hypotension and the venous umbilical cord blood pH (Spearman's $r = -0.461$; $P = .031$) as well as between the total dose of ephedrine administered and arterial umbilical cord blood pH (Spearman's $r = -0.451$; $P = .035$). There was no correlation between all other variables and umbilical cord blood pH.

4. Discussion

Our results show that a volume of hydroxyethyl starch 130/0.4 of 733 mL contributed to prevent maternal hypotension in 50% of our subjects under the conditions of this study. To our knowledge, this is the first trial which has investigated a variable volume of fluid to prevent spinal anesthesia-induced hypotension in healthy term pregnant women undergoing elective caesarean section. Although an effective volume in 90% of the patients may be more clinically relevant than an effective volume in 50% of patients (MEFV), this study confirms the feasibility and usefulness of the up-down sequential allocation design in investigating the role of fluid preloading to prevent maternal hypotension.

The up-down sequential allocation design provides a significant advantage by centering the mass of the observations around the target dose (in our case, MEFV of HES). This statistical approach is characterized by the fact that, as opposed to dose–response finding studies, significantly fewer patients are needed to obtain a stable estimate of the target dose. The estimator of the ED50 is obtained by isotonic regression which is extensively described by Pace and Stylianou [14]. The up-down sequential allocation method has proven useful in comparing local anesthetic potencies for epidural analgesia in labor [16]. Our study

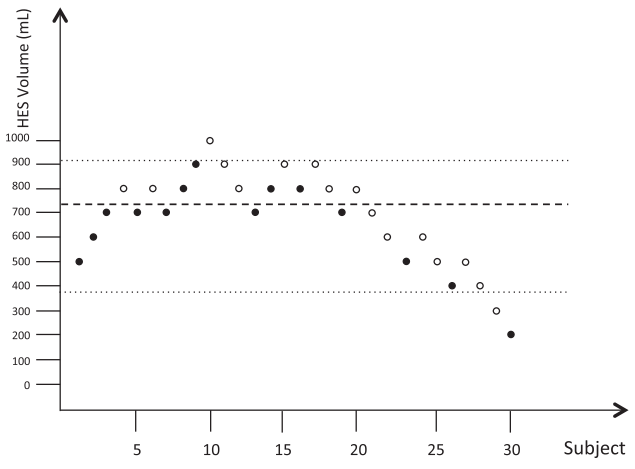

Fig. 2. Patient treatment response up-down sequence Volume infused for each patient (*y-axis*) ordinally ordered from first to last (*x-axis*). Successful (○) and failed fluid treatment (●) are represented. Bold dotted line (–) represent the minimal effective fluide volume (ED50) and fine dotted lines (···) represent upper and lower 95% confidence interval limits.

suggests that this design may become a useful tool to compare different fluid loading regimen in parturients undergoing caesarean section under spinal anesthesia.

The infusion of the HES study volume in the 10 minutes before spinal anesthesia increased maternal cardiac output, stroke volume and heart rate. These results confirm conclusions from previous investigations and suggest a benefit from pre-spinal colloid rapid infusion: a moderate intravenous bolus of HES may optimize stroke volume and cardiac output [8]. The maternal cardiac output and heart rate decreased over the 20 minutes following the onset of spinal anesthesia and phenylephrine infusion, while the stroke volume remained constant throughout the study period. Here as well, our results support previous data by Dyer etal who showed that there was a statistically significant correlation between the cardiac output and heart rate in mothers receiving spinal anesthesia for caesarean section [17]. The decrease in maternal cardiac output was not associated with a decrease in stroke volume. Our results confirm the conclusions from McDonald etal study in which the investigators showed, using a suprasternal Doppler device, that the maternal stroke volume remained constant during the 20 minutes following the onset of spinal anesthesia for elective caesarean section [11]. The absence of correlation between the timing of the spinal anesthesia and the incidence of maternal hypotension or its surrogates suggests that preoperative fasting has no significant hemodynamic effects. Altogether, this results support the postulate that spinal anesthesia-induced maternal hypotension is caused by a decrease in systemic vascular resistance, more specifically arterial resistance rather than by a decrease in cardiac preload and cardiac output [18].

Although fluid infusion improved maternal hemodynamic parameters as described above, there was no correlation between the infused volume of HES and the magnitude of changes in the hemodynamic parameters measured before nor after the spinal anesthesia. It is possible that maternal autoregulation could alter venous tone in order to minimize extreme variations in stroke volume and cardiac output. Alternatively, we postulate that at term gestation, the low-resistance uteroplacental vascular bed, the increased vascular distensibility, the extensive collateral venous network and the pregnancy-associatedneurohumoral-induced vasodilation may result in a high central venous capacitance [19-21]. Rapid increases in central volume, as would occur after a rapid infusion of a fluid bolus, could sufficiently raise central venous pressure and venous return to result in an improved stroke volume and cardiac output. However, owing to this high capacitance, greater fluid bolus volumes may not result in correlated changes in central venous pressure and in maternal cardiac output. Under the influence of the spinal anesthesia-induced sympathetic blockade, which impedes on vascular autoregulation, our « high capacitance » hypothesis remains valid and would support our hypothesis for this absence of correlation between maternal hemodynamic measurements and HES bolus volumes. Lastly, our study, with a sample size of 30 subjects, has not been powered to show a correlation between these variables.

The clinical advantage of a fluid bolus on maternal and neonatal outcome is matter of debate. Jackson etal compared a pre-spinal bolus of 1000 mL of crystalloid solution with a 200 mL bolus in mothers undergoing a caesarean section under spinal anesthesia [4]. All patients received a prophylactic infusion of ephedrine to prevent maternal hypotension. The investigators showed that there was no statistically significant difference between the two groups in terms of the incidence of hypotension, vasopressor consumption, cord blood pH and Apgar scores. In contrast, Ngan Kee etal showed that a combination of a crystalloid solution bolus of 2 L with a prophylactic phenylephrine infusion compared to no fluid bolus reduced the incidence of maternal hypotension from 30% to 2% [22]. One could conclude from these studies that in healthy mothers receiving a phenylephrine infusion, the benefit of a fluid bolus may be limited to an improvement in maternal blood pressure without any clinical advantage for the neonate. It is becoming more accepted that the drop in maternal cardiac output associated with a prophylactic phenylephrine infusion is probably associated

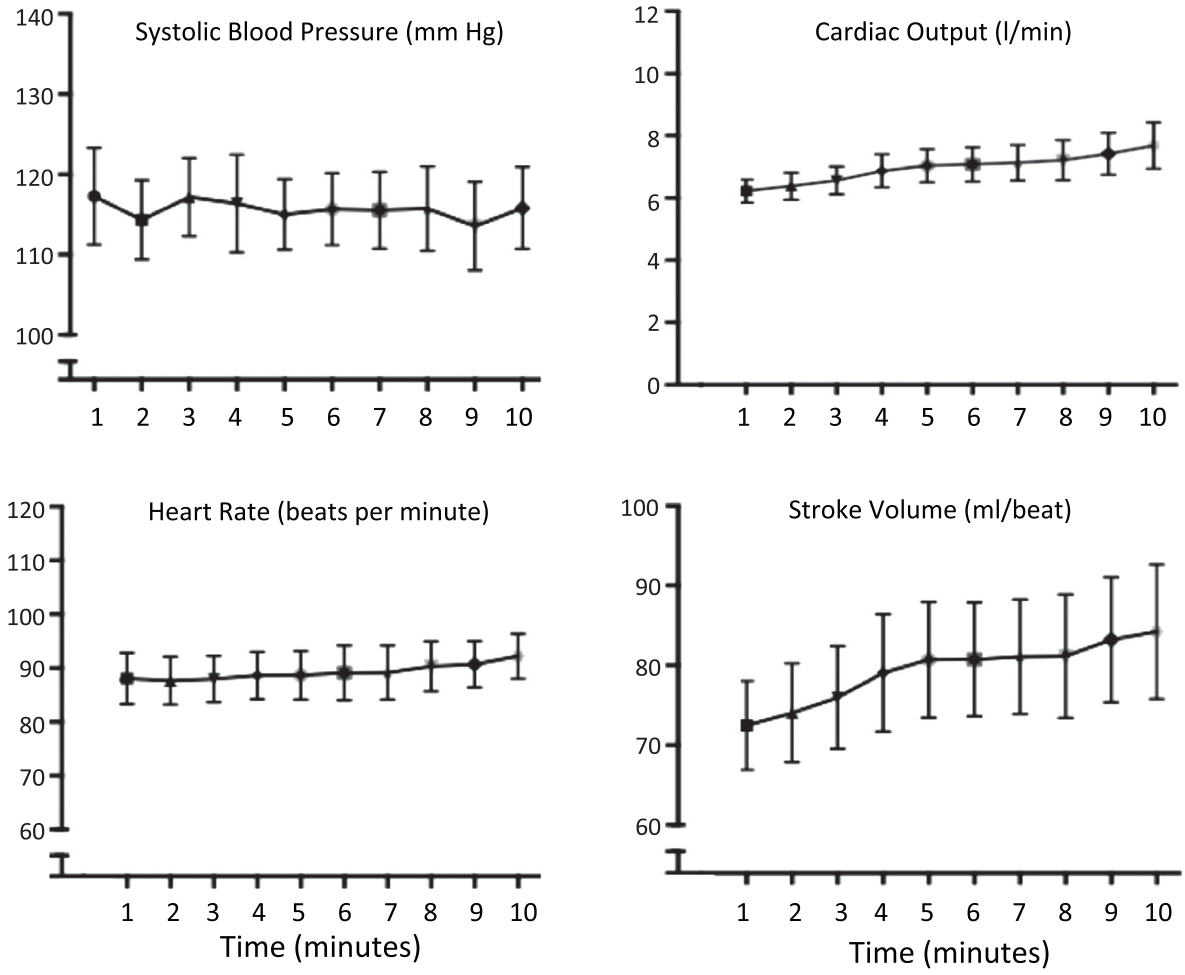

Fig. 3. Hemodynamic measurements during the 10 minutes following the initiation of the HES bolus. Dots represent mean value and whiskers represent 95% confidence intervals.

with a reflex bradycardia rather than a decrease in ventricular filling or in stroke volume [11,17]. Further investigations on the benefit of optimizing stroke volume and cardiac output with a fluid bolus when alpha-agonist infusions are used are warranted.

We decided to use hydroxyethyl starch rather than a crystalloid for several reasons. First, Ueyama etal determined that 100% of HES volume remain in the intravascular space 30 minutes after the infusion as opposed to 28% of the infused volume of crystalloid [23]. Because the time interval of our measurements spanned over 30 minutes, we favored HES as we were fairly confident that the infused fluid would still impact on the mothers' hemodynamics throughout this timeframe. Second, the literature has shown that there is no advantage of colloid coloading over preloading in terms of the preventing hypotension [9,24-28]. Since our trial design included hemodynamic measurements in the 10 minutes preceding the spinal injection, we considered it more appropriate to use colloids. Recently, the Food and Drug Administration and the European Medicines Agency have issued black box warnings with regards to the use of HES in critically ill adult patients, owing to an associated increased risk of kidney injury and mortality, as well as in patients undergoing cardiac surgery with cardiopulmonary bypass because of an increased risk of post-operative coagulopathy. They howeverallowed for their continuous use for treatment of acute hypovolemia due to acute blood loss in limited settings, including elective caesarean deliveries. Although some trials suggest HES-induced mild coagulation impairement as measured by thromboelastrography, the clinical relevance of these findings is probably negligible in elective caesareandeliveries [29,30].

The bioreactance technology used by the NICOM to measure hemodynamic parameters provides the significant advantage of being completely non-invasive. The validation of this device is currently limited to non-pregnant patients although there are a few reports of its use in the pregnant population [31-33]. Trends in ejection volume and cardiac output observed in our study are similar to those reported in other studies on the same population using different cardiac output monitoring devices [8,11,17]. Therefore the NICOM machine may be useful to assess trends in hemodynamic parameters. Validation of the NICOM in the obstetric population is nevertheless needed before introducing this device into routine clinical practice.

Our study has some limitations. Firstly, the strict definition of hypotension, which was defined as a single episode of systolic hypotension below 80% baseline value, may have overestimated our primary outcome results. In half of our subjects, a hypotensive episode occurred only once and was resolved with a single phenylephrine bolus. Had we used a less stringent definition of hypotension in which, for example, two consecutive hypotensive episodes below 80% baseline were necessary to confirm failure of treatment, the successful response rate to the study treatment might have been higher, and consequently our estimated MEFV lower? Secondly, baseline vital signs were taken minutes before the caesarean procedure in the waiting area. This stressful environment may have caused an overestimation of the true baseline values. Lower baseline vital signs could have led to a lower incidence of hypotension and, as noted above, lowered the MEFV. Thirdly, the shape of the up-down curve suggests a higher rate of hypotension at the beginning of the study than at the end. The IV tubing sets available

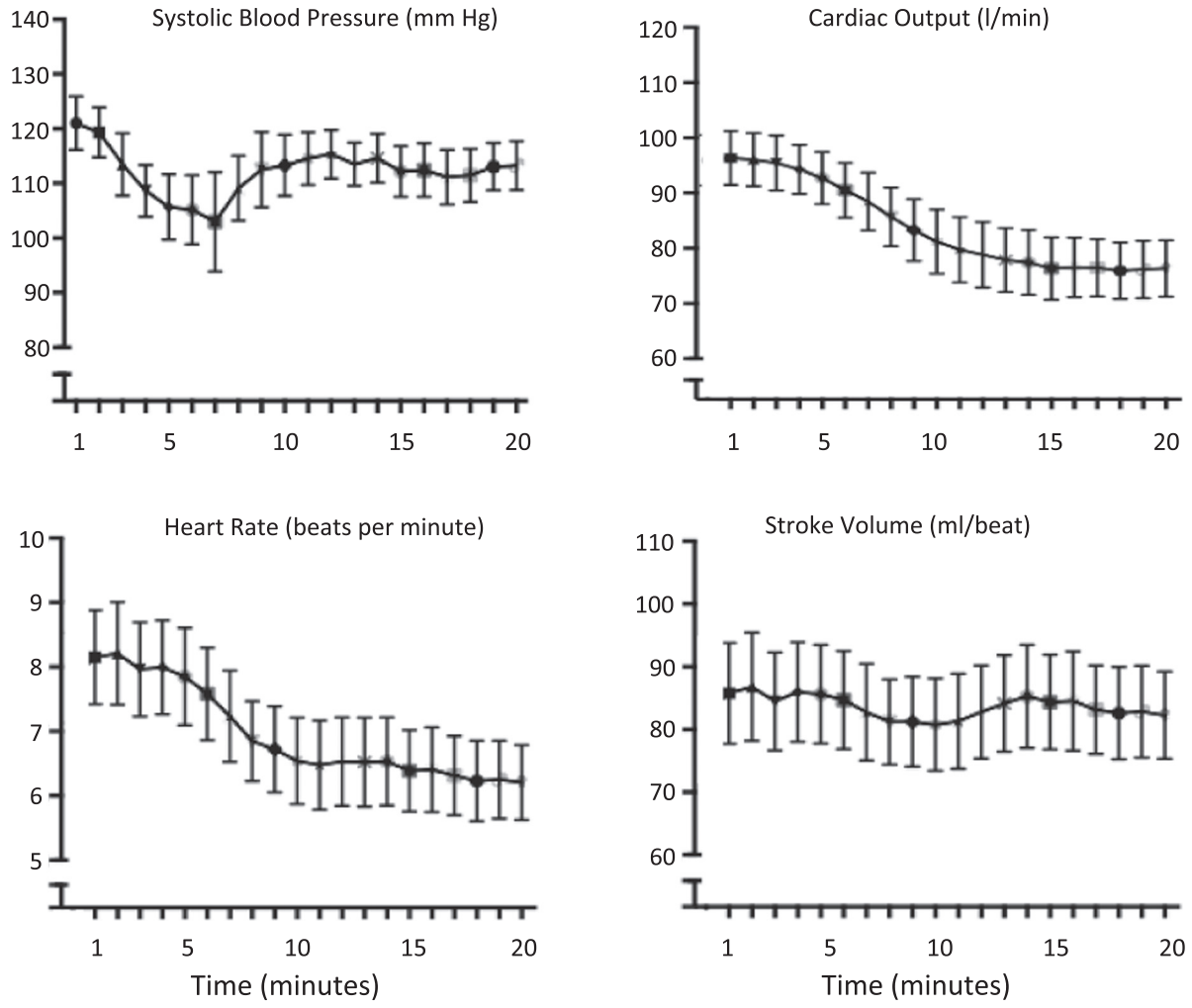

Fig. 4. Hemodynamic measurements during the 20 minutes following the adoption of the patient's left-wedged supine position after the injection of spinal anesthesia Dots represent mean value and whiskers represent 95% confidence intervals.

in our institution are provided with an injection port (to which was connected the phenylephrine infusion) located at 20cm distal to the patient and another port located at 50cm to the patient. In the first 10 patients, we did not control for the port to which we connected the phenylephrine infusion. It might have taken significantly more time for the phenylephrine to reach the subject's intravenous space when the infusion was connected to the 50cm port, potentially leading to an increased maternal hypotension rate However, a close analysis of our data (data not shown) shows that the episodes of hypotension occurred during the first 8 minutes after the spinal injection in all patients but 2, suggesting that connecting the perfusion line to the 20cm port did not prevent early maternal hypotension. Therefore, this limitation cannot completely explain the shape of the curve in Fig. 2. Finally, The literature suggests that including between 20 and 40 patients will provide a stable estimate of the target volume [14]. Our sample size of 30 patients did not allow for our results to be centered around the MEFV, as reflected by the absence of a plateaued curve in Fig. 2. Our data cannot refute that the up-down curve – and the MEFV– might have been sustained down toward lower values had we included at least 40 patients.

In conclusion, the effective dose of HES 130/0,4 that would prevent hypotension in 50% of mothers undergoing a caesarean section under spinal anesthesia while receiving a prophylactic phenylephrine infusion was 733 mL. Our study confirms the feasibility and usefulness of the up-down sequential allocation design in investigating the role of fluid infusion and in comparing different fluid loading regimens as part of an overall strategy to reduce maternal hypotension.

Table 3
Neonatal outcomes.

Variable	Value
Apgar 1[a]	9 [9–9]
Apgar 5[a]	9 [9–10]
Cord blood arterial pH[b]	7,25 (0,04)
Cord blood venous pH[b]	7,31 (0,03)

[a] Data expressed as median [interquartile range].
[b] Data expressed as mean (standard deviation).

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The authors would like to acknowledge the dedication of Mrs. Nadia Godin, registered research nurse, without whom the completion of this study would have not been possible. The authors would also like to thank Drs Nathan Pace and Mario Stylianou for their invaluable help providing additional information which allowed the authors to perform the isotonic regression analysis on their data.

Appendix A

The Non Invasive Cardiac Output Monitor (NICOM) (Cheetah Medical Inc., Boston, MA, USA) is a completely noninvasive device which uses the principle of bioreactance to measure beat to beat phase variations of subclinical electrical waves passing through the patient's thorax. The

NICOM is comprised of a high-frequency (75 kHz) sine wave generator and four dual-electrode "stickers" that are used to establish electrical contact with the body. Within each sticker, one electrode is used by the high-frequency current generator to inject the high-frequency sine wave into the body, while the other recording electrode is connected to a voltage input amplifier. Two electrodes are placed on the right side of the body, and two stickers are placed on the left side of the body. The stickers on a given side of the body are paired, so that the currents are passed between the outer electrodes of the pair and voltages are recorded from between the inner electrodes. A noninvasive cardiac output value signal, derived from the electrical wave shift phase, is thus determined separately from each side of the body, and the final noninvasive CO result signal is obtained by averaging these two signals [13].

Appendix B

Isotonic regression technique uses the implicit assumption in dose–response determination that the drug effect will increase with increasing doses. However, biologic and experimental variability may produce unexpected ups and downs in the observed response rate as dose increase. Therefore, the observed response rate may not be necessarily monotonically increasing with increasing concentration. Isotonic regression is a variant of restricted least squares regression which constrains the point estimates to be either monotonically increasing (never decreasing or monotonically decreasing (never increasing). This monotonic response rate is obtained by means of an adjusted response probability (p_k) easily calculated for each assigned dose by the pooled-adjacent-violators algorithm (PAVA) [14].

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