Effects of colloid preload on placenta stereology and cord blood S100β protein during cesarean section under spinal anesthesia

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Abstract: Objective To determine the optimal dose of colloid preload, which is both safe and effective, for preventing hypotension in parturients undergoing cesarean section under spinal anesthesia. Methods Forty-five healthy, term parturients scheduled for cesarean delivery under spinal anesthesia were randomly assigned to 3 colloid preload groups to receive gelofusine infusion at the rates of 5, 10, or 15 ml·kg⁻¹·h⁻¹ (groups I, II, and III, respectively). Colloid preload was administered 10 min before spinal anesthesia and maintained until the delivery. Blood pressure (BP) and heart rate (HR) of the parturients were monitored during the operation, and Apgar scores at 1 and 5 min after birth were recorded. S100β protein concentration and blood gas values of the umbilical artery were also measured. The vascular adaptation in the placental villous capillary was evaluated stereologically. Results At each time point of measurement, BP and HR showed no significant differences among the 3 groups during the operation (P>0.05), but within the same group, BP and HR underwent significant variations during the operation; groups II and III maintained more stable hemodynamics compared to group I. Apgar scores and blood gas analysis, pH value, and S100β protein in the umbilical artery showed no significant differences among the 3 groups (P>0.05). The 3 groups exhibited no significant differences in the length and volume density of the placental villous capillaries (P>0.05). Conclusion Colloid preload with gelofusine administered at the rate of 10 ml·kg⁻¹·h⁻¹ can reduce the incidence and severity of hypotension in cesarean section under spinal anesthesia with the least adverse maternal and fetal effects.

Key words: cesarean section; spinal anesthesia; maternal hypotension; stereology; S100β protein; volume preload; colloid preload

INTRODUCTION

Spinal anesthesia is currently the most desirable anesthetic technique during cesarean section in China, but it is associated with a high risk of maternal hypotension, which may result in both maternal and neonatal morbidities. Severe hypotension may cause maternal unconsciousness, pulmonary aspiration, apnea, cardiac arrest, and impairment of the placental perfusion, which can very likely lead to fetal hypoxia, acidosis and neurological injuries.

Several prophylactic measures can be taken to prevent maternal hypotension, such as the administration of vasopressors (epinephrine, phenylephrine, etc.), uterine displacement, and volume loading. Colloid preload is well recognized for its efficacy in preventing maternal hypotension and has become the method of choice by many anesthesiologists. But so far no definite criteria have been established in regard to the optimal dose of colloid administration. Colloid over-loading may potentially cause serious adverse effects including excessive hemodilution, pulmonary edema and increased cardiac burden, whereas insufficient loading can attenuate the efficacy. We hence conducted this study to determine the optimal dose of colloid preload, which is both effective and safe, for spinal anesthesia during cesarean delivery.

PATIENTS AND METHODS

Patients and study design

This prospective, randomized, double-blinded trial was conducted with approval by Jinan University Ethics Committee. All the participants in this study gave their written informed consents after being informed of the procedural details, and the sample size was estimated statistically.

Inclusion criteria: women aged 20 to 35 years with ASA class I and II who had uncomplicated, singleton, term pregnancy and were scheduled for elective cesarean delivery under spinal anesthesia were enrolled. Exclusion criteria: women with diabetes, chronic or pregnancy-induced hypertension, cardiac disease,
height <155 cm, a booking body mass index >40 kg/m², contraindications to spinal anesthesia, or any known fetal abnormality were excluded.

The parturients were randomized, using a computer-generated randomization list, into 3 treatment groups (15 in each group) to receive colloid preload with gelofusine administered at the rate of 5 ml·kg⁻¹·h⁻¹ (group I), 10 ml·kg⁻¹·h⁻¹ (group II), and 15 ml·kg⁻¹·h⁻¹ (group III). All the parturients were allocated before being transferred to the theatre, where the baseline measurements of heart rate (HR), systolic blood pressure (SBP) and oxygen saturation (SpO₂) (measured by a Mairui monitor) were recorded. The preload was administered 10 min prior to spinal block, and the loading was discontinued after the delivery. Supplemental oxygen was administered through a nasal catheter at the rate of 2 L/min.

Anesthetic protocol

Lumbar puncture for combined spinal epidural anesthesia (CSEA) was performed with the patient in the left-lateral position. After skin disinfection, a skin wheal was raised with 1% lidocaine at the L₃-L₄ interspace where a 26 G Atracana spinal needle (B. Braun Melsungen, Germany) was inserted. All the parturients received a standard dose of spinal solution (8 mg of 0.5% bupivacaine) after confirmation of the free flow of cerebrospinal fluid. After the injection, the parturients were immediately turned supine with a 30° left-lateral tilt. Sensory and motor measurements were recorded at 8 time points, namely before CSEA (T₀), at 1 min (T₁), 3 min (T₂), 5 min (T₃), and 10 min (T₄) after CSEA, at delivery (T₅), and after the operation (T₆).

Hypotension was defined as a decrease in systolic pressure by >20% of baseline or to <90 mmHg, and was managed with 6-mg increments of ephedrine until resolution. Smaller decreases in blood pressure were similarly treated if accompanied by nausea, vomiting or dizziness. The Apgar scores of the infants at 1 and 5 min after birth were also recorded.

Umbilical artery blood gas analysis

After the umbilical cord was clamped during delivery, the blood from the umbilical artery was drawn into 1-ml heparinized syringes. Gas analysis was performed immediately using a Rapid Lab348 blood gas analyzer (Bayer, Germany). Five milliliters of blood samples from the umbilical artery without heparin treatment were centrifuged at 3000 × g for 10 min, and the supernatants were stored at -80 °C for later assay of S100β protein.

S100β protein concentration analysis

S100β protein levels were measured by indirect enzyme-linked immunoabsorbent assay (ELISA) using an ELISA kit (Zhongshan Company of China) according to the manufacturer’s instructions.

Statistical analysis

The data was presented as Mean±SD. The data was tested for statistical significance by One-way ANOVA and repeated measures ANOVA. A P value less than 0.05 was considered to indicate a statistically significant difference. All the analyses were performed using SPSS 16.0.

RESULTS

According to the inclusion criteria, 45 parturients were enrolled and all of them completed this study. The general maternal demographic data, time of spinal anesthesia to delivery, and incidence of maternal hypotension in the 3 groups were summarized in Tab.1 and Fig.1. The 3 groups were similar in age, weight, height, BMI, gestational week, time from spinal anesthesia to delivery, and neonatal Apgar score at 1 min and 5 min after birth (P>0.05). Seven parturients in group I experienced hypotension episodes during the delivery, a rate significantly higher than that in the other two groups.

Maternal hemodynamics

The hemodynamic data were shown in Tab.2. The baseline SBP was similar among the 3 groups. SBP dropped significantly from the baseline at T₁ in group I and at T₂ in groups II and III, but increased at T₃ and became normal afterwards in all the groups. A significant increase in HR was found in all the 3 groups after CSEA at T₃. At T₄ and T₅, a significant decrease of HR was noted in all the 3 groups.

Neonatal data

Stereological evaluation of vascular adaptation in placental villous capillary

Shortly after the delivery of the fetus, the placenta near the umbilical cord was collected and fixed in 10% formalin for over 24 h. CD34 was used to mark the endothelial cells of the placental villous capillary, and the specimen was sliced for analysis with the Envision technique. The three-dimensional (3D) structural parameters (Qₓ, Pₓ, Pᵧ) and the sectional images were obtained using Leica MPS 60 stereological microscope. Finally, the length density (Lv) and volume density (Vᵥ) were calculated using the equations below:

\[
Lv = \frac{\sum_{i=1}^{n} Qxi}{\sum_{i=1}^{n} Aci} = \frac{2 \sum_{i=1}^{n} Qxi}{a \sum_{i=1}^{n} Pci} \quad Vᵥ = \frac{\sum_{i=1}^{n} Pxi}{a \sum_{i=1}^{n} Pci}
\]

(Qₓ=the quantity of the capillaries; Pₓ=the test point fall down on the villus; Pᵧ=the test point fall over the cross section of capillaries).

Statistical analysis

The data was presented as Mean±SD. The data was tested for statistical significance by One-way ANOVA and repeated measures ANOVA. A P value less than 0.05 was considered to indicate a statistically significant difference. All the analyses were performed using SPSS 16.0.
The results of umbilical cord blood gas analysis and S100β levels are summarized in Tab.3, which showed no significant differences among the 3 groups (P>0.05). The infants had Apgar scores of 8 to 9 at 1 min after delivery and 9 to 10 at 5 min, showing no significance difference among the 3 groups neither (P>0.05).

**Histological and stereological analysis**

As shown in Fig.2, the vascular endothelial cells (VEC) were visualized by immunohistochemistry for CD34 with the nuclei stained blue under Leica MPS 60 stereological microscope. The length density (Lv) and volume density (Vv) of the villous capillaries calculated from these images, as shown in Tab.4, were comparable among the 3 groups (P>0.05).

**DISCUSSION**

Our previous work demonstrated that the preloading volume with colloid (Voluven) could maintain stable hemodynamics during spinal anesthesia [7]. In addition, the procedure efficiently improved tissue perfusion, microcirculation, and utero-placental blood flow, and increased oxygen supply to the fetus. Colloid preload is an effective means to prevent hypotension associated with spinal anesthesia in patients undergoing cesarean delivery. Preloading with gelofusine at a higher loading rate (in groups II and III), as we found in this study, obviously lowered the incidence of hypotension compared to a low loading rate of 5 ml·kg⁻¹·h⁻¹ in group I. This result is in agreement with several published studies which demonstrated the efficacy of volume loading with colloids in preventing hypotension [5,8-9].

Low-dose colloid preload might not be able to prevent hypotension, while large-volume infusions may potentially increase the risk of cardiac overload [10] and excessive hemodilution to result in physiological anemia [11]. Sudani et al tried to establish a correlation between the amount of colloid load and urine specific gravity but failed [12]. Currently there are few, if any, investigations conducted to address the effective preloading volume. In this study, we found that SBP in group I showed unstable changes compared to that in groups II and III, suggesting that a greater preloading volume achieves better hemodynamic stability.

We found no differences in neonatal outcomes in regard to cord blood gases and Apgar scores between the 3 groups. This finding is consistent with that by Carvalho et al [13]. We presume that this may be attributed to increased blood flow of the placenta by volume preloading. However, the effects of maternal hypotension on neonatal outcomes remain unclear. We chose S100β protein as an index for evaluating the neonatal outcome for its sensitivity in reflecting brain damage [14] in the intrauterine fetus. Under normal circumstances, S100β protein is not highly expressed with a venous concentration ranging from 0.02 to 0.2 μg/L [15], and an increased expression of

<table>
<thead>
<tr>
<th>Index</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>1.6±0.0</td>
<td>1.6±0.1</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.0±9.0</td>
<td>70.3±6.1</td>
<td>63.8±7.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>27.9±3.1</td>
<td>27.4±2.2</td>
<td>26.3±1.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6±8.0</td>
<td>27.6±9.0</td>
<td>26.3±1.7</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>30.0±7.9</td>
<td>29.6±5.8</td>
<td>29.6±5.8</td>
</tr>
<tr>
<td>Number of patients with hypotension</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are Mean±SD if not indicated otherwise. *P<0.05 vs group I.

**Tab.2 Hemodynamic data of the 3 groups during the operation (Mean±SD)**

<table>
<thead>
<tr>
<th>Index</th>
<th>Group</th>
<th>T₀</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
<th>T₄</th>
<th>T₅</th>
<th>T₆</th>
<th>T₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (min⁻¹)</td>
<td>I</td>
<td>82.6±8.7</td>
<td>92.2±20.2</td>
<td>100.4±15.0</td>
<td>92.6±20.2</td>
<td>87.6±19.7</td>
<td>86.5±15.7</td>
<td>87.4±15.1</td>
<td>86.4±14.7</td>
</tr>
<tr>
<td>II</td>
<td>81.9±10.3</td>
<td>89.9±16.1</td>
<td>100.1±14.3</td>
<td>93.6±20.0</td>
<td>90.6±14.5</td>
<td>85.5±12.0</td>
<td>83.2±12.3</td>
<td>83.0±12.2</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>81.5±7.1</td>
<td>90.9±22.6</td>
<td>99.0±18.5</td>
<td>90.2±17.9</td>
<td>85.3±18.6</td>
<td>83.8±15.1</td>
<td>84.3±16.5</td>
<td>85.7±16.3</td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>I</td>
<td>120.9±9.2</td>
<td>111.0±12.4</td>
<td>101.3±11.9</td>
<td>103.0±14.2</td>
<td>115.8±19.3</td>
<td>112.3±20.7</td>
<td>111.1±13.0</td>
<td>111.4±11.2</td>
</tr>
<tr>
<td>II</td>
<td>122.5±10.6</td>
<td>114.2±13.4</td>
<td>109.5±13.4</td>
<td>107.8±10.3</td>
<td>100.7±13.3</td>
<td>113.5±13.6</td>
<td>115.7±12.4</td>
<td>111.1±11.6</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>122.2±9.8</td>
<td>116.9±11.4</td>
<td>111.4±10.6</td>
<td>109.1±10.6</td>
<td>114.0±13.0</td>
<td>111.2±17.0</td>
<td>114.7±12.8</td>
<td>112.2±10.7</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 vs T₀; *P<0.05; **P<0.01 vs T₀.
S100β protein in the cerebrospinal fluid often indicates brain cell damages. In our case, the concentration of S100β protein was comparable between the 3 groups, which, we presume, could be a result of sympathetic block occurring within minutes following the initiation of spinal anesthesia, and also of an unsynchronized timing of the maximum intravascular volume expansion by colloid co-load and the maximum S100β protein variation.

Volume preloads at different rates can have different effects on placental perfusion. Stereology offers a toolkit of transparent sampling rules and simple estimation tools which allowed 3D quantities to be calculated from 2D images appearing on slice planes [16].

It can measure the ability of a placenta to transfer oxygen and nutrients by passive diffusion. The samples from each group demonstrated a great abundance of placental villi, yet there were no significant differences among the groups either in length or volume density of the villous capillaries. This can be the consequence of good placental perfusion after colloid preload which ensures sufficient oxygen supply to the fetus. The length and volume densities of the villous capillaries reflect the status of uteroplacental circulation and therefore the activity of oxygen exchange.

In conclusion, the tested volumes of colloid preload in this study resulted in similar maternal hemodynamics, umbilical artery blood gas values, S100β protein concentration and the placental indexes. Gelofusine preload demonstrated better buffer effect at the rate of 10 and 15 ml·kg⁻¹·h⁻¹ than at the rate of 10 ml·kg⁻¹·h⁻¹. Taking into account that 1L HES preload before anesthesia would potentially increase maternal cardiac output [37], we conclude that gelofusine infusion maintained at the rate of 10 ml·kg⁻¹·h⁻¹ initiated 10 min before anesthesia is optimal to ensure a steady oxygen supply for the best maternal and fetal benefits.

REFERENCES


