

# Comparison of Hemostasis Parameters in Vaginal versus Cesarean Deliveries

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Brooke Hendrick, BSN, RNAS  
Dawn Jones, BSN, RNAS  
Matt Williams, BSN, RNAS

Approved By:

Mohan Achwal 7/18/02  
Date  
Mohan Achwal, MD  
Anesthesiologist

Francis Gerbasi 8/7/02  
Date  
Francis Gerbasi, CRNA, PhD  
Director, Anesthesia Program

Cheryl Hopkins 7-25-02  
Date  
Cheryl Hopkins, CRNA, MS  
Staff Nurse Anesthetist

Abdelaziz Saleh 8-1-02  
Date  
Abdelaziz Saleh, MD  
Director of Maternal Fetal Medicine

Harland Verrill 7/11/02  
Date  
Harland Verrill, PhD  
Clinical Biochemist

University of Michigan-Flint/Hurley Medical Center  
Master of Science in Anesthesia Program  
Flint, Michigan

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### **Abstract**

Several studies have documented coagulation changes that occur with normal vaginal deliveries, while several others have shown the beneficial effects of epidural analgesia in vascular and orthopedic procedures. One study has documented the beneficial effects of epidural analgesia in normal vaginal deliveries. To date, no studies have been conducted examining *in vivo* hemostasis activity during cesarean delivery with spinal anesthesia in normal pregnancies.

This is a prospective longitudinal study measuring hemostasis markers in healthy parturients having a cesarean section with spinal anesthesia. The sample population consists of 15 patients, aged 18-45, ASA classification of II, and  $\geq 34$  weeks gestation scheduled for cesarean delivery. Four blood samples were drawn at the following times: prior to cesarean section, immediately following cesarean section, 3 hours and 24 hours postpartum. The hemostasis tests included: D-dimer, fibrin-fibrinogen degradation products (FDP), beta-thromboglobulin (BTG), platelet factor 4 (PF 4), platelet count, hemoglobin, hematocrit, prothrombin fragments 1+2 (PF 1+2), antithrombin III (AT III), alpha-2-antiplasmin (A2A), fibrinogen, and thrombin-antithrombin III (TAT) complexes. These results were compared to a previous study which examined healthy parturients who received epidural anesthesia and healthy parturients without epidurals having a vaginal delivery.

The sample consisted of a total of 75 patients to detect a 1 standard deviation difference between the three groups. Analysis of Variance for Repeated Measures with Post Hoc T-tests was used to detect differences in the descriptive variables and hemostasis markers. A p value  $< 0.05$  was accepted as significant. The power for this study with an effect size of .60 is .59.

In the cesarean group, PF 4 and BTG levels showed the greatest increase 3 hours postpartum. Antithrombin III was decreased at delivery and 3 hours postpartum, while PF 1+2 and TAT complex increased at delivery and 3 hours postpartum. Alpha-2-antiplasmin decreased at delivery and 3 hours postpartum. An increased frequency of a positive D-dimer was found with each blood draw, and the percent of FDP in the 10-40 ug/mL range increased successively in the first three draws. These findings suggest an increase in platelet, coagulation, and fibrinolytic activity at delivery and in the immediate postpartum period in the cesarean section group.

There was no significant difference in platelet activation among the groups. Platelet factor 4 levels were significantly greater in the non-epidural group at delivery. The cesarean group did show significantly lower AT III levels at delivery, 3 hours and 24 hours postpartum ( $p < 0.005$ ) when compared to both vaginal groups. While not statistically significant, the cesarean group demonstrated lower levels of fibrinogen, A2A, and platelets at delivery and 3 hours postpartum compared to the vaginal groups. Overall, similar changes in hemostasis occurred among the three groups. However, the greater decrease in AT III, fibrinogen, A2A and platelets in the cesarean group may reflect an increase in platelet, coagulation, and fibrinolytic activity with cesarean section as compared to vaginal delivery.

**Key Words:** hemostasis, epidural, spinal anesthesia, coagulation, cesarean section.

## Chapter I

### Introduction

#### **Hemostasis**

Hemostasis is a complex process that allows the formation of clots to prevent blood loss in the event of vessel damage. This involves three primary components: the blood vessel wall, platelets, and coagulation. The endothelial lining of the blood vessel wall contributes to hemostasis by synthesizing and secreting various substances such as prostaglandins, prostacyclin, von Willebrand factor, tissue plasminogen activator, thromboplastin, and thrombomodulin.<sup>1</sup> The endothelial lining inhibits platelet aggregation by prostacyclin, thrombomodulin, and protein S production, which promote fibrinolytic activity.<sup>1</sup> The vessel wall also promotes clot formation. Initial damage to a blood vessel results in release of thromboplastin from the endothelial cells, activating the clotting system.

Platelets are the second major component of hemostasis. Platelets are 2-3 micrometers in diameter and have a circulating life span of 9-12 days. Thirty percent of platelets are stored in the spleen, ready for release in response to stress. Platelets are initially activated by contact with exposed subendothelial collagen following damage to a blood vessel. This causes a conformational change in the platelets that results in multiple pseudopodia which both increase surface area and enhance platelet clumping. After this change in shape, platelets adhere to the exposed endothelium due to an interaction between

plasma von Willebrand factor and membrane glycoprotein Ib. Von Willebrand factor is a type of factor VIII that mediates platelet adherence.<sup>2</sup>

Once adhesion occurs, activation and aggregation follows. Platelet activation ends with the release or secretion of both dense and alpha granules. The release of these granules further amplifies the platelet activation process. Beta-thromboglobulin and platelet factor 4 are platelet alpha-granule proteins released during platelet activation. Both serve as markers for platelet activity *in vivo*.<sup>3,4</sup> Aggregation refers to the affinity of platelets for each other. Platelet aggregation results from the binding of fibrinogen to specific platelet surface receptors, the glycoproteins IIb and IIIa.<sup>2</sup> The ultimate result is the formation of the primary hemostatic plug.

In addition to forming the primary hemostatic plug, platelets also have specific coagulant activities. The prothrombinase complex that results in the activation of prothrombin is assembled and stabilized on the membrane surface of aggregated platelets. Thrombin is also formed on the platelet surface and is dispersed in the blood to convert fibrinogen to fibrin. Lastly, platelets can play a role in clot retraction by pulling back its pseudopodia.<sup>2</sup>

The third component of this complex process is the coagulation cascade which results in the formation of the fibrin clot. The coagulation cascade begins as two separate pathways and converges to one common pathway. The intrinsic system is initiated with the binding of factor XII to an exposed, negatively charged surface, such as damaged endothelium.<sup>3</sup> Activation of factor XII is followed by activation of factor XI. Platelet factor III is necessary for the reaction

in which factors IX, VIII, VII, and calcium activate factor X.<sup>2</sup> The result is activated factor X.<sup>1,3,5</sup> All of these plasma factors are produced by the liver, with the exception of factor VIII-von Willebrand factor, which is produced by vascular endothelium. (Appendix A) In the extrinsic pathway, thromboplastin release activates factor VII. The thromboplastin-factor VII complex accelerates the activation of factor X.<sup>3</sup> Activated factor X from both the intrinsic and extrinsic pathways then enters the common pathway.

In the common pathway of the coagulation cascade, prothrombin is cleaved to thrombin in the presence of activated factor Xa and, in the process, prothrombin fragments 1+2 are produced.<sup>4</sup> Prothrombin fragments 1+2 signify activation of the coagulation system. Thrombin-antithrombin III complex is an additional parameter that serves as a marker for thrombin formation.<sup>6</sup> Thrombin then interacts with fibrinogen to form fibrin monomers. Fibrinogen is a protein composed of a pair of alpha, beta, and gamma chains, and is soluble prior to thrombin binding to it. The fibrin monomers then combine to form polymers, which cross-link and surround the mass of platelets, forming an insoluble clot.<sup>1,2</sup> Fibrin is the endpoint of the coagulation cascade. Antithrombin III is a regulatory protein that inactivates thrombin and factor X, thus inhibiting coagulation. A decrease in antithrombin III level indicates increased thrombin binding secondary to increased thrombin generation.<sup>3</sup>

An important counter to the coagulation process is fibrinolysis. During clot formation, plasminogen is integrated into the fibrin mass, where it is later converted into plasmin. Since plasmin is integrated into the clot, fibrinolytic



activity is localized and disseminated intravascular coagulation (DIC) is prevented. Plasmin is an enzyme responsible for the degradation of factor VIII, fibrinogen, and fibrin. This results in the breakdown of clots and formation of fibrin-fibrinogen degradation products.<sup>2,3</sup> Alpha-2-antiplasmin, which is made by the liver, is a rapid inhibitor of plasmin activity.<sup>4</sup> After plasmin has lysed the clot, there are four degradation products: fragments X, Y, D, and E. Fragments X and Y are detected first, followed by D-dimer and E fragments.<sup>2</sup> These products impair hemostasis by inhibiting normal fibrin polymerization and by competing with fibrinogen for receptor binding sites on platelets. Detection of fibrin-fibrinogen degradation products in the circulation reflect evidence of clot formation and lysis of that clot.<sup>4</sup> D-dimer is a specific fibrin-fibrinogen degradation product and is associated with DIC.<sup>3</sup> (Appendix B)

Normal hemostasis is a complex process involving the blood vessel wall, platelet activation, and coagulation with fibrinolysis. Each of the three stages involve numerous steps with a variety of plasma factors and proteins. Because of its complexity, normal hemostasis can be affected and altered in many ways. Knowledge of normal hemostasis is thus essential in understanding the hemostatic effects of normal pregnancy and cesarean sections.

## Chapter II

### Definition of Terms

Term	Definition
<b>Alpha-2-antiplasmin</b>	Inactivates plasmin, interferes with absorption of plasminogen to fibrin, and undergoes cross-linking with the alpha chains of fibrin during clotting. <sup>4</sup>
<b>Antithrombin III</b>	Major inhibitor of blood coagulation by neutralization of thrombin, factor IXa, and factor Xa. <sup>7</sup>
<b>Beta-thromboglobulin</b>	An alpha granule protein secreted by activated platelets. BTG also serves as a marker for platelet activation. <sup>7</sup>
<b>Bupivacaine (Marcaine, Sensorcaine)</b>	A local amide anesthetic frequently used for regional analgesia. Bupivacaine 0.75% is used for spinal anesthesia (1-1.6 cc), max dose 3mg/kg).
<b>D-dimer</b>	One of four fibrin degradation products signifying impaired hemostasis. It is associated with Disseminated Intravascular Coagulation. <sup>3</sup>
<b>Fibrin-fibrinogen Degradation Products</b>	The result of fibrin clot degradation. FDP act as anti-coagulants by inhibition of thrombin and fibrin polymerization, and by interfering with platelet aggregation. The presence of FDP signify clot formation and lysis of that clot. <sup>1</sup>
<b>Fibrin monomers</b>	Molecules that polymerize into long fibrin strands that form the reticulum of the fibrin clot. <sup>4</sup>
<b>Fibrinogen</b>	A complex glycoprotein consisting of three polypeptide chains. Thrombin cleaves to fibrinogen to remove the polypeptide chains, forming fibrin monomers. <sup>4</sup>
<b>Platelet Factor 4</b>	An alpha granule protein absent from plasma until secreted by activated platelets. PF4 helps to promote blood coagulation at the site of vessel wall injury and is a marker for platelet activation. <sup>7</sup>
<b>Prothrombin Fragments 1+2</b>	Produced when prothrombin is cleaved to thrombin signifying activation of the coagulation system. <sup>4</sup>
<b>Thrombin-antithrombin III complex</b>	Formed when antithrombin III binds to thrombin to inhibit coagulation. Persons predisposed to thrombosis, such as in preeclampsia, have elevated concentrations of TAT complexes.

## Chapter III

### Literature Review

#### **Coagulation in Normal Pregnancy**

Several hemodynamic changes occur in normal pregnancy. Plasma volume increases by 50-55% during the second trimester. There is a dilutional decrease in red blood cell volume initially, followed by an increase of 30% above the prepregnant volume with an overall increase in total blood volume of 45% by the end of the third trimester. This increase is due to an increase in maternal hormone production.<sup>8</sup> Pregnancy also represents a state of accelerated intravascular coagulation due to increased platelet turnover, clotting, and fibrinolysis. Such changes in hemostasis that occur during normal pregnancy create a state of hypercoagulability to protect the parturient from hemorrhage at delivery.<sup>6</sup>

Increases during pregnancy are seen in platelet factor 4 and beta-thromboglobulin, both of which signify platelet activation.<sup>3</sup> Gerbasi, et. al. measured *in vivo* hemostasis activity in 70 healthy pregnant women by measuring specific hemostatic markers simultaneously during the delivery period. These markers included platelet factor 4, beta-thromboglobulin, platelet count and volume, fibrinopeptide A, antithrombin III, fibronectin, fibrinogen, alpha-2-antiplasmin, D-dimer, and fibrin degradation products. They found significant increases in fibrinopeptide A, beta-thromboglobulin, and platelet factor 4 indicating platelet activation at the time of delivery. These values normalized by 24-48 hours postpartum.<sup>9</sup> Their data also indicated an increase in the clotting

system as alpha-2-antiplasmin and antithrombin III levels were decreased at the time of delivery. This study, compared to previous studies on hemostasis during normal pregnancy, held greater significance because a large sample size was studied and each study participant was sampled at each time point. In addition, it was the first study to measure specific molecular markers for detection of *in vivo* hemostatic activity during the delivery period.

Increases in platelet width and volume are seen during pregnancy signifying increased platelet consumption. However, thrombocytopenia is generally not seen during normal pregnancy. Gerbasi, et. al. found slightly elevated platelet volumes during labor and at delivery. Platelet counts were only slightly decreased at delivery and slightly increased during the postpartum period.<sup>9</sup> Baker, et. al. cited several studies that found no significant changes in platelet count during normal pregnancy.<sup>5</sup> It has therefore been suggested that an increase in platelet production occurs to compensate for increased platelet activation.<sup>8</sup>

Changes in coagulation factors also occur in normal pregnancy. Increased concentrations of Factors I, VII, VIII, IX, X, and XII are seen at term gestation. Fibrinogen (Factor I) levels approximately double by 20 weeks gestation and remain elevated throughout pregnancy. Factor VII increases two-fold during the second trimester and remains elevated through the third trimester. A decrease in Factor XI and XIII to approximately 70% occurs in the third trimester. Prothrombin time and partial thromboplastin time decrease by 20%. Bleeding time is decreased by 10%. An increase is seen in fibrinopeptide A,

fibrin degradation products and plasminogen, while decreases are seen in antithrombin III.<sup>3</sup> The increase in coagulation factors and increased fibrinopeptide A signify activation of the clotting system.

Dahlman, et. al. studied 16 healthy parturients longitudinally from the first to the sixth week postpartum to determine if thrombosis prophylaxis should be discontinued after delivery. Factor VIII, fibrinogen, fibrinopeptide A, antithrombin III, plasminogen, tissue plasminogen activator, alpha-2-antiplasmin, urokinase inhibitors, fragment B 15-42 and kallikrein inhibitor were analyzed. Significant increases in fibrinogen, factor VIII, and fibrinopeptide A at one week postpartum were noted indicating increased coagulation and fibrinolysis. At 3 weeks postpartum, these parameters normalized.<sup>10</sup> This study, while indicating changes in hemostasis in the postpartum period, did not address significant changes that occur during pregnancy and the delivery period.

Previous studies have indicated a significant, gradual rise in prothrombin fragments 1+2 and thrombin-antithrombin III complexes throughout gestation which are markers for thrombin formation. Bremme, et. al. studied 26 normal pregnant women through gestation, at delivery, and during the puerperium. Platelets, fibrinogen, prothrombin complex, antithrombin III, Proteins C and S, fibrin, thrombin-antithrombin III complexes, D-dimer, plasminogen activator inhibitors 1+2, and cardiolipin antibodies were measured. Results indicated increased levels of thrombin-antithrombin complex as well as increases in D-dimer and FDP signifying thrombin formation.<sup>6</sup> This study also indicates a balance in fibrinolysis activity that occurs in pregnancy. An increase in

plasminogen activator inhibitors 1+2 was demonstrated indicating fibrinolysis inhibition countered by the presence of D-dimer and increased fibrin-fibrinogen degradation products.

Eichinger, et. al. studied 113 healthy pregnant women at 12, 22, and 34 weeks gestation and then 3 months after delivery. Their results indicate significant increases in prothrombin fragments 1+2 as well as increases in thrombin-antithrombin complex and D-dimer.<sup>11</sup> However there was no data obtained from the sample participants at the time of delivery.

Studies have also suggested an increase in fibrinolysis activity at the time of delivery in normal pregnancy. An increase occurs in D-dimer and fibrin-fibrinogen degradation products signifying increased fibrin formation and breakdown. Gerbasi, et. al. found similar results of increased fibrinolytic activity immediately after placental delivery. These results were reflected by increased FDP levels, D-dimer, and a decrease in alpha-2-antiplasmin.<sup>9</sup>

### **Epidurals and Hemostasis**

Thromboembolism due to hypercoagulability, although rare, is a complication of the postpartum period and a major cause of maternal morbidity and mortality, especially in preeclampsia.<sup>8,9</sup> Several studies suggest that the administration of epidural anesthesia improves surgical outcome by decreasing the incidence of post-operative coagulopathies including deep vein thrombosis.<sup>12</sup> Borg, et. al. showed that factors involved in preventing the development of deep vein thrombosis and subsequent pulmonary embolism include sympathetic

blockade. Sympathetic blockade from epidural anesthesia results in increased blood flow to the lower extremities, thus preventing a hypercoagulable condition by preserving baseline fibrinolysis.<sup>12</sup>

Rosenfield, et. al. studied 95 patients undergoing lower extremity vascular reconstruction and found that administration of epidural anesthesia decreased the incidence of post-operative arterial thrombosis as well as attenuated the cortisol and catecholamine responses to surgery.<sup>13</sup> Plasminogen activator inhibitors (PAI) inhibit the conversion of plasminogen to plasmin. Plasmin is responsible for degradation of fibrin clots. It was demonstrated that PAI-1 levels increase postoperatively in patients who received general anesthesia, as compared to no increase in PAI-1 levels with epidural anesthesia. Elevated levels of PAI-1 are known to lead to thrombosis.<sup>13</sup> Thus, it can be said that there is a so-called fibrinolytic shutdown following surgery. The results of the study suggest a reduced incidence of arterial thrombosis in patients who receive intraoperative epidural analgesia followed by postoperative epidural fentanyl. However, they were unable to determine whether this effect was due to the intraoperative anesthesia or the postoperative analgesia regimen.

Epidural anesthesia has been shown to have an inhibitory effect on platelet aggregation and release while increasing blood flow to the lower limbs. Borg, et. al. found that local anesthetics have a direct effect on platelet aggregation leading to a lower frequency of deep vein thrombosis.<sup>14</sup> Platelet rich plasma was incubated in an aggregometer with lidocaine, bupivacaine and tocainimide in various concentrations for different incubation times. Lidocaine

had the greatest inhibitory effect on collagen and adenosine-diphosphate induced platelet aggregation, followed by bupivacaine and tocainimide. These *in vitro* effects of local anesthetics were with higher levels than used in clinical conditions. However, clinically there is contact for a longer period of time with the platelets making them less likely to aggregate when stimulated.<sup>14</sup>

Tuman, et. al. conducted a study of 80 patients with atherosclerotic disease undergoing major vascular surgery. One group received general anesthesia with postoperative epidural analgesia, and the other group received general anesthesia with intravenous narcotics for postoperative pain. A third group of 40 patients without atherosclerosis undergoing non-cardiovascular procedures served as the control. They found that vascular patients were hypercoagulable compared with control patients. Postoperatively, those who received epidurals had a lower incidence of thrombotic events, less postoperative complications, and a decreased length of hospital stay.<sup>15</sup>

Henny, et. al. studied the effects of epidural bupivacaine on platelet function in patients undergoing transurethral resection of the prostate. Twenty patients were divided into two groups. One group received general anesthesia while the other received lumbar extradural anesthesia. The extradural anesthesia group demonstrated inhibitory effects on platelet aggregation with a subsequent decrease in alpha-2-antiplasmin, suggesting the thromboprophylactic effect of epidural analgesia.<sup>16</sup> This, as noted previously, plays a role in decreasing embolic complications postoperatively. No such effect was demonstrated in the general anesthesia group.



It is documented that the incidence of thromboembolic complications after various orthopedic procedures is decreased in patients operated on under regional anesthesia when compared to those operated on under general anesthesia. Modig, et. al. showed the incidence of deep vein thrombosis and pulmonary embolism to be significantly lower among patients operated on under lumbar epidural anesthesia involving total hip replacements as compared to general anesthesia.<sup>17</sup> The coagulative and fibrinolytic responses to surgery are altered by the use of afferent and efferent nerve blockade from epidural anesthesia. The capacity for activation of factor VIII as determined by a modified clotting time test was significantly lower postoperatively in patients given continuous epidural anesthesia rather than general anesthesia. The fibrinolytic inhibition activity in serum was significantly lower in patients given continuous epidural anesthesia versus those given general anesthesia.<sup>17</sup>

Modig, et. al. conducted two similar studies of patients undergoing total hip replacement receiving continuous epidural administration and concluded that these patients had increased fibrinolytic activity through alterations in alpha-2-antiplasmin and lower levels of factor VIII. The capacity of the venous endothelium to release plasminogen activators and the resting level of plasminogen activators were significantly greater during and after epidural anesthesia thus allowing a more efficient lysis of thrombi.<sup>18,19</sup> These coagulative and fibrinolytic differences, causing the epidural group to be less susceptible to the development of thrombi, may be partially explained by an altered neuroendocrine response to surgery related to neural blockade. One explanation

of this response to surgical stress is that regional anesthesia decreases the cortisol, renin, aldosterone, and catecholamine response to surgery. Steele, et. al. conducted a retrospective review of epidural coagulation studies and noted that cortisol, catecholamines, corticotropin, antidiuretic hormone, and von Willebrand factor were blunted by epidurals.<sup>20</sup>

### **Epidurals and Pregnancy**

Studies have been conducted regarding the safety of epidural analgesia for both mother and fetus. It is now known that hypotension usually does not develop with moderate fluid pre-loading, and that uteroplacental blood flow moderately increases following epidural block in preeclampsia, thus providing satisfactory intrauterine oxygen exchange.<sup>21</sup> When comparing epidural to local anesthesia in preeclamptics, no difference was found in Apgar scores or cord blood gas values, and the incidence of hypotension was almost the same. When epidural anesthesia was compared to general anesthesia in preeclamptic women, the incidence of hypotension in the epidural group was less than half that found in the general anesthesia group. In fact, the general anesthesia group demonstrated a significant increase in blood pressure with induction, thus placing them at risk for cerebral hemorrhage and pulmonary edema.<sup>21</sup> It has also been demonstrated that circulating catecholamines are significantly higher in preeclampsia than in normal parturients and that epidural analgesia decreases the level of catecholamines in preeclamptic patients, thus lowering blood pressure and improving blood flow overall, including uteroplacental blood flow.<sup>21</sup> Epidural analgesia decreases catecholamine levels in normal pregnancies also,

demonstrating a block of the stress response that normally occurs with labor and delivery.<sup>22</sup>

Very limited research has been done on the effects of epidural analgesia on hemostatic parameters during labor and delivery in normal pregnancies. A study by Bressler and Hoskey in 1994 studied the effects of epidural analgesia on *in-vivo* hemostasis parameters during labor, delivery, and the postpartum period in normal pregnancy.<sup>23</sup> Group I consisted of 30 healthy patients receiving epidurals, while group II consisted of 30 healthy patients not receiving epidurals. Blood samples were drawn prior to epidural placement, post-epidural, at time of delivery, three hours postpartum, and 24 hours postpartum. They found platelet factor 4 and beta-thromboglobulin showed peaked activity at the time of delivery and platelet counts were decreased, reflecting platelet consumption. Although platelet factor 4 and beta-thromboglobulin levels between the two groups were not statistically significant, overall lower values were found in the epidural group. The epidural group also demonstrated increased fibrin-fibrinogen degradation products and D-dimer along with decreased alpha-2-antiplasmin, indicating increased fibrinolytic activity as compared to the non-epidural group. Overall, they concluded this may be related to a decreased stress response and that the use of epidural analgesia for patients at increased risk for deep vein thrombosis, such as in preeclampsia, may benefit from its use.<sup>23</sup> The results of this study will be used for comparison in the current study.

## **Spinal Anesthesia and Cesarean Delivery**

Regional anesthesia has been associated with a reduced incidence of thromboembolic events, and local anesthetics have been shown to have an inhibitory effect on platelet aggregation. However, little research has been conducted on the effects of spinal anesthesia on hemostasis during cesarean delivery. A study by Sharma and Philip in 1997 compared the effects of spinal versus general anesthesia using thromboelastography (TEG) in normal pregnant women undergoing cesarean section. A total of 30 patients were sampled, 15 in each group. Values measures with TEG included clot formation time, coagulation time, clot formation rate, and coagulation index. These values were measured before induction and in the immediate postanesthesia period. Values in the preanesthesia period were similar between the two groups. However, in the postanesthesia period, the general anesthesia group showed a decrease in coagulation time by 30%, an increase in the speed of clot formation by 25%, and no change in clot strength. Overall, general anesthesia when used for cesarean section accelerates blood coagulation in the immediate postoperative period but has no effect on the final clot strength. Cesarean sections with spinal anesthesia, on the contrary, did not have significant changes in thromboelastographic variables in the postanesthesia compared with the preanesthesia period. Sharma and Philip concluded that the use of general anesthesia for cesarean section is associated with accelerated coagulability when compared to spinal anesthesia.<sup>24</sup>

In summary, the increased platelet activation with delivery and increased fibrinolysis in the immediate postpartum is well documented in vaginal deliveries. The beneficial effects of regional anesthesia, including decreased platelet activation due to decreased stress response resulting in decreased incidence of deep vein thrombosis, is also well documented in vascular and orthopedic surgeries. Limited research has been conducted on *in-vivo* coagulation changes that occur with epidural analgesia in normal vaginal deliveries. In vaginal deliveries, BTG and PF4 increase the most at delivery, signifying platelet activation. There is less of an increase in BTG and PF4 suggesting decreased platelet activation when an epidural is used for delivery. Minimal research is available on coagulation changes that occur with cesarean section. To date, no studies have been done on *in-vivo* coagulation changes that occur with cesarean delivery. Since cesarean section poses an increased risk of postoperative thrombosis, more research on the specific hemostatic changes that occur is needed.

## Chapter IV

### Significance and Aim

#### **Statement of the Problem**

Coagulation changes occur with normal pregnancy causing a hypercoagulable state. Previous studies have suggested beneficial effects of epidural anesthesia in decreasing platelet activation and thromboxane release. There is an increased risk of post-delivery thrombosis with cesarean sections compared to vaginal deliveries. To date, there have been no studies comparing hemostatic changes that occur in normal vaginal deliveries versus cesarean sections.

#### **Purpose**

The purpose of this study is to measure the effects of cesarean delivery on *in vivo* coagulation, fibrinolytic, and platelet hemostatic components in healthy parturients.

#### **Hypothesis**

We hypothesize that cesarean deliveries will increase hemostatic activity compared to vaginal deliveries.

## Chapter V

### Materials and Methods

This is a prospective longitudinal study measuring hemostasis markers in healthy parturients having a cesarean section with spinal anesthesia. Institutional Review Board approval was obtained. The sample population for this study consists of 15 patients, aged 18-45, ASA classification of II, and  $\geq 34$  weeks gestation scheduled for cesarean delivery. Exclusion criteria consist of the following: ASA classification  $>II$ , pre-existing coagulation abnormality/bleeding disorder, patients taking anticoagulants, use of illegal substances, or prior history of thrombosis. (Appendix C)

The total sample population included three groups. Group one consists of 15 healthy parturients scheduled for cesarean section with spinal anesthesia. Group two consists of 30 healthy parturients having a vaginal delivery with epidural anesthesia. Group three consists of 30 healthy parturients having a vaginal delivery without regional anesthesia. Data from the Hoskey, et. al. study was used for group two and group three.<sup>23</sup>

Data collection and blood sampling for group one was performed by the three primary researchers. After obtaining informed consent (Appendix D), four blood samples were drawn from the same patient at the following times: prior to cesarean section, immediately following cesarean section (within one hour of delivery), 3 hours postpartum, and 24 hours postpartum. (Appendix E) Free-flowing technique for blood collection was performed using a 19 gauge winged needle attached to a 3-way stopcock. The first 2cc of blood was discarded. The

remaining 12 cc of blood was placed into the following tubes: 2ml into an EDTA tube for hemoglobin, hematocrit, and platelet count; 2.7ml into a vacuum broken CTAD tube (.3ml Buffered Sodium Citrate 0.109M, Theophylline 15mM, Adenosine 3.7mM, Dipyridamole 0.198mM) for platelet factor 4 and beta-thromboglobulin measurement; 2ml into a tube containing Soybean Tripsin Inhibitor 3,670 NF units and Thrombin 20 NIH units for measurement of fibrin-fibrinogen degradation products; and 4.5ml into a tube containing Buffered Sodium Citrate 3.2% for measurement of antithrombin III, alpha-2-antiplasmin, prothrombin fragments 1+2, thrombin-antithrombin III complex, D-dimer, and fibrinogen. The CTAD tube was immediately placed in an ice bath and cooled for 15 minutes. It was then centrifuged at 2,500 g for 30 minutes at 6 degrees Celsius. The other two tubes were also centrifuged at 2,500 g for 30 minutes at 6 degrees Celsius. The plasma samples for platelet factor 4, beta-thromboglobulin, prothrombin fragments 1+2, thrombin-antithrombin III complex, antithrombin III, fibrin-fibrinogen degradation products, and alpha-2-antiplasmin were separated and transferred in small aliquots into plastic tubes and frozen immediately at minus seventy degrees Celsius. The lab tests were processed in batches.

The hemostatic parameters were measured using state of the art analytical techniques. (Appendix F) Prothrombin fragments 1+2 and thrombin-antithrombin III complex test kits were obtained from Dade Behring, Deerfield, Illinois. Assay kits for beta-thromboglobulin and platelet factor 4 were obtained from Diagnostica Stago, Parsippany, New Jersey. Assay kits for antithrombin III



and alpha-2-antiplasmin were obtained from Instrumentation Laboratory, Lexington, Massachusetts. The accuracy of these tests have been demonstrated in previous studies involving *in-vivo* hemostatic activity, including the 1994 study of the effects of epidurals on hemostatic activity in normal pregnancies.<sup>23</sup> Lab samples were processed according to the standard instructions from the commercial test kits and the clinical laboratory standards at the hospital. The six aforementioned tests were processed at Wayne State University Hematology Laboratory in Detroit, Michigan under the supervision of Dr. Eberhardt Mammen. The remainder of the lab tests were processed using standard procedures at the research hospital.

### **Statistical Analysis**

A power of .59 was obtained with a total of 75 patients to detect a 1 standard deviation difference between the three groups. The Analysis of Variance for Repeated Measures with Post Hoc T-tests ( $p < 0.05$ ) was used to detect differences in descriptive variables and the hemostasis markers obtained during parturition between the three groups. This test was used because the same hemostasis variables were measured on all three groups. The Post Hoc T-test was used to specify where the difference was among the three groups. The descriptive variables compared include the following: age, weight, parity, blood loss, and APGAR scores. Chi-Square analysis was used on the nominal level data, which included ethnicity and history of smoking. A  $p < 0.05$  was accepted as significant. (see Appendices G-K for data collection tools) For clinical

significance, this study needed an effect size of .60. The power for this study with an effect size of .60 is .59. A total of 35 study subjects per group would be needed to obtain a power of .80 with an effect size of .60.

## Chapter VI

### Results

A total of 75 patients, 15 in group one (cesarean section), 30 in group two (vaginal delivery with epidural anesthesia) and 30 in group three (vaginal delivery without regional anesthesia) were enrolled in the study. The samples in group one were collected by the same three primary researchers. The data from groups two and three were obtained from the previous study by Hoskey, et. al. study.<sup>23</sup> Demographic and descriptive data is shown in Table 1.

Several differences were identified between the groups. Ethnicity between the three groups varied significantly ( $p = 0.0003$ ). In group one, 33.3% were African-American, 60% were Caucasian, and 6.7% Hispanic. In group two, 40% were African-American and 60% were Caucasian. In group three, 87% were African-American and 13% were Caucasian. Group one had a significantly older population and higher gravida as compared to groups two and three ( $p = 0.0134$  and  $p = 0.0001$ , respectively). The ages for group one ranged from 19 to 39 years and all 15 participants were 38-40 weeks gestation. There was no significant difference among the three groups for weight or history of smoking.

In total, 52% of the patients had type O blood, 31% had type A, 10% had type B, and 7% had type AB. The cesarean group had significantly greater blood loss compared to both vaginal groups ( $p = 0.0$ ). The mean estimated blood loss in the cesarean group was 787 ml, compared to 360 ml in the epidural group and 291 ml in the non-epidural group. The birth weights of the cesarean group ranged from 2613 grams to 4200 grams. The birth weights for the two vaginal

groups ranged from 2608 grams to 5430 grams. None of the infants required resuscitation, and there was no significant difference between APGAR scores at one and five minutes between the three groups.

Platelet count in the cesarean section group decreased from the initial level of 227,000 to 187,000 at delivery. Although not statistically significant, platelet counts decreased the most at delivery in the cesarean section group. (Table 2, Figure 3) No significant difference was found in beta-thromboglobulin levels among the three groups. However, beta-thromboglobulin levels showed the greatest increase at delivery in the vaginal groups, whereas the cesarean group had the greatest increase three hours postpartum. Platelet factor 4 levels were significantly higher in the non-epidural group at delivery (94 ng/ml) when compared to the cesarean group (27 IU/ml,  $p = 0.0001$ ) and epidural group (54 ng/ml,  $p = 0.0003$ ). Once again, the cesarean group had the highest platelet factor 4 level at three hours postpartum, whereas the other two groups peaked at delivery. (Table 2, Figures 1 & 2)

In regard to coagulation activity, the cesarean group showed decreased fibrinogen levels at delivery (407 mg/dL) and three hours postpartum (421 mg/dL). Fibrinogen levels in the cesarean group were statistically significantly lower than the non-epidural group at delivery, three hours postpartum, and 24 hours postpartum. The cesarean group also showed decreased antithrombin III levels at delivery (78.5%) and three hours postpartum (77.9%). Antithrombin III levels of the cesarean group were statistically significantly lower than the other

two groups at delivery, three hours postpartum, and 24 hours postpartum. (Table 3, Figures 4 & 5)

Additional assessment of coagulation activity included measuring prothrombin fragments 1+2 and thrombin-antithrombin III complex, both of which signify thrombin formation, in the cesarean group. In this group, prothrombin fragments 1+2 levels increased continuously then decreased at 24 hours postpartum (Table 3, Figure 7). Thrombin-antithrombin III complex levels increased with delivery then decreased in the postpartum period (Table 3, Figure 8). These two parameters were not tested in the vaginal groups. The vaginal groups tested fibrinopeptide A, which also assesses coagulation activity. The test for fibrinopeptide A is no longer available.

Fibrinolytic activity was assessed by measurement of D-dimer, fibrin-fibrinogen degradation products, and alpha-2-antiplasmin. The cesarean group demonstrated a positive D-dimer at an increased frequency with each blood draw. A positive D-dimer indicates a level > 120 ug/L. Fibrin-fibrinogen degradation products (FDP) also increased with each blood draw in the cesarean group. Fibrin-fibrinogen degradation products were measured with an agglutination test and reported in ranges. The three ranges were < 10 ug/mL (normal range), 10-40 ug/mL, and > 40 ug/mL. (Table 4) The percent of patients within the 10-40 ug/mL range increased with each blood draw. Only at delivery did any of the patients reach > 40 ug/mL in the cesarean group. All three groups showed decreased alpha-2-antiplasmin at delivery and three hours postpartum.

Although the cesarean group decreased the most, there was no statistically significant difference. (Table 4, Figure 6)

## Chapter VII

### Discussion

The results of our study suggest an increase in platelet activation and coagulation at delivery and in the immediate postpartum, with an increase in fibrinolysis three hours following cesarean section. *In-vivo* hemostasis parameters then returned to baseline 24 hours postpartum. These results are supported by previous research conducted on vaginal deliveries.<sup>9,11,23</sup>

There were several differences between the three groups. However, these differences did not effect coagulation activity. The cesarean section group was significantly older than the other two groups. The age difference may be due in part to the fact that group one consisted of multi-gravidas scheduled for repeat cesarean sections. The cesarean section group also had significantly greater blood loss than both vaginal groups. This is anticipated with the incision required for cesarean deliveries and parturients are fluid bolused prior to the cesarean section. No blood transfusions were required.

A slight decrease was noted in hemoglobin and hematocrit in the cesarean group prior to delivery. This draw was taken prior to any fluid bolusing, therefore can not be attributed to hemodilution. Though the difference in hemoglobin levels were found to be statistically significant ( $p < 0.02$ ) the difference between the groups was only one gm/dL. The mean hemoglobin of the cesarean group was slightly below normal (11 mg/dL), whereas the mean hemoglobin for both vaginal groups was low normal (12 mg/dL). The normal range for hemoglobin in the adult female is 12-16 mg/dL. The greatest decrease

in hemoglobin and hematocrit in the cesarean group was seen at the time of delivery. This is most likely due to an increase in blood loss and hemodilution with fluid bolusing. Only a slight decrease was seen in the epidural group at delivery. At 24 hours postpartum, hemoglobin and hematocrit levels began to normalize in the cesarean group. No significant difference in hemoglobin and hematocrit was found between the three groups 24 hours postpartum. (Table 3)

Platelet changes were similar in the cesarean and epidural groups. Platelet count decreased at the time of delivery with a gradual increase at 3 hours and 24 hours postpartum in the cesarean group. (Figure 3) This suggests platelet consumption with cesarean section and a decrease in consumption by 24 hours postpartum. Only a slight decrease in platelet count was seen in the epidural group at the time of delivery with a gradual increase in the postpartum period. The non-epidural group, on the contrary, demonstrated steady increases in platelet counts throughout the puerperium. The platelet count in the non-epidural group actually increased at delivery.

We hypothesized that beta-thromboglobulin and platelet factor 4 levels would peak at delivery in the cesarean group. However, in the cesarean group platelet factor 4 and beta-thromboglobulin showed the greatest increase at 3 hours postpartum and decreased at 24 hours postpartum. The epidural and non-epidural groups peaked at delivery and returned to pre-delivery values by 24 hours postpartum. One possible explanation for the increased platelet activation at delivery in the vaginal groups is that these parturients have been in labor and have had a greater time of stress. Stress has been shown to increase platelet



activation. Also, several studies have suggested that regional anesthesia decreases platelet activation. In addition, the profound peripheral vasodilation that accompanies subarachnoid block is normally no longer present at 3 hours postpartum. It is possible that platelet activation is greatest at this time in the cesarean group because of this change.

Fibrinogen levels in the cesarean group were above the normal range (200-400mg/dL) at all time points. Previous studies have demonstrated increased fibrinogen levels throughout pregnancy which is consistent with our findings at the pre-delivery time. Fibrinogen levels decreased slightly at delivery, with return to pregnancy baseline by 24 hours postpartum. Similar results were found in the epidural group. The decrease in fibrinogen levels at delivery may be attributed to the activation of the final common pathway in which fibrinogen is converted to fibrin to form the clot. Prothrombin fragments 1+2 increased at delivery, with the highest levels seen at 3 hours postpartum in the cesarean group. This suggests increased activity at delivery and immediate postpartum. Prothrombin fragments 1+2 are produced when prothrombin is cleaved to thrombin in the final common pathway.

An increase in coagulation activity is further supported by a decrease in antithrombin III, the major inhibitor of coagulation, at delivery and 3 hours postpartum in the cesarean group. The cesarean group had statistically significant ( $p < 0.01$ ) lower levels of antithrombin III at delivery, 3 hours postpartum and 24 hours postpartum when compared to the epidural and non-epidural groups, suggesting increased coagulation activity. No statistically

significant difference in antithrombin III levels was found between the epidural and non-epidural groups. Both the epidural and non-epidural groups also showed a decrease in antithrombin III levels at delivery and 3 hours postpartum, however the change was minimal.

Antithrombin III inhibits coagulation by binding and inactivating thrombin. When this occurs, thrombin-antithrombin III complexes are formed. Thus, as antithrombin III levels decrease, an increase in thrombin-antithrombin III complex is normally seen. In the cesarean group, thrombin-antithrombin III complex levels were increased both at delivery and 3 hours postpartum. The inverse relationship between antithrombin III and thrombin-antithrombin III complex shown in the cesarean group suggests increased thrombus formation at delivery and postpartum.

An increase in fibrinolytic activity was demonstrated in the cesarean section group by decreased alpha-2-antiplasmin at delivery and three hours postpartum. This was supported by an increased frequency of a positive D-dimer. Forty-seven percent of D-dimer results were positive prior to cesarean delivery and increased to eighty percent positive by 24 hours postpartum. Positive D-dimer results prior to delivery have been shown in previous studies and reflect increased coagulation and fibrinolytic activity.<sup>9,11</sup> An increase in the frequency of a positive D-dimer was hypothesized in the postpartum period and is consistent with previous findings in the vaginal delivery groups. The percent of FDP in the 10-40ug/ml range increased successively in the first three draws, then decreased at 24 hours postpartum in the cesarean group. This data suggests increased

fibrinolytic activity in the cesarean group. Similar increases in FDP were seen in the vaginal groups with a decline at 24 hours postpartum. Due to differences in laboratory analysis and reporting of D-dimer and FDP between groups, we were unable to statistically analyze the significance of the results between the three groups.

No prior studies have assessed *in-vivo* hemostatic changes that occur with cesarean section, nor has anyone compared *in-vivo* changes that occur with cesarean section to vaginal delivery. Sharma and Philip compared the effects of spinal versus general anesthesia using thromboelastography in normal pregnant women undergoing cesarean section. They concluded that the use of general anesthesia for cesarean section is associated with accelerated coagulability when compared to spinal anesthesia. All patients in the current study received spinal anesthesia for cesarean section. A follow-up study is recommended comparing the *in-vivo* hemostatic changes that occur in cesarean section with spinal versus general anesthesia. This would help clarify the beneficial effects of regional anesthesia on decreasing platelet activation in the parturient, especially since increased hemostatic activity was demonstrated in the cesarean section group of this study.

Despite the fact that the technique of blood sampling and the methods were the same for all three groups, the data for each individual group was collected by different researchers during different time frames. Because the data for groups two and three was collected in 1994 and 1990, some of the variables tested in group one differ. Prothrombin fragments 1+2 and thrombin-antithrombin

III complex are newer tests used to detect thrombin formation and were not available when groups two and three were sampled. Therefore, these two variables could not be compared to the vaginal groups. Also, groups two and three reported D-dimer and FDP as a titer. The current method of testing used in group one does not report these results as titers. Instead, D-dimer is reported as positive or negative and FDP is reported as a range. Because of this, the cesarean section group could not be statistically compared to the vaginal groups in regard to these variables. All that could be analyzed was the trend found in the cesarean section group with D-dimer, FDP, prothrombin fragments 1+2, and thrombin-antithrombin III complex.

## Chapter VIII

### Conclusion

In summary, the results of this study support previous research suggesting an increase in platelet, coagulation, and fibrinolysis activity during the puerperium. Overall, platelet, coagulation, and fibrinolytic activity increased at the time of delivery and in the immediate postpartum period in the cesarean section group. (Figure 9) Increased platelet factor 4 and beta-thromboglobulin signify platelet activation, while the decrease in antithrombin III and increases in prothrombin fragments 1+2 and thrombin-antithrombin III complex demonstrate increased coagulation. Increased fibrinolytic activity is evidenced by decreased alpha-2-antiplasmin, increased FDP, and an increase in the frequency of a positive D-dimer. The changes we observed with cesarean section are in agreement with other investigations of term pregnancy and delivery measuring the same molecular markers.<sup>6,9,11,23</sup> However, these studies were conducted on patients having vaginal deliveries.

Although hemostasis changes demonstrated in the cesarean section group are similar to the vaginal groups, the cesarean section group showed significantly lower antithrombin III levels at delivery, three hours postpartum, and 24 hours postpartum ( $p < 0.005$ ) when compared to both vaginal groups. While not statistically significant, overall the cesarean section group demonstrated lower levels of fibrinogen, alpha-2-antiplasmin, and platelets at delivery and three hours postpartum compared to the vaginal groups. Despite the lack of a statistically significant difference in platelet activation, and considering the fact

that D-dimer and FDP could not be statistically compared, our results still suggest increased hemostatic activity in cesarean section compared to vaginal delivery.

There is an increased risk of post-delivery thrombosis with cesarean section compared to vaginal delivery. Sharma and Philip concluded that the use of general anesthesia for cesarean section is associated with accelerated coagulability when compared to spinal anesthesia.<sup>24</sup> In spite of the inhibitory effects of spinal anesthesia on platelet activation, the cesarean section group in this study still exhibited increased platelet, coagulation, and fibrinolysis activity at delivery and in the immediate postpartum. Therefore, even when using spinal anesthesia for cesarean section, measures should be utilized to prevent blood clots both preoperatively and postoperatively.

## Chapter IX

## DEMOGRAPHIC CHARACTERISTICS

Table 1: Demographic characteristics among the three groups.

VARIABLE	CESAREAN (n=15)	EPIDURAL (n=30)	NON-EPIDURAL (n=30)	P VALUE
Age (years)	28.9 ± 5.8	24 ± 5	25 ± 5	0.0134
Parity	2.1 ± 1.5	0.5 ± 0.9	1 ± 1	0.0001
Weight (Kg)	83.5 ± 18.2	77 ± 13	77 ± 14	N.S.
Ethnicity (%)	33.3 AA 60 C 6.7 H	40 AA 60 C	87 AA 13 C	0.0003
Smokers (%)	20	43	20	N.S.
Estimated Blood Loss (mL)	787 ± 142	360 ± 91	291 ± 84	0.0000
APGAR (1 minute)	8.7 ± 0.5	8 ± 2	8 ± 1	N.S.
APGAR (5 minutes)	9 ± 0.4	9 ± 1	9 ± 0	N.S.

AA = African American

C = Caucasian

H = Hispanic

-All values represent mean ±1 standard deviation

-N.S. = not significant

-Significance = p &lt; 0.05

## Platelet Activity

Table 2: Platelet activity among the three groups.

Marker	Cesarean (n=15) Epidural (n=30) Non- epidural (n=30)	Pre- spinal/epidural	Delivery	3 hrs postpartum	24 hrs postpartum
Platelet Count (x10/mm <sup>3</sup> )	Cesarean	227.13 <sub>±</sub> 71.41	186.5 <sub>±</sub> 63.16	196.73 <sub>±</sub> 69.91	202.53 <sub>±</sub> 69.26
	Epidural	221.35 <sub>±</sub> 52.91	204.0 <sub>±</sub> 63.22	210.91 <sub>±</sub> 61.06	211.25 <sub>±</sub> 51.31
	Non- epidural	149.28 <sub>±</sub> 128.69	217.97 <sub>±</sub> 67.04	237.0 <sub>±</sub> 55.91	240.28 <sub>±</sub> 73.87
	P Value	0.0054	N.S.	N.S.	N.S.
Platelet Factor 4 (IU/mL)	Cesarean	26.60 <sub>±</sub> 18.67	27.10 <sub>±</sub> 19.59	31.25 <sub>±</sub> 24.49	15.21 <sub>±</sub> 10.70
	Epidural	40 <sub>±</sub> 51	54 <sub>±</sub> 51	34 <sub>±</sub> 31	39 <sub>±</sub> 30
	Non- epidural	64 <sub>±</sub> 79	97 <sub>±</sub> 39	84 <sub>±</sub> 99	44 <sub>±</sub> 62
	P Value	N.S.	0.002	0.0378	N.S.
Beta- thrombo- globulin (IU/mL)	Cesarean	102.40 <sub>±</sub> 50.08	102.80 <sub>±</sub> 24.10	143.00 <sub>±</sub> 96.08	75.29 <sub>±</sub> 32.20
	Epidural	103 <sub>±</sub> 101	131 <sub>±</sub> 89	97 <sub>±</sub> 64	75 <sub>±</sub> 55
	Non- epidural	107 <sub>±</sub> 75	136 <sub>±</sub> 116	123 <sub>±</sub> 103	71 <sub>±</sub> 63
	P Value	0.0052	N.S.	0.0011	N.S.

-All values represent mean  $\pm$  1 standard deviation

-N.S. = not significant

-Significance =  $p < 0.05$



### Hemostasis Parameters

**Table 3: Hemostasis markers among the three groups.**

Marker	Cesarean (n=15) Epidural (n=30) Non-epidural (n=30)	Pre- spinal/epidural	Delivery	3 hrs postpartum	24 hrs postpartum
Fibrinogen (mg/dL)	Cesarean	472 $\pm$ 120	407 $\pm$ 62	421 $\pm$ 61	465 $\pm$ 77
	Epidural	457 $\pm$ 103	453 $\pm$ 146	473 $\pm$ 143	497 $\pm$ 155
	Non-epidural	553 $\pm$ 155	570 $\pm$ 161	543 $\pm$ 168	574 $\pm$ 138
	P Value	0.0141	0.0004	0.0289	0.0285
AT III (%)	Cesarean	94 $\pm$ 13.64	78.46 $\pm$ 8.61	77.92 $\pm$ 9.28	83.21 $\pm$ 10.16
	Epidural	108 $\pm$ 10	98 $\pm$ 12	95 $\pm$ 12	98 $\pm$ 12
	Non-epidural	96 $\pm$ 14	96 $\pm$ 13	93 $\pm$ 13	96 $\pm$ 12
	P Value	0.0003	0	0.0002	0.0007
PF 1+2 (nmole/L)	Cesarean	3.62 $\pm$ 1.05	4.45 $\pm$ 1.20	4.90 $\pm$ 1.18	3.08 $\pm$ 0.83
	Epidural	NA	NA	NA	NA
	Non-epidural	NA	NA	NA	NA
TAT (ng/L)	Cesarean	20.69 $\pm$ 22.92	26.15 $\pm$ 20.23	24.32 $\pm$ 23.72	8.54 $\pm$ 4.89
	Epidural	NA	NA	NA	NA
	Non-epidural	NA	NA	NA	NA
Hgb (mg/dL)	Cesarean	11.04 $\pm$ 1.35	9.83 $\pm$ 1.32	10.18 $\pm$ 1.26	10.2 $\pm$ 1.63
	Epidural	12 $\pm$ 1	12 $\pm$ 1	11 $\pm$ 1	11 $\pm$ 1
	Non-epidural	12 $\pm$ 1	NA	NA	11 $\pm$ 1
	P Value	0.0114	NA	NA	N.S.
Hct (%)	Cesarean	32.57 $\pm$ 3.74	28.72 $\pm$ 3.45	30.05 $\pm$ 3.34	29.87 $\pm$ 4.6
	Epidural	36 $\pm$ 3	35 $\pm$ 3	34 $\pm$ 2	31 $\pm$ 5
	Non-epidural	36 $\pm$ 3	NA	NA	33 $\pm$ 2
	P Value	N.S.	NA	NA	0.0317

-All values represent mean  $\pm$  1 standard deviation

-N.S. = not significant

-NA = not available

-Significance =  $p < 0.05$

### Fibrinolysis

**Table 4: Fibrinolysis activity among the three groups.**

Marker	Cesarean (n=15) Epidural (n=30) Non-epidural (n=30)	Pre-spinal/epidural	Delivery	3 hrs postpartum	24 hrs postpartum
D-dimer	Cesarean (% positive = % > 120 mcg/L)	46.7 % +	66.7 % +	73.3 % +	80.0 % +
	Epidural (titer: mg/L)	0.06±0.24	0.29±0.41	0.36±0.45	0.04±0.18
	Non-epidural (titer: mg/L)	0.07±0.25	0.13±0.35	0.66±0.71	0
FDP	Cesarean (% per range, units ug/mL)	86.7 % < 10 13 % 10-40 0% > 40	60 % < 10 27 % 10-40 6.7 % > 40	26.7 % < 10 60 % 10-40 0 % > 40	40 % < 10 53 % 10-40 0 % > 40
	Epidural (titer)	1.12±0.60	1.37±0.63	1.3±0.8	1.13±0.8
	Non-epidural (titer)	0.9±0.8	1.13±0.73	1.34±0.84	1.0±0.8
A-2-A (%)	Cesarean	93.21±11	81.85±12.14	81.46±13.6	91.86±15.18
	Epidural	91±20	86±17	83±18	92±17
	Non-epidural	96±13	88±12	84±16	89±12
	P Value	N.S.	N.S.	N.S.	N.S.

-All values represent mean ± 1 standard deviation

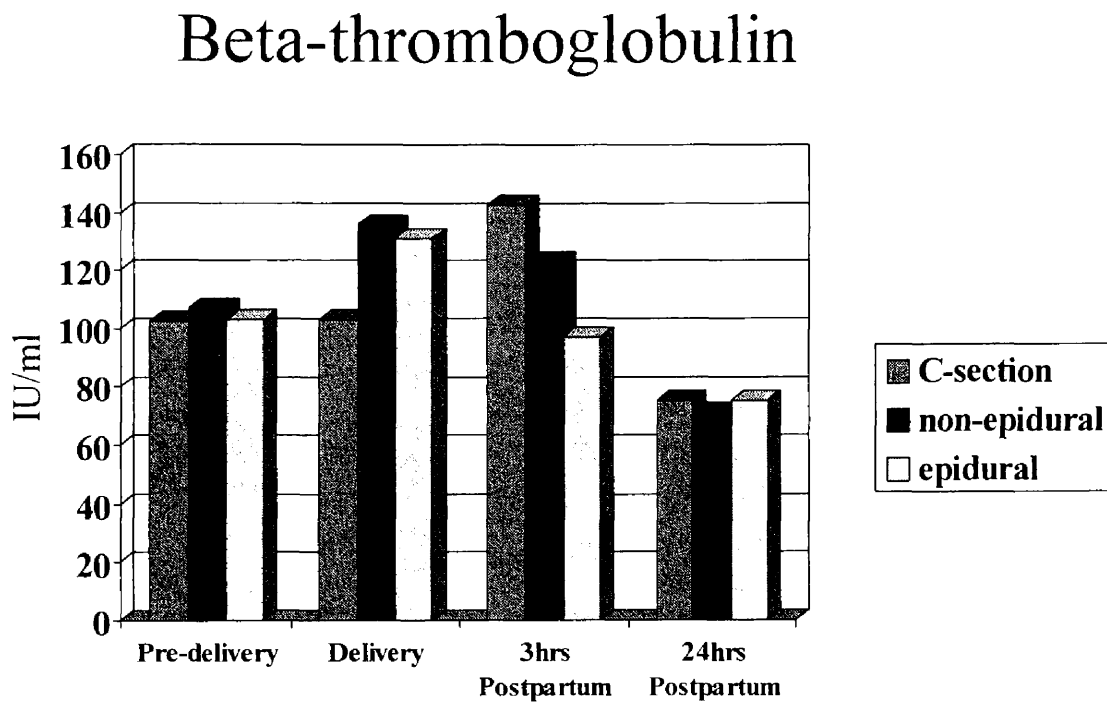
-N.S. = not significant

-Significance = p < 0.05



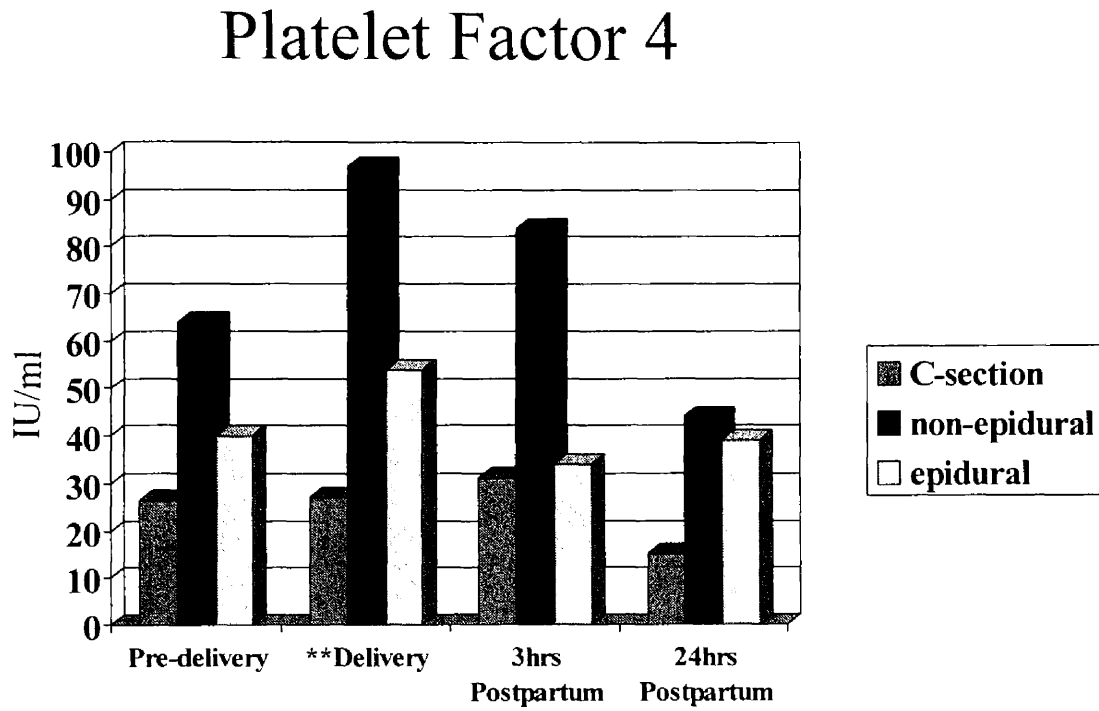
Chapter X

Figure 1: Beta-thromboglobulin levels of the three groups.



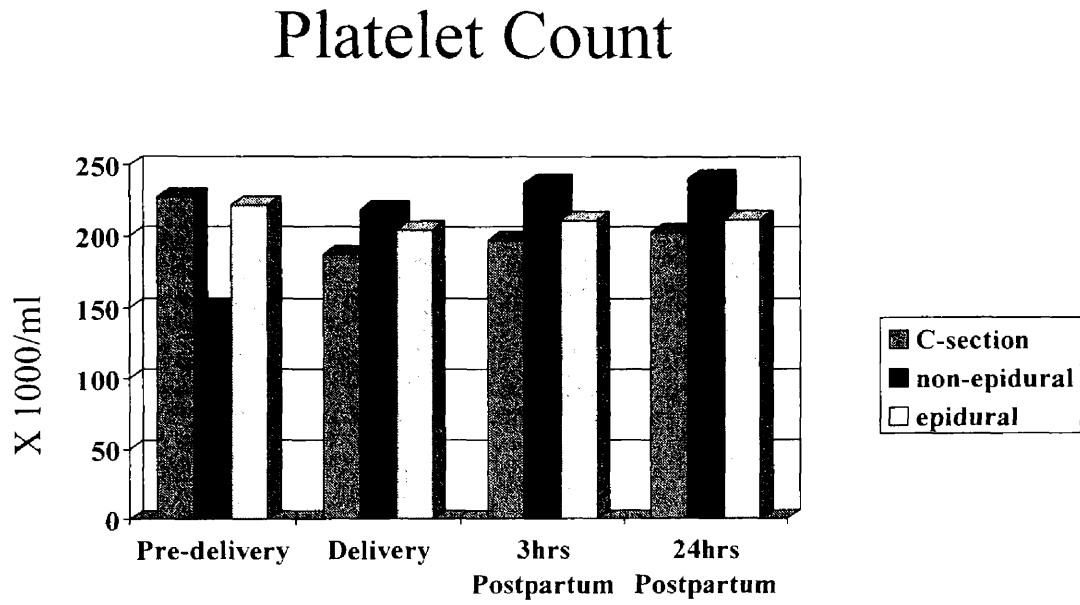
No statistical significance

Figure 2: Platelet factor 4 levels among the three groups.



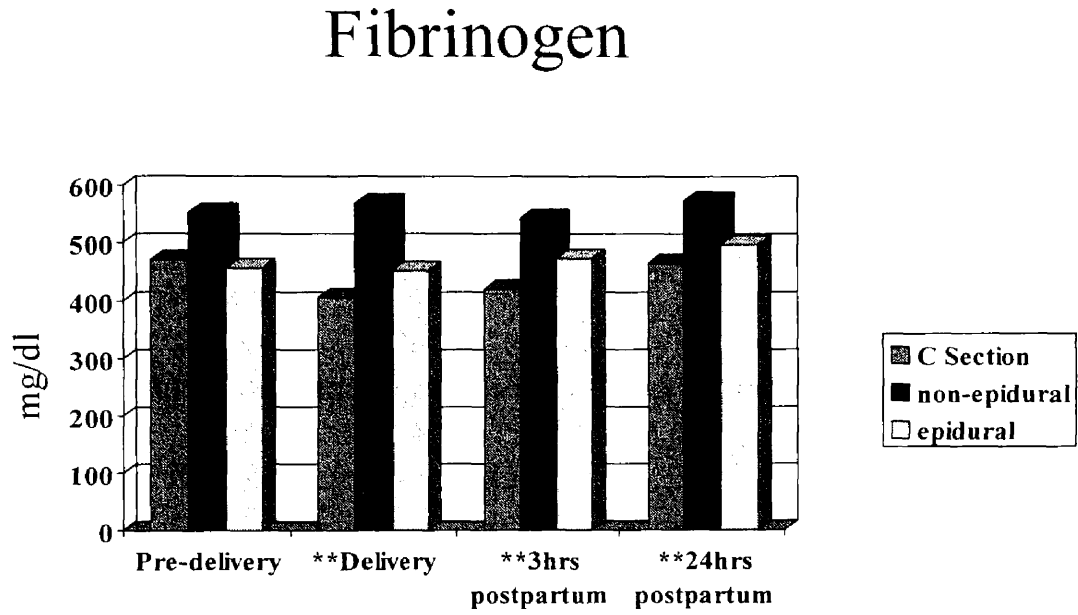
\*\* = statistical significance ( $p < 0.05$ )

**Figure 3: Platelet count among the three groups.**



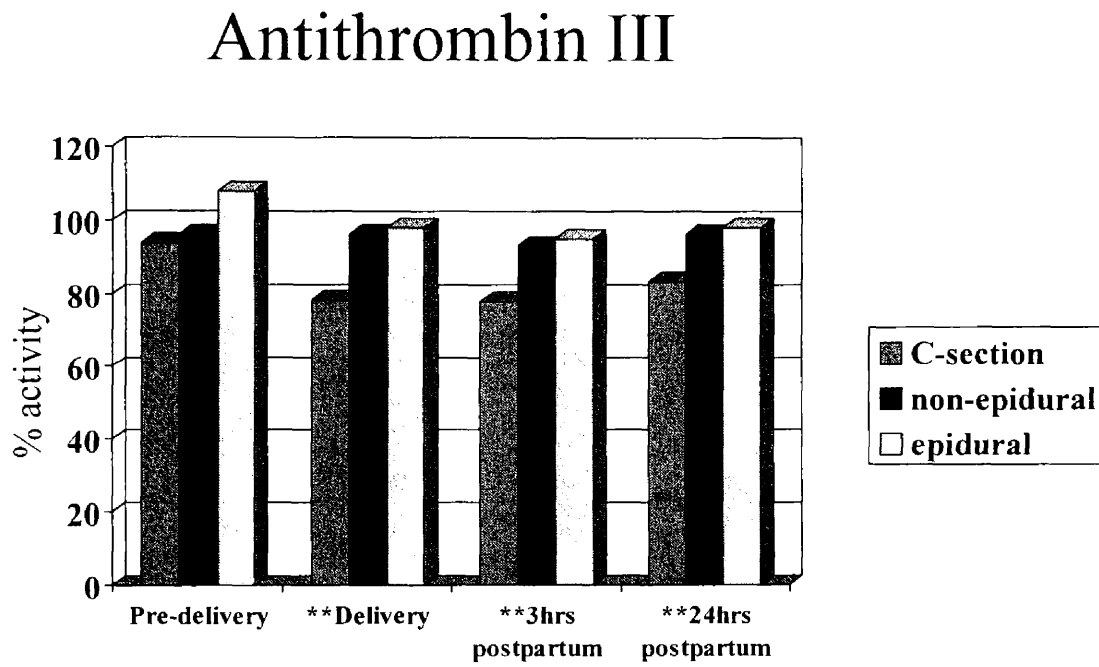
**No statistical significance**

Figure 4: Fibrinogen levels among the three groups.



\*\* = statistical significance (p < 0.05)

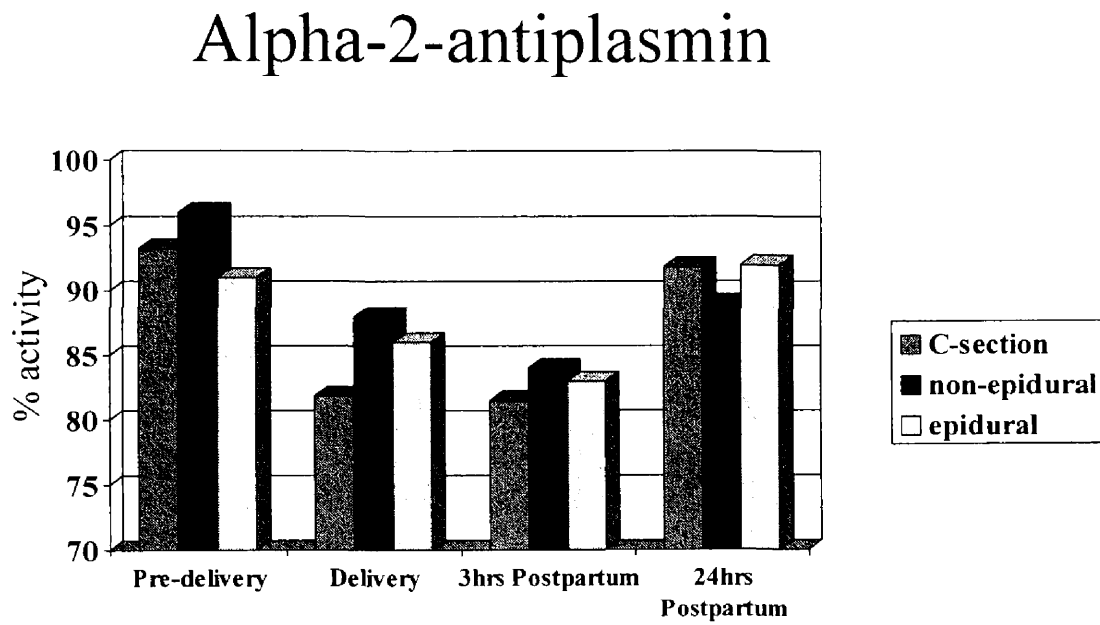
Figure 5: Antithrombin III levels among the three groups.



\*\* = statistical significance ( $p < 0.05$ )

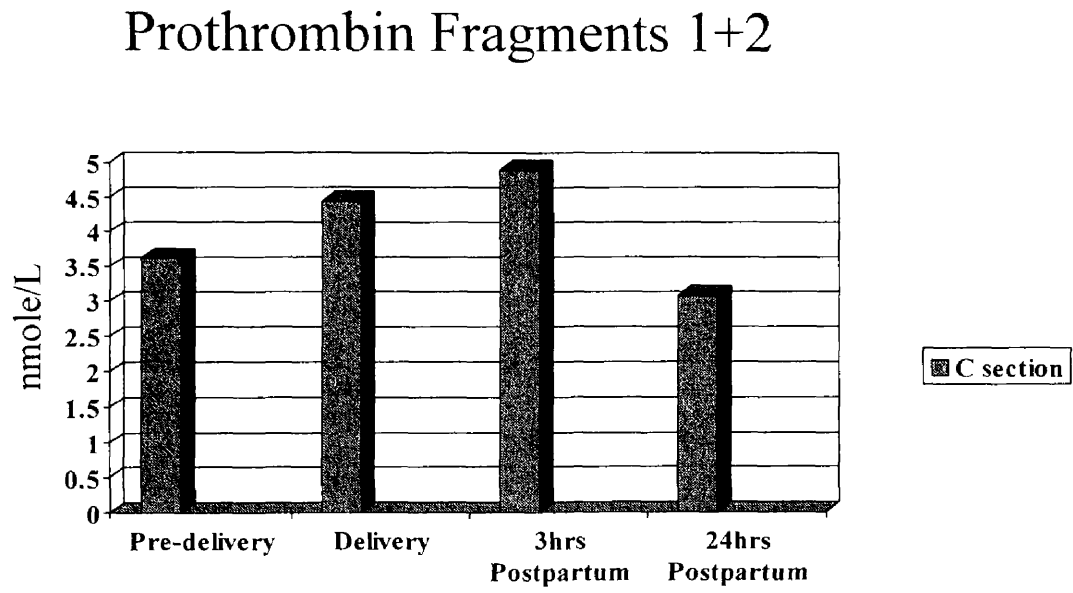


**Figure 6: Alpha-2-antiplasmin levels among the three groups.**

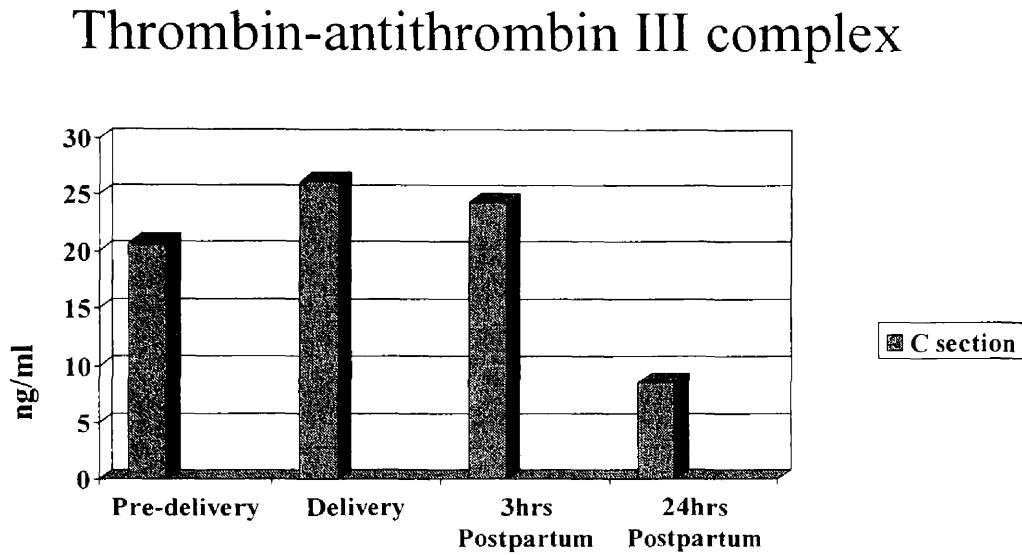


**No statistical significance**

**Figure 7: Prothrombin fragments 1+2 levels in the group one.**

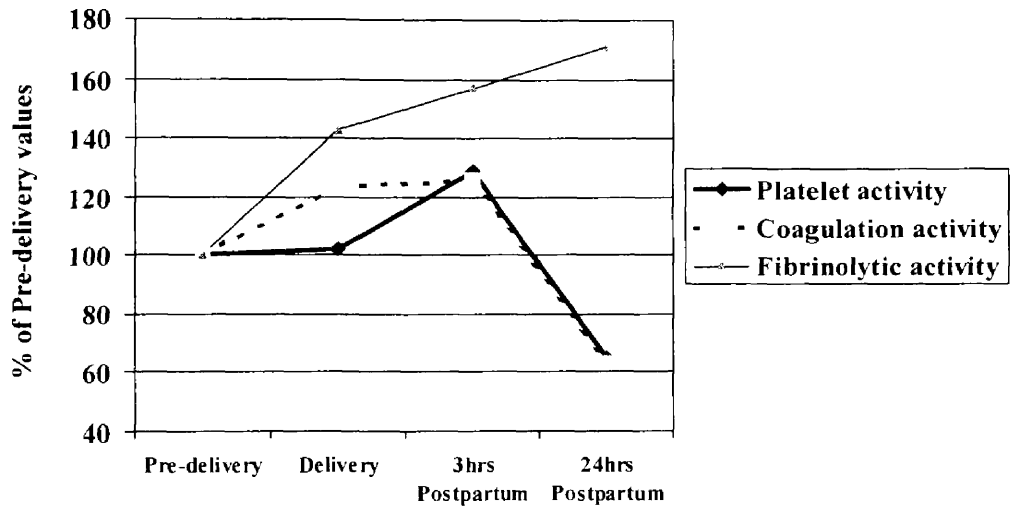


**Figure 8: Thrombin-antithrombin III complex levels in group one.**



**Figure 9: Platelet, coagulation, and fibrinolytic activity in the puerperium. Hemostasis activity is on basis of Beta-thromboglobulin and platelet factor 4 (platelet), prothrombin fragments 1+2 and thrombin-antithrombin III complexes (clotting), and D-Dimer (fibrinolysis).**

### Summary of Hemostasis Activity in Cesarean Group



Chapter XI

Appendices

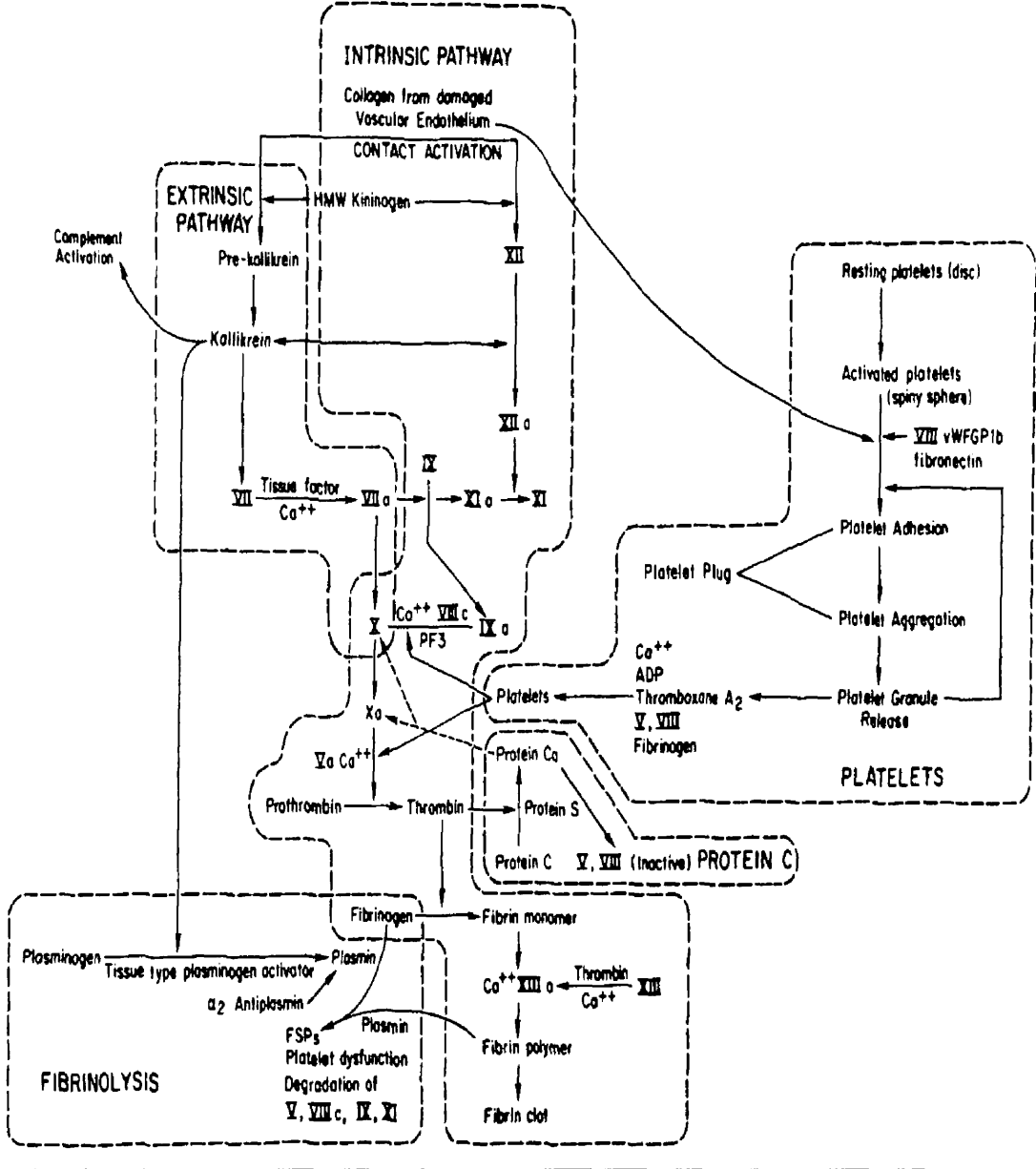
Appendix A

Hemostatic Factors<sup>2</sup>

Factor	Name	Type	Site of origin	Normal level	Minimal level (% NL)	Half-life (hr)
Factor I	Fibrinogen	—	Liver	150–300 mg%	50–100	72–144
Factor II	Prothrombin	Protease	Liver	100 µg/ml	20–40	72–120
Factor III	Tissue factor	Cofactor	Vasculature	Unknown	Unknown	Unknown
Factor IV	Calcium ions					
Factor V	Labile factor	Cofactor	Liver	5–12 µg/ml	5–10	12–36
Factor VII*	Stable factor	Protease	Liver	0.5 µg/ml	30	4–6
Factor VIII c	AHF	Cofactor		0.2 µg/ml	30	10–18
Factor VIII vWF	von Willebrand	Adhesion	Endothelium Megakaryocytes Platelet α granule	10 µg/ml	30	10
Factor IX	Christmas	Protease	Liver	5 µg/ml	20–25	18–36
Factor X	Stuart–Prower	Protease	Liver	10 µg/ml	10–20	24–60
Factor XI	PTA	Protease	Liver	5 µg/ml	20–30	40–80
Factor XII	Hageman	Protease	Liver	30 µg/ml	0	60
Factor XIII	Fibrin stabilizing	Transamidase	Liver, platelets	15 µg/ml	1–3	150

\* This is the only coagulation protein circulating in active form.

Appendix B



An overview of the hemostatic mechanism, which includes intrinsic and extrinsic pathways, the final common pathway, platelets, and the fibrinolytic system.<sup>2</sup>

### **Appendix C**

Inclusion criteria for our sample population consist of the following:

1. ages 18-45
2. ASA classification I or II
3.  $\geq 34$  weeks gestation

Exclusion criteria consist of the following:

1. ASA classification  $> II$
2. pre-existing coagulation abnormality/bleeding disorder
3. patients taking anticoagulants
4. use of illegal substances
5. prior history of thrombosis

## Appendix D

### Informed Consent University of Michigan-Flint/Hurley Medical Center Department of Anesthesia

Patient name: \_\_\_\_\_

Date: \_\_\_\_\_

**Project Title:** A Comparison of Hemostasis Parameters in Vaginal versus Cesarean Deliveries.

**Purpose:** The purpose of this study is to look at specific blood values in healthy pregnant women having a cesarean section (c-section). This will consist of four separate blood draws during the delivery period, 15ml (approx. 3 tsp) of blood for each draw. The times of these blood draws will be as follows: before delivery, within one hour of delivery, 3 hours after delivery, and 24 hours after delivery. There are normal clotting changes that occur with pregnancy. We will compare changes that occur with cesarean sections to vaginal deliveries to see if there is a difference. If you choose to participate in our study, it will not change the care you would normally receive. All information in this study will be available to the doctors involved in your care.

**Risks:** The risks with this study include discomfort during vein puncture, local swelling due to bleeding, and the remote possibility of local infection or blood clot in the vein that the blood was obtained from. Sterile technique will be used and every effort will be made to prevent the complications mentioned.

**Benefits:** The benefits will not be seen directly, but we hope to contribute to the body of knowledge of medical and anesthesia practice, specifically the use of regional anesthesia for delivery.

**Cost of the study:** This study is **voluntary**. There is no charge for your participation, nor is there reimbursement for participation.

**Consent:** I have been fully informed and I understand the procedure involved with this study along with the possible benefits and risks. My questions have been fully answered and I give my permission for participation in this study. I understand that I am free to withdraw my consent and discontinue my participation in this project at any time without prejudice to my medical care. I have received a copy of this informed consent document.



I understand that biomedical research such as this study involves the risk of injury. In the event of physical injury resulting from these procedures, no compensation or free medical care will be provided by Hurley Medical Center, the University of Michigan, or its affiliates.

In the event I believe that I have suffered any physical injury as a result of my participation in this study, I may contact Brooke Hendrick, RNAS (primary investigator), Dawn Jones, RNAS, Matt Williams, RNAS or Dr. Francis Gerbasi at the Department of Anesthesia, Hurley Medical Center at 810-257-9264. I may also contact the Institutional Review Board of HMC at 810-257-9963.

I agree to allow my name and study records to be available to other authorized physicians and researchers for the purpose of evaluating the results of this study. I consent to the publication of any data which may result from these investigations for the purpose of advancing medical knowledge, providing my name or any other identifying information is not used with such publication.

\_\_\_\_\_  
(Signature of Witness)

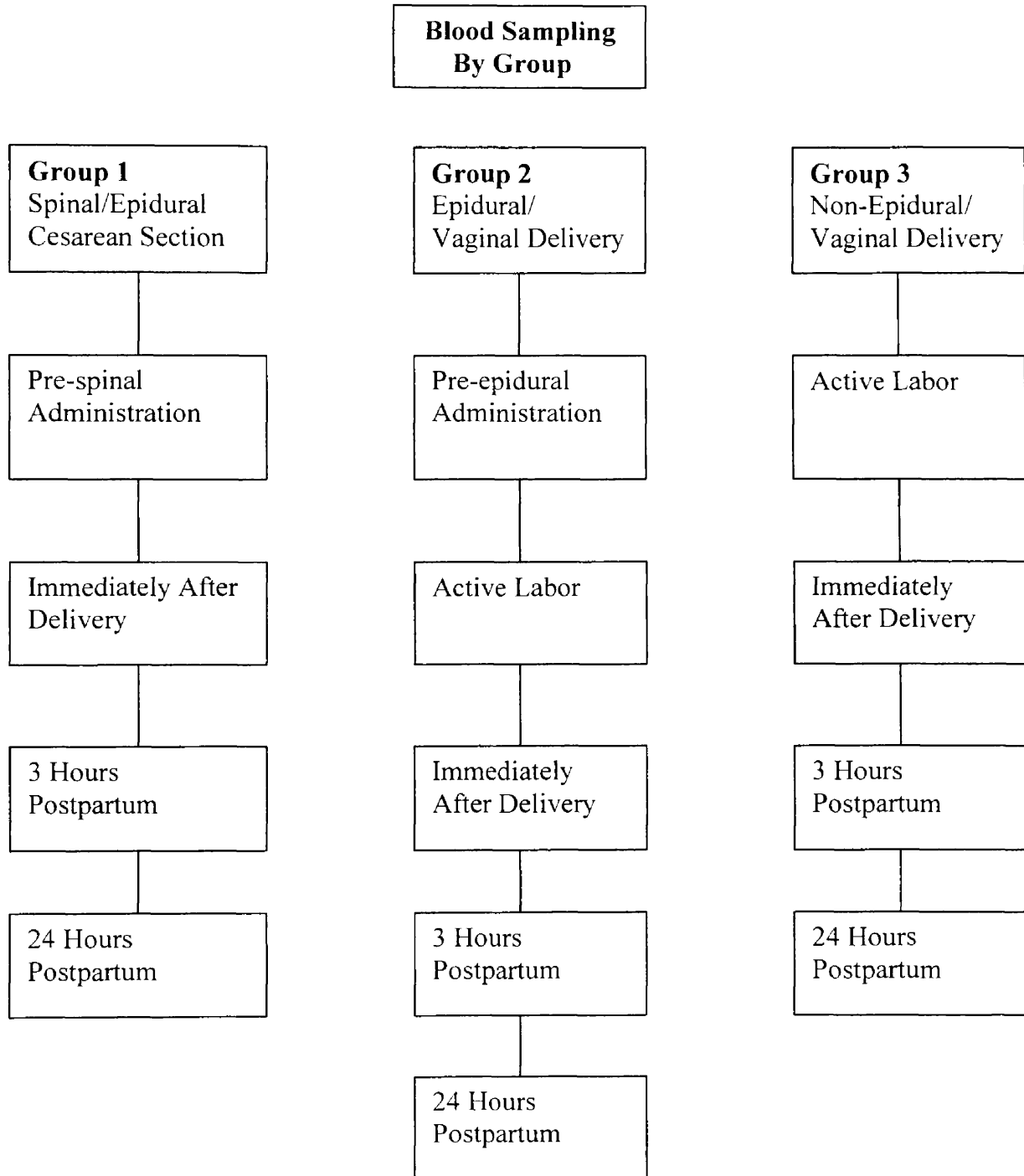
\_\_\_\_\_  
(Signature of Patient)

\_\_\_\_\_  
(Date)

I have fully explained to \_\_\_\_\_ the nature and purpose of the described study and the risks that are involved. I have answered and will answer all questions to the best of my ability.

\_\_\_\_\_  
(Signature of Investigator)

**Appendix E**



**Appendix F**

**Hemostasis Assays**

<b>Parameter</b>	<b>Instrumentation</b>	<b>Normal Range</b>
D-dimer	SimpliRED Agglutination Test American Diagnostica	< 120 ug/L (negative result)
Fibrin-fibrinogen degradation products	Thrombo Wellcotest Murex Biotech Limited	< 10 ug/mL
Platelet factor 4	EIA, Asserachrome Kit Diagnostica Stago	0-10 IU/mL
Beta-thromboglobulin	EIA, Asserachrome Kit Diagnostica Stago	10-40 IU/mL
Fibrinogen	Hemoliance fibrinogen detection kit	200-400 mg/dL
Antithrombin III	Chromogenic Assay Kit Instrumentation Laboratory	65%-105%
Alpha-2-antiplasmin	Chromogenic Assay Kit Instrumentation Laboratory	80%-115%
Prothrombin fragments 1+2	ELISA Assay Kit Dade Behring	0.2-1.2 nmole/L
Thrombin-antithrombin III complex	ELISA Assay Kit Dade Behring	0-3 ng/L

### Appendix G

#### Medical Information

Date: \_\_\_\_\_

Gravida: \_\_\_\_\_

Case #: \_\_\_\_\_

Para: \_\_\_\_\_

Name: \_\_\_\_\_  
\_\_\_\_\_

Blood type: \_\_\_\_\_

S.S. #: \_\_\_\_\_  
\_\_\_\_\_

Weeks gestation: \_\_\_\_\_

Age: \_\_\_\_\_

Weight (Kg): \_\_\_\_\_

Height (cm): \_\_\_\_\_

Ethnic origin: \_\_\_\_\_

1. History of medical problems prior to pregnancy? Y N  
If yes, explain: \_\_\_\_\_
2. History of thrombosis/embolism/bleeding disorder? Y N
3. Previous complications during pregnancy: Y N  
If yes, explain: \_\_\_\_\_
4. Present medical problems? Y N  
If yes, explain: \_\_\_\_\_
5. Present medications?  
\_\_\_\_\_
6. History of smoking? Y N PPD \_\_\_\_\_
7. History of illegal substance use? Y N

**Appendix H**

**Prior to C/S Data Collection Tool**

Date: \_\_\_\_\_

Case #: \_\_\_\_\_

S.S. #: \_\_\_\_\_

Group #: \_\_\_\_\_

Amount of IV fluid infused up to sampling: \_\_\_\_\_

**C-Section:**

..Time of spinal: \_\_\_\_\_

Medication used: \_\_\_\_\_

Time blood sample obtained: \_\_\_\_\_

## Appendix 1

### Delivery Data Collection Tool

Date: \_\_\_\_\_ Time blood sample drawn: \_\_\_\_\_

Case #: \_\_\_\_\_

S.S. #: \_\_\_\_\_

Time of delivery: \_\_\_\_\_

Time between previous sample and delivery of the baby: \_\_\_\_\_  
minutes

Estimated blood loss: \_\_\_\_\_ mls

Medication given between labor and delivery sample:  
Narcotic/pitocin/antiemetic?

Amount of IV fluid infused from period between previous sample and delivery of  
placenta: \_\_\_\_\_ ml

Infant sex:    male                  female

Birthweight: \_\_\_\_\_

APGAR 1 min. \_\_\_\_\_                  APGAR 5 min. \_\_\_\_\_

Gestation: \_\_\_\_\_ weeks

Baby's condition:    liveborn                  stillborn                  early death

Resuscitation required:    Y                  N

Criteria for disqualifying the patient from the study:

1. Administration of general anesthesia
2. Receiving anticoagulants
3. Patient receiving blood transfusion or plasma.

## Appendix J

### Postpartum Data Collection Tool

Date: \_\_\_\_\_

Case: \_\_\_\_\_

S.S. #: \_\_\_\_\_

Group #: \_\_\_\_\_

Sample # \_\_\_\_\_ 3 hrs postpartum \_\_\_\_\_ 24 hrs postpartum

Time of sample: \_\_\_\_\_

Blood pressure: \_\_\_\_\_

Presence of postpartum complications: Y N

If yes, explain: \_\_\_\_\_ thrombosis  
\_\_\_\_\_ bleeding  
\_\_\_\_\_ infection  
\_\_\_\_\_ other

Is the mother breast-feeding? Y N

Medications between last sampling and this sampling: \_\_\_\_\_ Pitocin  
\_\_\_\_\_ Demerol  
\_\_\_\_\_ Estrogen  
\_\_\_\_\_ Methergine  
\_\_\_\_\_ Other

IV infusion: Y N type \_\_\_\_\_

Amount of fluid since last sample: \_\_\_\_\_ mls

Criteria for disqualifying the patient from the study at three or twenty four hours postpartum:

1. Received anticoagulant.
2. Blood or plasma products.

**Appendix K**  
**Laboratory Data Sheet**

Case #: \_\_\_\_\_

S.S. #: \_\_\_\_\_

Sample #:

1. pre-C/S \_\_\_\_\_
2. delivery \_\_\_\_\_
3. three hours \_\_\_\_\_
4. twenty-four hours \_\_\_\_\_

	One	Two	Three	Four
<b>Time</b>				
<b>Date</b>				
<b>Hemoglobin</b>				
<b>Hematocrit</b>				
<b>Platelets</b>				
<b>Fibrinogen</b>				
<b>FDP's</b>				
<b>D-dimer</b>				
<b>PF 4</b>				
<b>BTG</b>				
<b>PF 1 + 2</b>				
<b>AT III</b>				
<b>A-2-A</b>				
<b>TAT complex</b>				



## Chapter XII

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