Trauma and pregnancy: is flow cytometry detection and quantification of fetal red blood cells useful? A retrospective cohort study

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Abstract

Objective: To assess whether positive flow cytometry quantification of fetal red blood cells is associated with adverse outcomes in cases of mild trauma during pregnancy. Design: A retrospective computerized database cohort. Population: Pregnant women with viable gestation involved in trauma who underwent flow cytometry. Flow cytometry was considered positive ([?]0.03/[?]30 ml). Methods: A univariate analysis was followed by a multivariate analysis. Main outcome measures: Composite adverse maternal and neonatal outcome was defined as one or more of the following: intrauterine fetal death, placental abruption, pre-term birth $<$ 37 weeks of gestation, immediate premature rupture of the membranes, and immediate delivery following trauma. Results: During the study 1023 women met inclusion and exclusion criteria. Among the cohort, 119 women (11.6%) had positive flow cytometry ([?]0.03/[?]30 ml) with median result of 0.03 [0.03-0.04], while 904 women (88.4%) had negative flow cytometry test result ([?]0.03/[?]30 ml) with median result of 0.01 [0.01-0.02]. Composite adverse outcome occurred in 8% of the women, with no difference in the groups with vs. without positive flow cytometry (4.2% vs. 8.5%; p=0.1). Positive flow cytometry was not associated with any adverse maternal or neonatal outcome. This was confirmed on a multivariate analysis. Conclusions: Flow cytometry result is not related to adverse maternal and fetal/neonatal outcome of women involved in minor trauma during pregnancy. We suggest that flow cytometry should not be routinely assessed in pregnant women involved in minor trauma.

Introduction

Trauma during pregnancy occurs in 1 to 12% of pregnancies[1]. In an attempt to optimize maternal and neonatal outcomes, predictors of serious perinatal consequences such as placental abruption, preterm delivery, lethal fetal injury, and uterine rupture, in addition to the full range of maternal injuries, have been sought[2–6]. The most significant risk factor for adverse maternal and neonatal outcomes is severe injury. Fortunately however, severe trauma occurs in only a minority of pregnant trauma patients[7].

As most trauma cases during pregnancy are mild, continuing exploration of risk factors for adverse maternal and neonatal outcomes in this setting is clinically important[8]. We hypothesized that feto-maternal hemorrhage (FMH), also called transplacental hemorrhage (TPH), could possibly be associated with the risk of adverse maternal and neonatal outcome.

FMH may be diagnosed via flow cytometry (FCM) for detection and quantification of fetal red blood cells, or by Kleihauer–Betke test (KB test). FCM is an accurate method to evaluate this type of bleeding[9–14] , based on the identification of fetal erythrocytes containing hemoglobin F (HbF) with an anti-HbF monoclonal antibody. The FCM method can analyze a very high number of erythrocytes, improving the sensitivity and specificity of the KB test[15,16]. Massive TPH associated with complete placental abruption is rare

and clinically evident, making FCM testing superfluous. FCM testing is commonly used to detect TPH in pregnant trauma women. Some studies have suggested that a positive KB test in the setting of trauma during pregnancy may be predictive of adverse perinatal outcomes, however other studies have shown differently[17,18]. We failed to find similar studies assessing the utility of FCM in this setting.

In this study, we aimed to assess whether positive FCM is associated with adverse perinatal outcomes in pregnant patients experiencing mild trauma.

Materials and Methods

Study design

Following Shaare Zedek Medical Center (SZMC) IRB approval (SZMC 0064-20) and given that the study was based on patient records, informed consent was waived. A retrospective cohort database study was conducted at Shaare Zedek Medical Center (SZMC), a tertiary obstetric and trauma center.

Clinical setting: The SZMC is a 1000-bed university-affiliated acute care hospital with a Division of Obstetrics that includes a high-risk pregnancy unit, two labor and delivery wards with 16 delivery rooms, two dedicated obstetric operating rooms and four maternity wards. There were approximately 15,000 annual delivery admissions during the study period. The SZMC is a level 2 trauma center in the center of Jerusalem, Israel, with annual trauma admissions of approximately 3000 patients. The trauma center serves a population of about 2 million including the city of Jerusalem and its surroundings, and patients from the West Bank. Our trauma center data is collected and analyzed as part of the National Israeli Trauma Database.

Study population

The medical records of all women that presented to the SZMC emergency room between January 2013 and December 2019 due to trauma during pregnancy were reviewed for the following inclusion criteria: viable pregnancy (gestational age >24.0 weeks) and at least one FCM test was obtained during their ER visit or hospitalization. Maternal trauma was defined as any blunt injury occurring during pregnancy resulting in evaluation in the emergency room or hospital admission. We excluded women with penetrating trauma as these are rare; we also excluded women with trauma in whom FCM test was not obtained, as our aim was to assess the predictive role of FCM, and finally we excluded women in whom a FCM test was obtained for reasons other than trauma.

Charts of all eligible women were reviewed and the following data was extracted: patient demographics, obstetric history, details of maternal injury, presence of preterm contractions, preterm labor (PTL), and placental abruption, surgical procedure(s) (if any), obstetric complications, and neonatal outcomes: fetal injury and death. We also devised an anatomic scoring system based on information in the medical chart, to provide an overall score for patients with multiple injuries: the Injury Severity Score (ISS).

The FCM data was extracted and recorded as both categorical values (FCM-positive or -negative) and continuous data (TPH volume when positive). According to our medical center protocol, FCM is obtained when the following criteria are met: direct abdominal injury, all cases of motor vehicle accident (MVA), and other blunt trauma cases where the attending physician deems it necessary.

Furthermore, according to local policy all such women undergo thorough evaluation including detailed history, past and current pregnancy information, trauma mechanism, acuteness/severity of the case, symptoms/signs of acute abdomen, vital signs, and assessment for uterine contractions and/or premature labor. Also, all women undergo fetal heart rate monitoring to detect fetal compromise, tocodynamometer for monitoring and recording uterine contractions, and sonographic examination of the fetus and placenta by certificated technician and/or obstetrician at the time of admission. Finally, decision regarding any surgical intervention is made by a senior physician.

Obstetric issues were managed by the perinatology team, consistent with the multidisciplinary approach at the SZMC and as recommended by the ATLS and ACOG [19,20]. Accordingly, women with any signs of regular uterine contractions (more than one in 10 minutes), vaginal bleeding, rupture of amniotic membranes, serious

maternal injury, significant abdominal/uterine pain or fetal tachycardia, decelerations, or non-reassuring fetal heart rate tracing, were monitored for a period of no less than 24 hours. When uterine contractions were associated with progressive cervical dilation, the definition of PTL was met and clinical management protocols implemented.

Following assessment by the obstetric team and judged necessary, five milliliters of maternal venous blood were collected into EDTA tubes, sent to the laboratory for FCM, stored at 4°C and processed within 72 hours of collection. The results of the FCM were typically available either at noon of the same day or the following day, and usually were not used for acute management decisions. Rather, FCM was used to decide whether to prolong observation time beyond 24 hours. Rarely, more than one blood sample was obtained. In women with a positive FCM test, another blood sample was obtained. The quantification of fetal blood hemorrhage by FCM has been described in detail elsewhere[13,21]. FCM was considered positive above 30 ml FMH, a volume that may trigger Rh sensitization [15].

Definition of outcomes

Postpartum hemorrhage (PPH) was defined as an estimated blood loss of > 500 mL and/or a hemoglobin drop of [?] 3 g% after a VD. PPH is often objectively measured according to the weight of the pads used after delivery.

Primary outcome was defined as a composite adverse maternal and neonatal outcome. The composite outcome included one or more of the following: intrauterine fetal death, placental abruption, pre-term birth < 37 weeks of gestation, immediate premature rupture of the membranes, and immediate delivery following trauma.

Secondary outcome was the occurrence of any of the individual complications that composed the composite adverse outcome.

Statistical analysis

Statistical analysis was performed with SPSS 25.0 (SPSS, Inc., Chicago, IL). Categorical variables are presented as percentages and were compared using the chi-square and Fisher's exact tests, as appropriate. Continuous variables are presented as mean and standard deviation; comparisons were made using Student's t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. All analyses were two sided, and a p value of < 0.05 was considered statistically significant.

Predictors of composite adverse maternal and neonatal outcomes were sought using a multinomial logistic regression model. For this analysis, composite maternal and neonatal outcomes were the dependent variable, whereas trauma scores, demographic details, and positive FCM test as a categorical variable, were independent variables. These confounders were identified according to their clinical significance and/or their statistical significance evaluated in the univariate analysis, and included in the multivariate analysis (vaginal bleeding at admission, uterine contractions at admission, multifetal gestation, and hospitalization). Adjusted Odds Ratios (aOR) and 95% confidence intervals (CI) were computed.

Results

During the study period, FCM test was obtained in 1296 pregnant women (Figure 1). Of these, 273 tests were performed for non- trauma indications and were excluded. Of the 1023 remaining, 387 (38%) were collected due to MVA, 367 (36%) due to fall, 353 (35%) had direct abdominal injury, and 14 (1%) had other mechanisms of trauma. (Some women had more than one mechanism of trauma recorded).

In 914 (89.3%) women who were evaluated due to a trauma incident during pregnancy some FMH was detected. In 119 (11.6%) women FCM test was considered positive ([?]0.03/[?]30 ml) with median result of 0.03 [0.03-0.04] and in 904 (88.4%) women FCM test was negative (([?]0.03/[?]30 ml) with median result of 0.01 [0.01-0.02].

Table 1 presents the demographic and obstetric characteristics of the study groups. The gestational age at

trauma did not differ between the groups. Also, no differences were noted in the gravidity/parity order or the number of previous miscarriages/cesarean deliveries. Accordingly, the rates of fertility treatments, multifetal gestation, and hypertensive or diabetic disorders of pregnancy were comparable between the groups.

The clinicopathological characteristics of the trauma event and short term maternal and neonatal outcomes, stratified by FCM negative or positive results, are presented in **Table 2.** Trauma mechanism did not differ between the groups. In addition, no differences were noted in the ISS and the maternal symptoms, including vaginal bleeding, uterine contractions, decreased fetal movements, rupture of the membranes, clinical or sonographic signs of placental abruption, or fetal death. However, the rate of trauma injury necessitating assessment by non-obstetric specialty was statistically higher in the positive FCM group (49.6% vs 40.5%, p=0.06). Nevertheless, the type of hospitalization, rate of non-obstetrical surgery, and the rate of delivery during hospitalization were also similar between the groups. However, length of stay (days) was statistically longer in the positive FCM group (1.4+-1.8 vs 1.1+-1.6, p=0.03).

Delivery information was available for 650/1023 (63.5%) women. The demographic and obstetric characteristics and the positive FCM rates of women who deliver in our medical center were mostly similar to those who did not deliver in our center. However, women who delivered in our medical center had higher rates of vaginal bleeding and uterine contractions at admission **(supplementary table 1)** .

Maternal and neonatal delivery outcomes stratified by FCM results is shown in **Table 3.** Of the women with available delivery information, 84 women (70.6%) had positive FCM results and 566 (62.6%) had negative FCM (p=0.09). No cases of fetal death were noted during trauma admission in both groups. The other maternal and neonatal delivery outcomes, including the composite adverse outcomes, were similar in both groups.

In order to evaluate the independent association between positive FCM and composite adverse outcome we fitted a multivariate model. We included all variables found to be significantly associated with composite adverse outcome in the univariate analysis (not presented): vaginal bleeding at admission, uterine contractions at admission, multifetal gestation, and hospitalization. The multivariate model revealed (in order of risk magnitude) that vaginal bleeding at admission, multifetal gestation and hospitalization were independently associated with the composite adverse outcome. However, the association between positive FCM and composite adverse maternal and neonatal outcome was not significant (adjusted OR 0.42, 95% CI 0.16-1.11, p=0.08), **Table 4** .

Comment:

Principal findings:

In this retrospective study, the role of FCM in predicting immediate and labor associated maternal and neonatal outcomes following trauma during pregnancy was examined. No independent association was found between positive FCM test results and immediate or delivery associated adverse maternal or neonatal outcome.

Results:

Upon assessment of pregnant women involved in trauma, one of the first steps is to assess trauma severity as determined by mechanism of injury, and signs and symptoms revealed by history taking, physical examination, and laboratory tests[22]. This is performed in order to triage women into high or low risk and hence determine their management and observation period[23]. Nevertheless, even minor trauma during pregnancy, which is the more common, may be associated with adverse outcomes. Several predictive factors for adverse maternal and fetal/neonatal outcomes have emerged over the years, these may include advanced maternal age and advanced gestational age (>35 weeks), primiparity and high-risk pregnancy, MVA characteristics such as ejection from the vehicle, pedestrian or motorcycle injury, lack of seatbelt use, and lastly, injury characteristics such as abdominal and pelvic injury, intracranial injury, internal organ injury, open injury, and loss of consciousness[23].

Transplacental passage of fetal blood into the maternal circulation is a common phenomenon, occurring in up to 98% of all pregnancies[24]. However, massive FMH is a rare condition during pregnancy. Possible causes include procedures of prenatal diagnosis, vaginal bleeding due to placental abnormalities (placenta previa or placental abruption), external cephalic version and maternal trauma [25].

Clinical implications:

Previous studies have shown that massive FMH (>150 mL) is associated with high perinatal mortality—ranging from 33% to 50%—because severe fetal anemia, hydrops, and stillbirth may occur[26–29]. The diagnosis of FMH is often difficult because it has nonspecific clinical symptoms and is associated with very specific sonographic signs, thus confirmation via laboratory testing such as the KB test or FCM is required [25].

Some have suggested that the KB test should be performed in every woman involved in major trauma during pregnancy to determine the degree of FMH, regardless of Rh status[30–33]. Studies performed to find an association between FMH diagnosed by positive KB test following trauma and adverse outcome have shown contradictory results[17,34–37]. Muench and colleagues, based on a study of 71 women, found that the KB test had a 100% sensitivity for the prediction of preterm labor[35], hence recommended its use as a predictor of risk for preterm labor after trauma. However, others have not found that the KB test has any predictive value and did not support the routine use of this test as a predictor of adverse outcome[34,36,38,39]. These reports did however find that the KB test has utility in women who are Rh-negative, in determining the need for additional Rh immune globulin to protect against isoimmunization but has little predictive value of other adverse pregnancy outcomes such as placental abruption, preterm birth, or fetal hypoxemia.

Interestingly, a recent survey by the College of American Pathologists demonstrated that the KB test is used for Rh positive women in 52% of the laboratories surveyed[40]. Given its labor intensity and the experience needed to perform the KB test, it is no wonder that some medical centers have started using the FCM. With the use of various known red blood cell group antigens, fluorescent antibodies are used to mark fetal erythrocytes and are quantified electronically. This is a more sensitive test, which may better identify severe FMH cases and predict perinatal outcome.

In our study, even though the vast majority of women involved in trauma during pregnancy had evidence of some FMH, only 11.6% had significant FMH (>30 ml). Our results are consistent with other studies[21,24].

As with the results of studies of the KB test, none of the demographic and prenatal risk factors and outcomes we studied seems to correlate with the positive FCM test. The only statistical differences between the study groups was the duration of hospitalization, which probably was affected by the FCM result itself and cannot indicate adverse outcome. In our literature review, we were unable to find studies that examined the relationship between FCM result and adverse outcome.

Despite the contradictory literature regarding the value of the KB test in Rh positive women involved in minor trauma, a recent report demonstrated many laboratories still perform the KB test in this setup[40]. It is possible that a positive KB test or a positive FCM result by themselves, do not necessarily indicate pathologic fetal-maternal hemorrhage in pregnant women with trauma[41]. As previously shown, a significant FMH occurred both in trauma and in a low-risk population during pregnancy, and among women with and without third trimester bleeding[38,42]. Hence diagnosis of placental abruption or fetal distress remains clinical, based on a combination of clinical signs and symptoms.

Strengths and limitations:

Our study had several notable strengths. This was a large population study and the first study to address FCM in maternal trauma. The SZMC computerized database is updated in real time and further validated, hence minimizing any potential bias. In addition, during the study period, our department followed a strict indication protocol for performing FCM testing with a uniform decision-making process and obstetric practice for maternal trauma. Accordingly, our study is appropriate for generalization.

Our study had some limitations, mainly attributable to its retrospective design and inherent pitfalls. In this

study, we did not include women with trauma during pregnancy in whom FCM test was not obtained. For many women, delivery information was not available in their medical files because they delivered in other medical centers. Nevertheless, when comparing maternal, pregnancy and trauma characteristics between those who delivered in our medical center and those who did not, we did not find major differences. A positive FCM was determined by our laboratory, however, it may be possible that only higher FCM is associated with adverse outcome.

In conclusion, in this retrospective cohort study FCM result was not related to adverse maternal or fetal/neonatal outcomes of women involved in minor trauma during pregnancy or their offspring. We suggest that FCM should not be routinely assessed in pregnant women involved in minor trauma, hence reducing patients' and physicians' unjustified expectations and the cost of care.

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MR: protocol development, data analysis, and manuscript writing/editing. RM: study design, protocol development, data collection, data analysis, IG and HA: literature search and data collection, ADS, ED and SGG: manuscript editing and critical review of the literature and the manuscript, HYS: study design, protocol development, and manuscript editing and critical revision of it.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required. This article does not contain any studies with animals performed by any of the authors. The Shaare Zedek Medical Center Institutional Review Board approved the study (0064-20), with waiver of informed consent due to the retrospective, observational design of the study**.**

Figure 1: Schematic Study Flowchart

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Table 1:

Table 1: Demographic and obstetric characteristics of women admitted with maternal trauma

	Negative FCM n=904
Maternal age (years)	29±5.6
Previous miscarriage	258 (28.5%)
Miscarriage > 3	30 (3.3%)
Gravidity	3.2±2.5
Parity	2.8±2
Primiparity	305 (33.7%)
Previous CD	103 (11.4%)
Fertility treatment	17 (3.3%)
Hypertensive disorders (of pregnancy)	12 (2.2%)
Diabetes (gestational or pre-gestational)	20 (3.9%)
Multifetal gestation	22 (2.4%)
Gestational age at trauma (weeks)	31.5±5
FCM - Flow cytometry. Data are mean± standard deviation; number (%);	FCM - Flow cytometry. Data are mean± stan

Table 2:

Table 2: The clinicopathological data of the trauma event and short term maternal and neonatal outcome

Mechanism of trauma*
Motor vehicle accident
Fall
Direct abdominal injury
Other
Injury severity score
Vaginal bleeding
Uterine contractions (more than 1 in 10 min)
Decreased fetal movements
Rupture of membranes
Sonographic placental abruption signs
Fetal death
Seen by other specialty: general surgeon / orthopedic / other
Other obstetric complications
ER admission only
Hospitalization in high-risk pregnancy department
Hospitalization in another department
Non-obstetric surgery
Length of stay
Delivery during hospitalization
Adverse maternal & neonatal outcome
Some women had more than one mechanism of trauma FCM - Flow cytometry. ER – Emergency Room. Data are mean± s

Table 3:

Table 3: The maternal and neonatal delivery outcomes

Trauma to delivery interval (days)
Gestational age at delivery (weeks)
Preterm delivery (<37 weeks)
Induction of labor
Epidural analgesia
Cesarean delivery
In labor cesarean delivery
Operative vaginal delivery
Anemia (Hb<11gr%) **at admission**
Neonatal birth weight (grams)
1-Minute Apgar score [?] 7
5-Minute Apgar score [?] 7
Neonatal ICU admissions
Maternal ICU admissions
Postpartum hemorrhage
Placental abruption
Severe postpartum hemorrhage (delta Hb> 4 gr%)
Blood products transfusion
Composite adverse maternal & neonatal outcome*

Data are mean± standard deviation; number (%); FCM - Flow cytometry. Hb-Hemoglobin
Composite adverse maternal and neonatal outcome was defined as one or more of the following complications: intrauterine

Table 4:

Table 4: Risk factors for composite adverse maternal and neonatal outcome*; multivariate analysis

Variables:
Vaginal bleeding
Multifetal gestation
Hospitalization in high-risk pregnancy department
Uterine contractions (more than 1 in 10 min)
Positive FCM

aOR adjusted odds ratio, CI Confidence interval, FCM Flow cytometry
Composite adverse maternal and neonatal outcome was defined as one or more of the following complications: intrauterine

Supplementary table 1

Supplementary table 1: Demographic, obstetric and clinicopathological characteristics of women with or without available delivery information

	Not delivered at SZM
Maternal age (years)	28.8±5.6
Previous miscarriage	99 (26.5%)
Miscarriage > 3	10 (2.7%)
Gravidity	3±2.4
Parity	2.6±2

Primiparity	131 (35.1%)
Previous CD	43 (11.5%)
Positive FCM	35 (9.4%)
Gestational age at trauma (weeks)	30.7±4.8
Mechanism of trauma*	**Mechanism of trau**
Motor vehicle accident	165 (44.2%)
Fall	121 (32.4%)
Direct abdominal injury	122 (32.7%)
Other	3 (0.8%)
Injury severity score	0.2±0.8
Vaginal bleeding	3 (0.8%)
Uterine contractions (more than 1 in 10 min)	69 (18.5%)
Decreased fetal movements	14 (3.8%)
Rupture of membranes	1 (0.3%)
Sonographic placental abruption signs	4 (1.1%)
Fetal death	0 (0%)
ER admission only	86 (23.1%)
Hospitalization in high-risk pregnancy department	277 (74.3%)
Hospitalization in another department	4 (1.1%)
Non-obstetric surgery	1 (0.3%)
Length of stay	1±1.1
Seen by other specialty: general surgeon / orthopedic / other	174 (46.6%)
Delivery during hospitalization	3 (0.8%)
FCM - Flow cytometry. ER – Emergency Room. Data are mean± standard deviation; number (%);	FCM - Flow cytomet

Figure 1: Schematic Study Flowchart