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Fetal thrombocytopenia in pregnancies with fetal human parvovirus-B19 infection

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CONDENSATION

Severe fetal thrombocytopenia is relatively common in fetal hParvo-B19 infection and may increase the risk of procedure related fetal loss following fetal blood sampling (36.4% vs. 11.1%).

Short title: Parvovirus-B19 and fetal thrombocytopenia

ABSTRACT

Objective: Fetal infection with human parvovirus B19 (hParvo-B19) has been mainly associated with fetal anemia, while data regarding other fetal hematological effects are limited. Our aim was to assess the rate and consequences of severe fetal thrombocytopenia following fetal hParvo-B19 infection.

Methods: We conducted a retrospective study of pregnancies complicated by fetal hParvo-B19 infection which underwent fetal blood sampling (FBS). The characteristics and outcome of fetuses with severe thrombocytopenia (< 50 $x10^9$/L) were compared with those of fetuses with a platelet concentration ≥ 50 $x10^9$/L. (controls). Fetuses in whom 3 FBS's were performed (N=4) were analyzed to assess the natural history of platelet levels following fetal hParvo-B19 infection.

Results: A total of 37 pregnancies affected by fetal hParvo-B19 infection were identified. Of the 29 cases which underwent FBS and had information regarding fetal platelets, 11 (38%) were complicated by severe fetal thrombocytopenia. Severely thrombocytopenic fetuses were characterized by a lower hemoglobin concentration (2.6±0.9 vs. 5.5±3.6 g/dL, p=0.01), lower reticulocyte count (9.1±2.8% vs. 17.3±10.6%, p=0.02), and lower gestational age (GA) at the time of diagnosis (21.4±3.1 vs. 23.6±2.2 wks, p=0.03). Both the fetal death rate within 48 hrs of FBS (27.3% vs. 0%, p=0.02), and the risk of prematurity (100.0% vs. 13.3%, p<0.001) were higher in fetuses with severe thrombocytopenia. Fetal thrombocytopenia was more common during the 2nd trimester, but in some cases persisted into the 3rd. Intrauterine transfusion (IUT) of RBCs resulted in a further mean decrease of 40.1±31.0% in fetal platelet concentration.

Conclusion: Severe fetal thrombocytopenia is relatively common following fetal hParvo-B19 infection, can be further worsened by IUT and may be associated with an increased risk of procedure related fetal loss following either FBS or IUT.

Key words: Parvovirus, fetal, pregnancy, thrombocytopenia

INTRODUCTION

Human Parvovirus B19 (hParvo-B19) infection complicates up to 1-2% of pregnancies and is associated with a fetal vertical transmission rate of 30-50% [1-4]. hParvo-B19 infection is one of the most common infectious causes of fetal anemia, hydrops and death [5].

The most studied clinical consequence of fetal hParvo-B19 infection is the induction of apoptosis of erythroid precursors cells [6, 7], leading to fetal anemia which, in severe cases, can result in high output fetal heart failure, hydrops and death [8, 9]. Recent data suggest that fetal hParvo-B19 infection can also be associated with fetal thrombocytopenia in 15-54% of cases [10-15], thought to be secondary to a direct cytotoxic effect of hParvo-B19 on megakaryocytes [16]. This is of major importance, as it can lead to fetal hemorrhagic complications, including hemorrhage and even exsanguination following fetal blood sampling (FBS) and/or intrauterine transfusion (IUT) of red blood cells (RBC) [10, 12, 17].

Unfortunately, data on hParvo-B19 induced fetal thrombocytopenia are relatively sparse, and have been reported in few studies [10-15], most of which included only a small number of cases (n=8-13) [10, 12, 13, 15]. In only two of those studies was fetal thrombocytopenia the main focus of the study [12, 14]. Furthermore, data on factors that are predictive of severe thrombocytopenia, the impact of severe thrombocytopenia on perinatal outcome, the natural history of fetal thrombocytopenia, and the possible impact of RBC IUT on the severity of fetal thrombocytopenia are even more limited.

The aims of this study were to address several questions related to severe fetal thrombocytopenia in pregnancies complicated by fetal hParvo-B19 infection, including: 1) the

rate of severe thrombocytopenia, 2) the possible impact of severe thrombocytopenia on perinatal mortality, 3) the factors predictive of severe thrombocytopenia, 4) the natural history of such thrombocytopenia, and 5) the effect of RBC IUT on platelet concentration.

METHODS

This was a retrospective study of all pregnancies complicated by fetal hParvo-B19 infection which underwent FBS +/- IUT at a single tertiary care fetal medicine referral centre from August 1992 to January 2014 (Figure 1). The study was approved by the institutional research ethics board (#13-0090-C).

Cases were identified using the fetal medicine unit database and the following data were retrieved: demographics, medical and obstetric history, characteristics of the hParvo-B19 infection, including gestational age (GA) at diagnosis, maternal symptoms, maternal serology, fetal manifestations (ultrasound (US) and laboratory) and management.

The diagnosis of fetal hParvo-B19 infection was made during the investigation of fetal anemia and/or ascites or hydrops detected either on routine US examination or as a result of fetal monitoring due to maternal exposure to and/or infection with hParvo-B19. Doppler US evaluation of the middle cerebral artery peak systolic velocity (MCA-PSV) was used to assess for fetal anemia [18]. Fetal hParvo-B19 infection was confirmed either by positive PCR for DNA on amniotic fluid or fetal ascites, or the presence of IgM antibodies in fetal blood. All women with fetal hParvo-B19 infection in whom fetal anemia was suspected were offered FBS. The most common sampling route was the intrahepatic segment of the umbilical vein (IHV, 83.7%) or the umbilical vein (UV, 18.6%) at the placental cord root. Rarely, if fetal vascular access was difficult, such as at very early GA's, an intra-cardiac (9.3%) or intra-peritoneal (2.3%) approach was used. Fetal blood was sent for rapid analysis of Hb (g/dL), platelets ($x10^9$/L), and reticulocyte count (%). If fetal anemia was confirmed, an IUT was performed at the same session of the FBS, as described previously [19]. The level of thrombocytopenia at which platelets were

transfused was at the discretion of the fetal medicine staff physician. Ultrasound examinations were usually repeated on the following day and then weekly thereafter. Subsequent FBS +/- IUT's were determined by MCA-PSV values. Fetal thrombocytopenia was defined as a platelet concentration < 150 $x10^9$/L, and was classified as mild (100-149 $x10^9$/L), moderate (50-99 $x10^9$/L) or severe (<50 $x10^9$/L).

Data analysis was performed with IMB SPSS (Statistical Package for the Social Sciences) v19.0 (Chicago, IL). Assuming that it is the presence of severe fetal thrombocytopenia which has the most important clinical implications, we compared the characteristics and outcome of severely thrombocytopenic fetuses (<50 $x10^9$/L) with those with platelet concentrations ≥ 50 $x10^9$/L (controls). The Student's t-test and Mann-Whitney U test were used to compare continuous variables with and without a normal distribution. The Chi-square and Fisher's exact tests were used for categorical variables. Spearman's correlation coefficient was used to assess the correlation between platelet concentration and other continuous variables such as fetal Hb concentration and GA at diagnosis. Fetuses in whom three FBS's were performed were analyzed to assess the natural history and rate of recovery of platelets. Comparison of platelet levels before and immediately after RBC IUT was performed to assess the dilutional effect of RBC transfusion on fetal platelets. Differences with $p < 0.05$ were considered to be significant.

RESULTS

Characteristics of the study and control groups

Of 37 fetuses with hParvo-B19 infection, 31 underwent FBS, in 29 of whom fetal platelets results were available (Figure 1). Of these, 11 (37.9%) were severely thrombocytopenic and constituted the study group (Figure 1). The controls (n=18) included 10 (34.5%) moderately thrombocytopenic, one (3.4%) mildly thrombocytopenic and 7 (24.1%) fetuses with a normal platelet count (Figure 1).

The characteristics of the severely thrombocytopenic and control fetuses are presented in Table 1. Women whose fetuses were severely thrombocytopenic were more likely to be > 35 years old, and GA at diagnosis of fetal hParvo-B19 infection was earlier. There were no differences between the severely thrombocytopenic and control groups with respect to parity, fetal sex, maternal symptoms of hParvo-B19 infection or fetal US findings (Table 1).

Severely thrombocytopenic fetuses had significantly lower Hb and reticulocyte levels compared to controls (2.6 vs. 5.5 g/dL, and 9.1% vs. 17.3%, respectively) (Table 1). Platelets were transfused to 8 (72.7%) severely thrombocytopenic fetuses compared with 5 (27.8%) controls (Table 1).

There was a significant correlation between fetal platelet and Hb concentrations ($r=0.67$, $p<0.001$) (Figure 2), such that fetuses with lower Hb's were more likely to have lower platelet concentrations. There was also a significant correlation between fetal platelets and GA at diagnosis ($r=0.51$, $p=0.006$) (Figure 3). Mean fetal platelet concentration was significantly lower

in cases diagnosed with hParvo-B19 infection ≤ 22 wks gestation compared to those diagnosed later in pregnancy (52.7 vs. 152.1 x10^9/L, respectively, p=0.015) (Figure 3).

Perinatal outcome in study and control groups

Severely thrombocytopenic fetuses were more likely to die *in-utero*, and this difference was significant for fetal deaths within 48 hours of the procedure (Table 2). The rate of preterm delivery was significantly higher for fetuses which were severely thrombocytopenic compared to controls (Table 2). There were no statistically significant differences in neonatal Hb or platelet concentrations between the groups (Table 2).

Table 3 presents the details of the cases complicated by fetal death. In severely thrombocytopenic fetuses, two deaths occurred due to hemorrhage from the puncture site in the umbilical cord at the time of the FBS procedure. The two other cases of fetal death were diagnosed 2 and 3 days after the procedure (Table 3). The two cases of fetal death in the control group occurred 5 and 7 days following the procedure, one of which was attributed to chorioamnionitis (Table 3).

Natural history of thrombocytopenia

To address the natural history and rate of recovery of fetal thrombocytopenia in fetal hParvo-B19 infection, we analyzed platelet levels in 4 cases in which three serial FBS's were performed (Figure 4). In two, there was a rapid resolution of fetal thrombocytopenia after 23 wks

gestation (Figure 4 A & B), whereas in the others, platelet concentrations continued to fall, even into the 3rd trimester (Figure 4 C & D). In the latter, the fall in platelet concentration in the 3rd trimester was accompanied by a similar decrease in Hb concentration, possibly as a result of ongoing bone marrow suppression.

Effect of RBC transfusion on platelet concentration

Another practical question relates to the possible dilutional effect of transfused RBC's in fetal hParvo-B19 infection. This might further lower platelet concentrations in fetuses which are already severely thrombocytopenic. To address this question we compared platelet counts before and immediately after IUT in fetuses which were only transfused with RBC's (i.e. no platelets). RBC transfusion was associated with a mean decrease of 40.1±31.0% (-45±69 $x10^9$/L) in platelet concentration.

COMMENT

The aims of our study were to assess the incidence, consequences and predictive factors of severe fetal thrombocytopenia in pregnancies complicated by fetal hParvo-B19 infection. Our study has several key findings: 1) severe fetal thrombocytopenia complicates almost 40% of cases of fetal hParvo-B19 infection undergoing FBS; 2) severe fetal anemia, low reticulocyte count and earlier GA at diagnosis (≤22 wks) are associated with an increased risk of severe fetal thrombocytopenia; 3) severe fetal thrombocytopenia appears to be associated with an increased risk of fetal death within 48 hrs of FBS; 4) although severe fetal thrombocytopenia following fetal hParvo-B19 is more common during the 2nd, trimester it may persist into the 3rd; 5) IUT of RBC's may result in a further decrease in fetal platelet concentration.

Most studies on fetal hParvo-B19 infection have focused on the risk of fetal anemia secondary to the viral apoptotic effects on erythroid precursors [6, 7]. Some studies have reported that fetal hParvo-B19 may also be associated with fetal thrombocytopenia, which has been postulated to be secondary to a direct cytotoxic effect of hParvo-B19 on megakaryocytes [16]. However, data on hParvo-B19 induced fetal thrombocytopenia are from a limited number of studies, most of which included only small numbers of cases [10-15], and in only two of these was fetal thrombocytopenia the main focus of the study [12, 14] (Table 4). The incidence of thrombocytopenia varied widely in these studies, with the range being 71%-100%, 57%-87%, and 18%-55% for platelets concentration of less than $150x10^9$/L, $100x10^9$/L and $50x10^9$/L (severe thrombocytopenia), respectively (Table 4). This variation may be attributable to differences in the severity of hParvo-B19 infection in the study populations. We suggest that the rate of severe thrombocytopenia in our study (38%) probably reflects that in fetuses at the severe end of the spectrum, as 86% (25/29) were hydropic. It should be emphasized that these rates

relate only to hydropic fetuses, while information on the rate of thrombocytopenia in non-hydropic fetuses is lacking. . In fact, we were able to identify only 2 cases of non-hydropic fetuses with information on platelets concentration - in the study of Schild et al. only 2 out of the 37 women that underwent FBS involved non-hydropic fetuses, and fetal platelets concentrations in these cases were 161 x109/L and 223 x109/L, respectively [11].

Identifying fetuses with hParvo-B19 infection which are at particular risk of severe thrombocytopenia is of clinical importance, as this would allow the appropriate preparation of platelets for transfusion prior to FBS or IUT and may affect the decision regarding the site of FBS [20]. This information may also serve to counsel patients regarding the risk of procedure-related complications. Indeed, several studies have reported that severe fetal thrombocytopenia is associated with an increased risk of fetal death due to severe hemorrhage or exsanguination following FBS and/or IUT [10, 12, 17]. However, data on risk factors for severe fetal thrombocytopenia in hParvo-B19 infection are limited. We have found several factors that are associated with severe fetal thrombocytopenia: 1) severe fetal anemia, 2) a low reticulocyte count, and 3) diagnosis at ≤22 wks gestation. Similar to our findings, Schild *et al.* [11] reported a significant correlation between Hb and platelet concentration with a correlation coefficient of r=0.67, which is identical to that found in our study. This probably implies that the cytotoxic effect of hParvo-B19 on fetal megakaryocytes correlates with the viral apoptotic effect on erythroid precursors. Whether these findings are independent of one other could not be determined since our sample size was not large enough to allow for multivariate analysis. Although fetal Hb concentration and reticulocyte count cannot be assessed prior to FBS, MCA-PSV on Doppler US is a very reliable predictor of the severity of fetal anemia [18].

We have found that the risk of fetal death within 48 hrs of FBS is higher in severely thrombocytopenic fetuses. The proximity of fetal death to the FBS procedures suggests that fetal hemorrhage/exsanguination secondary to severe thrombocytopenia may have been causative. Indeed, we clearly documented procedure related exsanguination on US in one of the cases of fetal death. While two previous studies reported an increased rate of fetal loss due to exsanguination from the umbilical cord puncture site in thrombocytopenic fetuses [10, 12, 17], a more recent study [14] did not find severe thrombocytopenia to be associated with an increased risk of fetal death. This may relate in part to the routine administration of platelets to all severely thrombocytopenic fetuses in the latter study. Such an increase in the risk of procedure-related complications in the presence of fetal thrombocytopenia has been also observed in pregnancies complicated by alloimmune thrombocytopenia [21, 22]. Although it is usually considered unlikely that significant fetal hemorrhage will occur at platelet counts > 20 x 10^9/L, it has been our practice in recent years to transfuse platelets if the fetal count is < 50 x 10^9/L. Of concern, one fetus died from a procedure related hemorrhage from the cord root with a platelet count of 49 x 10^9/L (Table 3). Similarly, whenever we anticipate fetal thrombocytopenia, to minimize the procedure related risk, we sample fetal blood from the IHV rather than the UV at the placental cord root, with the rationale that any extravasation would result in an intraperitoneal transfusion and, in addition, there are no adjacent arteries that can be compressed by such extravasation [20].

There are no data regarding the natural history or rate of recovery of fetal thrombocytopenia in hParvo-B19 infection. In cases where 3 serial FBS's were performed, we found that, while in some cases fetal platelets recovered rapidly after 22 wks gestation, in others thrombocytopenia persisted into the early third trimester. Thus, despite the small numbers, this

suggests that it is prudent to have platelets prepared at the time of subsequent FBS procedures as well.

Another factor that should be taken into account with respect to fetal thrombocytopenia in the context of hParvo-B19 infection is that IUT of RBC's can cause a further drop in platelets, probably due to a dilutional effect. This implies that the severity of thrombocytopenia may even transiently worsen following RBC transfusion, which might further increase the risk of fetal hemorrhage. Indeed, we have found that platelet concentration decreases by an average of 40% following RBC transfusion. In concordance with our findings, de Haan *et al*. [14] reported that fetuses who were transfused with RBC's only (n=14) showed a significant decrease in platelets following transfusion. Similarly, Segata *el al.* [12] reported that platelet count decreased in all 6 fetuses with hParvo-B19 infection following RBC transfusion.

The main limitations of our study relate to its retrospective nature, as well as the lack of more detailed laboratory information, including viral load and megakaryocyte count. Neither could we determine whether the association of severe thrombocytopenia with fetal death following FBS was independent of other fetal hematologic aberrations (e.g., severe fetal anemia), since the sample size was not large enough to allow for adjustment for potential confounding factors. Nevertheless, this study represents one of the largest series focusing on fetal thrombocytopenia secondary to hParvo-B19 infection managed at a single center, and provides unique data with respect to the natural history of fetal thrombocytopenia in these cases.

In summary, we have found that severe fetal thrombocytopenia is a relatively common finding in pregnancies complicated by fetal hParvo-B19 infection that require FBS and may be associated with an increased risk of fetal death following FBS. We suggest that platelets should

always be available for transfusion at the time of any FBS in suspected or proven fetal hParvo-B19 infection, especially in cases diagnosed at ≤ 22 wks gestation or when severe fetal anemia is suspected on the basis of MCA-PSV Doppler US. Furthermore, the additional drop in fetal platelets concentration following RBC transfusion should be taken into consideration in these cases. Further studies are necessary to provide more insight with respect to the mechanisms responsible for hParvo-B19 induced fetal thrombocytopenia and the contribution of severe fetal thrombocytopenia to perinatal morbidity and mortality in these pregnancies.

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TABLES

Table 1: Characteristics of study and control groups

Characteristic	Severe thrombocytopenia [Platelets <50 x10^9/L] (n=11)	Controls [Platelets ≥50 x10^9/L] (n=18)	p
Maternal age (yr)	32.5±6.1	30.3±5.3	0.3
> 35 yrs	5 (45.5)	2 (11.1)	**0.04**
Parity	2 (1-2)	1 (0-2)	0.3
Nulliparity	1 (9.1)	7 (38.9)	0.08
Fetal male sex	6 (54.5)	11 (61.1)	0.7
Characteristics of infection			
Gestational age at diagnosis (wks)	21.4±3.1	23.6±2.2	**0.03**
≤22 wks	8 (72.7)	7 (38.9)	0.08
Maternal symptoms	4 (36.4)	6 (33.3)	0.9
Fetal findings			
Hydrops	10 (90.9)	15 (83.3)	0.6
Ascites	10 (90.9)	17 (94.4)	0.7
Pericardial effusion	6 (54.5)	10 (55.6)	0.9
Pleural effusion	2 (18.2)	3 (16.7)	0.9
Subcutaneous edema	5 (45.5)	6 (33.3)	0.5
Placentamegaly / thickening	3 (27.3)	3 (16.7)	0.5
Echogenic bowel	2 (18.2)	5 (27.8)	0.6
Cardiomegaly	7 (63.6)	5 (27.8)	0.06

Degree of thrombocytopenia			N/A
Severe (<50 x10^9/L)	11 (100.0)		
Moderate (50-99 x10^9/L)		10 (55.6)	
Mild (100-149 x10^9/L)		1 (5.6)	
Normal (≥150 x10^9/L)		7 (38.9)	
Management			
Total number of FBS's	17	28	0.9
Fetuses undergoing 2 FBS's	2 (18.2)	4 (22.2)	0.8
Fetuses undergoing 3 FBS's	2 (18.2)	3 (16.7)	0.9
Platelet count (x10^9/L)	31.6±10.2	144.2±120.6	**0.005**
Platelet transfusion	8 (72.7)	5 (27.8)	**0.02**
Hemoglobin (g/dL)	2.6±0.9	5.5±3.6	**0.01**
Hemoglobin <4.0 g/dL	10 (90.9)	9 (50.0)	**0.03**
Reticulocytes (%)	9.1±2.8	17.3±10.6	**0.02**

Data are presented as mean ± SD, n (%), or median (inter-quartile ranges)

FBS, fetal blood sampling; RBC, red blood cells.

Table 2: Perinatal outcome of study and control groups

Outcome	Severe thrombocytopenia [Platelets <50 x10^9/L] **(n=11)**	Controls [Platelets ≥50 x10^9/L] **(n=18)**	P
Gestational age at first transfusion (wks)	21.7±3.3	23.7±2.2	0.07
TOP	1 (9.1)	0 (0.0)	0.2
Fetal death	4 (36.4)	2 (11.1)	0.1
Within 48 hrs of procedure	3 (27.3)	0 (0.0)	**0.02**
Neonatal death	0 (0.0)	1 (5.6)	0.4
Live birth	6 (54.5)	15 (83.3)	0.09
Gestational age at delivery (wks) *	32.9±2.6	37.8±2.8	**0.003**
<37 wks	6 (100.0)	2 (13.3)	**<0.001**
<34 wks	4 (66.7)	1 (6.7)	**0.004**
Neonatal Hemoglobin (g/dL)	20.3±1.4	18.4±5.7	0.6
Neonatal platelet count (x10^9/L)	115.0±70.7	164.5±111.5	0.6

Data are presented as mean ± SD or n (%).

TOP, termination of pregnancy.

* Refers only to live births

Table 3: Cases complicated by fetal death

Case #	Gestational age (wks^{+days})	Access	Plt conc. ($x10^9/L$)	Hb conc. (g/dL)	RBC transfusion	Plt transfusion	Outcome
Severely thrombocytopenic fetuses							
1	27^{+3}	UV	49	4.2	Yes	No	Significant bleeding from umbilical vein following transfusion, fetal death at the time of procedure
2	19^{+0}	IHV+ UV+ cardiac	13	1.4	Yes	Yes	Fetal death 3 days post procedure
3	19^{+3}	IHV	9	1.2	Yes	Yes	Fetal death at the time of procedure
4	18	IP	37	3.0	Yes	Yes	Fetal death 2 days post procedure
Controls							
5	21	IHV	53	2.0	Yes	Yes	PPROM, chorioamnionitis and fetal death at 22 wks, 5 days post procedure
6	19^{+6}	IHV	57	3.4	Yes	No	Fetal death at 21 wks, 7 days post procedure

Plt, platelets; Hb, hemoglobin; RBC, red blood cells; UV, umbilical vein – placental insertion; IVH, intrahepatic umbilical vein; IP, intra-peritoneal; PPROM, preterm premature rupture of membranes

Table 4: Summary of studies reporting on fetal thrombocytopenia in pregnancies with fetal parvovirus-B19 infection

Reference	**Main focus of study**	**Fetuses with hydrops (N)**	**Rate of thrombocytopenia (N[%])**		
			<150 x 10^9/L	**<100 x 10^9/L**	**<50 x 10^9/L**
Smoleniec et al., 1994 [15]	Management	8 (only 5 cases had data on platelets concentration)	5 (100.0%)	4 (80.0%)	1 (20.0%)
Florestier et al., 1999 [10]	Haematological parameters	13	11 (84.6%)	8 (61.5%)	2 (18.2%)
Schild et al., 1999 [11]	Management	35	25 (71.4%)	20 (57.1%)	11 (31.4%)
Segata et al., 2007 [12]	**Thrombocytopenia**	11	9 (81.8%)	7 (63.6%)	6 (54.5%)
de Haan et al., 2008 [14]	**Thrombocytopenia**	30	29 (96.7%)	26 (86.7%)	14 (46.7%)
Simms et al., 2009 [13]	Outcome	8	7 (87.5%)	6 (75.0%)	2 (25.0%)
Current study	**Thrombocytopenia**	29	22 (75.9%)	21 (72.4%)	11 (37.9%)

FIGURES

Figure 1: Selection of the study and control groups

Severe thrombocytopenia – platelet concentration <50 $x10^9$/L

Moderate thrombocytopenia – platelet concentration 50-99 $x10^9$/L

Mild thrombocytopenia – platelet concentration 100-149 $x10^9$/L

Figure 2: Correlation between platelet and hemoglobin concentrations

The relationship between platelets and hemoglobin concentrations was best described using a linear equation.

r, Spearman's coefficient of correlation

Red circles represent platelets concentration < $50x10^9$/L

Figure 3: Correlation between platelet concentration and gestational age at diagnosis

The relationship between platelet concentration and gestational age at diagnosis was best described using a quadratic equation.

r, Spearman's coefficient of correlation

The mean platelet concentration for cases diagnosed ≤ 22 wks was significantly lower compared to cases diagnosed > 22 wks.

Red circles represent platelet concentrations < $50x10^9$/L

Figure 4: Natural history of platelet count

The natural history of platelet concentration is presented for 4 fetuses who underwent 3 serial fetal blood sampling procedures, and is compared to the natural history of hemoglobin

concentration. The Y-axes reflect platelets concentration ($x10^9$/L) and hemoglobin concentrations (g/dL). The letter 'T' represents red blood cells transfusion (blue) or platelets transfusion (red).