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# Thrombocytopenia in hydropic fetuses with parvovirus B19 infection: incidence, treatment and correlation with fetal B19 viral load

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**Objective** To examine (1) the incidence of fetal thrombocytopenia in hydropic fetuses with congenital B19 virus infection, (2) the effect of intrauterine platelet transfusions and (3) the correlation between fetal B19 viral load and severity of thrombocytopenia.

**Design** Retrospective analysis of data from prospectively collected fetal blood samples.

**Setting** Leiden University Medical Centre, the national centre for management of intrauterine fetal disease in the Netherlands.

**Population** Thirty hydropic fetuses treated with intrauterine red blood cell and platelet transfusions for human B19 virus-induced severe fetal anaemia and thrombocytopenia over a 10-year period.

**Methods** Fetal blood samples ($n$ = 30) taken before and after intrauterine transfusion were investigated. No cases were excluded, and there was no loss to follow up.

**Main outcome measures** Parameters recorded were gestational age, experienced fetal movements, gravidity and parity, severity of fetal hydrops, severity of fetal anaemia and thrombocytopenia and megakaryocyte and reticulocyte counts. Survival and procedure-associated complications were documented. Quantitative B19 viral load measurements were performed on all fetal samples.

**Results** Forty-six percent of all hydropic fetuses showed severe thrombocytopenia. No antenatal intracerebral haemorrhage or procedure-associated bleeding occurred. Overall, survival was 77%. Platelet counts increased following platelet transfusion and decreased significantly following red blood cell transfusion alone. No correlation was found between fetal viral loads and platelet counts.

**Conclusion** Thrombocytopenia was frequently encountered in fetal B19V infection, but fetal bleeding complications were not noted. Absence of a direct relationship between fetal B19 viral load and platelet counts suggests a temporal dissociation between these findings. Dilutional thrombocytopenia is frequently seen in the fetus following red blood cell transfusion alone. The clinical significance of this phenomenon is unclear. The risk of fluid overload by fetal platelet transfusion in a severely hydropic fetus should be weighed against the low incidence of fetal bleeding complications.

**Keywords** Human parvovirus B19, intrauterine transfusion, thrombocytopenia, viral load.

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## Introduction

Congenital human parvovirus B19 (B19V) infection may lead to fetal hydrops, anaemia or fetal demise. In a series of 485 cases of fetal hydrops, 7% of all cases were due to congenital B19V infection.[1] Therapeutic intrauterine erythrocyte and platelet transfusion for severe fetal anaemia or thrombocytopenia is feasible using percutaneous umbilical cord blood sampling techniques.[2] The fetal loss rate due to this procedure is approximately 1.6% in experienced hands.[3,4] An increased rate of fetal loss due to exsanguination from the umbilical cord puncture site has been noted in thrombocytopenic fetuses.[5,6] Three studies describe the incidence of fetal thrombocytopenia in B19V infection during gestation.[1,5,7] Segata *et al.*[7] reported demise of two severely thrombocytopenic fetuses due to exsanguination from the umbilical cord puncture


 

site. None of these studies evaluated platelet counts before and after transfusion for each individual case.

The cause of thrombocytopenia in human B19V infection is unclear. B19V shows primary erythroid tropism in bone marrow through the cellular globoside receptor (blood group P antigen).[8–11] Viral genome has been shown in megakaryocytes *in vitro*, but viral replication of B19V in megakaryocytes has not been shown as yet. A significant inhibitory effect of B19V on megakaryocyte colony formation has been observed *in vitro*. The NS1 gene of B19V may be responsible for this direct cytotoxicity to megakaryocytes.[12] An inhibitory effect of this NS1 protein on the colony-forming ability of megakaryocytic progenitor cells *in vivo* was confirmed in an animal model of parvovirus infection. The degree of inhibitory effect was dependent on the parvovirus infective load, and the depletion of megakaryocyte progenitor cells in the bone marrow appeared to coincide with erythroid progenitor depletion.[13] Lesions in cell chromatin and possible induction of apoptotic events may be responsible for this cytotoxic effect.[14]

No studies on correlates of the B19V viral load and B19V-induced fetal thrombocytopenia has been published yet. This study was conducted to evaluate: (1) the incidence of thrombocytopenia in hydropic fetuses with congenital B19V infection, (2) the correlation between B19 viral load and pre-intrauterine transfusion (IUT) fetal platelet counts, (3) the effect of intrauterine platelet transfusions in congenital parvovirus B19 infection and (4) the incidence of procedure-associated complications due to thrombocytopenia.

## Methods

### Clinical data

The Leiden University Medical Centre, Leiden, is the single national referral centre for the management and intrauterine treatment of fetal anaemia in the Netherlands. We retrospectively studied the prospectively collected clinical and laboratory data from severely hydropic and anaemic fetuses, with a proven B19V infection during gestation from January 1997 until August 2006. As this study was performed on materials collected during intention-to-treat sessions and did not involve any new subject contact, the study was approved by the institutional ethics committee.

From this cohort, all cases requiring an IUT were included. Maternal parameters noted were: maternal age in years, gravidity and parity, fetal movements (or any reduction in fetal movements) as experienced by the mother, gestational age at the time of the IUT session and outcome. The indication to perform fetal blood sampling in all cases was a combination of signs of fetal hydrops and an increased middle cerebral artery peak systolic flow velocity (MCA-PSV) (>1.5 MoM).[15,16]

All fetal blood sampling procedures were performed by inserting a 22- or 20-G needle under continuous ultrasound guidance into the umbilical vein. From the sample, 0.2 ml was immediately aspirated into a Sysmex F800 micro cell counter (Goffin, IJsselstein, The Netherlands), present in the procedure room, for immediate assessment of haemoglobin concentration and platelet values and for immediate decisions on transfusion. Another 0.5 ml was immediately sent to the hospital's central haematology laboratory for confirmation of this measurement and manual differential counts. Fetal haematological parameters, that is haemoglobin (in g/dl), total leucocyte count ($\times 10^9$/l), erythroblast count (/100 white blood cells), reticulocyte count (%), platelet count ($\times 10^9$/l) and the number of circulating megakaryocytes (/100 white blood cells), were noted before and after IUT.

In all cases of this series, red blood cell transfusion was given first. Our protocol for IUT in anaemic and hydropic fetuses known or likely to be due to parvovirus B19 infection includes having platelets ready for transfusion in all cases. Platelet transfusion is performed when the pretransfusion platelet count is below $50 \times 10^9$/l using the following equation to determine the volume required:

$$\text{Transfused volume (in ml)} = \frac{\text{Fetal Placental Blood Volume (FPBV)} \times \left(\text{desired} - \text{current platelet count}\right) \times 2}{\text{Platelet count in transfused concentrate}}$$

The FPBV was calculated by multiplying the estimated fetal weight (in g) by 0.14. The factor 2 is used in the numerator of the equation to allow for possible platelet sequestration in the spleen or liver.[6] Since both fetal haemoglobin concentrations and platelet counts vary with gestational age, reference ranges published by Forestier *et al.*[17] were used. These reference ranges were used to be able to compare our results with those of Schild's group. Mild thrombocytopenia was defined as platelet counts $<150 \times 10^9$/l, moderate thrombocytopenia as counts $<100 \times 10^9$/l and severe thrombocytopenia was defined as fetal platelet counts $<50 \times 10^9$/l.

### Virological investigations

All fetal blood samples were evaluated for quantitative B19 viral load. The quantitative real-time polymerase chain reaction (PCR) used in this study has been described before.[18,19] Briefly, DNA was isolated from 200 μl serum using a QIAamp DNA Mini Blood Kit (Roche Applied Science 51106; Roche Diagnostics, Almere, the Netherlands) or a MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche Molecular Diagnostics 3038505). For quantitative detection of B19V DNA in blood, a real-time PCR assay using Taqman polymerase was developed. Primers were selected on the NS part of the B19V genome. Sensitivity of the real-time B19V DNA PCR was 100 iu/ml by duplo measurements of ten-fold dilutions of WHO B19V DNA international standard 16.

### Statistical analysis

Statistical analysis was performed using the SPSS version 11.5.1 data manager program (SPSS 11.5.1; SPSS Inc., Chicago, IL, USA). A $P$ value of $<0.05$ was considered statistically significant. As parameters were not distributed normally, correlations were computed using Spearman's ranking coefficient, and differences between pretransfusion and posttransfusion values were calculated using the Wilcoxon signed ranks test. All values are reported as median with minimum and maximum values.

## Results

### Clinical parameters

During the study period, a total of 30 consecutive cases were included. All cases were diagnosed as congenital parvovirus B19 infection by serology and PCR on maternal and fetal blood samples. Population characteristics are depicted in Table 1. The original data series are shown in Table 2. Twenty-five out of 30 (83%) women reported decreased fetal movements before the IUT session and clearly increased fetal movements after IUT. Median gestational age at IUT was 22.5 weeks (minimum: 18 weeks; maximum: 28 weeks). At ultrasound investigation before IUT, all cases showed a MCA-PSV >1.5 MoM, indicative of severe fetal anaemia. Eight fetuses were diagnosed as mildly hydropic and 22 fetuses were diagnosed as severely hydropic. No fetuses were noted with a raised MCA-PSV without signs of hydrops, indicating the clinical severity of cases in this cohort. Two fetuses died of heart failure and irreversible bradycardia during the IUT session directly following the red blood cell transfusion. There were no cases of haemorrhage from the cordocenteses puncture site in our series. Three fetuses died *in utero* following IUT due to progressive cardiac decompensation (range: 1 day to 4 weeks following IUT). Two fetuses died after a failed neonatal resuscitation at 29 and 33 weeks of gestational age following spontaneous premature delivery and initial stabilisation on the neonatal unit. The total mortality in this cohort was 7/30 (23%).

**Table 1.** Maternal characteristics and median fetal blood values at IUT

| | Median | Minimum and maximum values (range) |
|---|---|---|
| Maternal age (years) | 28.0 | 19.0–39.0 |
| Gravidity | 3 | 1–4 |
| Parity | 1 | 0–3 |
| Gestational age (weeks) | 22.5 | 18.0–28.0 |
| Hb pre-IUT (g/dl) | 10.8 | 0.9–11.0 |
| Hb post-IUT (g/dl) | 17.6 | 1.1–18.7 |
| Plt pre-IUT ($\times 10^9$/l) | 57 | 4–238 |
| Plt post-IUT ($\times 10^9$/l) | 392 | 14–406 |
| Platelets <50 ($\times 10^9$/l) ($n = 14$) | 29 | 4–49 |
| Platelets 50–100 ($\times 10^9$/l) ($n = 12$) | 77 | 54–96 |
| Platelets >100 ($\times 10^9$/l) ($n = 4$) | 134 | 128–238 |
| Leucocytes pre-IUT ($\times 10^9$/l) | 2.1 | 0.8–20.0 |
| Megakaryocytes (/100 WBC) | 2 | 0–32 |
| Erythroblasts (/100 WBC) | 314 | 24–4208 |
| Reticulocytes (in %) | 10.1 | 1.8–79.7 |
| Viral load (log value) | 8.1 | 3.7–11.3 |

Hb, haemoglobin value; Platelet group, values depicted for all cases pre-IUT; Plt, platelet value; WBC, white blood cells.
Median values and range.

### Haematological parameters

*Platelets*

Table 1 shows the haematological parameters. At blood sampling before IUT, 14/30 (46%) fetuses had severe thrombocytopenia (median platelet value $29 \times 10^9$/l), 12/30 (40%) had moderate thrombocytopenia (median platelet value $77 \times 10^9$/l), and 3/30 (10%) had mild thrombocytopenia (median platelet value $131 \times 10^9$/l). In only one case, normal values were noted. All severely thrombocytopenic fetuses received a platelet transfusion to prevent procedure-associated bleeding. In two cases with moderate thrombocytopenia, the operator chose to transfuse platelets (with platelet counts of 54 and $59 \times 10^9$/l). Mild thrombocytopenic cases received no transfusions. Although the median platelet value following platelet transfusion exceeded the pretransfusion value, this difference was not statistically significant ($P = 0.143$). This is probably due to the small sample size. In three cases, platelet values after IUT were unavailable due to needle displacement.

All fetuses receiving red blood cells only ($n = 14$) showed a significant decrease in platelet values following transfusion ($P = 0.001$). In 8/14 (57%) cases, platelet values dropped below $50 \times 10^9$/L, but in no cases was a platelet transfusion given after red blood cell transfusion for posttransfusion thrombocytopenia. In 22 of all 30 (73%) cases, megakaryocytes were detected in the differential counts. We did not observe a statistically significant correlation between pre-IUT platelet counts and megakaryocyte counts/100 WBC ($P = 0.123$).

*Haemoglobin and leucocyte counts*

All cases were anaemic with a median pre-IUT haemoglobin value of 3.6 g/dl (minimum: 0.96 g/dl; maximum: 11.0 g/dl). In one case, a pretransfusion haemoglobin level of 11 g/dl was noted in the presence of a raised MCA-PSV and signs of fetal hydrops. We speculated that in this case, the contribution of fetal myocarditis may have outweighed possible earlier effects of fetal anaemia. All remaining 29 fetuses received a red blood cell transfusion with a median post-IUT haemoglobin value of 12.3 g/dl (minimum: 1.12 g/dl; maximum: 18.7 g/dl). The rise in haemoglobin value following transfusion was statistically significant ($P < 0.001$). A statistically significant correlation existed between the severity of fetal anaemia and


 

**Table 2.** Individual fetal haematological data

| GA (weeks) | Pretransfusion values | | | | | | Volume transfused (ml) | | Posttransfusion values | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hb (g/dl) | Plt ($10^9$/l) | Leuc ($10^9$/l) | Ery (/100 WBC) | Mkc (/100 WBC) | B19 viral load (log value) | RBC | Plt | Hb post (g/dl) | Plt post ($\times 10^9$/l) | S/D |
| 20 | 6.5 | 96 | 1.2 | 314 | 21 | NK | 16 | – | 13.9 | 62 | S |
| 22 | 8.3 | 76 | 3.1 | 842 | 6 | 8.80 | 6 | – | 13.9 | 39 | S |
| 23 | 2.4 | 88 | 2.8 | 76 | 9 | 9.80 | 27 | – | 9.7 | 68 | S |
| 27 | 11.0 | 37 | 3.8 | 24 | 1 | 6.20 | – | 14 | 12.8 | 214 | S |
| 20 | 4.9 | 87 | 1.1 | 824 | NK | 7.40 | 13 | – | 18.7 | 19 | S |
| 23 | 3.2 | 70 | 3.1 | 1180 | 32 | 11.30 | 28 | NK | 11.3 | 406 | S |
| 25 | 9.4 | 238 | 2.0 | 303 | 3 | 7.00 | 21 | – | 12.9 | 208 | S |
| 20 | 2.4 | 80 | 2.0 | 66 | 1 | NK | 20 | 2 | 11.8 | 40 | S |
| 22 | 5.4 | 54 | 1.8 | 479 | 4 | 7.20 | 27 | – | NK | NK | S |
| 18 | 4.0 | 128 | 1.4 | 28 | 0 | 11.00 | 19 | – | 17.6 | 40 | S |
| 21 | 2.7 | 37 | 2.3 | 0 | 1 | 8.10 | 25 | 5 | NK | NK | S |
| 22 | 4.4 | 48 | 2.7 | 83 | 2 | 9.60 | 28 | NK | 13.4 | 207 | S |
| 20 | 7.5 | 38 | 4.3 | 180 | 1 | 8.00 | 42 | 20 | 12.3 | 87 | S |
| 23 | 4.0 | 7 | 2.1 | 73 | 1 | 9.80 | 40 | 3 | 9.7 | 23 | S |
| 28 | 4.9 | 137 | 2.3 | 213 | 5 | NK | 87 | – | 10.8 | 107 | S |
| 23 | 4.4 | 79 | 4.3 | 0 | 0 | 6.40 | 24 | – | 9.7 | 70 | S |
| 26 | 2.2 | 32 | 1.9 | 481 | 2 | 10.00 | 52 | 5 | 10.5 | 86 | D |
| 25 | 2.5 | 45 | 1.6 | 864 | 2 | 9.20 | 69 | 7 | 9.7 | 158 | D |
| 23 | 4.6 | 70 | 20.0 | 411 | 10 | 10.70 | 38 | – | 13.4 | 33 | D |
| 24 | 0.96 | 36 | 1.2 | 27 | 3 | 7.70 | NK | – | 1.1 | 14 | D |
| 26 | 1.4 | 4 | 1.1 | 2115 | 0 | 7.80 | 52 | 7 | 8.9 | 106 | S |
| 25 | 2.5 | 12 | 0.8 | 4208 | 0 | 3.74 | 50 | 5 | 7.2 | 50 | D |
| 19 | 1.4 | 39 | 1.3 | 24 | 0 | 6.40 | 16 | 2 | 12.3 | 122 | S |
| 25 | 7.3 | 96 | 1.3 | 64 | 14 | 8.00 | 42 | 10 | 13.9 | 194 | S |
| 22 | 2.8 | 60 | 2.6 | 753 | 2 | 8.00 | 28 | 5 | 12.3 | 182 | S |
| 21 | 5.1 | 73 | 2.0 | 1900 | 7 | 7.50 | 20 | 4 | 14.2 | 72 | S |
| 21 | 3.0 | 49 | 3.6 | 2311 | 5 | 10.40 | 29 | 4 | 11.5 | 217 | S |
| 21 | 1.9 | 6 | NK | NK | NK | 8.60 | 10 | 5 | NK | NK | S |
| 27 | 2.0 | 24 | 2.9 | 3196 | 2 | 8.10 | 62 | – | 10.7 | 17 | D |
| 20 | 3.2 | 131 | 1.7 | 205 | 0 | 9.60 | 24 | – | 15.0 | 35 | D |

D, died; Ery, erythroblast count; GA, gestational age at time of IUT; Hb, haemoglobin value; Leuc, leucocyte count; Mkc, megakaryocyte count; NK, missing (not known) value; Plt, platelet count; RBC, red blood cells; S, survived; WBC, white blood cells.

the MCA-PSV values ($P = 0.007$). In 18/30 (60%) cases, erythroblastosis was present according to gestational age corrected reference ranges.[17] Leucocyte and neutrophil counts were all normal when compared with gestational age adjusted reference values.[17]

*Quantitative viral load measurements*

To evaluate any correlation between the severity of thrombocytopenia and fetal B19 viral load values, all fetal blood samples were tested for B19 viral load (Table 2). Twenty-seven samples were tested; in three samples, insufficient material had been preserved for reliable testing. We found no correlation between fetal quantitative B19 viral load values and fetal pre-IUT platelet counts ($P = 0.580$) nor did we detect any significant correlation between haemoglobin values and fetal B19 viral load ($P = 0.459$).

## Discussion

In this cohort of pregnancies complicated by parvovirus B19 infection and hydrops, we found a 46% incidence of severe concomitant thrombocytopenia. Combined intrauterine treatment with both red blood cell and platelet transfusion was associated with a survival rate of 77%. These results are in accordance with the few other series reporting survival rates ranging from 54 to 85% after IUT for parvovirus B19 infection in pregnancy.[1,5,20] Although the mechanisms of disease cannot be fully compared, intrauterine treatment for severe fetal anaemia with hydrops due to red blood cell alloimmunisation is associated with a survival rate of 55%.[16]

Our findings confirm the limited existing data on the association between B19V infection and fetal thrombocytopenia, with incidences ranging from 29 to 64%.[5,7] We realise that

our data only apply to the selected group of hydropic fetuses treated with intrauterine blood transfusion. Whether thrombocytopenia occurs in all fetuses affected by parvovirus B19 infection is unknown and difficult if not impossible to determine.

In our report, all 30 cases were evaluated on one occasion only. There were no follow-up transfusions performed. The presented data show that platelet transfusions resulted in an adequate direct increment of the platelet count. However, whether this increase is sustained remains uncertain, as this would require serial sampling procedures with inherent risks.

A clinically important observation was the decrease in platelet count after transfusion of red blood cells alone. In 8/14 cases, posttransfusion platelet counts dropped to values below $50 \times 10^9$/l. Red blood cell transfusion-related thrombocytopenia has been reported previously, before the routine use of leucocyte depletion of blood components. Platelet counts dropped after transfusion due to adherence of platelets to microaggregates (composed of leucocytes and platelets) in transfused blood.[21] Most centres, including ours, nowadays use leucocyte-depleted and irradiated donor blood to eliminate any leucocyte activity. In this cohort, therefore, another explanation must be considered. In adult patients, dilutional thrombocytopenia during massive erythrocyte transfusion is a common finding.[22–24] This mechanism is counteracted by endogenous platelet release, and standard prophylactic platelet transfusion is considered not warranted.[23,24] In B19V infection with bone marrow depression and a possible reduction in platelet production, this endogenous platelet release may be insufficient. We do think that dilutional thrombocytopenia in B19V-infected fetuses with an already lower platelet count is responsible for the low platelet counts in some of the posttransfusion samples. The clinical significance of this 'dilutional thrombocytopenia' remains to be elucidated. In the cases with severe dilutional thrombocytopenia, 2/8 (25%) fetuses died following intrauterine red blood cell transfusion compared with 5/14 (35%) fetuses with severe thrombocytopenia before IUT. Fetal demise was not due to bleeding complications in any case.

When performing any invasive intervention, benefits should always be weighed against possible complications. Evidence for the need of fetal platelet transfusion for human B19 virus-induced thrombocytopenia is limited. Reviewing the literature on complications of severe thrombocytopenia in fetal B19 virus infection, we found only one report by Segata *et al.*[7] reporting exsanguination of two thrombocytopenic fetuses following cordocentesis for B19V-induced severe fetal anaemia. In a study by Kiefel *et al.*,[25] bleeding complications of cord vessel puncture in fetuses with normal platelet counts were seen in 5% of cases. Paidas *et al.*[26] reported an increased risk of fetal loss related to cordocenteses in alloimmune thrombocytopenic fetuses. However, this is an entirely different condition with a high risk of fetal haemorrhage. Therefore, the need for fetal platelet transfusion in congenital B19V infection remains a matter of debate. In our series of 30 cases, we did not observe any procedure-associated haemorrhage. We also did not note any cases of antenatal or postnatal bleeding complications. As it is our current practice to perform platelet transfusions in all fetuses with pre-IUT platelet counts $<50 \times 10^9$/l, we are not able to provide evidence on the natural course of severely thrombocytopenic cases in this disease. As the fetus is in a state of severe cardiovascular compromise possibly due to a combination of fetal myocarditis and severe fetal anaemia, platelet transfusion may prove hazardous due to the extra fluid overload. We conclude that the risk of exacerbating cardiovascular compromise should be carefully weighed against the risk of procedure-associated bleeding as described by Segata *et al.*[7] Larger studies are needed to evaluate the incidence of bleeding from the sampling site.

In contrast with reports that speculate about an association between the B19V viral load and thrombocytopenia [12–14], we found no such correlation in our series. This may be due to a temporal dissociation between the peak value of viral DNA and the resulting decline of platelet counts. Another possible result of platelet and megakaryocyte destruction may be the release of internalised thrombopoietin (Tpo) and the decrease of Tpo receptors, resulting in an increased level of free circulatory thrombopoietin. This could compensate for fetal platelet destruction by B19 virus at the time of treatment.[26–30]

We were unable to show any correlation between the pretransfusion platelet count and megakaryocyte count. We speculate that this might be explained to a reduced fetal response to thrombopoietin, since in preterm infants, the response to thrombopoietin was shown to be reduced.[31] This hypothesis warrants further studies. In our series, we did not observe leucopenia or neutropenia, and fetal white blood cell progenitors do not seem to be influenced by congenital B19V infection.

A limitation of our study is the fact that all values were obtained at one single time point of the total infectious course. Serial fetal sampling would be extremely interesting. However, given the risks, this is not acceptable in continuing pregnancies, and future investigations should be directed towards development of an animal model of congenital B19V infection, with the possibility of serial fetal sampling sessions to answer questions on the course of haematological and virological parameters.

In conclusion, we found that anaemic and hydropic fetuses with a fetal B19V infection have a high risk of concomitant severe thrombocytopenia. There was no correlation between the fetal B19V viral load and the severity of thrombocytopenia. Fetal haemorrhage was not seen as a complication of thrombocytopenia. Intrauterine platelet transfusion can be performed relatively safely, although the risk of fluid overload in the hydropic fetus may outweigh possible benefits. If fetal treatment consists of transfusion of red blood cells only, posttransfusion


 

dilutional thrombocytopenia occurs in a majority of cases. The clinical significance of this dilutional thrombocytopenia needs to be elucidated. ■

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