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Authors(s):	Stefano Ghirardello (Co-author), Genny Raffaeli (Corresponding Author), Beatrice Letizia Crippa (Co-author), Silvia Gulden (Co-author), Ilaria Amodeo (Co-author), Dario Consonni (Co-author), Giacomo Cavallaro (Co-author), Federico Schena (Co-author), Fabio Mosca (Co-author)
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Research Article

The Thromboelastographic profile at birth in very preterm newborns with patent ductus arteriosus

Stefano Ghirardello[1], Genny Raffaeli[1]*, Beatrice Letizia Crippa[1,2], Silvia Gulden[1,2], Ilaria Amodeo[1,2], Dario Consonni[3], Giacomo Cavallaro[1], Federico Schena[1], Fabio Mosca[1,2]

1. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neonatal Intensive Care Unit, Milan, Italy
2. Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy
3. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Epidemiology Unit, Milan, Italy.

Short title: Thromboelastography and patent ductus arteriosus

Corresponding author: Genny Raffaeli MD

Neonatal Intensive Care Unit

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

E-mail: genny.raffaeli@gmail.com

Tel +39/2-55032234; Fax +39/2-5503221

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Abstract

Background: The role of hemostasis on the closure of a patent ductus arteriosus (PDA) in preterm infants is controversial.

Objective: To assess thromboelastography (TEG) at birth in very low birth-weight (VLBW) infants affected by PDA.

Methods: This is an ancillary study of a prospective observational study aimed at defining the TEG profile in healthy VLBW infants in the first month of life. In this analysis, we included neonates <33 weeks of gestation (GA) with PDA and compared TEG traces based on 1. spontaneous closure vs. need for pharmacological treatment; 2. treatment response. We collected blood samples in the 1st day of life to perform *recalcified native blood TEG* [reaction time (R), maximum amplitude (MA), lysis (LY30)], standard coagulation tests and blood count.

Results: We enrolled 151 infants with a PDA at first echocardiogram: 111 experienced spontaneous PDA closure while 40 required treatment. Mean GA and birth-weight were 29.7 ± 1.7 vs. 27.6 ± 2.1 weeks and 1158 ± 256g vs. 933 ± 263g in the two groups, respectively (p<0.001). The hemostatic profile was similar between groups. Median hematocrit (44.6-vs-48.7%; p=0.01) and platelet count (187-vs-216 x $10^3/\mu L$; p=0.04) were lower in the treated group, although differences lost significance after controlling for GA and illness severity at multivariate analysis. Responders to PDA treatment (n=20) had a significantly lower median Ly30 than non-responders (0-vs-0.7%; p=0.02).

Conclusion: Thromboelastography at birth does not predict spontaneous PDA closure in preterm newborns. Fibrinolysis is enhanced in non-responders to PDA treatment; this observation warrants further investigation.

Introduction

Patent ductus arteriosus (PDA) is a common issue of prematurity. Approximately 5-15% of infants born below twenty-nine weeks of gestational age (GA) show ductal patency after the first week of life [1].

Prolonged ductal patency, especially in the most preterm newborns, increases the risk of death and co-morbidities [2]. Pharmacological treatment of a hemodynamically significant PDA (hsPDA) includes indomethacin, ibuprofen or paracetamol. However, the rate of failure of the first treatment cycle is around fifty percent [1].

Ductal closure occurs in two sequential but overlapping phases. In term newborns, postnatal constriction of the DA leads to narrowing of the ductal lumen and compression of vasa vasorum, resulting in a hypoxic stimulus within the vessel wall [3,4]. Differently, in preterm newborns, DA seems less sensitive to hypoxia; a preclinical study demonstrated that permanent closure requires platelet aggregation in the ductal lumen within the first minutes after birth, followed by the formation of occlusive thrombi [4].

Therefore, the ability of platelet count to predict spontaneous DA closure or response to pharmacological treatment in preterm newborns has been explored, but results are conflicting [5–9]. A meta-analysis confirmed a marginal but significant association between platelet count below 100,000/µL and hsPDA [10].

Moreover, in preterm newborns platelet aggregation is reduced by the first days of life and improves by two post-natal weeks [11,12]. Indeed, impaired platelet function, rather than platelet count, was proposed to contribute to ductal patency [13,14]. Of note, platelets have a pivot role in both primary and secondary hemostasis, through interactions with almost all coagulation factors [15].

In this context, thromboelastography (TEG), which is a whole blood-based viscoelastic test, could be valuable as it evaluates the clot kinetics, from its initiation up to lysis, considering the contribution of both cellular and enzymatic factors involved in clotting.

With this in mind, we hypothesized that preterm newborns with spontaneous DA closure might show a different TEG profile compared to those requiring pharmacological treatment.

To this purpose, we compared the hemostatic profile in the first day of life of preterm very-low-birth-weight (VLBW) infants affected by PDA, based on 1. spontaneous versus pharmacological PDA closure; 2. treatment response.

Material and Methods

Study design and setting

This study is a secondary analysis of a blinded prospective observational study conducted at the NICU Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (07/2015-06/2018) and aimed at defining the TEG profile in healthy VLBW infants in the first month of life [16].

The Institutional Review Board approved the study protocol, and the written informed consent was obtained from patients' tutors. All procedures were carried out following the Helsinki Declaration.

All consecutive VLBW infants were assessed at birth for eligibility. Exclusion criteria and demographic data were detailed elsewhere [16].

Study population

We included all patients from the original cohort with GA below 33 weeks and for whom a TEG trace was available on the first day of life.

Exclusion criteria included: 1. platelet transfusion before spontaneous DA closure or before and during pharmacological treatment; 2.major bleeding; 3. platelet count <50,000/µL.

Patients were daily evaluated for the occurrence of sepsis, bleeding (intraventricular-IVH, pulmonary, gastrointestinal hemorrhage), platelet or plasma transfusion, Ibuprofen, Indomethacin, or Paracetamol administration for PDA closure. Cardiac ultrasound was performed daily during the first three days of life in all neonates with GA < 33 weeks and, afterward, every 48-72 hours, according to clinical signs of hsPDA, until spontaneous closure or pharmacological treatment decision. The hsPDA was defined as a DA with an internal diameter ≥ 1.5mm with exclusive left-to-right shunt, a left atrium/aortic root ratio ≥ 1.5 and evidence of pulmonary over-circulation or systemic shunt effect, according to Mc Namara et al. [17]. Patients with persistently hsPDA were treated with intravenous Ibuprofen or Paracetamol, according to the internal

protocol, based on international recommendations [18]. A cardiac ultrasound was performed the day after treatment. Demographic data included multiple pregnancies, steroid prophylaxis, chorioamnionitis, birth-weight, delivery mode, Apgar score, arterial and venous pH, Clinical Risk Index for Babies (CRIB-II score), mechanical ventilation, sepsis, respiratory distress syndrome, bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of prematurity, IVH.

Blood collection and measurements

Blood samples were collected at birth through non-heparinized arterial or venous lines or direct venipuncture in EDTA and citrated tubes (3.2% trisodium citrate; 9:1 vol/vol ratio), as previously described [19]. Laboratory tests included Prothrombin Time (PT; 100µL plasma), Activated Partial Thromboplastin Time (APTT; 100µL plasma), fibrinogen (100µL plasma) and full blood cell count (500µL whole blood).

We performed citrated-native TEG assays (340µL whole blood) using a two-channel thromboelastograph (TEG5000 Hemoscope/Haemonetics®, Niles, IL), by adding 20 µL of 0.2mol/L $CaCl_2$ followed by citrated blood into the TEG cup (37°C). A detailed description of the procedure is available elsewhere [20]. We measured the following parameters (figure 1):

- Reaction time (R): time (minutes) to initial clot formation (2mm)
- Kinetics (K): time (minutes) to significant clot strength (20mm)
- Alpha angle (α): angle (degrees) between the midline and the tangent to the trace
- Maximum amplitude (MA): widest trace amplitude (millimeters) representing maximum clot strength
- Lysis at 30 minutes (LY30): rate of clot dissolution (%) 30 minutes after MA attainment

Statistical analysis

Quantitative variables were expressed as median with Interquartile Range (IQR).

Demographic characteristics, TEG parameters, full blood cell count and standard coagulation tests of patients with spontaneous DA closure were compared to those requiring pharmacological treatment; within the latter, we evaluated for differences based on treatment response. For comparison between groups, we used

the Mann–Whitney U test for continuous variables and the χ2-test for categorical variables. To control for potential confounders, we performed a multivariable analysis with logistic regression to calculate the odds ratio [OR and 95% confidence intervals (CI)] of PDA spontaneous closure, including, as covariates, GA, birth-weight and CRIB. Among treated patients, association of lysis with variables associated with a positive response (gender, being small for gestational age, gemellarity, delivery mode, ventilation, prenatal steroids, chorioamnionitis, and sepsis) were evaluated through Mann-Whitney or Kruskal-Wallis tests. Statistical analysis was performed with Stata 16 (StataCorp LLC, College Station, TX, USA, 2019).

Results

The initial cohort included 201 VLBW infants; fifty of them were excluded for unmet inclusion criteria. The analysis was performed on 151 infants with PDA, forthy of them requiring pharmacological treatment. This latter group was divided between responders and non-responders, equally distributed. Table 1 summarizes demographic and laboratory data by comparing patients with spontaneous versus pharmacological PDA closure. Patients whose duct closed spontaneously had higher GA and BW, higher Apgar score, and were less likely ventilated. The hemostatic profile was similar between groups. Red blood cells (15.1-vs-16.4 gr/dL; p=0.01), hematocrit (44.6-vs-48.7%; p=0.01) and platelet count (187-vs-216 x $10^3/\mu L$; p=0.04) were lower among patients with hsPDA, compared to those with spontaneous PDA closure. However, these results were not confirmed when corrected for potential confounders [adjusted OR 95%: 0.52 (0.24-1.17); 0.98 (0.92-1.04); 0.70 (0.40-1.27)]. Table 2 reports comparisons between responders and non-responders to pharmacological treatment. We found no statistical difference for any of the parameters considered, except for the increase in lysis in the non-responders group (Lys30=0.7-vs-0%; p=0.02), which remained significant after correction for confoundings factors. In treated patients, associations between lysis and clinical variables (gender, being small for gestational age, gemellarity, mode of delivery, ventilation, prenatal steroids, chorioamnionitis, and sepsis) were negligible, with p-values ranging from 0.11 to 0.92 (data not shown).

Discussion

This study is the first that evaluated the role of thromboelastography in predicting spontaneous ductal closure and response to pharmacological treatment in preterm newborns.

Our results showed no thromboelastographic differences at birth between patients with spontaneous DA closure and those requiring treatment. Moreover, although this latter group had significantly lower platelet count and hematocrit, both variables seem to depend upon GA and illness severity.

In mice, platelet-triggered ductal sealing was shown to be a relevant mechanism for ductal closure [4]. In this respect, the role of platelets has been explored in preterm infants, but conclusions were conflicting [5–9]

A meta-analysis observed an association between platelet count below 100,000/μL and DA patency [10]. Similarly, thrombocytopenia on the first day of life was associated with delayed ductal closure and the risk of hsPDA on day seven of life [21]. In this context, platelet transfusions to maintain platelets count above 100,000/μL did not improve PDA closure rate or hasten PDA closure [22] and were associated with increased risk of IVH [22]. Moreover, lowering the transfusion threshold from 50,000 to 25,000 platelets/μL reduced the rates of death or major bleeding in preterm newborns [23].

Platelet function rather than count might predict hsPDA or pharmacological response; indeed, higher platelet distribution width, a marker of platelet activation, has been associated with ductal patency [5].

A PFA-100-based study has shown that prolonged closure time (collagen-ADP > 130), a marker of impaired platelet function, was independently associated with PDA [14]. However, hematocrit differences may have affected results as red blood cells play a direct role in hemostasis and anemia is an independent risk factor for PDA [24, 25].

Platelets and clotting factors combine to sustain thrombin generation, a platelet-activating agonist, while fibrin stabilizes the platelet plug during the hemostatic process [15]. Of note, immunohistochemical studies demonstrated that fibrinogen, von Willebrand factor, and collagen were expressed in the mice DA lumen [4]. The involvement of the coagulation cascade in DA closure has been postulated in children with persistent ductal patency [26]. Plasma proteomic analysis revealed that the concentration of fibrinogen, platelet factor 4, von Willebrand factor, collagen and mannose-binding lectin-associate serine protease-1 were significantly lower in children with persistent PDA, compared to controls.

If these observations hold, thromboelastography may disclose differences between patients with spontaneous DA closure compared to those with persistent PDA.

Specifically, MA reflects the interaction between platelets (number and function) and fibrin(ogen), which are both essential for clot formation and stabilization. Platelets are known to contribute up to 80% of clot strength in healthy adults when platelet function is mature, differently to preterm newborns. [11,12].

The interplay among endothelium, humoral, and cellular factors involved in the hemostatic process may explain the lack of correlation between MA and ductal closure. Indeed, thromboelastography cannot discriminate the contribution of individual factors to clot formation.

Another explanation may be related to the timing of thromboelastographic evaluation. We compared MA at birth, but it increased significantly after the first days of life as a result of platelet maturation and early-postnatal inflammation that triggers platelet activation [16].

Although MA at birth was similar between newborns with spontaneous closure compared to those with PDA, platelet count was significantly higher in the former, fibrinogen being equal. These observations suggest that in preterm newborns 1. the contribution of platelet function to clot strength may vary according to GA and co-morbidities; 2. in the absence of severe thrombocytopenia, clotting seems adequate, despite hypofunctional platelets. Similarly, Deschmann et al. [27] demonstrated that preterm newborns with platelet count > 90,000/μL had substantially normal clot-time evaluated by PFA 100.

The response of PDA to pharmacological treatment changes depending on antenatal and postnatal factors. Extremely preterm infants have the highest rate of failure to the first treatment cycle, reaching 70% at 23-24 GA [1]. Our observation confirms that non-responder patients were younger and sicker than those with spontaneous closure.

The relationship between platelet count and the response to COX inhibitors is still undetermined, as results from retrospective studies provided mixed results [1,7]. A meta-analysis [28] showed that platelet counts were significantly lower in preterm infants who failed to close DA after pharmacological treatment and that pre-treatment thrombocytopenia was associated with a lower success rate.

We found a significantly higher degree of fibrinolysis in neonates non-responders to pharmacological therapy compared to responders. The median fibrinolysis was higher even when compared to thromboelastographic reference ranges [16]. Fibrinolysis is an enzymatic system responsible for fibrin degradation by two opposing drivers, that regulate the conversion of plasminogen to plasmin, the enzyme that dissolves the fibrin clot.

The enhanced fibrinolysis observed in our cohort is intriguing. Fibrinolysis is inhibited in the first ten days of life, accounted by higher plasminogen activator inhibitors-1 (PAI-1) activity and lower tissue-type plasminogen activator (t-PA)/PAI-1 activity ratio, compared with full-term neonates [29, 30].

If the coagulation system may favor ductal closure, the increased fibrinolysis may reduce clot strength and counteract clot formation, possibly contributing to treatment failure.

We tried to explain our findings by considering eight out of the twenty newborns with the highest degree of fibrinolysis (> 2%, data not shown). Seven were extremely low birth-weight with GA < 28 weeks without fetal growth restriction, chorioamnionitis, or sepsis; all were mechanically ventilated; one developed intraventricular hemorrhage. As all samples were drawn a few hours after birth, no acquired clinical conditions could explain the enhanced fibrinolysis.

This observation could be due to chance, although unlikely. Indeed, according to previous studies [28], we detected lower rates of fibrinolysis in preterm newborns in the first ten days of life, compared to values observed one month after birth [16].

We recognize some study limitations. First, it was not powered to identify thomboelastographic differences between patients with spontaneous versus pharmacological ductal closure. This is a secondary analysis, and the aim was to explore the potential role of TEG in the setting of PDA management. Further prospective evaluations will be performed, to define the contribution of both clot lysis and platelet function in ductal closure, through tailored TEG and bedside evaluation of primary hemostasis.

Secondly, we compared thromboelastographic profiles at birth but hemostasis evolves rapidly after birth in preterm neonates, and the contribution of platelet function to clot strength could have been different after the first day of life, especially in critically-ill patients. Nevertheless, the blood sparing policy and the difficulty in sampling preterm neonates prevented us from repeating measurements at the time of PDA diagnosis.

In conclusion, thromboelastography at birth does not predict the spontaneous closure of DA in preterm infants. Its role in anticipating spontaneous DA closure and the association between enhanced fibrinolysis and treatment failure require further investigations.

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Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The Institutional Review Board approved the study protocol, and the written informed consent was obtained from patients' tutors.

Disclosure Statement

The authors have no conflicts of interest to declare.

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None.

Author Contributions

SG was involved in the conception and planning of the study, the acquisition, analysis, and interpretation of data, writing of the first draft, and critical revision of the manuscript. GR contributed to conception and planning of the study, acquisition, analysis, interpretation of data, cowriting, and critical revision of the article. BLC was involved in data analysis and interpretation, cowriting and critical revision of the article. IA and SG were involved in the acquisition and interpretation of the data, and the critical revision of the article. DC was involved in the analysis of the data and the critical revision of the article. GC and FS were involved in the acquisition, analysis, interpretation of the data, and critical revision of the article. FM was involved in the conception and planning of the study, the interpretation of the data, and the critical revision of the article. All authors revised and gave final approval of the version to be submitted.

Tables' Legend

Table 1: Demographic features, outcomes and hemostatic profile of preterm infants affected by PDA: focus on pharmacological versus spontaneous closure

BPD: bronchopulmonary dysplasia; CRIB: clinical risk index baby score; GA: gestational age; IVH: intraventricular hemorrhage; ROP: retinopathy of prematurity; RDS: respiratory distress syndrome; SGA: small for gestational age; *Mann Whitney test; ** Chi-squared test.

Table 2: Demographic features, outcomes and hemostatic profile of preterm infants affected by PDA requiring pharmacological treatment: focus on responders versus non-responders.

CRIB: clinical risk index baby score; GA: gestational age; RDS: respiratory distress syndrome; SD: standard deviation; SGA: small for gestational age; *Mann Whitney test; ** Chi-squared test

Figures' Legend

Figure 1: Summary of the hemostatic process (partially modified from Raffaeli et al, ADC 2019, copyright license n° 4733720500744).

1a. Cell-based model of hemostasis occurs through three overlapping phases: I) initiation phase in the tissue-factor bearing cells, which generates small amounts of thrombin; II) amplification phase on the platelet surface, which enables the procoagulant complex interaction through activation of platelets cofactors and III) propagation on the activated platelet surface, which contributes to the burst of thrombin generation.

1b. Thromboelastographic trace. A representation of the TEG main parameters and their determinants (§).

1c. The cascade model of coagulation, which involves the "extrinsic" and "intrinsic" pathways, respectively depicted by PT and APTT conventional tests.

> Increase; a: activated; TF: tissue factor.

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Research Article

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Stefano Ghirardello[1], Genny Raffaeli[1]*, Beatrice Letizia Crippa[1,2], Silvia Gulden[1,2], Ilaria Amodeo[1,2], Dario Consonni[3], Giacomo Cavallaro[1], Federico Schena[1], Fabio Mosca[1,2]

1. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neonatal Intensive Care Unit, Milan, Italy
2. Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy
3. Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Epidemiology Unit, Milan, Italy.

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Corresponding author: Genny Raffaeli MD

Neonatal Intensive Care Unit

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

E-mail: genny.raffaeli@gmail.com

Tel +39/2-55032234; Fax +39/2-5503221

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Conclusion: Thromboelastography **at birth** does not predict spontaneous PDA closure in preterm newborns. Fibrinolysis is enhanced in non-responders to PDA treatment; this observation warrants further investigation.

Introduction

Patent ductus arteriosus (PDA) is a common issue of prematurity. Approximately 5-15% of infants born below twenty-nine weeks of gestational age (GA) show ductal patency after the first week of life [1].

Prolonged ductal patency, especially in the most preterm newborns, increases the risk of death and co-morbidities [2]. Pharmacological treatment of a hemodynamically significant PDA (hsPDA) includes indomethacin, ibuprofen or paracetamol. However, the rate of failure of the first treatment cycle is around fifty percent [1].

Ductal closure occurs in two sequential but overlapping phases. In term newborns, postnatal constriction of the DA leads to narrowing of the ductal lumen and compression of vasa vasorum, resulting in a hypoxic stimulus within the vessel wall [3,4]. Differently, in preterm newborns, DA seems less sensitive to hypoxia; a preclinical study demonstrated that permanent closure requires platelet aggregation in the ductal lumen within the first minutes after birth, followed by the formation of occlusive thrombi [4].

Therefore, the ability of platelet count to predict spontaneous DA closure or response to pharmacological treatment in preterm newborns has been explored, but results are conflicting [5–9]. A ~~**recent**~~ meta-analysis confirmed a marginal but significant association between platelet count below 100,000/**µL** and hsPDA [10].

Moreover, ~~**platelets are hypofunctional**~~ in preterm newborns ~~**; specifically,**~~ platelet aggregation is reduced by the first days of life and improves by two post-natal weeks [11,12]. Indeed, ~~**some authors proposed that**~~ impaired platelet function, rather than platelet count, **was proposed to** ~~**may**~~ contribute to ductal patency [13,14]. Of note, platelets have a pivot role in both primary and secondary hemostasis, through interactions with almost all coagulation factors [15].

In this context, thromboelastography (TEG), which is a whole blood-based viscoelastic test, could be valuable as it evaluates the clot kinetics, from its initiation up to lysis, considering the contribution of both cellular and enzymatic factors involved in clotting.

With this in mind, we hypothesized that preterm newborns with spontaneous DA closure might show a different TEG profile compared to those requiring pharmacological treatment.

To this purpose, we compared the hemostatic profile in the first day of life of preterm very-low-birth-weight (VLBW) infants affected by PDA, based on 1. spontaneous versus pharmacological PDA closure; 2. treatment response.

Material and Methods

Study design and setting

This study is a secondary analysis of a blinded prospective observational study conducted at the NICU Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (07/2015-06/2018) and aimed at defining the TEG profile in healthy VLBW infants in the first month of life [16].

The Institutional Review Board approved the study protocol, and the written informed consent was obtained from patients' tutors. All procedures were carried out following the Helsinki Declaration.

All consecutive VLBW infants were assessed at birth for eligibility. Exclusion criteria and demographic data were detailed elsewhere [16].

Study population

We included all patients from the original cohort with GA below 33 weeks and for whom a TEG trace was available on the first day of life.

Exclusion criteria included: 1. platelet transfusion before spontaneous DA closure or before and during pharmacological treatment; 2.major bleeding; 3. platelet count <50,000/~~ml~~µL.

Patients were daily evaluated for the occurrence of sepsis, bleeding (intraventricular-IVH, pulmonary, gastrointestinal hemorrhage), platelet or plasma transfusion, Ibuprofen, Indomethacin, or Paracetamol administration for PDA closure. Cardiac ultrasound was performed daily during the first three days of life in all neonates with GA < 33 weeks and, afterward, every 48-72 hours, according to clinical signs of hsPDA, until spontaneous closure or pharmacological treatment decision. The hsPDA was defined as a DA with an internal diameter ≥ 1.5mm with exclusive left-to-right shunt, a left atrium/aortic root ratio ≥ 1.5 and evidence of pulmonary over-circulation or systemic shunt effect, according to Mc Namara et al. [17]. Patients with persistently hsPDA were treated with intravenous Ibuprofen or Paracetamol, according to the internal

protocol, based on international recommendations [18]. A cardiac ultrasound was performed the day after treatment. Demographic data included multiple pregnancies, steroid prophylaxis, chorioamnionitis, birth-weight, delivery mode, Apgar score, arterial and venous pH, Clinical Risk Index for Babies (CRIB-II score), mechanical ventilation, sepsis, respiratory distress syndrome, bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of prematurity, IVH.

Blood collection and measurements

Blood samples were collected at birth through non-heparinized arterial or venous lines or direct venipuncture in EDTA and citrated tubes (3.2% trisodium citrate; 9:1 vol/vol ratio), as previously described [19]. Laboratory tests included Prothrombin Time **(PT; 100µL plasma)**, Activated Partial Thromboplastin Time **(APTT; 100µL plasma)**, fibrinogen **(100µL plasma)** and full blood cell count **(500µL whole blood).**

We performed citrated-native TEG assays **(340µL whole blood)** using a two-channel thromboelastograph (TEG5000 Hemoscope/Haemonetics®, Niles, IL), by adding 20 µL of 0.2mol/L $CaCl_2$ followed by citrated blood into the TEG cup (37°C). A detailed description of the procedure is available elsewhere [20]. We measured the following parameters (figure 1):

- Reaction time (R): time (minutes) to initial clot formation (2mm)
- Kinetics (K): time (minutes) to significant clot strength (20mm)
- Alpha angle (α): angle (degrees) between the midline and the tangent to the trace
- Maximum amplitude (MA): widest trace amplitude (millimeters) representing maximum clot strength
- Lysis at 30 minutes (LY30): rate of clot dissolution (%) 30 minutes after MA attainment

Statistical analysis

Quantitative variables were expressed as median with Interquartile Range (IQR).

Demographic characteristics, TEG parameters, full blood cell count and standard coagulation tests of patients with spontaneous DA closure were compared to those requiring pharmacological treatment; within the latter, we evaluated for differences based on treatment response. For comparison between groups, we used

the Mann–Whitney U test for continuous variables and the χ2-test for categorical variables. To control for potential confounders, we performed a multivariable analysis with logistic regression to calculate the odds ratio [OR and 95% confidence intervals (CI)] of PDA spontaneous closure, including, as covariates, GA, birth-weight and CRIB. **Among treated patients, association of lysis with variables associated with a positive response (gender, being small for gestational age, gemellarity, delivery mode, ventilation, prenatal steroids, chorioamnionitis, and sepsis) were evaluated through Mann-Whitney or Kruskal-Wallis tests. Statistical analysis was performed with Stata 16 (StataCorp LLC, College Station, TX, USA, 2019).**

Results

The initial cohort included 201 VLBW infants; fifty of them were excluded for unmet inclusion criteria **~~(Figure 2).~~** The analysis was performed on 151 infants with PDA, forthy of them requiring pharmacological treatment. This latter group was divided between responders and non-responders, equally distributed. Table 1 summarizes demographic and laboratory data by comparing patients with spontaneous versus pharmacological PDA closure. Patients whose duct closed spontaneously had higher GA and BW, higher Apgar score, and were less likely ventilated. The hemostatic profile was similar between groups. Red blood cells (15.1-vs-16.4 gr/dL; p=0.01), hematocrit (44.6-vs-48.7%; p=0.01) and platelet count (187-vs-216 x $10^3/\mu L$; p=0.04) were lower among patients with hsPDA, compared to those with spontaneous PDA closure. However, these results were not confirmed when corrected for potential confounders [adjusted OR 95%: 0.52 (0.24-1.17); 0.98 (0.92-1.04); 0.70 (0.40-1.27)]. Table 2 reports comparisons between responders and non-responders to pharmacological treatment. We found no statistical difference for any of the parameters considered, except for the increase in lysis in the non-responders group (Lys30=0.7-vs-0%; p=0.02), which remained significant after correction for confoundings factors. **In treated patients, associations between lysis and clinical variables (gender, being small for gestational age, gemellarity, mode of delivery, ventilation, prenatal steroids, chorioamnionitis, and sepsis) were negligible, with p-values ranging from 0.11 to 0.92 (data not shown).**

Discussion

This study is the first that evaluated the role of thromboelastography in predicting spontaneous ductal closure and response to pharmacological treatment in preterm newborns.

Our results showed no thromboelastographic differences at birth between patients with spontaneous DA closure and those requiring treatment. Moreover, although this latter group had significantly lower platelet count and hematocrit, both variables seem to depend upon GA and illness severity.

In ~~a~~ mice ~~model~~, platelet-triggered ductal sealing was shown to be a relevant mechanism for ductal closure [4]. ~~Besides, a relationship between platelet count below 100.000/ml and ductal patency was found in preterm newborns. Thereafter,~~ **In this respect,** the role of platelets ~~in ductal closure~~ has been explored in preterm infants, but conclusions were conflicting [5–9]

~~The retrospective nature of data and the variability in the definition of thrombocytopenia, timing of platelets count, GA and comorbidities of infants included may account for discrepancies among results.~~

A ~~recent~~ meta-analysis observed an association between platelet count below 100,000/**µL** and DA patency [10]. Similarly, thrombocytopenia on the first day of life was associated with delayed ductal closure and the risk of hsPDA on day seven of life [21]. In this context, platelet transfusions to maintain platelets count above 100,000/**µL** did not improve PDA closure rate or hasten PDA closure [22] **and were associated with increased risk of IVH [22]. Moreover, lowering the transfusion threshold from 50,000 to 25,000 platelets/µL reduced the rates of death or major bleeding in preterm newborns [23].**

~~Some authors observed that~~ Platelet function rather than count might predict hsPDA or pharmacological response; indeed, higher platelet distribution width, a marker of platelet activation, has been associated with ductal patency [5]. ~~Results from~~ A PFA-100-based study ha**s** shown that prolonged closure time (collagen-ADP > 130), a marker of impaired platelet function, was independently associated with PDA [14]. However, hematocrit differences may have affected results as red blood cells play a direct role in hemostasis and anemia is an independent risk factor for PDA **[24, 25].**

Platelets and clotting factors combine to sustain thrombin generation, a platelet-activating agonist, while fibrin stabilizes the platelet plug during the hemostatic process [15]. Of note, immunohistochemical studies demonstrated that fibrinogen, von Willebrand factor, and collagen were expressed in the mice DA lumen [4].

The involvement of the coagulation cascade in DA closure has been postulated in children with persistent ductal patency **[26]**. Plasma proteomic analysis revealed that the concentration of fibrinogen, platelet factor 4, von Willebrand factor, collagen and mannose-binding lectin-associate serine protease-1 were significantly lower in children with persistent PDA, compared to controls.

If these observations hold, thromboelastography may disclose differences between patients with spontaneous DA closure compared to those with persistent PDA.

Specifically, MA reflects the interaction between platelets (number and function) and fibrin(ogen), which are both essential for clot formation and stabilization. Platelets are known to contribute up to 80% of clot strength in healthy adults when platelet function is mature, **differently to preterm newborns~~. Differently, in preterm newborns, platelet function is reduced in the first days of life and improves in the next two weeks~~** [11,12].

The interplay among endothelium, humoral, and cellular factors involved in the hemostatic process may explain the lack of correlation between MA and ductal closure ~~**observed in our study**~~. Indeed, thromboelastography cannot discriminate the contribution of individual factors to clot formation.

Another explanation may be related to the timing of thromboelastographic evaluation. We compared MA at birth, but it increased significantly after the first days of life as a result of platelet maturation and early-postnatal inflammation that triggers platelet activation [16].

~~**Interestingly,**~~ Although MA at birth was similar between newborns with spontaneous closure compared to those with PDA, platelet count was significantly higher in the former, ~~**while**~~ fibrinogen ~~**concentration was similar between the two groups**~~ **being equal**. These observations suggest that **in preterm newborns 1.** the contribution of platelet function to clot strength may vary according to GA and co-morbidities; **2. in the absence of severe thrombocytopenia, clotting seems adequate, despite hypofunctional platelets.** ~~**Moreover, although re hypofunctional in preterm newborns in the first days of life, in the absence of severe thrombocytopenia, clotting seems adequate.**~~ Similarly, Deschmann et al. **[27]** demonstrated that preterm newborns with platelet count > 90,000/**µL** had substantially normal clot-time evaluated by PFA 100.

The response of PDA to pharmacological treatment changes depending on antenatal and postnatal factors.

Extremely preterm infants have the highest rate of failure to the first treatment cycle, reaching 70% at 23-24 GA [1]. Our observation confirms that non-responder patients were younger and sicker than those with spontaneous closure.

The relationship between platelet count and the response to COX inhibitors is still undetermined, as results from retrospective studies provided mixed results [1,7]. A ~~**recent**~~ meta-analysis **[28]** showed that platelet counts were significantly lower in preterm infants who failed to close DA after pharmacological treatment and that pre-treatment thrombocytopenia was associated with a lower success rate.

~~**Interestingly, w**~~**W**e found a significantly higher degree of fibrinolysis in neonates non-responders to pharmacological therapy compared to responders. The median fibrinolysis was higher even when compared to thromboelastographic reference ranges [16]. Fibrinolysis is an enzymatic system responsible for fibrin degradation by two opposing drivers, that regulate the conversion of plasminogen to plasmin, the enzyme that dissolves the fibrin clot.

The enhanced fibrinolysis observed in our cohort is intriguing. ~~**Only a few studies addressed the fibrinolytic system in neonates In extremely preterm newborns,**~~ Fibrinolysis is inhibited in the first ten days of life, accounted by higher plasminogen activator inhibitors-1 (PAI-1) activity and lower tissue-type plasminogen activator (t-PA)/PAI-1 activity ratio, compared with full-term neonates [29, **30**].

If the coagulation system may favor ductal closure, the increased fibrinolysis may reduce clot strength and counteract clot formation, possibly contributing to treatment failure.

We tried to explain our findings by considering eight out of the twenty newborns with the highest degree of fibrinolysis (> 2%, data not shown). Seven were extremely low birth-weight with GA < 28 weeks without fetal growth restriction, chorioamnionitis, or sepsis; all were mechanically ventilated; one developed intraventricular hemorrhage. As all samples were drawn a few hours after birth, no acquired clinical conditions could explain the enhanced fibrinolysis.

This observation could be due to chance, although unlikely. Indeed, according to previous studies [28], we detected lower rates of fibrinolysis in preterm newborns in the first ten days of life, compared to values observed one month after birth [16].

We recognize some study limitations. First, it was not powered to identify thomboelastographic differences between patients with spontaneous versus pharmacological ductal closure**. This is a secondary analysis, and the aim was to explore the potential role of TEG in the setting of PDA management. Further prospective evaluations will be performed, to define the contribution of both clot lysis and platelet function in ductal closure, through tailored TEG and bedside evaluation of primary hemostasis.**

Secondly, we compared thromboelastographic profiles at birth but hemostasis evolves rapidly after birth in preterm neonates, and the contribution of platelet function to clot strength could have been different after the first day of life, especially in critically-ill patients. **Nevertheless, the blood sparing policy and the difficulty in sampling preterm neonates prevented us from repeating measurements at the time of PDA diagnosis.**

In conclusion, thromboelastography at birth does not predict the spontaneous closure of DA in preterm infants. Its role in anticipating spontaneous DA closure and the association between enhanced fibrinolysis and treatment failure require further investigations.

Acknowledgment

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Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The Institutional Review Board approved the study protocol, and the written informed consent was obtained from patients' tutors.

Disclosure Statement

The authors have no conflicts of interest to declare.

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None.

Author Contributions

SG was involved in the conception and planning of the study, the acquisition, analysis, and interpretation of data, writing of the first draft, and critical revision of the manuscript. GR contributed to conception and planning of the study, acquisition, analysis, interpretation of data, cowriting, and critical revision of the article. BLC was involved in data analysis and interpretation, cowriting and critical revision of the article. IA and SG were involved in the acquisition and interpretation of the data, and the critical revision of the article. DC was involved in the analysis of the data and the critical revision of the article. GC and FS were involved in the acquisition, analysis, interpretation of the data, and critical revision of the article. FM was involved in the conception and planning of the study, the interpretation of the data, and the critical revision of the article. All authors revised and gave final approval of the version to be submitted.

Tables' Legend

Table 1: Demographic features, outcomes and hemostatic profile of preterm infants affected by PDA: focus on pharmacological versus spontaneous closure

BPD: bronchopulmonary dysplasia; CRIB: clinical risk index baby score; GA: gestational age; IVH: intraventricular hemorrhage; ROP: retinopathy of prematurity; RDS: respiratory distress syndrome; SGA: small for gestational age; *Mann Whitney test; ** Chi-squared test.

Table 2: Demographic features, outcomes and hemostatic profile of preterm infants affected by PDA requiring pharmacological treatment: focus on responders versus non-responders.

CRIB: clinical risk index baby score; GA: gestational age; RDS: respiratory distress syndrome; SD: standard deviation; SGA: small for gestational age; *Mann Whitney test; ** Chi-squared test

Figures' Legend

~~**Figure 1: Study population. PDA: patent ductus arteriosus, VLBW: very low birth weight**~~

Figure 1: Summary of the hemostatic process (partially modified from Raffaeli et al, ADC 2019, copyright license n° 4733720500744).

1a. Cell-based model of hemostasis occurs through three overlapping phases: I) initiation phase in the tissue-factor bearing cells, which generates small amounts of thrombin; II) amplification phase on the platelet surface, which enables the procoagulant complex interaction through activation of platelets cofactors and III) propagation on the activated platelet surface, which contributes to the burst of thrombin generation.

1b. Thromboelastographic trace. A representation of the TEG main parameters and their determinants (§).

1c. The cascade model of coagulation, which involves the "extrinsic" and "intrinsic" pathways, respectively depicted by PT and APTT conventional tests.

> Increase; a: activated; TF: tissue factor.

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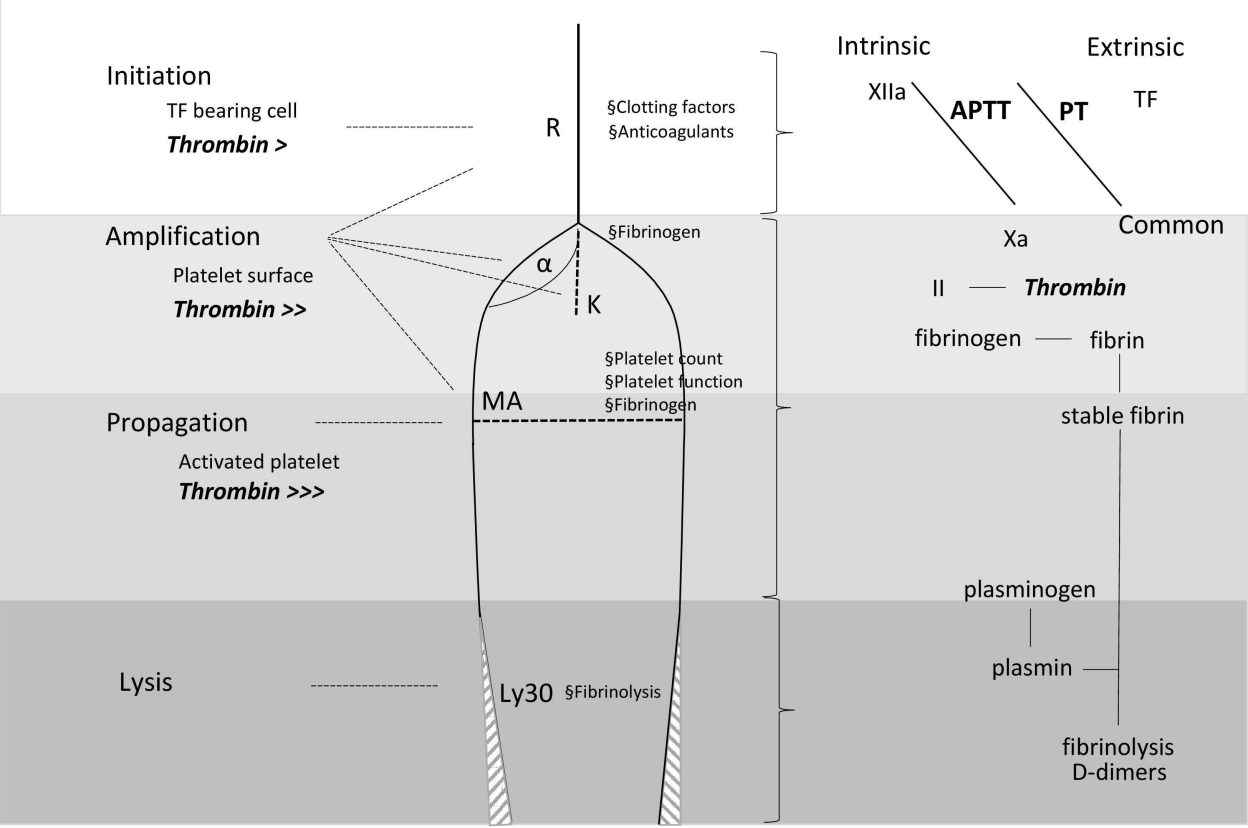

1a. Cell-based hemostasis 1b. Thromboelastography 1c. Coagulation cascade

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The Thromboelastographic profile at birth in very preterm newborns with patent ductus arteriosus

Stefano Ghirardello[1], Genny Raffaeli[1]*, Beatrice Letizia Crippa[1,2], Silvia Gulden[1,2], Ilaria Amodeo[1,2], Dario Consonni[3], Giacomo Cavallaro[1], Federico Schena[1], Fabio Mosca[1,2]

Table 1R (For Reviewers only). Association between Lysis and clinical variables.

Variable (n°observations)	Lysis (median, IQR)	p-value
Gender		0.612
Male (16)	0.2 (0.0 – 1.9)	
Female (24)	0.1 (0.0 – 0.8)	
Weight for age		0.523
SGA (4)	0.0 (0.0 – 7.0)	
No SGA (36)	0.2 (0.0 – 1.2)	
Gemellarity		0.873
Singleton (24)	0.2 (0.0 – 1.1)	
Twin (16)	0.1 (0.0 – 1.5)	
Mode of delivery		0.355
Vaginal (7)	0.0 (0.0 – 0.5)	
C-section (33)	0.1 (0.0 – 1.8)	
Ventilation		0.505
Yes (24)	0.2 (0.0 – 1.6)	
No (16)	0.1 (0.0 – 1.0)	
Prenatal steroids		0.112
Yes (32)	0.1 (0.0 – 1.9)	
No (8)	0.3 (0.1 – 0.4)	
Chorioamnionitis		0.819
Yes (2)	1.1 (0.0 – 2.3)	
No (38)	0.1 (0.0 – 1.1)	
Sepsis		0.924
Yes (4)	0.1 (0.0 – 1.2)	
No (36)	0.3 (0.0 – 1.4)	
IQR: interquartile range; SGA: small for gestational age		

Table 1. Demographic features, outcomes and hemostatic profile of preterm infants affected by PDA: focus on pharmacological versus spontaneous closure.

Demographic features and outcomes			
	Treated for PDA (n = 40)	**Spontaneous PDA closure (n =111)**	
	mean ± SD (min-max)	**mean ± SD (min-max)**	***p* –Value***
GA (weeks)	27.6 ± 2.1 (23 –31.9)	29.7 ± 1.7 (25.7 – 32.9)	< 0.001
Birth weight (g)	933 ± 263 (500 – 1490)	1158 ± 256 (500 – 1495)	< 0.001
Apgar 1’	5.6 ± 1.5 (2 – 8)	6.4 ± 1.8 (1 – 9)	0.003
Apgar 5’	8 ± 0.8 (6 – 9)	8.3 ± 0.9 (6 – 10)	0.04
Venous pH	7.3 ± 0.1 (6.9 – 7.4)	7.3 ± 0.1 (7.1 – 7.5)	0.90
Arterious pH	7.3 ± 0.1 (6.9 – 7.4)	7.3 ± 0.1 (6.9 – 7.4)	0.20
CRIB	10.1 ± 0.1 (4 – 17)	6.9 ± 2.8 (2 – 14)	< 0.001
	n (%)	**n (%)**	***p* –Value****
Male gender	16 (40)	51 (45.9)	0.52
SGA < 10^{th} percentile	4 (10.1)	24 (21.6)	0.10
Pairs of twins	16 (40)	58 (52.3)	0.18
Spontaneous delivery	7 (17.5)	11 (9.9)	0.23
Steroid prophylaxis	32 (80)	87 (78.4)	0.42
Chorioamnionitis	2 (5)	4 (3.6)	0.69
Mechanical ventilation	24 (60)	17 (15.3)	< 0.001
Sepsis	4 (10)	4 (3.6)	0.12
RDS (grade 3 and 4)	31 (77.5)	30 (27)	< 0.001
BPD (severe)	19 (47.5)	26 (23.4)	0.004
NEC (>2 grade)	9 (22.5)	14 (12.6)	0.13
ROP (all stages)	14 (35)	13 (11.7)	0.001
IVH (all grades)	10 (25)	16 (14.1)	0.13
Hemostatic profile			
	Treated for PDA (n = 40)	**Spontaneous PDA closure (n =111)**	
	Median (IQR)	**Median (IQR)**	***p* –Value***
Standard coagulation profile			
PT (seconds)	16.2 (14.7 – 18.7)	15.8 (13.4 – 18.5)	0.48
PT (ratio)	1.4 (1.2 -1.6)	1.6 (1.4 -1.8)	0.22
PTT (seconds)	60.2 (50 – 64)	58.3 (47.1 – 67.3)	0.65
PTT (ratio)	1.9 (1.7 -2.3)	1.9 (1.5 -2.2)	0.69
Antithrombin (%)	32 (20 – 33)	35 (29 – 39)	0.09
Protein C (%)	10.5 (9 – 13)	12 (9 -20)	0.33
Fibrinogen (mg/dl)	160 (107 – 306.5)	156.5 (121.5 -234)	0.59
Tromboelastography			
Reaction time (min)	8.4 (4.5 – 12)	8.8 (4.8 – 12)	0.98
Kinetics (min)	2.5 (1.7 – 3.8)	2.8 (2 - 4.6)	0.16
Alpha angle (°)	57 (43.7 – 66)	50.5 (40.3 – 62.1)	0.10
Maximum amplitude (mm)	55.3 (51 -62)	55.3 (47.9 – 60)	0.34
Lysis 30minutes (%)	0.1 (0 – 1.2)	0.2 (0 -0.7)	0.83
Blood Test			
Red blood cells (10^6/µL)	3.8 (3.6 – 4.1)	4.2 (3.9 – 4.7)	< 0.001
Hemoglobin (g/dl)	15.1 (14.4 – 17.1)	16.4 (15.2 – 17.9)	0.01
Hematocrit (%)	44.6 (41.8 – 50.1)	48.7 (44.6 – 51.7)	0.01
White blood cells (10^3/µL)	6.9 (5 -8.9)	7.9 (6 – 11.1)	0.12
Platelet count (10^3/µL)	187 (134 – 235)	216 (156 -252)	0.04

BPD: bronchopulmonary dysplasia; CRIB: clinical risk index baby score; GA: gestational age; IVH: intraventricular hemorrhage; ROP: retinopathy of prematurity; RDS: respiratory distress syndrome; SGA: small for gestational age; *Mann Whitney test; ** Chi-squared test

Table 2. Demographic features, outcomes and hemostatic profile of preterm infants affected by PDA requiring pharmacological treatment: focus on responders versus non-responders.

Demographic features and outcomes			
	Responders (n = 20)	**Non – responders (n = 20)**	
	mean ± SD (min-max)	**mean ± SD (min-max)**	***p* –Value***
GA (weeks)	27.7 ± 2.1 (23-31)	26.5 ± 2 (23-29)	0.08
Birth weight (g)	955.7 ± 280 (600-1490)	910.2 ± 250 (500-1400)	0.74
CRIB	9.3 ± 3.6 (4-17)	10.8 ± 3.6 (5-16)	0.16
	n (%)	**n (%)**	***p* –Value****
Male gender	6 (30)	10 (50)	0.2
SGA < 10^{th} percentile	1 (5)	3 (15)	0.3
Pairs of twins	7 (35)	9 (45)	0.5
Spontaneous delivery	5 (25)	2 (10)	0.2
Cesarean section	15 (75)	18 (90)	0.2
Steroid prophylaxis	18 (90)	14 (70)	0.08
Chorioamnionitis	0 (0)	2 (10)	0.1
Mechanical ventilation	9 (45)	15 (75)	0.05
Sepsis	1 (5)	3 (15)	0.3
RDS (grade 3 and 4)	13 (65)	18 (90)	0.06
Hemostatic profile			
	Responders (n = 20)	**Non – responders (n = 20)**	
	Median (IQR)	**Median (IQR)**	***p* –Value***
Standard coagulation profile			
PT (seconds)	16.6 (14.8 – 18.5)	15.6 (13.6 – 18.9)	0.40
PT (ratio)	1.4 (1.4 – 1.4)	1.4 (1 – 1.7)	1
PTT (seconds)	62.5 (50 – 70)	56.9 (49.2 – 62.3)	0.27
PTT (ratio)	1.7 (1.6 – 1.8)	2 (1.3 – 2.5)	0.18
Antithrombin (%)	32 (18.5 – 32.5)	30 (20 – 35)	1
Protein C (%)	11 (9 – 12)	10 (10 – 13)	0.94
Fibrinogen (mg/dl)	112.5 (91 – 303)	257.5 (134 -337)	0.11
Tromboelastography			
Reaction time (min)	8 (4.3 – 10.2)	9.7 (4.8 – 14.6)	0.28
Kinetics (min)	2.1 (1.6 – 3.5)	2.7 (1.7 – 3.9)	0.46
Alpha angle (°)	60.6 (47.4 – 67.8)	54.7 (39.7 -64)	0.30
Maximum amplitude (mm)	58.3 (52.2 – 61.5)	53.9 (50.8 – 62.2)	0.38
Lysis 30minutes (%)	0 (0 – 0.2)	0.7 (0 – 2.5)	0.02
Blood Test			
Red blood cells (10^6/µL)	3.8 ± 0.4 (3.4 – 4.8)	3.9 ± 0.5 (2.7 – 4.7)	0.22
Hemoglobin (g/dl)	15.5 ± 2 (11.2 – 19.5)	15.6 ± 2.4 (10.6 – 19.9)	0.66
Hematocrit (%)	45.5 ± 5.4 (36.1 – 57.9)	45.7 ± 6.7 (31.5 – 55.6)	0.80
White blood cells (10^3/µL)	7.7 ± 3.9 (3.6 – 18.7)	8.7 ± 5.6 (2.9 – 24.7)	0.63
Platelet count (10^3/µL)	202.8 ± 63.5 (63 – 352)	165.8 ± 73.8 (57 – 276)	0.09

CRIB: clinical risk index baby score; GA: gestational age; RDS: respiratory distress syndrome; SD: standard deviation; SGA: small for gestational age; *Mann Whitney test; ** Chi-squared test