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Reference intervals of citrated-native whole blood thromboelastography in premature neonates

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ABSTRACT

Background: Bleeding due to acquired coagulation disorders is a common complication in premature neonates. In this clinical setting, standard coagulation laboratory tests might be unsuitable to investigate the hemostatic function as they reflect the concentration of pro-coagulant proteins but not of anti-coagulant proteins. Thromboelastography (TEG), providing a more complete assessment of hemostasis, may be able to overcome some of these limitations. Unfortunately, experience on the use of TEG in premature neonates is very limited and, in particular in this population, reference ranges of TEG parameters have not been yet evaluated.

Aims: To evaluate TEG in preterm neonates, and to assess their reference ranges.

Methods: One hundred and eighteen preterm neonates were analyzed for TEG in a retrospective cohort study. Double-sided 95% reference intervals were calculated using a bootstrap method after Box-Cox transformation. TEG parameters were compared between early-preterm and moderate-/late-preterm neonates and between bleeding and non-bleeding preterm neonates.

Results: Comparing early-preterm with moderate-/late-preterm neonates, TEG parameters were not statistically different, except for fibrinolysis which was significantly higher in early preterm neonates. Platelet count significantly correlated with α angle and MA parameters. Bleeding and non-bleeding neonates had similar TEG values.

Conclusions: These results reinforce the concept that in stable preterm neonates, in spite of lower concentration of pro- and anti-coagulants proteins, the hemostasis is normally balanced and well functioning.

1. Introduction

Bleeding is a common complication in premature neonates admitted to intensive care unit. The risk of developing a severe bleeding, such as intraventricular hemorrhage, increases significantly with decreasing gestational age at birth and with increasing level of illness during the first days after birth [1,2]. In this clinical setting, the assessment of coagulation state is required to evaluate the cause of bleeding and to decide the appropriate treatment. Historically, the approach of bleeding disorders in neonates included platelet count, standard coagulation tests, such as prothrombin time (PT), activated partial prothrombin time (APTT), and fibrinogen level [3]. However, the neonatal hemostatic function is a developing and dynamic system that includes a series of interactions among endothelial cells, plasma proteins and platelets. During infancy, the concentration of some pro- and anti-coagulant proteins are increasing with gestational and postnatal age, therefore, PT and APTT might be unsuitable to investigate acquired coagulation disorders which frequently occur in sick or preterm neonates [4].

Viscoelastic tests of coagulation, such as thromboelastography (TEG), may be able to overcome some of these limitations [5]. TEG differs significantly from conventional coagulation tests, as it evaluates the kinetics of the whole hemostatic process, providing a rapid global assessment of clotting, platelet function and fibrinolysis, and reflecting the in vivo condition more closely than the conventional tests do [6].

Unfortunately, experience on the use of TEG in high-risk for bleeding premature neonates is very limited and reference ranges of TEG parameters are available only in term neonates [7–9].

The aim of this study was to evaluate TEG parameters in preterm neonates at birth, and to organize obtained data into reference intervals.

2. Materials and methods

2.1. Patients and data collection

TEG analysis was implemented in our Neonatal Unit (ASST Spedali Civili, Children's Hospital, Brescia, Italy) from September 2013 to

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Inclusion criteria for the study were preterm neonate (gestational age at birth < 37 weeks) combined with blood sample taken for TEG analysis within 36 h after birth. To obtain TEG reference ranges, TEG analysis acquired in the following settings were excluded: death within 7 days of life, early-onset sepsis confirmed by a positive blood culture, suspected sepsis with raised C-reactive protein defined as values > 10 mg/L, presence of bleeding (intracranial, gastrointestinal, cutaneous and pulmonary hemorrhage), platelet count < 100×10^9/L.

Selection and identification of neonates to be included in the study were retrospectively given by using electronic healthcare databases of our Institution. Clinical, laboratory and TEG data were reviewed and collected by using Milos 1.0 (Gruppo Finmatica, Italy), Galileo 1.4.3.2.107 (NoemaLife, Italy) and TEG Analytical Software 4.2.3 (Haemoscope, USA), respectively.

A written informed consent for TEG analysis and, subsequently, for the inclusion in the study was obtained from both parents.

The Ethical Committee of the ASST Spedali Civili, Brescia, Italy, approved the study.

2.2. Operator training, blood sampling and thromboelastography assessment

Before starting the study, during a period of three months' time, the procedure of TEG analysis was standardized with particular respect to blood sampling and collection. In addition, the operators were specifically trained in the method, the analytical technique and the instrument to be used. The collection of blood citrated sample was preferred to native blood sample because of the lesser incidence of pre-analytical errors. After the training activity, the qualification of each operator to carry out a TEG analysis was to successfully overcome a proficiency test which included the assessment of accuracy and reproducibility performances.

According to our Unit Guidelines, in preterm neonates admission laboratory tests including blood culture, full blood count and blood group are drawn at birth from the umbilical cord/placenta blood. Standard coagulation tests are not performed routinely at birth. Full blood count and C-reactive protein are repeated by taking a vein blood sample within 36 h after birth. At this time, during the study period, a 0.5 mL blood sample for TEG analysis was obtained and directly collected into test tubes with 3.2% sodium citrate (0.129 mol/L) as anticoagulant (9:1 vol/vol ratio) by a vacutainer system. TEG analysis was performed immediately after blood collection using a TEG hemostasis analyzer (Haemoscope, Skokie, IL, US) and the following parameters of clot formation and lysis, directly measured during the instrument analysis, were evaluated as a representation of hemostasis:

- Clotting time (R). The time, expressed in seconds, from the start of a sample run until the first significant levels of fibrin formation, arbitrarily defined as the trace amplitude of 2 mm, which corresponds to the initial clot formation.
- Clot kinetics (K). The time, expressed in seconds, from the measurement of R until 20 mm of amplitude are reached. This time is a measure of the clot kinetics to reach a fixed level of clot strength. It correlates with fibrinogen level and, to a lesser extent, with both platelet function and values.
- α angle (α). The angle formed between the midline and the tangent to the main body of the trace. Similarly to K, α correlates with fibrinogen level and with platelet function and values.
- Maximum amplitude (MA). The amplitude, expressed in mm, at the widest point of the trace representing the maximum clot strength as the result of the modest contribution of fibrin and the much more significant contribution of platelets.
- Fibrinolysis (LY30). The rate of clot breakdown measured as the reduction of the area under the TEG tracing from the time MA is reached to 30 min after the MA.

In addition, others derived TEG parameters determining the kinetics, the strength and the stability of clot were evaluated:

- G. The shear elastic modulus strength of clot when MA is reached, measured in dyn/cm^2 divided by 1000 (displayed by the software as Kd/sc).
- Coagulation Index (CI). Derived from the R, K, MA and α of native whole blood tracings. This parameter describes the overall coagulation performance [10].
- Thrombodynamic Potential Index (TPI). The normalized shear elastic modulus strength divided by the kinetics of clot formation. Similarly to CI, TPI gives information on the overall measure of coagulation performance [11].

2.3. Statistical analysis

The characteristics of neonates, TEG and platelet values were analyzed using descriptive statistics. Continuous variables were presented as the median and interquartile range (Q1-Q3), and were compared by Mann-Whitney *U* test. Categorical variables were presented as absolute number and percentage, and were compared by Fisher's exact test. Correlations among continuous variables were obtained by Pearson's correlation test. Double-sided 95% reference intervals were calculated using a bootstrap method after Box-Cox transformation [12–14], and possible outliers were detected by Tukey test [15]. All statistical tests were considered significant for *P* values of < 0.05. Computations were performed using a commercial statistical package: MedCalc for Windows (MedCalc Software, Mariakerke, Belgium).

3. Results

One hundred and eighteen preterm neonates were inborn at < 37 weeks of gestational age and received TEG analysis within 36 h after birth. The distribution and the grouping of neonates according to the different clinical conditions and the given data analysis are shown in Fig. 1. Fifty-three neonates were not included in the assessment of reference intervals because of: death within 7 days (n = 16 cases), sepsis with positive blood culture (n = 4 cases), suspected sepsis with elevated C-reactive protein defined as values > 10 mg/l (n = 18 cases), bleeding (IVH, n = 22 cases, pulmonary hemorrhage, n = 2 cases), PLT count < 100×10^9/L (n = 8 cases).

Sixty-five preterm neonates were included in the analysis of reference intervals and organized into two categories according to gestational age at birth (Fig. 1). Thirty-two neonates were allocated in the group of < 32 weeks' gestation (early-preterm neonates) and 33 neonates in the group 32 to < 37 weeks' gestation (moderate- and late-preterm neonates). Characteristics of the two groups along with TEG values are summarized in Table 1.

Among these neonates, the occurrence of respiratory distress syndrome was significantly higher in early-preterm neonates rather than in late-preterm neonates (Table 1). The number of neonates small for gestational age was significantly lower in early-preterm neonates rather than in late-preterm neonates (Table 1). The two groups had similar R, K, α, MA values. We found lower values of TPI and CI and higher values of G in the moderate-/late-preterm neonates, although differences were not statistically significant. Statistically significant higher values of LY30 were found in early-preterm neonates.

By running the Pearson's correlation test we found the lower the gestational age, the higher is the percentage of LY30 ($P = 0.038$, $r = -0.267$). Furthermore there was a statistically significant correlation between platelet count and α ($P = 0.023$, $r = 0.291$), platelets count and MA ($P = 0.000$, $r = 0.538$). Double-sided 95% reference intervals for early-preterm and moderate-/late-preterm neonates are showed in Table 2.

Twenty-four bleeding neonates were compared with 94 non-bleeding neonates (Fig. 1) and their TEG parameters resulted similar