Early Human Development 127 (2018) 48–52

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

The intra-assay reproducibility of thromboelastography in very low birth weight infants

Stefano Ghirardello[a,*,1], Genny Raffaeli[a,1], Erica Scalambrino[b], Veena Chantarangkul[b], Giacomo Cavallaro[a], Andrea Artoni[b], Fabio Mosca[a], Armando Tripodi[b]

[a] NICU, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Italy
[b] Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Università degli Studi di Milano, Italy

ARTICLE INFO

Keywords:
Coagulation
Global hemostatic assay
Point-of-care testing
TEG
Analytical variability

ABSTRACT

Background and aims: Despite the potential benefits of thromboelastography (TEG) for bedside hemostatic assessment in critical care settings, its accuracy remains to be determined, especially in critically ill neonates. We determined the intra-assay reproducibility of TEG parameters: Reaction time (R), clot kinetics (K) and Maximum Amplitude (MA) in a cohort of very low birth weight (VLBW) infants.

Study design: Observational study.

Subjects: One hundred VLBW newborns.

Outcome measures: We performed TEG duplicate measurements for blood samples from VLBW newborns. To assess for correlation, we calculated the coefficients of correlation by plotting the values of the first vs the second measurement. Paired samples were compared with *t*-test and the coefficient of variation (CV) on paired results was also calculated as a measure of variability. To evaluate the agreement between duplicates, Bland-Altman (BA) analysis was performed.

Results: We evaluated 228 TEG pairs. Both the coefficient of correlation and the BA analysis showed an acceptable level of agreement between duplicates.

TEG variability (CV, mean ± SD) was highest for K (10.4%, ± 12.9), lowest for MA (3.6%, ± 8.0) and moderate for R (7.9%, ± 9.0). The results from ANOVA one-way analysis describe different variability trends: K-CV increased at higher values, while MA-CV and R-CV increased at lower values.

Conclusions: In VLBW newborns, the agreement between TEG duplicate measurements for R and MA parameters is adequate for clinical purposes. TEG is a promising tool to quickly assess hemostasis ensuring a significant blood sparing in critically ill neonates.

1. Introduction

Viscoelastic hemostatic tests are increasingly used both in surgical and critical care settings for the bedside management of hemostasis [1–5]. Thromboelastography (TEG® Hemoscope/Haemonetics, Niles, IL) was firstly developed in 1948 by Hartert as a method for near-patient global hemostatic evaluation, by allowing a real-time assessment of the viscoelastic clot strength in whole blood [6]. This method provides a detailed visual trace of the entire hemostatic process (initiation, amplification, propagation, and dissolution), thus supporting caregivers with a rapid and broad qualitative assessment of hemostasis potential [7]. The dynamic viscoelastic tests, in fact, explore the entire hemostatic process, including procoagulants (and possibly anticoagulants), and the contribution of cellular components (erythrocytes, leukocytes and platelets), whose role in the cell-based hemostasis has been proved to be relevant [8]. Historically, viscoelastic tests have been applied during liver and cardiac surgery but, in recent years, this technology has gained renewed interest, especially in critical care settings [1–3,9].

Despite their indisputable role in congenital hemorrhagic diseases, prothrombin (PT) and activated partial thromboplastin time (aPTT) exhibit a series of limitations, especially in the neonatal period [10]. The traditional coagulation tests are mainly responsive to the

* Corresponding author at: Neonatal Intensive Care Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Università degli Studi di Milano, Via Commenda 12, 20122 Milano, Italy.

E-mail addresses: stefano.ghirardello@mangiagalli.it (S. Ghirardello), giacomo.cavallaro@mangiagalli.it (G. Cavallaro), andrea.artoni@policlinico.mi.it (A. Artoni), fabio.mosca@mangiagalli.it (F. Mosca), armando.tripodi@unimi.it (A. Tripodi).

[1] Co-first authorship. These authors contributed equally to the work.

https://doi.org/10.1016/j.earlhumdev.2018.10.004
Received 3 April 2018; Received in revised form 26 July 2018; Accepted 4 October 2018

procoagulant factors, thus providing static information on the initiation of the clot formation, which is equivalent to the Reaction time (R) of TEG technology [10]. However, PT and aPTT are poorly responsive to the anticoagulants (i.e., antithrombin, protein C and protein S). Hence, they are not as efficient as expected to evaluate hemostatic abnormalities in the neonatal period, when both pro- and anticoagulants are decreased [10]. In the above conditions, the PT and aPTT prolongations hardly explain the normal thrombin generation observed in affected subjects [11,12]. In contrast, TEG is a promising complementary diagnostic tool in critical care settings. As a point-of-care test, TEG provides two clear advantages over the standard coagulation tests: the shorter turnaround time and the lower amount of blood required, the latter particularly significant in the neonatal population [13].

A growing body of literature supports the role of TEG for a more rational goal-directed transfusion and hemostatic therapy in children and adults [1–3,14]. The rapid and complete assessment of ex-vivo hemostasis with viscoelastic techniques has been associated with a reduction of plasma, fibrinogen and platelet transfusions and improved clinical outcomes [1,14,15]. Currently in the neonatal and pediatric population, the use of TEG is mainly addressed to the coagulation monitoring and management related to the extracorporeal life support (ECLS) and cardio-pulmonary bypass [16–18].

Although the precision assessment of the viscoelastic tests has been performed during the FDA validation process [19], the reproducibility of TEG may be altered in selected populations. A relevant degree of variability has been previously reported in veterinary and human studies [20–23]. While the role of pre-analytical errors (related to the collection, handling, and storage of samples) is well established [24], the intrinsic variability of the analytical phase requires further investigation.

As the use of TEG might be beneficial to critically ill neonates [25,26], the evaluation of its reproducibility is needed to make neonatologists more aware of the reliability of this test. Currently, no data are available on the reproducibility of TEG in the NICU setting. Specifically, no information is available to decide on whether testing should be performed as single or duplicate measurements. This knowledge could be highly relevant in the context of blood sparing policies, commonly applied for newborns, especially those with birth weight < 1500 g (VLBW).

The present study aimed to assess the agreement of duplicate TEG measurements performed on samples from VLBW newborns.

2. Materials and methods

We performed an observational study at the Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan with the approval of the Institutional Review Board and written informed consent from patients' parents. All procedures were carried out in accordance with the Helsinki Declaration of 1975, as revised in 2008. Testing was performed using TEG (Hemoscope/Haemonetics®, Niles, IL).

TEG measurements were performed at the Neonatal Intensive Care Unit by a restricted skilled staff. Each operator has been thoroughly trained in samples handling and TEG setup before starting patients' enrollment. We performed all tests following manufacturer's instructions for re-calcified native blood; the instrument underwent regular maintenance checks throughout the study period.

The evaluation of reproducibility of thromboelastography is part of a research project, aimed at identifying the reference ranges of TEG parameters in a cohort of 200 VLBW infants in their first month of life.

We analyzed either arterial or venous blood samples, based on sample availability. Arterial blood samples were collected from a non-heparinized umbilical arterial line. Venous blood samples were collected either from a non-heparinized umbilical venous catheter or direct venipuncture. Sample timing schedule was: 1st, 3rd–5th, 10th and 30th day of life, to be matched with routine laboratory investigations. Blood samples were immediately placed in test tubes containing 3.2% trisodium citrate as anticoagulant (9:1 vol/vol ratio) and stored at room temperature until testing. Sample handling was standardized to minimize variation in storage time (30 min up to 2 h) [27]. Before TEG analysis, samples were visually inspected for hemolysis or clot formation and possibly discarded. Immediately before running the test, both TEG channels passed electronic quality controls.

Each TEG analysis was performed in duplicates for each sample. We used a two-channel TEG hemostasis analyzer enabling for the duplicates' simultaneous evaluation. Twenty microliters of 0.2 mol/L $CaCl_2$ were pipetted into the warmed TEG cup. Citrated blood sample was gently mixed, and then 340 μL of blood was pipetted into each of the two TEG cups. All measurements were performed at 37 °C and were allowed to run until a stable maximum clot amplitude (MA) was reached.

For comparative analysis (first vs second measurement), we recorded the following parameters: Reaction time (R), clot kinetics (K) and maximal amplitude (MA). These parameters will be named as TEG-R, TEG-K and TEG-MA across the manuscript.

2.1. Data recording

TEG-R refers both to the time (in minutes) the trace requires to reach an amplitude of 2 mm. TEG-K is defined as the time required for clot amplitude to increase from 2 to 20 mm. TEG-MA identifies the clot strength. The measurements were stored automatically in the database of the device and further extracted for statistical analysis.

2.2. Data analysis

To assess for comparability of duplicate measurement, we made the following analyses. Paired samples *t*-tests to compare means obtained with duplicate measurements. Correlation between duplicate measurements was evaluated by scatter plots (first vs second measurement) and calculation of coefficients of correlation (r). Bland-Altman (BA) analysis was performed, to assess for between-pair agreement by plotting percentage of between-pair differences versus the mean value. We also calculated the coefficient of variation (CV) between duplicate values for TEG parameters. Additionally, we explored the variation of the CV values along different quartiles, by means of one-way ANOVA. Quartiles were defined into four categories (< 25°, 25–50°, 50–75°, > 75°), based on the average between duplicates. All values are reported as mean (± SD). We used IBM SPSS statistics 24 software for data analysis. A p-value < 0.05 was considered statistically significant.

2.3. Sample size

With a sample size of 220 duplicates (n = 440 TEG traces), a two-sided paired *t*-test achieves 80% power to detect an effect size of 0.2 between duplicate measurements, with an actually achieved significance level of 0.05.

3. Results

3.1. TEG

We evaluated 100 VLBW infants between July 2015 and February 2017. We excluded 19 (7.7%) paired measurements due to technical procedural errors; a total of 228 duplicates of TEG traces (n = 456) were eligible for analysis. Table 1 summarizes the characteristics of the study population.

Paired samples *t*-tests performed on duplicates revealed slightly different results: mean R (95% CI) was 6.9 (6.4 to 7.4) vs 6.7 (6.2 to 7.2) (p = 0.047); mean K was 2.6 (2.3 to 2.9) vs 2.7 (2.3 to 3.0) (p = 0.573); mean MA was 60.8 (59.3 to 62.3) vs 61.5 (60.0 to 63.1) (p = 0.008).

As shown in Fig. 1A we observed a statistically significant