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Thromboelastometry measurements in severe and non-severe COVID-19 patients

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TARGET

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ABSTRACT

In patients with severe forms of COVID-19, thromboelastometry has been reported to display a hypercoagulant pattern. However, an algorithm to differentiate severe COVID-19 patients from nonsevere patients and healthy controls based on thromboelastometry parameters has not been developed. Forty-one patients over 18 years of age with positive qRT-PCR for SARS-CoV-2 were classified according to the severity of the disease: nonsevere (NS, n=20) or severe (S, n=21). A healthy control (HC, n=9) group was also examined. Blood samples from all participants were tested by extrinsic (EXTEM), intrinsic (INTEM), non-activated (NATEM) and functional assessment of fibrinogen (FIBTEM) assays of thromboelastometry. The thrombodynamic potential index (TPI) was also calculated. Severe COVID-19 patients exhibited a thromboelastometry profile with clear hypercoagulability, which was significantly different from the NS and HC groups. Nonsevere COVID-19 cases showed a trend to thrombotic pole. The NATEM test suggested that nonsevere and severe COVID-19 patients presented endogenous coagulation activation (reduced clotting time and clot formation time). TPI data were significantly different between the NS and S groups. The maximum clot firmness profile obtained by FIBTEM showed moderate/elevated accuracy to differentiate severe patients from NS and HC. A decision tree algorithm based on the FIBTEM-MCF profile was proposed to differentiate S from HC and NS. Thromboelastometric parameters are a useful tool to differentiate the coagulation profile of nonsevere and severe COVID-19 patients for therapeutic intervention purposes.

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KEYWORDS

thromboelastometry, COVID-19, NATEM

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GUIDELINES

1) ROTEMdelta2.8.0.01.EN_.mail_ [Internet]. 2016 [cited june of 2020]. Available from: https://www.rotem.de/wp-content/uploads/2016/08/ROTEMdelta2.8.0.01.EN_.mail_.pdf.

ROTEM-delta-US-Operating-Manual-Part-12(1).pdf

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ROTEM-delta-and-platelet_EN_2016_V01-1(3).pdf

3) Adamzik M, Eggmann M, Frey UH, Gorlinger K, Brocker-Preuss M, Marggraf G, et al. Comparison of thromboelastometry with procalcitonin, interleukin 6, and C-reactive protein as diagnostic tests for severe sepsis in critically ill adults. Crit Care. 2010;14(5):R178.

Adamzik-2010-Comparison of thromboelastometry.pdf

MATERIALS TEXT

Reagents and materials used in all tests.

Purchased from WERFEN Diagnostics Solutions for Life in Brazil.

1) star-tem 10: Batch 42280701

2) r ex-tem 10x10: Batch 42311401

3) in-tem: Batch 42340101

4) fib-tem 10x5: Batch 42331701

5) ROTROL N: Batch 42318601

6) ROTROL P: Batch L 42304601

7) Cup & Pin pro: Batch M19061

8) TIPTRAY Box eLine 10-320 µL: Batch PR155278

SAFETY WARNINGS

As dealing with COVID-19 infected blood samples, maintain during the complete measurement time, biological hazards protection devices: gloves, masks and aprons.

BEFORE STARTING

Check availability of blood sample
Check availability of reagents and actual temperature
Check availability of cups, pins, pipette tips and pipette

Inclusion criteria

1 COVID-19 patients treated during the pandemic in midwestern Brazil (Hospital Regional da Asa Norte and Hospital Universitário de Brasília, Brasilia, DF, Brazil (between August 1st and September 30th, 2020) were included. Confirmatory diagnosis was based on positive SARS-CoV-2 infection results in oropharyngeal swabs by quantitative real-time polymerase chain reaction (qRT-PCR).

Exclusion criteria

2
1. under 18 years old.
2. pregnancy.
3. thrombophilia or previous thromboembolic events.
4. previous use of anticoagulants.
5. previous use of antiplatelet drugs
6. surgical procedures in the last 4 weeks.
7. hereditary coagulopathies and
8. psychiatric diseases that impaired the understanding of the informed consent form.

Sample calculation

3 The sample size calculation was carried out based on a previous study of NATEM curves in septic patients and healthy controls (Adamzik, 2010).

The G*Power software version 3.1.9.6 was used:

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- Test family: t tests
- Statistical test: Means: Difference between two independent means (two groups)
- Type of power analysis: A priori: Compute required sample size - given alfa, power, and effect size
- Tail: Two
- alfa err prob: 0,05
- power (1-beta err prob): 0,95,
- effect size: d = 1.664101
- Determine:
  - n1=n2
  - Mean group 1: 229
  - Mean group 2: 259
  - SD group 1: 19
  - SD group 2: 17
- yielded a minimal sample of 11 patients in each group.
- No data was excluded.

Patient inclusion 8w

4 Patients with confirmed COVID-19 infection by SARS-CoV-2 PCR were offered participation in the study.
Participation in the study was not necessary for receving the proper disease treatment.
Those who decided to participate were informed about the study, the possibility to abandon it at any time and received all information for a proper Informed Consent, which was sent by electronic form.

Patient classification

5 Patients were classified according to diseae severity as follows:

Severe: patients needing hospitalization due to at least one of the following: dyspnea (respiratory rate >30 respiratory incursions per minute), SpO2 <93% in room air, PaO2/FiO2<300mmHg, admission to the intensive care unit or need for mechanical ventilation.

Non-severe: patients not necessitating hospitalization

Blood sampling

6 Citrated whole blood is collected of each research participant (enough to complete standard coagulation tests tubes:4 ml) and kept at room temperature.

When not possible to make measurements immediately, the samples must be preheated for 5–10 min before measurement in the sample preheating station of the ROTEM® delta.

Quality control

7 1) Quality control must be performed weekly, using standardized system controls ROTROL N and ROTROL P.

2) Daily maintenance: outer surface, cup holder, pipette and filter

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8

9 3) Weekly maintenance: replace pipette filter

10 4) Quarterly maintenance: device temperature (between 36oC and 38oC) and CCD chip (amplitude, centre and variance)

Instrument preparation

11 1) All reagents must be taken from refrigerator prior to use

12 2) The tests must be started only after target temperature are achieved

13 3) Take the cup with the pin in it from the storage box

14 4) Push the pin in the cup onto the axis chosen for the measurement.

15 5) Place the cup with its opening facing upwards into the appropriate preheated cupholder.

16 6) Place the cup holder onto the temperature-controlled work area again.

17 7) Push and fix the cup in the cup holder using the MC Rod.

EXTEM exam

18 1) Reagents: r ex-tem® and star-tem®

19 2) Pipetting steps are dysplayed on the screen

INTEM exam

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20 1) Reagents: in-tem® and star-tem®

21 2) Pipetting steps are dysplayed on the screen

FIBTEM exam

22 1) Reagents: r ex-tem, star-tem® and fib-tem®

23 2) Pipetting steps are dysplayed on the screen

NATEM exam

24 1) Reagents: star-tem®

25 2) Pipetting steps are dysplayed on the screen

Parameters analyzed

26 CT: clotting time, expressed in seconds
ALPHA: alpha angle, expressed in o
CFT: clot formation time, expressend in seconds
MCF: Maximum clot firmness, expressed in mm
ML: maximum lysis, expressed in %
TPI: thrombodynamic potential index: calculated as [(100 x MCF) / (100 - MCF)] / CFT

Statistics

27 1. GraphPad Prism version 8.0.0 for Windows software (GraphPad Software, San Diego, California USA, www.graphpad.com) was used for the descriptive statistical analysis.
A Normal distribution was tested with the Shapiro-Wilk test.
Categorical variables were described as absolute and relative frequencies and analyzed with the Chi-square or Fisher exact test.
Continuous variables were described as the mean ± SD.
Multiple comparisons among groups were performed using one-way ANOVA followed by Tukey's test for pairwise comparisons.
Two-tailed Student's t test was used for comparisons between nonsevere and severe patients.
In all cases, p values < 0.05 indicated statistical significance.

2. ROC curves were constructed using MedCalc software, Version 7.3.0.0 (Ostend, Belgium, URL https://www.medcalc.org/), to define the cutoff values and estimate the global accuracy based on the area under the ROC curve (AUC).
Performance indices expressed as percentages (sensitivity and specificity) were obtained for each thromboelastometric parameter in all ROTEM tests.
TG-ROC curves were assembled to confirm the selected cutoffs.

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3. Decision trees were built using WEKA software (Waikato Environment for Knowledge Analysis, version 3.6.11, University of Waikato, New Zealand, URL https://www.cs.waika to.ac.nz/ml/weka/) to classify COVID patients and healthy controls based on selected thromboelastometric parameters.
   Leave-one-out cross-validation (LOOCV) was applied to estimate the classification accuracy and test the generalizability of the model.

4. The graphics (bar diagrams, scatter charts and decision trees) were generated using Microsoft Office Package version 2012 and GraphPad Prism software, Version 8.0

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