Value of Rotational Thromboelastometry and Impedance Aggregometry for Evaluating Coagulation Disorders in Patients With Cyanotic and Nongenetic Congenital Heart Disease

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Adults with cyanotic congenital heart diseases (CCHD) have a higher risk for bleeding, but also for thrombosis. Rotational thromboelastometry (RT), using tissue factor (EXTEM), a contact activator (INTEM) or cytochalasin (FIBTEM), assesses coagulation by determining the time to initiation of clotting (CT) and clot firmness (MCF) including platelet-fibrin-interaction. The aim of this study was to evaluate RT and whole blood impedance aggregometry (IA) in CCHD compared with a control group without chronic cyanosis (NCCHD). These were used to establish normal reference ranges. We prospectively included 124 patients (76 CCHD, 48 NCCHD). Mean oxygen saturation in CCHD was 81.5%, and 98% in NCCHD ($p < 0.001$). Fifty-five CCHD and 1 NCCHD had pulmonary hypertension. Eisenmenger syndrome was present in 39 CCHD (51.3%). Hemoglobin, hematocrit, and reticulocyte levels were significantly higher in CCHD, and they also showed more thrombocytopenia. Platelet aggregation was under normal range in 89.5% of CCHD after triggering with ADP, in 85.5% after triggering with arachidonic acid (ASPI) and in 73.7% after TRAP-6. RT showed significantly longer clotting times and reduced clot firmness in both EXTEM and INTEM tests. FIBTEM-MCF was also significantly reduced. Moderate inverse correlation was found between platelet count and erythrocytes ($r = -0.608$, $p < 0.001$). Significant correlations were found between platelet number and RT-parameters as well as with all IA parameters. In conclusion, according to RT and IA, CCHD present hypocoagulable disorders. No signs of hypercoagulability were found. **(Am J Cardiol 2019;123:1696−1702)**

Cyanotic congenital heart disease (CCHD) is a complex, multiorganic disorder with several hemostatic abnormalities.[1] These include prolonged bleeding time, prolongation of prothrombin or partial thromboplastin time, deficiency in vitamin K-dependent clotting factors, and abnormal fibrinolysis.[2,3] Moreover, the number of platelets is reduced,[4] and also platelet aggregability[5] may be altered. As a consequence, these patients have a bleeding tendency, and the use of antiaggregants and anticoagulants may increase the risk for life-threatening hemorrhage. Rotational thromboelastometry (RT) is useful to assess coagulation by determining the time to initiation of clotting, the rate of clot formation and clot firmness including platelet-fibrin-interaction.[6] In contrast, platelet function can be assessed using whole blood impedance aggregometry (IA). Both techniques are useful to evaluate the antithrombotic effects of drugs.[7] The primary aim of our study was to determine global coagulation with RT and platelet function using IA in adults with CCHD compared with CHD adults without cyanosis (NCCHD), and second, to evaluate the correlation of platelet count and hematocrit, and platelet count and abnormal platelet function.

Methods

The study population was based on adults with CHD presented in the outpatient clinic of our center. All patients provided informed written consent. Exclusion criteria were <18 years, refusal to consent and lack of cognitive competency to understand the aim of the study. The ethic committee approved the design of the study. This study complies with the Declaration of Helsinki.

Clinical characteristics were registered. Blood tests included whole blood count, proteins, ferritin, and transferrin receptor saturation. Blood samples were obtained from the antecubital vein and analyzed with EDTA-anticoagulated blood on a Sysmex XE2100 analyzer.

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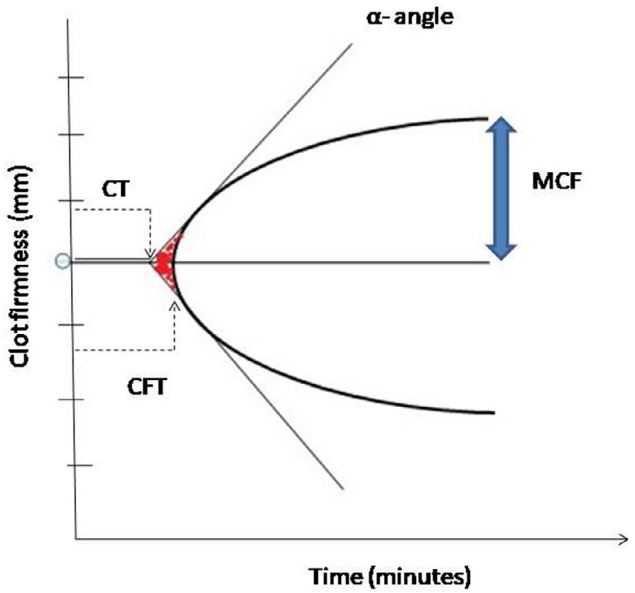

Figure 1. Diagram showing the different parameters of ROTEM. α-angle = alpha-angle; CFT = clot formation time; CT = clotting time; MCF = maximal clot firmness.

RT[6] was performed in citrated whole blood using the ROTEM system. The ROTEM system allowed rapid whole blood coagulation testing with different activators of coagulation additives reaching the maximal amplitude (or maximal clot firmness-MCF) typically after 15 to 20 minutes. In contrast to the classical thromboelastometry, the sample-containing cup is stationary and the pin is moving, fixed on a rotating shaft, which is guided by a ball bearing system. When a clot is formed inside the cup, it hinders the movement of the pin and the data are processed in real time by the software. Because of the method of activation and the different technique, the results are different than those of the classic thromboelastometry, but they correlate well. Parameters recorded after recalcification and activation with tissue factor (EXTEM), with partial thromboplastin phospholipid (INTEM) or with additional platelet inhibition by cytochalasin (FIBTEM) were: clotting time (CT) and maximum clot firmness (MCF) (Figure 1). FIBTEM also allowed a qualitative assessment of fibrinogen status.

Whole blood IA[8] was measured with the Multiplate system. Platelet aggregation was triggered using adenosine diphosphate (ADP), arachidonic acid (ASPI), and thrombin-receptor activating protein (TRAP-6). Platelet aggregation is recorded by the increase of impedance through attachment of platelets onto the sensors and is transformed to arbitrary aggregation units and plotted against time.

All statistical analyses were performed using SPSS v. 15.0 (SPSS Inc, Chicago, IL). Continuous variables were expressed by mean value ± SD. Mann-Whitney U test was used for nonparametric analyses. Otherwise Student's *t* test was used. Statistical significance was set at $p < 0.05$ with 2 tails. Categorical variables were compared using the chi-square test. Spearman's rank correlation was used when looking for correlation between quantitative variables.

Table 1
Clinical characteristics of patients with cyanotic congenital heart disease (CCHD) compared with noncyanotic patients (NCCHD)

	NCCHD (n = 48) [min; max]	CCHD (n = 76) [min; max]	p
Female (n)	25 (52%)	39 (51%)	n.s.
Age (years, median)	37 [22; 45]	34 [26; 40]	n.s.
Oxygen-saturation (%, median)	98 [97; 99]	82 [78; 85]	<0.001
Functional class (n):			<0.001
Perloff 1,2	46 (96%)	46 (60%)	
Perloff 3,4	2 (4%)	30 (39%)	
Eisenmenger (n)	0	39 (51%)	<0.001
Pulmonary hypertension (n)	1 (2%)	55 (72%)	<0.001
Previous intervention (n)	7 (14%)	15 (20%)	n.s.
Previous operation (n)	24 (50%)	44 (58%)	n.s.
Fontan operation (n)	0	8 (10%)	0.023
Total cavo-pulmonary connection (n)	0	3 (4%)	n.s.
Aspirin (n)	4 (8%)	4 (5%)	n.s.
Vitamin-K antagonists (n)	8 (17%)	20 (26%)	n.s.

Results

A total of 124 adults (76 CCHD patients and 48 NCCHD patients) were included. Clinical characteristics and general blood tests are shown in Tables 1 and 2, respectively.

IA showed significant lower platelet aggregability in the CCHD versus the NCCHD group. Platelet aggregation was below the lower limit of the reference range based on the results of healthy blood donors in 90% of CCHD after triggering with ADP (mean 359 ± 190 vs 682 ±

Table 2
Results of the hole blood analysis. Comparison between groups

	NCCHD (mean ± SD)	CCHD (mean ± SD)	p
Hemoglobin (g/dl)	14 ± 1.3	21 ± 2.8	<0.001
Erythrocytes (n × 10^{12}/L)	5 ± 0.5	6.7 ± 1	<0.001
Hematocrit (%)	40.4 ± 4.4	60.8 ± 8	<0.001
MCV (L/cell)	86.6 ± 4.4	91.5 ± 8.8	0.001
MCH (g/cell)	29.7 ± 1.9	31.6 ± 3.7	0.001
Thrombocytes (×10^9/L)	237.6 ± 78.8	133 ± 55	<0.001
Leucocytes (×10^9/L)	7.2 ± 1.9	6.8 ± 1.9	n.s.
Reticulocytes (%)	10.6 ± 4.5	16.2 ± 5.9	<0.001
INR (n)	1.3 ± 0.6	1.7 ± 0.9	0.01
Creatinine (mg/dl)	0.8 ± 0.2	0.9 ± 0.3	0.04
GOT (IU/L)	25 ± 7.1	28.9 ± 10.3	n.s.
GPT (IU/L)	25.2 ± 16.8	25.4 ± 12.6	n.s.
Uric acid (mg/dl)	5.5 ± 1.7	8.2 ± 2.8	<0.001
Proteins (g/dl)	7.2 ± 0.4	8.3 ± 8.4	n.s.
C reactive protein (mg/L)	4.2 ± 13.8	5.8 ± 7.6	n.s.
Ferritin (ng/ml)	137 ± 231	117 ± 128	n.s.
sTfR (mg/L)	3.4 ± 2.8	7.5 ± 4.9	<0.001
NT-proBNP (pg/ml)	565 ± 1323	1383 ± 2438	0.03

GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; INR = international normalized ratio; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; NT-proBNP = N-terminal pro-brain natriuretic peptide; sTfR = soluble transferrin receptor.

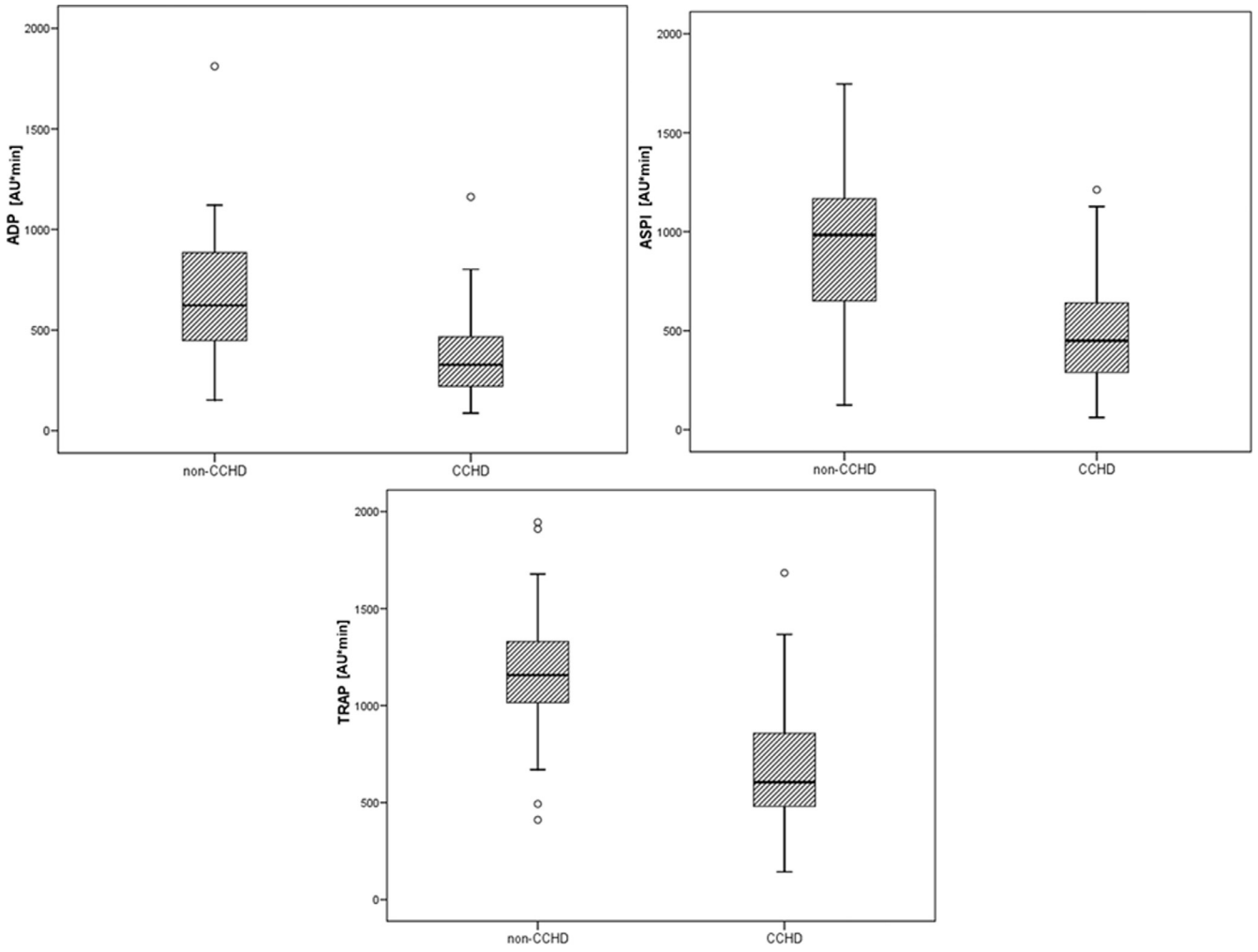

Figure 2. Comparison in mean IA values between CCHD and non-CCHD.

307AU*min, p <0.001), in 86% after ASPI (477 ± 240 vs 907 ± 348 AU*min, p <0.001), and in 74% after TRAP-6 (664 ± 293 vs 1169 ± 313 AU*min, p <0.001). NCCHD presented values under normal range in 39.6% with ADP test, 31% with ASPI test, and 13% with TRAP-6 test (Figure 2). Most patients taking acetylsalicylic acid (n = 8) showed low platelet aggregation with ASPI test (296 ± 257 AU*min).

RT showed significantly longer clotting times (EXTEM: 129 ± 68 vs 63 ± 24 s, p <0.001; INTEM: 241 ± 70 vs 194 ± 42 s, p >0.001) and reduced clot firmness (EXTEM: MCF 47 ± 9 vs 61 ± 7 mm, p <0.001; INTEM: 48 ± 9 vs 56 ± 8 mm; p <0.001) in the CCHD group compared with NCCHD. MCF in FIBTEM was also significantly reduced (7 ± 4 mm vs 15 ± 13 mm, p <0.001; Figure 3).

Moderate inverse correlation was found between platelet count and erythrocytes (r = −0.6, p <0.001). EXTEM-CT correlated positively with hematocrit (r = 0.5, p <0.001), but it correlated inversely with MCF (r = −0.5, p <0.001) correlated inversely. The same behavior was seen with INTEM parameters: a positive correlation with CT (r = 0.4, p <0.001) and an inverse correlation with MCF (p = −0.7, p <0.001). FIBTEM-MCF (r = −0.4, p <0.001) also correlated inversely (Figure 4). A significant inverse correlation was also found with all IA parameters: ADP (r = −0.4, p <0.001), ASPI (r = −0.6, p <0.001), and TRAP-6 (r = −0.5, p <0.001).

Platelet number also correlated positively with EXTEM alpha (r = 0.6, p <0.001) and MCF (r = 0.6, p <0.001), as well as with INTEM-MCF (r = 0.7, p <0.001). A significant inverse correlation was found between platelet count and EXTEM-CT (r = −0.5, p <0.001). An inverse, but not significant correlation was found between INTEM-CT and thrombocytes (r = −0.1, p = 0.3). A positive correlation was found with FIBTEM-MCF (r = 0.3, p = 0.006; Figure 5). A positive correlation was also found between thrombocytes and ADP test (r = 0.6, p <0.001), ASPI test (r = 0.7, p <0.001), and TRAP-6 test (r = 0.7, p <0.001).

Discussion

This study demonstrates hypocoagulable disorders using RT and IA in CCHD patients. Moreover, IA shows that not only is the number of platelets reduced,

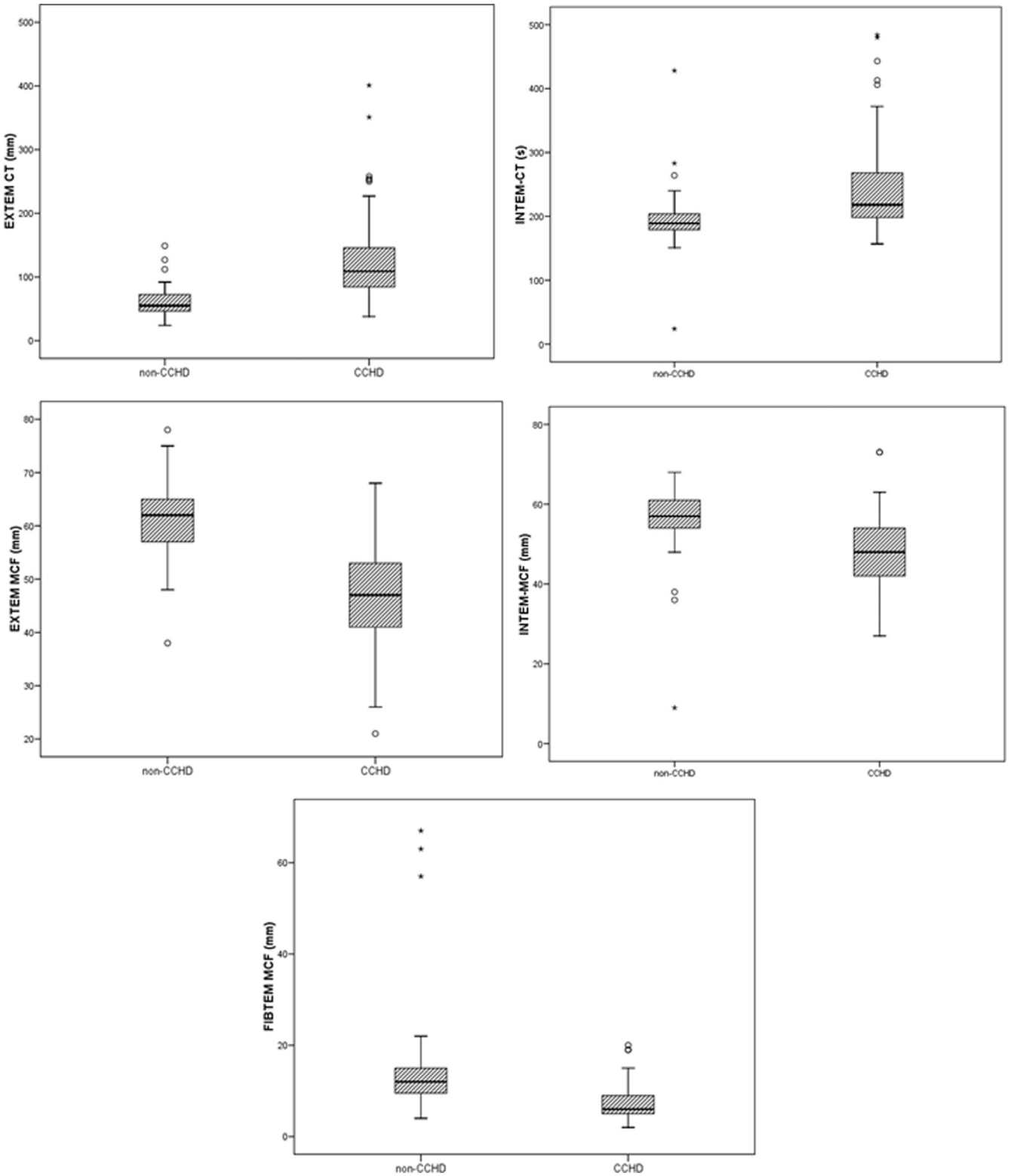

Figure 3. Comparison in mean RT values between CCHD and non-CCHD.

but also platelet function is impaired in this group of patients. All measurements correlate with platelet number and hematocrit. Finally, we demonstrate that platelet count and hematocrit correlate inversely as well.

Hemostatic abnormalities are among the most common consequences of CCHD in adults, are well known, and have been previously described.[3,5] Particularly the Eisenmenger syndrome has been associated with both bleeding

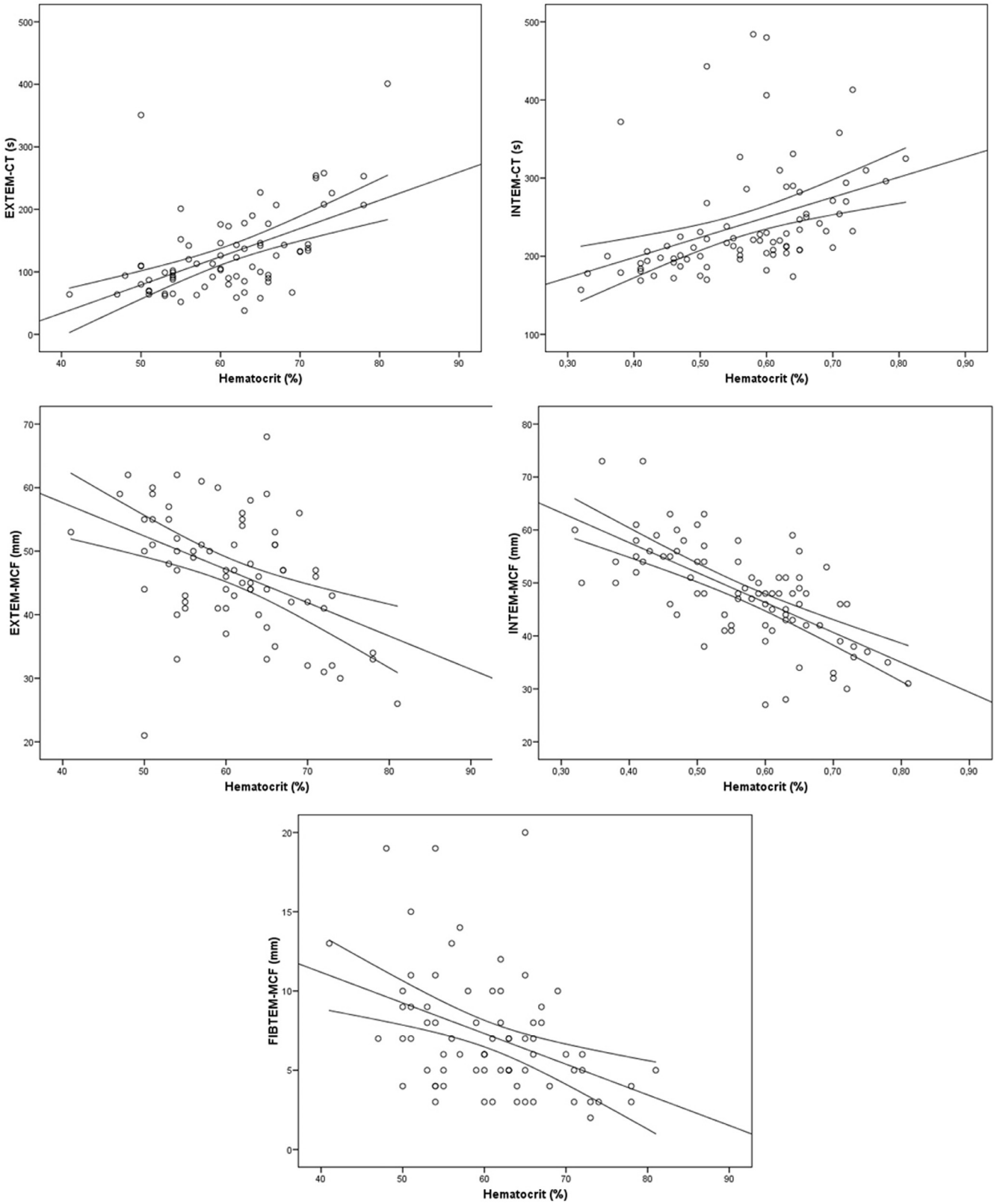

Figure 4. Correlation between RT parameters and hematocrit in CCHD. CT = clotting time; MCF = maximum clot firmness.

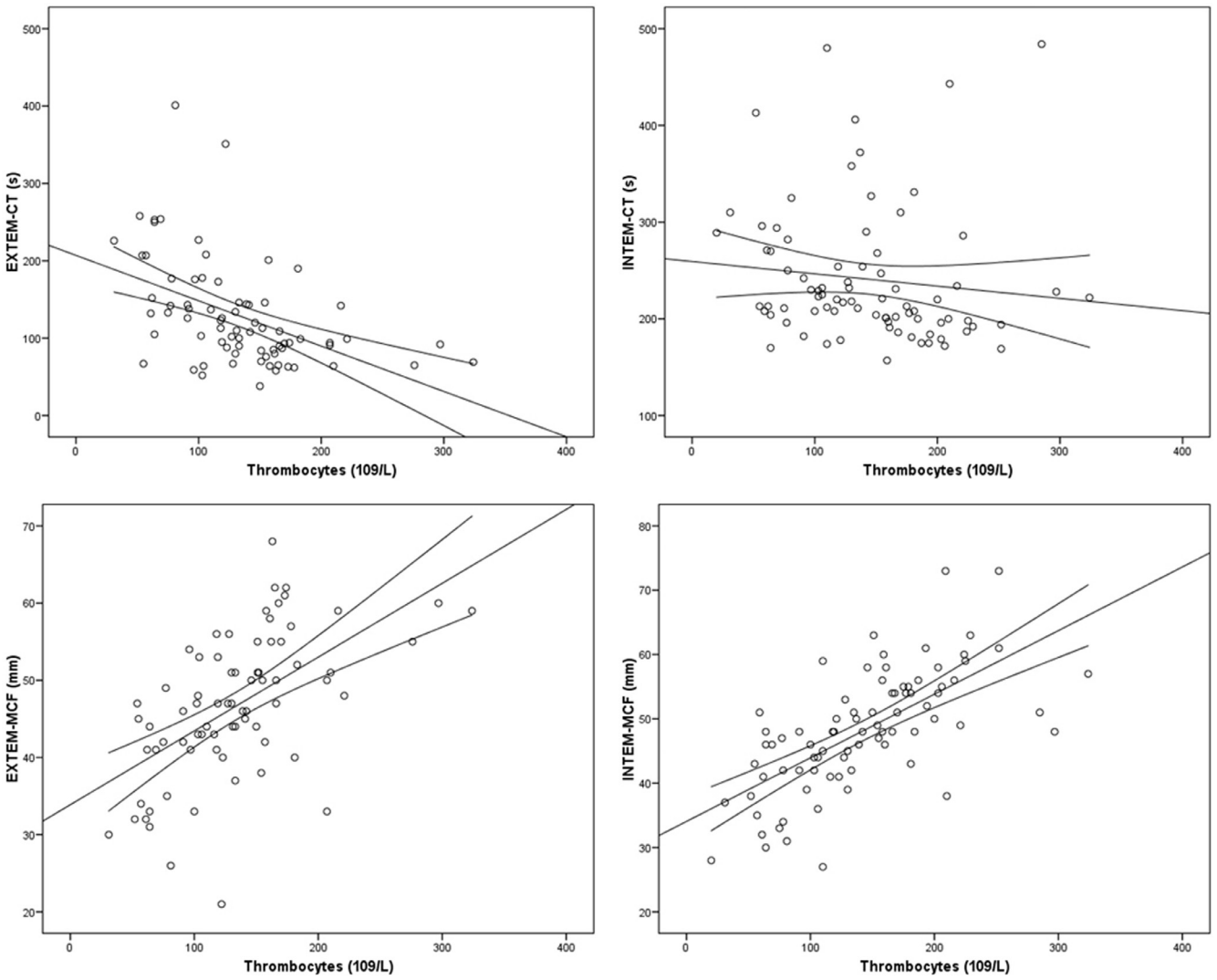

Figure 5. Correlation between RT parameters and platelet count in CCHD patients. CT = clotting time; MCF = maximum clot firmness.

and clotting tendencies.[9] In our study, both IA and RT showed that both CCHD and NCCHD present hypocoagulation, but CCHD have it more often and are at higher risk than NCCHD. Alexander et al already published that RT was impaired in infants with both CCHD and NCCHD compared with children without CHD.[10] Even though they demonstrated that clot formation, stability, and stabilization were impaired, they did not quantify platelet function. With the inclusion of the IA, we contributed in the study of platelet function. IA is also useful to predict coronary events in already revascularizated patients.[11] In our study, ADP, TRAP-6, and ASPI tests are significantly reduced in CCHD, hence manifesting a hypocoagulable disorder at platelet level.

We also showed a correlation between RT and hematocrit. These results were already described by Jensen et al[12] who showed a strong negative correlation between RT and hematocrit. After phlebotomy to reduce hematocrit, coagulation improves; inversely, iron substitution increases hematocrit and favors hypocoagulability.

In contrast, reduced platelet count can also increase bleeding risk. Patients with CCHD often have mild to moderate thrombocytopenia. The mechanisms for this thrombocytopenia might probably be multifactorial. First of all, the more erythroblasts are produced from stem cells in the bone marrow, the less megakaryocytes are formed, thus less platelets are found in the blood stream. Second, a disseminated intravascular coagulation due to an increase of blood viscosity might be present, which could substantially reduce platelet number.[1] This hypothesis is supported by an increased production of platelet-derived microparticles in blood, originated by an increased shear stress caused by erythrocytosis.[13] Finally, because platelets are also formed in the pulmonary bed, the presence of a right-to-left shunt could reduce the number of megakaryocytes and substantially reduce platelet number.[4]

Our results did not show signs of hypercoagulability. However, it is known that CCHD have a higher risk for thromboembolism, especially in cerebral and pulmonary vascular bed.[9,14] The prevalence of such events rises up to 30% to 40% of patients, and in many cases occurs without clinical manifestations.[14] The risk of thrombosis is supposed to be multifactorial. The combination of secondary

erythrocytosis and iron deficiency in CCHD, which originate rigidity and lack of deformability of the erythrocytes at capillary level,[1] increases blood viscosity and helps thrombus formation. Our study shows that CCHD patients have significant higher hematocrit levels and reticulocyte counts. Even though MCV and MCH are not reduced, the high-soluble transferrin receptor levels point toward iron deficiency in this group. This result agrees with previous publications, in which it was highlighted that iron deficiency in CCHD patients do not have to be associated with hypochromia and microcytosis.[15]

In our study, platelet count inversely correlates with hematocrit ($r = -0.713$, $p < 0.001$). This finding has already been described in previous publications[4,12] but, compared with them, this article provides more information about platelet function. IA shows low ADP, TRAP-6, and ASPI values, which are concordant with an impaired platelet function and a hypocoagulable state. We have found a moderate correlation between these tests and platelet count. However, platelet aggregation measured by IA is substantially influenced by platelet count, especially out of the range between $150 \times 10^9\ L^{-1}$ and $600 \times 10^9\ L^{-1}$[16]. Even though thrombocytopenia was not very pronounced in our study group, it could probably be enough to impair aggregation.

Last but not least, a recent publication of Haas et al[17] reflected that underfilling of blood tubes in healthy donors has an impact in clot firmness, but it does not influence CFT or CT. The differences between groups were rather small, so that they probably have a mild clinical impact. Unluckily, this data is missing in our study. Furthermore, we do not have data about filling tubes in chronic cyanosis.

In conclusion, RT and IA analysis facilitates measurement of coagulation and platelet function in CCHD. Both methods show a hypocoagulable state in this group. No tendency for hypercoagulability has been found with any of the techniques applied. All parameters correlate inversely with hematocrit and directly with platelet count.

Disclosures

The authors have no conflicts of interest to disclose.

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