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HEMATOLOGY: BRIEF REPORT

Are children with SARS-CoV-2 infection at high risk for thrombosis? Viscoelastic testing and coagulation profiles in a case series of pediatric patients

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Abstract

The coagulopathy of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is well documented in adults, with increases in D-dimer and prothrombin time found to be strong predictors of mortality, and anticoagulation shown to decrease this mortality. Viscoelastic parameters such as elevations in maximum clot firmness (MCF) on rotational thromboelastometry (ROTEM) have correlated with a hypercoagulable state in adults with SARS-CoV-2. We report our experience in children infected with SARS-CoV-2, with noted elevations in D-dimer and MCF on ROTEM (indicating hypercoagulability). Exploration of viscoelastic testing to provide additional laboratory-based evidence for pediatric-specific risk assessment for thromboprophylaxis in SARS-CoV-2 is warranted.

KEYWORDS
coagulation, COVID-19, rotational thromboelastometry, ROTEM, SARS-CoV-2, thrombosis, viscoelastic testing

1 | INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was found to induce an increased incidence of thrombosis[1,2] and strokes[3] in adults. They developed a recognizable coagulopathy, characterized by increased thrombin generation,[4] decreased fibrinolysis, elevated D-dimers, and a prolonged prothrombin time (PT), which was found to be a strong predictor of mortality, with pulmonary microthrombi contributing significantly.[5] Anticoagulation has been shown to decrease this mortality.[6]

Rannucci et al[7] reported viscoelastic testing (using the Quantra Hemostasis analyzer system) in SARS-CoV-2-infected adults admitted to the intensive care unit (ICU), demonstrating increased clot strength, fibrinogen and D-dimer, with significant time-related decrease in these parameters with escalating thromboprophylaxis. Spieza et al[8] used another viscoelastic tool, rotational thromboelastometry (ROTEM), also showing elevated fibrinogen and D-dimer, along with increased maximum clot firmness (MCF) in all ROTEM parameters in patients compared to controls.

Children develop thromboembolic complications in the face of catheter, anatomical and disease-related predisposing factors. However, the prevalence of thromboembolic complications in children with SARS-CoV-2 infection has not been well documented, and there are no pediatric-specific thromboprophylaxis guidelines. Major hematology organizations, including the American Society of Hematology[9] and the International Society of Thrombosis and Haemostasis,[10] have published recommendations for anticoagulation of hospitalized symptomatic adults with SARS-CoV-2, which have been largely extrapolated to the pediatric population. A recent report by Loi et al[11] has made

Abbreviations: CFT, clot formation time; CT, Clotting Time; ICU, intensive care unit; MA, maximal amplitude; MCF, maximum clot firmness; PT, prothrombin time; ROTEM, rotational thromboelastometry; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

TABLE 1 Demographics of pediatric patients admitted with SARS-CoV-2 infection

Patient	1	2	3	4	5	6	7	8	Mean (range) or % positive/abnormal
Age (years)	14	20	2	9	16	17	17	8	12.9 (2-20)
Sex	F	F	M	M	F	M	F	M	F:M 50:50
Race	Black	Black	Other	Other	Black	n/a	Other	Asian	–
BMI (kg/m^2)	21.9	20.4	13.3	**25.1**	20.1	**27.6**	**31.9**	14.9	21.9 (13.3-31.9)
PMH	CP, Seizures, CLD	HbSBeta^{0}Thal	None	None	Asthma	None	MS, ADHD	None	50%
History of thrombosis	Yes	No	No	No	No	No	No	No	13%
Oxygen	**Yes**	No	No	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	75%
PICU	**Yes**	No	No	No	Yes	**Yes**	**Yes**	**Yes**	63%
Central line (days)	None	None	None	None	RFV (5)	None	None	LIJ (7)	25%
Length of Stay (days)	16	9	6	8	10	11	25	14	12.4 (6-25)
Enoxaparin	Tx	None	None	Ppx	Tx	Tx	Tx	Tx	75%

Abbreviations: ADHD, attention deficit hyperactivity disorder; CLD, chronic lung disease; CP, cerebral palsy; F, female; HbSBeta^{0}Thal, hemoglobin S beta-zero thalassemia; LIJ, left internal jugular vein; M, male; MS = multiple sclerosis; n/a, not available; PICU = pediatric intensive care unit; PMH = past medical history; Ppx = enoxaparin prophylactic dosing (0.5 mg/kg/dose subcutaneously every 12 h); RFV, right femoral vein; Tx = enoxaparin treatment dosing (1 mg/kg/dose subcutaneously every 12 h).
Parameters outside range for age are in bold.

some diagnostic and therapeutic anticoagulation recommendations in children with SARS-CoV-2 based on risk stratification of a single institutional experience. However, there remains a need for laboratory-based risk-assessment tools to guide clinical decision making on the use of anticoagulant prophylaxis in this population.

In an effort to explore the utility of viscoelastic testing, we added ROTEM to routine coagulation testing in children admitted with SARS-CoV-2. The objective was to determine if standard coagulation tests and ROTEM testing could be obtained in these children to assess its feasibility in determining thrombosis risk; if so, were changes in clot strength in children during an acute SARS-CoV-2 infection comparable to that seen in adults. We report our experience in this retrospective case series of eight children with SARS-CoV-2 infection.

2 | METHODS

Data were collected retrospectively for patients younger than 21 years of age admitted to Cohen Children's Medical Center between April 13 and April 29, 2020, with varying illness severity associated with SARS-CoV-2 infection documented by a positive polymerase chain reaction test. Patients were excluded for known coagulopathy or chronic anticoagulation. In addition to clinical demographics, laboratory data including baseline coagulation and inflammatory markers along with results of ROTEM analysis (ROTEM delta, Instrumentation Laboratory–Werfen, Barcelona, Spain) were collected. Whole blood for ROTEM analyses was obtained within 1-4 days of admission and included EXTEM (evaluation of the extrinsic pathway), INTEM (intrinsic pathway), FIBTEM (fibrinogen activity), and APTEM (fibrinolysis) as previously described.[12] The following ROTEM parameters were analyzed: (1) clotting time (CT): corresponding to the initiation phase of the clotting process; (2) clot formation time (CFT): propagation phase of clot formation; (3) MCF: maximum amplitude in millimeters.

Means and ranges were calculated as appropriate for normalcy of data. Comparisons were performed with Spearman's correlation and Mann-Whitney testing using SPSS Statistics (Version 1.0.0.1347, Armonk, NY). The Northwell Health Institutional Review Board approved this case series as minimal-risk research using data collected for routine clinical practice and waived the requirement for informed consent.

3 | RESULTS

The demographics and laboratory values of eight hospitalized children diagnosed with SARS-CoV-2 infection are shown in Table 1. Average age was 12.9 years, with equal sex distribution and 38% were overweight or obese (BMI ≥25 kg/m^2 or ≥30 kg/m^2, respectively). Seventy-five percent required oxygen supplementation, 63% required ICU admission, and 25% had central lines, which remained in place for an average of 6 days. Prophylactic enoxaparin (0.5 mg/kg/dose subcutaneously every 12 h) was initiated in patients based on institutional adult guidelines of oxygen requirement and elevated D-dimer levels. It was escalated to therapeutic enoxaparin (1 mg/kg/dose subcutaneously every 12 h) in 63 % of patients for clinical deterioration. There were no observed bleeding events, thromboembolic complications, or deaths.

Abnormal laboratory data included lymphopenia (37.5%), mild thrombocytopenia (13%), prolonged PT (50%), elevated ferritin (37%) and C-reactive protein (88%). Elevations in D-dimer levels (75%) and fibrinogen (88%) were observed on the day ROTEM was drawn for analyses.

TABLE 2 Laboratory data of pediatric patients admitted with SARS-CoV-2 infection

Lab results		Patient	1	2	3	4	5	6	7	8	Mean (range) and % positive/abnormal
Admission labs		**WBC** (K/μL)	4.97	**14.5**	**21.2**	6.54	7.02	9.94	5.23	**18.77**	11 (4.97-21.2), 38% leukocytosis
		ALC (μL)	1100	1900	5110	**580**	**490**	1680	**710**	1630	1650 (490-5110), 38% lymphopenia
		Hb (g/dL)	12.3	**8**	**9.3**	12	13	14	11.6	11.1	11.4 (8-14), 40% anemia
		Plt (K/μL)	166	**422**	**446**	168	**104**	281	292	186	258 (104-446), 25% thrombocytosis, 13% thrombocytopenia
		PT (s)	12.6	**14.2**	**13.7**	12.4	n/a	n/a	12.1	n/a	13 (12.1-14.2), 40% prolonged PT
		PTT (s)	**29.9**	**25**	34.5	34.8	n/a	n/a	32.5	n/a	31.3, 40% shortened PTT
		LDH (U/L)	**294**	**522**	**250**	**301**	n/a	**285**	**564**	**482**	384 (250-564), 88% elevated
		Ferritin (ng/mL)	61.5	**458.3**	160.2	**569.2**	**1147**	133.3	**563.7**	**4739**	979 (61.5-4739), 63% elevated
		Creatinine (mg/dL)	0.24	0.62	0.24	0.42	2.15	0.78	0.66	0.64	0.72 (0.24-2.41), 13% elevated
		AST (U/L)	35	40	25	**43**	**86**	**91**	30	**126**	59.5 (25-126), 50% elevated
		ALT (U/L)	22	25	8	29	**227**	19	13	**63**	50.6 (8-227), 25% elevated
		Bilirubin (mg/dL)	0.2	**4.2**	0.3	0.4	0.5	0.6	0.2	0.4	0.85 (0.2-4.2), 13% elevated
		Albumin (g/dL)	3.4	5.2	4	3.9	**2.4**	4.9	3.5	**2.6**	3.74 (2.4-4.9), 25% reduced
		Procalc (ng/mL)	0.05	**0.2**	n/a	**2.41**	n/a	0.05	n/a	**35.23**	7.59 (0.05-35.23)
		CRP (mg/L)	**16.3**	**51.8**	**30**	**130**	**206**	<4	**88.9**	**77**	85.7 (4-130), 88% elevated
ROTEM (>/< = upper/ lower limit of normal for age)	**Labs**	**Fibrinogen** (mg/dL)	**536**	**701**	**732**	329	**619**	**383**	**589**	**427**	540 (329-732), 88% elevated
		D-dimer (ng/mL)	151	**1281**	**497**	**1033**	**2451**	342	**630**	**1075**	932 (151-2451), 75% elevated
	EXTEM	**CT** (s)	57	70	68	76	105	52	59	64	25% elevated
		CFT (s)	75	55	43	77	90	103	63	**47 (<49)**	13% decreased
		A10/A20 (mm)	56	**76 (>70)**	**74 (>68)**	53	56	63	70	64	25% elevated
		MCF (mm)	64	**76 (>70)**	**79 (>70)**	60	57	64	**72 (>70)**	**69 (>68)**	50% elevated
	INTEM	**CT** (s)	143	**96 (<122)**	188	175	213	152	153	169	13% decreased
		CFT (s)	60	47	52	70	88	77	60	**39 (<48)**	13% decreased
		A10/A20 (mm)	58	**76 (>72)**	**71 (>68)**	53	54	62	69	65	25% elevated
		MCF (mm)	62	**76 (>72)**	**76 (>73)**	60	54	63	69	**70 (>69)**	38% elevated
	FIBTEM	**A10/20** (mm)	**27 (>22)**	**40 (>24)**	**41 (>22)**	**28 (>21)**	24	17	**39 (>24)**	**26 (>21)**	75% elevated
		MCF (mm)	**29 (>24)**	**40 (>24)**	**44 (>23)**	**30 (>22)**	24	17	**39 (>24)**	**29 (>22)**	75% elevated

Abbreviations: A10/20, amplitude at 10 or 20 minutes; ALC, absolute lymphocyte count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CFT, clot formation time; CRP, C-reactive protein; CT, clotting time; Hb, hemoglobin; LDH, lactate dehydrogenase; MCF, maximal clot firmness; Plt, platelet count; Procal, procalcitonin; PT, prothrombin time; PTT, partial thromboplastin time. ROTEM, rotational thromboelastometry machine; WBC, white blood cells.

Parameters outside range for age are in bold.

ROTEM analysis (Table 2) showed a predominance of hypercoagulable profiles with elevated EXTEM MCF (50%), elevated INTEM MCF (38%), and both elevated FIBTEM A10/20 and elevated FIBTEM MCF (in 75% of patients) comparable to adults. Averages and ranges cannot be reported due to variability of age-based reference ranges in pediatrics.[12] There was no statistically significant correlation between fibrinogen and EXTEM MCF ($P = .116$), INTEM MCF ($P = .232$), or FIBTEM MCF($P = .130$) nor was there correlation of D-dimer levels with these parameters [D-dimer and EXTEM MCF ($P = .949$), INTEM MCF ($P = .731$), or FIBTEM MCF ($P = 0.748$)]. However, we observed that all patients admitted to the ICU had a three- to 10-fold elevation in D-dimer levels, a two-fold elevation in fibrinogen levels, and 80% had elevated FIBTEM parameters.

4 | DISCUSSION

Most revealing from this study, we observed increased evidence of clot strength in ROTEM parameters of EXTEM MCF and FIBTEM MCF (increased clot firmness with contribution from fibrinogen) in children with SARS-CoV-2 infection, similar to that reported by Rannucci[7] and Spieza.[8] This paralleled a report by Mortus et al[13] who noted on thromboelastography (TEG), another viscoelastic tool, a significantly elevated maximal amplitude (MA, comparable to MCF in ROTEM) in all patients with two or more thrombotic events. Panigada et al[14] also noted shorter *R* and *K* values (comparable to CT and CFT, respectively) and higher MA values in patients with SARS-CoV-2 infection similar to our pediatric cohort (see Figure S1 for a temogram of patient #8).

In addition to increased hypercoagulability, Nougier et al[4] demonstrated significantly increased thrombin generation in SARS-CoV-2 patients admitted to the ICU versus non-ICU patients, along with higher tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), and thrombin activatable fibrinolysis inhibitor levels. It is hypothesized that local tPA is overwhelmed by high PAI-1 levels, tipping the balance toward hypercoagulability. There is limited availability of measuring thrombin generation at institutions, but the study team used a modified ROTEM parameter that correlated with thrombin generation, and could be more accessible to clinicians and researchers.

We further noted that in the early stages of infection, children under age 21 had elevated fibrinogen, D-dimer and CRP (all suggestive of a highly inflammatory state), in addition to lymphopenia and prolonged PT (similar to those observed in adults). However, our pediatric cohort did not develop symptomatic thromboembolic events or increased mortality, despite demonstration of a comparable hypercoagulable state.

Although our patient population was heterogeneous with respect to clinical course and level of coagulopathy, our small sample size precludes a demonstrable correlation between fibrinogen, D-dimers or viscoelastic testing or its predictive value in assigning risk for thrombosis on prophylactic anticoagulation in children. However, we demonstrated that ROTEM testing is feasible and recommend that its utility in determining the hypercoagulable state merits further study in children, who we and others, have shown can exhibit clinical severity and laboratory evidence of a coagulopathy identical to that seen in adults with SARS-CoV-2.

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AUTHOR CONTRIBUTIONS

M. Al-Ghafry performed research, analyzed data, and wrote the paper; B. Aygun, A. Appiah-Kubi, and A. Vlachos analyzed and interpreted data; G. Ostovar, C. Capone, T. Sweberg, N. Palumbo, and P. Goenka took care of patients; L.C. Wolfe and J.M. Lipton interpreted data and revised the paper; S.S. Acharya designed research, interpreted data, and wrote the paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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