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Coinfection with Staphylococcus Aureus Increases Risk of Severe Coagulopathy in Critically Ill Children with Influenza A (H1N1) Virus Infection

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Abstract

Objective—H1N1 influenza with coinfections has been implicated to have high morbidity and mortality. We hypothesized that critically ill children with 2009 H1N1 and coinfections are at a higher risk for developing disseminated intravascular coagulation (DIC).

Design—The chart review included demographics, length-of-stay (LOS), severity of illness score (PRISM III), clinical labs, and outcomes at hospital day 90 (D-90) data. Patients were classified as having methicillin-sensitive or -resistant Staphylococcus aureus (MSSA/MRSA), other, or no coinfections.

Patients—66 consecutive patients with 2009 H1N1 and influenza A infection

Setting—Single-center Pediatric Intensive Care Unit

Main Results—There were 12, 22, and 32 patients with MSSA/MRSA, other, and no coinfections respectively. PICU LOS was 11, 10, and 5.5 days (median), and survival at D-90 was 83, 96, and 91 % in patients with MSSA/MRSA, other, and no coinfections. Patients with MSSA/MRSA coinfections compared to patients with other, and no coinfections had higher PRISM III scores (14 [6–25], vs 7 [2–10], $p = 0.052$ and 6 [2.5–10] $p = 0.008$, (Median [Interquartile Range])), higher D-dimer (16.1 [7.9–19.3], vs 1.6 [1.1–4], $p = 0.02$ and 2.3 [0.8–8.7] mcg/ml, $p = 0.05$), longer prothrombin time (19.3 [15.4–25.9] vs 15.3 [14.8–17.1], $p = 0.04$ and 16.6 [14.7–20.4] seconds, p<0.39) on admission, and lower day-7 platelet counts (90K [26–161K] vs 277K [98–314], $p = 0.03$ and 256K [152–339]/mm3, p<0.07). Patients with MSSA/MRSA coinfections compared to patients without coinfections were more likely to be sicker with PRISM III score >10 vs <10 (RR 2.4; CI 1.2–4.7, p=0.035) (Relative Risk; 95% Confidence Interval), and have overt DIC (RR 4.4; CI 1.3–15.8, p=0.025)

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Conclusions—During the 2009–2010 H1N1 pandemic, pediatric patients with influenza A and MSSA/MRSA coinfections were sicker and more likely to develop DIC than patients with other or no coinfections.

Keywords

H1N1; influenza A; disseminated intravascular coagulation; DIC; coinfection; Staphylococcal aureus

INTRODUCTION

In April 2009, influenza A(H1N1) virus was first detected in the United States. The US Influenza Surveillance System noted two peaks nationwide between April, 2009 and March, 2010 [1]. During this period, there were 338 pediatric deaths from influenza, of which 83% (282 cases) were confirmed H1N1. This mortality was higher than the 153 pediatric deaths from seasonal influenza during 2003–2004. [2]

An adult study reported that those with pandemic H1N1 had higher rates of extrapulmonary complications, intensive care unit (ICU) admissions and/or death compared to those with seasonal influenza [3]. In this study, bacterial superinfection was associated with an increased risk of ICU admission and/or death. Pediatric studies reported that 7% of the pediatric deaths from 2003–2004 seasonal influenza had coinfections with Staphylococcus aureus (*S aureus*) [2]. Bacterial superinfection also occurred frequently (23%) in pediatric patients with H1N1 [4]. Our parent study reported that children with 2009 pandemic H1N1 and methicillin-resistant *S aureus* (MRSA) pulmonary coinfection had a higher risk of death than those without MRSA. Additionally, thrombocytopenia at admission was associated with an increased risk of death [5].

Despite published data regarding the epidemiology of H1N1 infection [1, 2], there are limited data on the mechanisms of increased mortality [2]. Data from our parent study suggest a role of both secondary bacterial infections and coagulopathy in H1N1-associated mortality [5]. Disseminated intravascular coagulation (DIC) occurs in 50% of pediatric patients with severe sepsis and is associated with high morbidity and mortality [6–9]. The purpose of this study was to evaluate the association between H1N1 pandemic influenza A infection, bacterial coinfections and DIC.

METHODS

Study Design

This single site data collection was a sub-study of a one-year multicenter observational cohort study. The parent study [5] was initiated as a collaborative effort among the U.S. National Institutes of Health, Centers for Disease Control and Prevention (CDC), the Office of the Assistant Secretary for Preparedness and Response, U.S. Department of Health and Human Services and the Pediatric Acute Lung Injury and Sepsis Investigators network to provide a rapid clinical knowledge-base of 2009 H1N1-associated critical illness in children. Data coordination and management was provided by the National Heart, Lung, and Blood Institute's ARDSNet coordinating center. We reviewed all PICU admissions for patients with any positive influenza A test result from April 15th through August 31st, 2009 and any positive influenza test or suspected case of influenza from September 1, 2009 through April 15th, 2010. Since the Institutional Review Board (IRB) approval was received November 20, 2009, data for two thirds of patients were collected retrospectively.

Data Collection

Following the parent study closure, we extracted our site's data from the multicenter database and, under a separate IRB protocol, supplemented these with coagulation parameters not collected in the parent data set. We thought that collecting data on days 1, 3 and 7 would give us a good clinical picture of whether or not the infections would trigger a severe coagulopathy as others have reported that 75% of critically ill children progressed to the highest DIC score within 6 hours of PICU admission [9].

Baseline demographic information, duration of illness, presenting symptoms, initial PICU admission findings, selected clinical events and outcomes were recorded in a REDCap [10] web-based electronic case report form. Data were collected on pathogens identified from blood and respiratory secretions throughout the PICU hospitalization. Mortality was defined as death occurring anytime during the PICU stay and up to hospital day 90 for patients transferred to the ward.

Inclusion/Exclusion

Criteria Inclusion Criteria: 1) Confirmed (defined as a person with an acute illness admitted to an ICU with laboratory confirmed influenza A or B virus infection) or suspected influenza infection (defined as a person admitted to the ICU without a positive influenza test but where the clinical team's suspicion for influenza was enough to treat empirically with anti-virals for influenza for the lesser of 5 days or until death). If another diagnosis was found to explain the patient's acute illness (e.g. RSV) then the person was NOT considered a suspected case for this registry); 2) Only patients with community-acquired infections, documented by positive viral or bacterial cultures sampled ≤ 72 hours of admission from a sterile site (respiratory secretions or blood) were eligible for inclusion in the study; 3) Admission to an ICU at a participating site.

Exclusion Criteria: Influenza-like illness due to non-influenza disease and negative testing for influenza.

Definitions

Patients were categorized as previously healthy if they were healthy prior to the index illness, without underlying medical conditions and not dependent on any medications or medical devices. Pediatric Risk of Mortality III acute physiology score (PRISM III) [11] was used to measure severity of illness within the first 24 hours of admission.

Shock requiring inotropes/vasopressors was defined as dopamine infusion >5 mcg/kg/min or any epinephrine, or norepinephrine infusion to maintain adequate blood pressure [12].

A clinically relevant bacterial coinfection was defined as 1) a clinical diagnosis of bacterial pneumonia for which antibiotics were started, 2) evidence of bacterial superinfection within 72 hours of the initial PICU admission from a sterile site (respiratory secretions or blood), and 3) a bacterial pathogen identified in their respiratory secretions.

A confirmed case of 2009 H1N1 was defined as a respiratory specimen that tested positive for 2009 H1N1 virus infection by real-time polymerase chain reaction (RT-PCR) testing using primers specific for 2009 H1N1 virus or viral culture. A patient with influenza A was defined as a PICU patient with a respiratory specimen that tested positive for influenza A virus infection by any influenza testing without further identification of subtype. These patients were possibly also H1N1, as early testing by PCR was found to be inaccurate as the primers used were not specific for H1N1.

Oseltamivir-regular dose was defined by the 2009 CDC recommendations [13], as follows: <12 months: 3 mg/kg/dose twice daily; ≤15 kg: 30 mg twice daily; >15 kg to ≤23 kg: 45 mg twice daily; >23 kg to ≤40 kg: 60 mg twice daily; >40 kg: 75 mg twice daily. Oseltamivir-high dose was defined as twice the regular dose.

Disseminated Intravascular Coagulation (DIC)

DIC was defined according to the Scientific Subcommittee on DIC of the International Society on Thrombosis and Haemostasis (ISTH) [14]. Coagulation tests were scored as follows: Platelet count: >100,000/m^3 = 0; ≤100,000/mm^3 = 2; D-Dimer: no increase (<0.4 mcg/ml of Fibrinogen Equivalent Unit [FEU]) = 0, moderate increase (0.4–4.0 mcg/ml FEU) = 2; strong increase (≥4 mcg/ml FEU) = 3; prothrombin time (PT): <18 seconds = 0, >18 seconds by <24 seconds = 1, if >24 seconds = 2; fibrinogen level: ≥1.0g/L = 0, <1.0g/L = 1. If the score was ≥5, it was considered overt DIC. If the score was <5, it was suggestive (not affirmative) for non-overt DIC.

Statistics

Patients were classified into three groups based on their initial coinfection results. Patients with methicillin-sensitive or -resistant *S aureus* (MSSA/MRSA) and other coinfections were compared to patients without coinfections. Normally distributed continuous variables were compared using paired and un-paired t-test and ANOVA and reported as mean±SD. Non-normally distributed variables were compared by Mann-Whitney *U*-test or Kruskal-Wallis test, reported as median with interquartile range (IQR). Categorical variables were compared using the Chi^2 test or Fisher's exact test. Relative risk, with 95% confidence intervals (CI) was calculated by Fisher Exact Test for a 2 x 2 contingency table. Statistical significance was defined as $P < 0.05$. Statistical analysis was performed with Statview and JMP (SAS Institute, Inc, Cary, NC).

RESULTS

We enrolled 66 patients (59% male; age 5.9 ± 5.4 yr [mean + standard deviation]) (Table 1). Sixty-four percent of patients were PCR-confirmed H1N1 and 36% of patients were influenza A positive (not-sub-typed by PCR, rapid or culture). Fifty-one percent of the patients had bacterial coinfections. Eighteen percent, 33 %, and 48 % of the patients had MSSA/MRSA, other pathogens, and no coinfections respectively. Fifty-five percent of patients had comorbidities.

The bacterial coinfections identified were *S aureus* in 35%, Pseudomonas aeruginosa in 18%, Moraxella catarrhalis in 18%, Haemophilus influenza (non-typable) in 9%, Streptococcus pneumonia in 6%, Group A Streptococcus in 6%, and Serratia marcescens in 9% (Table 2).

Fifty-nine percent and 20% of patients were treated with high and regular dose oseltamir, respectively. Two patients did not receive any antivirals. The remaining patients were treated with amantadine, peramivir, ribavirin and/or zanamivir. Of the patients with MSSA/MRSA, one patient received regular dose oseltamir, 6 received high dose oseltamir and 5 received other antivirals. None of the patients with MSSA/MRSA coinfections received steroids on admission, whereas 19% of patients without coinfections received steroids.

PRISM III scores were significantly higher in patients with MSSA/MRSA than patients with no coinfections (p <0.05;Mann-Whitney) (Table 3). Although patients with MSSA/MRSA had longer length-of-stay than patients with other and no coinfections, this did not reach statistical significance. Need for ECMO or inotropes/vasopressors or survival was not significantly different between groups. Three patients (two with MSSA/MRSA and one with

no coinfections) were on ECMO. Survival at d-90 was 83.3%, 95.5% and 90.6% in patients with MSSA/MRSA, other and no coinfections ($p = 0.51$).

International Normalized Ratio (INR), D-Dimer and PT were significantly higher on day 1 and day 3 in patients with MSSA/MRSA than in patients with other coinfections respectively ($p < 0.05$;Mann-Whitney) (Table 4). There was no significant difference for platelet counts between the three groups.

DIC scores on day 1, 3, and 7 are calculated in (Table 4). DIC scores on days 1 and 3 were significantly higher in MSSA/MRSA coinfections compared to other coinfections respectively ($p < 0.05$;Mann-Whitney). DIC scores on day 7 were significantly higher in MSSA/MRSA coinfections compared to no coinfections ($p < 0.05$).

Patients with MSSA/MRSA had a significantly higher risk of having a PRISM III score ≥10, and of having DIC compared to patients with no coinfections (Table 5).

Interestingly, patients with other coinfections were not significantly more likely to have higher PRISM III score nor have DIC compared to patients with no coinfections.

DISCUSSION

In our observational study, we demonstrate that MSSA/MRSA coinfection is associated with a higher risk of developing DIC in pediatric patients with H1N1 which may partially explain the increased mortality seen worldwide from pandemic H1N1.

Since the inception of the CDC surveillance for laboratory-confirmed influenza-related deaths among children in 2004 [2], the reports of pediatric hospitalization and death were the highest during the 2009–2010 pandemic H1N1 influenza season [15]. Bacterial coinfections with influenza have been increasing over the past years from 6% in 2004–2005 to 34% in 2006–2007 [16]. These coinfections had been associated with higher morbidity and mortality [17]. In our study, we report that 51% of critically ill children with H1N1 have bacterial coinfections, of which 35% were *S aureus*. The overall mortality was 9% for our study, but the mortality associated with *S aureus* was 33%. This is higher than the 2004–2007 CDC report wherein 13% of the pediatric influenza deaths were associated with *S aureus* coinfections [16].

S aureus has been previously associated with severe pneumonia in the 1918 influenza pandemic [18], with an increased incidence of pneumonia [19] and toxic shock syndrome in the 1968–69 Hong Kong flu epidemic [20] and increased mortality [21]. We found that children with H1N1 influenza and *S aureus* coinfections presented sicker to our ICU as evidenced by having a higher PRISM III score and a higher risk of having DIC. Two of the three children who required ECMO for hypoxic respiratory failure and catecholamine-refractory shock were coinfected with *S aureus*. A number of mechanisms have been proposed to why influenza A infection may make the host more susceptible to bacterial superinfection. Previous murine models of influenza A suggested that epithelial damage and changes in surface receptor caused by the virus put the host at risk for bacterial superinfection [22, 23]. In a recent H1N1 influenza murine model, investigators found that mice with prior influenza infection were significantly susceptible to secondary *S aureus* superinfection due to impaired natural killer cell responses in the lung [24].

We found that children with *S aureus* coinfections were four times more likely to develop overt DIC based on the ISTH criteria [14]. Autopsy findings from DIC patients revealed extensive fibrin deposition in small and mid-size vessels in all organs [25]. Neutrophil extracellular traps (NETs) are webs of DNA fibers which contain histones and neutrophil

antimicrobial proteins [26] and are proposed to be an innate response to trap and kill invasive bacteria [27]. Histones from NETs have been shown to activate platelets, promote thrombin formation and fibrin deposition, cause microvascular thrombosis, and become a major mediator of death in sepsis [28–31]. *S aureus* can rapidly induce neutrophils to release their DNA to form NETs via release of Panton-Valentine leukocidin, a pore-forming toxin. Furthermore, *S aureus* is a more potent and rapid inducer of NETs release compared to other bacteria [26]. Thus, NETs could be the link between *S aureus* infection and DIC.

Study limitations

We retrospectively reviewed the association between influenza A and bacterial coinfections in 66 patients admitted at a single center. As with all association studies, cause and effect cannot be determined from our data. Only 64% of our patients were PCR-confirmed H1N1. The other 36% of influenza A patients came early in the season during which testing by PCR was found to be inaccurate as the primers used were not specific for H1N1. However, later in the season all of our influenza A patients were PCR-confirmed H1N1.

CONCLUSIONS

During the 2009–2010 H1N1 pandemic, pediatric patients with influenza A and MSSA/MRSA coinfections had a higher risk of developing DIC. This higher risk for DIC may partially explain the increased mortality seen worldwide from pandemic H1N1. Further studies are warrant to evaluate the association between with influenza A and DIC.

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Table 1

Characteristics of children with influenza A (n = 66)

	No. of patients (%)
Sex	
Male	39 (59.1)
Female	27 (40.9)
Age (yr, mean±SD)	5.9 ± 5.4
Race	
White	19 (28.8)
Black	10 (15.2)
Hispanic	28 (42.4)
Other	9 (13.6)
Influenza testing (PCR)	
H1N1	42 (63.6)
Influenza A[a]	24 (36.4)
Coinfections	34 (51.5)
MSSA/MRSA	12 (18.2)
Other pathogens	22 (33.3)
None	32 (48.5)
Clinical presentation	
Suspected lower respiratory infection	31 (47.0)
Suspected central nervous system infection	1 (1.5)
Shock requiring inotropes/vasopressors	9 (13.6)
espiratory failure	39 (59.0)
Cardiac arrest	2 (3.0)
Co-Morbidities	36 (55.5)
Asthma	9 (13.6)
CLD/BPD[b]	7 (10.6)
Congenital Heart Disease	5 (7.6)
Developmental Delay	9 (13.6)
Miscellaneous (cancer, GI, diabetes)	6 (9.1)
None	30 (45.5)

SD, Standard Deviation; PCR, Polymerase Chain Reaction;

[a]not sub-typed by PCR, rapid or viral culture;

MSSA/MRSA, Methicillin-sensitive/resistant Staphylococcus aureus; CLD/BPD, Chronic Lung Disease/Bronchopulmonary Dysplasia; GI, gastrointestinal

Table 2

Bacterial coinfections in 34 (52%) out of 66 children with influenza A

	No. of patients
Staphylococcus aureus	12
Methicillin-resistant	5
Methicillin-sensitive	7
Moraxella catarrhalis	6
Pseudomonas aeruginosa	6
Haemophilus influenza (non-typable)	3
Streptococcus pneumonia	2
Group A Streptococcus	2
Serratia marcescens	3

Table 3

Clinical parameters of children with influenza A and bacterial coinfections

	None		Other infections		MSSA/MRSA	
	n	**Median (IQR)**	***n***	**Median (IQR)**	***n***	**Median (IQR)**
Mechanical ventilation (days)	32	8 (4–11)	22	8.5 (4–14)	12	5 (2.5–20.5)
LOS PICU (days)	32	5.5 (2.5–13)	22	10 (5–18)	12	11 (3.5–23.5)
LOS hospital (days)	32	10.5 (6–28.5)	22	14.5 (9–30)	12	20.5 (5–49)
PRISM III score[a]	32	6 (2.5–10)	22	7 (2–10)	12	14.0 (6–25)[b]
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
	% (n)		**% (n)**		**% (n)**	
ECMO[c]	3.1 (1)	96.9 (31)	0 (0)	100 (22)	16.7 (2)	83.3 (10)
Inotropes/Vasopressors[c]	21.9 (7)	78.1 (25)	27.3 (6)	72.7 (16)	58.3 (7)	41.7 (5)
Survival[cd]	90.6 (29)	9.4 (3)	95.5 (21)	4.5 (1)	83.3 (10)	16.7 (2)

MSSA/MRSA, Methicillin-sensitive/resistant Staphylococcus aureus; LOS, length-of-stay; PICU, Pediatric Critical Care Unie; ECMO, extracorporal membrane oxygenation;

[a]PRISM, Pediatric Risk of Mortality;

[b]Mann Whitney: none vs MSSA/MRSA, $p < 0.05$;

[c]Chi^2 not significant;

[d]at 90 days; IQR, interquartile range

Table 4

Coagulation parameters in influenza patients with coinfections at Day 1, 3 and 7

	None		Other infections		MSSA/MRSA	
Day-1	***n***	**Median (IQR)**	***n***	**Median (IQR)**	***n***	**Median (IQR)**
Platelets ($10^3/\mu l$)	29	262 (132–294)	22	234 (175–314)	12	225 (134–348)
PT (sec)	12	16.6 (14.7–20.4)	13	15.3 (14.8–17.1)	8	19.3 (15.4–25.9)[c]
PTT (sec)	12	37.2 (32.6–46.1)	13	36.2 (30.7–39.5)	8	39.1 (30.2–64.1)
Fibrinogen (mg/dl)	12	302 (253–404.5)	10	352 (265–438)	7	469 (250–561)
INR	12	1.25 (1.1–1.5)	11	1.2 (1.1–1.4)	8	1.6 (1.2–2.4)[c]
D-Dimer (μg/ml FEU)	12	2.3 (0.8–8.7)	11	1.6 (1.1–4.0)	7	16.1 (7.9–19.3)[bc]
DIC score	12	3.0 (3.0–4.0)	11	3.0 (2.0–3.8)	7	5.0 (3.3–6.8)[c]
Day-3						
Platelets ($10^3/\mu l$)	18	191 (136–272)	15	164 (121–253)	8	163 (74–210)
PT (sec)	10	14.9 (14.4–17.8)	6	14.2 (13.8–14.6)	4	16.9 (16.0–19.1)[c]
PTT (sec)	10	44.5 (32.1–50.8)	6	34.4 (32.9–35.2)	4	64.6 (37.9–120.6)[c]
Fibrinogen (mg/dl)	10	321.5 (224–494)	5	349 (271–389)	4	446.5 (272–609)
INR	10	1.1 (1.1–1.4)	5	1.1 (1.0–1.1)	4	1.4 (1.3–1.5)[c]
D-Dimer (μg/ml FEU)	9	8.1 (5.6–15.8)	5	2.00 (1.6–6.2)	4	11.4 (7.3–17.6)[c]
DIC score	9	4.0 (3.0–4.3)	5	3.0 (2.0–4.0)	5	4.0 (4.0–6.3)[c]
Day-7						
Platelets ($10^3/\mu l$)	10	256 (152–339)	6	277 (98–314)	5	90 (26–161)
PT (sec)	7	15.6 (13.9–17.4)	2	14.0 (13.4–14.5)	6	15.3 (14.7–15.8)
PTT (sec)	7	40.6 (33.7–44.8)	2	42.4 (37.1–47.7)	6	41.2 (34.9–46.5)
Fibrinogen (mg/dl)	7	282 (130–471)	2	474 (233–715)	5	549 (424–665)
INR	5	1.2 (1.1–2.2)	2	1.1 (1.0–1.1)	5	1.1 (1.1–1.2)
D-Dimer (μg/ml FEU)	7	6.0 (2.3–7.7)	2	10.9 (1.9–20.0)	5	7.8 (4.5–16.0)
DIC score	5	3.0 (2.8–3.0)	2	3.0 (2.0–4.0)	5	4.0 (3.8–4.3)[b]

MSSA/MRSA, Methicillin-sensitive/resistant Staphylococcus aureus; PT, prothrombin time; PTT, partial prothromboplastin time; INR, international normalized ratio; FEU, Fibrinogen Equivalent Unit; DIC disseminated intravascular coagulation; Mann Whitney:

[a] none vs other;

[b] none vs MRSA/MSSA;

[c] other vs MRSA/MSSA; IQR, interquartile range; $p < 0.05$

Table 5

Relative risk (RR) (95 % CI) for clinical parameters in patient with coinfections compared to patients without coinfections

	% (n)	% (n)	RR (95% CI)	*p*
Co-Infections				
D-Dimer	**<4 μg/ml**	**≥4 μg/ml**		
None	66.7 (8)	33.3 (4)	1	
Other	81.8 (9)	18.2 (2)	0.5 (0.1–2.4)	0.64
MSSA/MRSA	14.3 (1)	85.7 (6)	2.6 (1.1–6.0)	0.057
INR	**Ratio <1.5**	**Ratio ≥1.5**		
None	75.0 (9)	25.0 (3)	1	
Other	81.8 (9)	18.2 (2)	0.7 (0.1–3.6)	1.00
MSSA/MRSA	37.5 (3)	62.5 (5)	2.5 (0.8–7.6)	0.17
PRISM III	**Score <10**	**Score ≥10**		
None	71.9 (23)	28.1 (9)	1	
Other	72.7 (16)	27.3 (6)	1.0 (0.4–2.3)	1.00
MRSA/MSSA	33.3 (4)	66.7 (8)	2.4 (1.2–4.7)	0.035
DIC	**No**	**Yes**		
None	90.6 (29)	9.4 (3)	1	
Other	95.5 (21)	4.5 (1)	0.5 (0.05–4.4)	0.64
MSSA/MRSA	58.3 (7)	41.7 (5)	4.4 (1.3–15.8)	0.025

MSSA/MRSA, Methicillin-sensitive/resistant Staphylococcus aureus; INR, international normalized ratio; PRISM, Pediatric Risk of Mortality; DIC, disseminated intravascular coagulation; Fisher exact test; RR, relative risk; CI, confidence interval; INR, international normalized ratio; DIC disseminated intravascular coagulation