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Preeclampsia and pregnancies with small-for-gestational age neonates have different profiles of complement split products

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Abstract

Objective. The activation of the complement system results in the generation of split products with pro-inflammatory properties. The objective of this study was to determine whether preeclampsia and small-for-gestational age (SGA) are associated with changes in the maternal plasma concentrations of anaphylatoxins C3a, C4a and C5a.

Methods. A cross-sectional study was conducted in the following groups: (a) normal pregnant women ($n = 134$); (b) women who delivered an SGA neonate ($n = 53$); (c) preeclampsia with ($n = 52$) and without SGA ($n = 54$). Maternal plasma anaphylatoxin concentrations were determined by enzyme-linked immunoassay.

Results. (1) Women with preeclampsia with or without SGA had a significantly higher median plasma C5a concentration than that of normal pregnant women and those with SGA alone (all $P < 0.01$); (2) women with SGA alone did not have an increase in plasma C5a concentration; (3) in contrast, the median maternal plasma concentration of C4a was lower in women with preeclampsia and SGA than that of those with a normal pregnancy ($P = 0.001$); (4) no changes in C3a were observed among the study groups.

Conclusion. Preeclampsia is associated with increased plasma concentration of C5a, regardless of the presence or absence of an SGA fetus. In contrast, there was no difference in the plasma C3a, C4a and C5a concentration in patients with SGA.

Keywords: *Complement system, anaphylatoxins, innate immunity, inflammation, C3a, C4a, C5a, SGA, hypertension, pregnancy*

Introduction

Preeclampsia and small-for-gestational age (SGA) are two of the 'great obstetrical syndromes' [1] and considered leading causes of maternal and perinatal morbidity and mortality [2,3]. Both conditions share similar mechanisms of disease such as abnormal physiologic transformation of the spiral arteries [4–12], chronic uteroplacental ischemia [13–27], increased trophoblast apoptosis [28,29], anti-angiogenic state [30–66] and endothelial cell dysfunction [67–87]. Common risk factors observed in both syndromes include advance maternal age [88–90], renal disease [91–93], systemic lupus erythematous [94,95] and chronic hypertension [96–99].

In addition, activation of the innate immune system and an exaggerated maternal systemic inflammatory response has been described in preeclampsia [67,71,73,75,100–106] and, to a lesser extent, in SGA [72,75,104,106–108]. Evidence to support this view includes the following: (1) leukocyte activation [72,73], (2) increased maternal serum

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concentration of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-alpha [108] and (3) increased plasma markers of endothelial cell activation [75,76].

As part of innate immunity, the complement system participates in recognition and elimination of microorganism and foreign cells and in the inflammatory response. It also constitutes a bridge between innate and adaptive immunity [109]. Complement activation by the classical, alternative or lectin pathway results in the generation of split products C3a, C4a and C5a with pro-inflammatory properties. These glycopeptides, also referred as 'anaphylatoxins', induce vascular permeability [110–112], smooth muscle contraction [110,112,113] and chemotaxis of inflammatory cells [114–116]. In addition, proteolytic enzymes from phagocytic cells can also cleave C5 and release C5a [117–120]. The importance of complement activation in pregnancy complications has become evident from recent studies on animal models of antiphospholipid antibody syndrome and pregnancy loss, demonstrating that complement activation, especially C5a and C3a, are directly related to vascular injury, growth restriction and fetal demise [52,121–129].

Normal pregnancy is characterized by increased complement components in maternal circulation [130–132]. Indeed, higher plasma anaphylatoxins concentrations are found during normal pregnancy compared to nonpregnant women [133]. Previous studies have described an enhanced deposition of native complement proteins, split products and membrane attack complex (MAC) in trophoblast tissue of patients with preeclampsia [134,135]. However, the association between complement system and preeclampsia, through the determination of various complement proteins, has been addressed in the past with conflicting results [136–139]. Although preeclampsia and SGA share common features, there is a paucity of data on maternal plasma complement activation in women with SGA with or without preeclampsia. This study was designed to determine if the maternal plasma anaphylatoxin C3a, C4a and C5a concentrations in women with SGA neonates are different from those with preeclampsia.

Table I. Clinical characteristics of the study population.

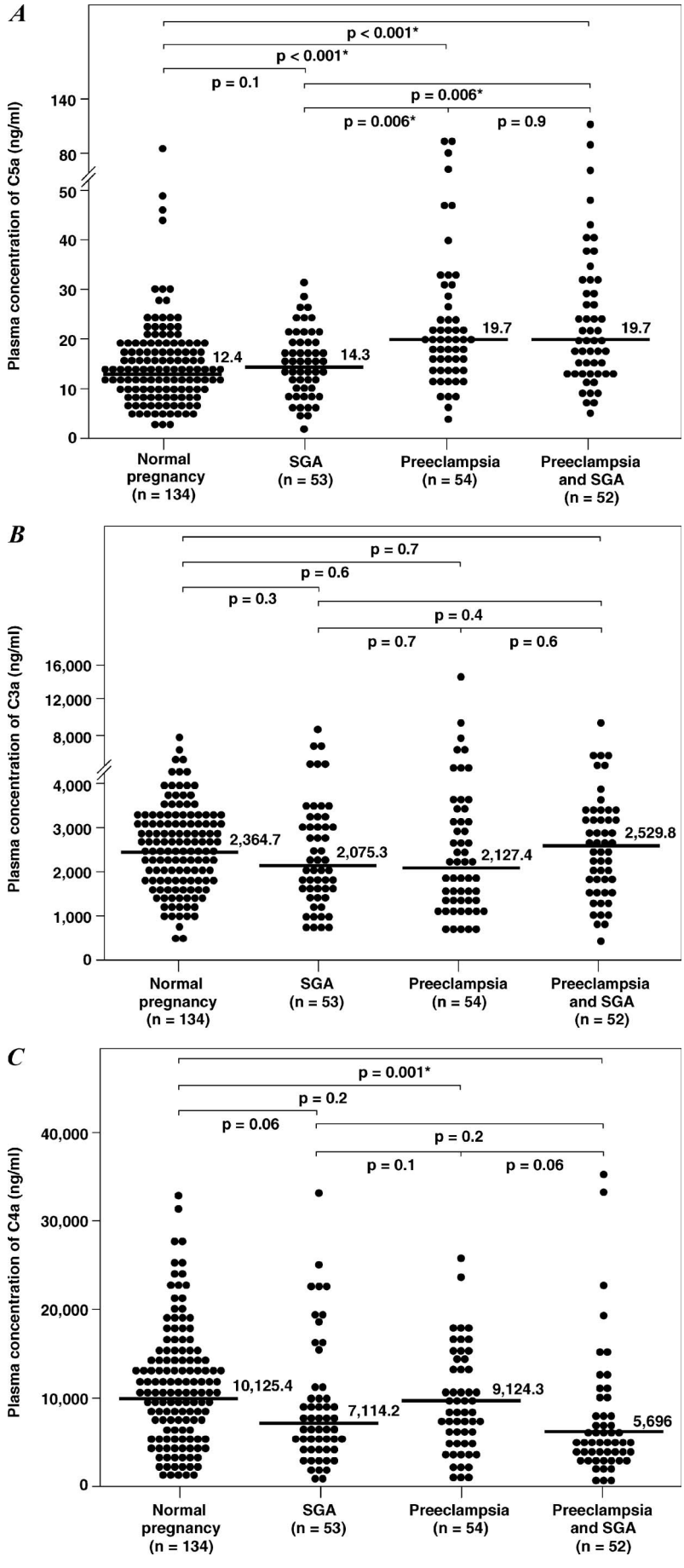
	Normal pregnancy $n=134$	Small for gestational age $n=53$	Preeclampsia without SGA $n=54$	Preeclampsia with SGA $n=52$
Maternal age (years)	25 (17–40)	24 (15–43)	24 (13–43)	23 (16–40)
Smoking⋆	23 (17.2)	17 (32)†,‡	6 (11.1)	8 (15.3)§
Drugs¶	14 (10.6)	7 (13.4)	4 (7.4)	3 (5.7)
Nulliparity	37 (27.6)	26 (49.1)†	36 (66.6)†	30 (57.6)†
Gestational age at venipuncture (weeks)	37.5 (20–41.7)	36.8 (25–39.7)	36.5 (23.4–42.4)	35.5 (25–41)
Gestational age at delivery (weeks)	39.2 (37–42.4)	37.1 (25–39.7)†	36.4 (23.7–42.4)†	35.6 (26.2–41.1)†
Birthweight (g)	3345 (2610–4080)	2050 (300–2880)†,‡	2195 (530–4460)†	1770 (550–2880)†,‡
Adjusted birthweight for gestational age (MOM)	−0.01 (−0.16 to 0.18)	−0.35 (−0.61 to −0.18)	−0.22 (−0.49 to 0.37)	−0.32 (−0.49 to −0.17)

Values expressed as median (range) or number (percent).
⋆Normal pregnancy, $n=133$.
†$P<0.05$ compared with normal pregnancy.
‡$P<0.05$ compared with preeclampsia without SGA.
§$P<0.05$ compared with SGA.
¶Normal pregnancy, $n=132$.

Table II. Plasma concentration of C3a, C4a and C5a of the study population.

	C3a	C4a	C5a
Normal pregnancy ($n=134$)	2364.7 (557.9–6642.7)	10125.4 (850.7–32,640)	12.4 (1.2–87.1)
Small for gestational age ($n=53$)	2075.3 (585.1–8155.6)	7114.2 (973.4–33,460)	14.3 (1.1–30.5)
Preeclampsia ($n=54$)	2127.4 (698.6–15,820)	9124.3 (1020.3–25,940)	19.7⋆,† (4.3–94.1)
Preeclampsia with SGA ($n=52$)	2529.8 (289.4–9080.8)	5696‡ (389.9–35,690)	19.7⋆,† (4.5–119)

Values expressed as median and range (ng/ml).
⋆$P<0.05$ compared with normal pregnancy.
†$P<0.05$ compared with SGA.
‡$P<0.05$ compared with preeclampsia without SGA.


A
p < 0.001*
p < 0.001*
p = 0.1
p = 0.006*
p = 0.006*
p = 0.9
Plasma concentration of C5a (ng/ml)
140
80
50
40
30
20
10
0
12.4
14.3
19.7
19.7
Normal pregnancy (n = 134)
SGA (n = 53)
Preeclampsia (n = 54)
Preeclampsia and SGA (n = 52)
B
p = 0.7
p = 0.6
p = 0.3
p = 0.4
p = 0.7
p = 0.6
Plasma concentration of C3a (ng/ml)
16,000
12,000
8,000
4,000
3,000
2,000
1,000
0
2,364.7
2,075.3
2,127.4
2,529.8
Normal pregnancy (n = 134)
SGA (n = 53)
Preeclampsia (n = 54)
Preeclampsia and SGA (n = 52)
C
p = 0.001*
p = 0.2
p = 0.06
p = 0.2
p = 0.1
p = 0.06
Plasma concentration of C4a (ng/ml)
40,000
30,000
20,000
10,000
0
10,125.4
7,114.2
9,124.3
5,696
Normal pregnancy (n = 134)
SGA (n = 53)
Preeclampsia (n = 54)
Preeclampsia and SGA (n = 52)

Material and methods

Study design and population

A cross-sectional study was designed by searching our clinical database and bank of biological samples including 293 women in four groups: (1) normal pregnant women ($n = 134$); (2) women with SGA neonates ($n = 53$); (3) patients with preeclampsia with SGA ($n = 52$) and (4) patients with preeclampsia without SGA ($n = 54$). Eligible patients were approached at the Detroit Medical Center/Hutzel Women's Hospital in Detroit/Michigan. All women provided written informed consent prior to the collection of the samples. The collection of samples was approved by the Human Investigation Committees and its utilization for research purposes by the Institutional Review Boards of Wayne State University and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, DHHS. Many of these samples have been previously used to study the biology of inflammation, hemostasis and growth factor concentrations in normal pregnant women and those with pregnancy complications.

Definitions

Women with normal pregnancies met the following criteria: no medical, obstetrical or surgical complications, not in labor, gestational age that ranged from 20 weeks until term and delivery of a normal term infant with a normal birth weight [140]. Patients in this group were enrolled from either a labor-delivery unit (in cases of scheduled cesarean section) or our antenatal clinic and followed until delivery. Preeclampsia was defined as hypertension (systolic blood pressure of $\geq$140 mmHg or diastolic blood pressure of $\geq$90 mmHg on at least two occasions, 4 h to 1 week apart) and proteinuria ($\geq$300 mg in a 24-h urine collection or one dipstick measurement of $\geq$2+). Severe preeclampsia was defined as severe hypertension (diastolic blood pressure $\geq$110 mmHg) and proteinuria, or mild hypertension and severe proteinuria (a 24 h urine sample that contained 3.5 g protein or one urine specimen of $\geq$3+ protein by dipstick measurement). SGA was defined as estimated fetal weight below the 10th percentile for gestational age, confirmed by neonatal birth weight using the reference range proposed by Alexander et al. [140].

Blood collection and human anaphylatoxins immunoassays

Samples of peripheral blood were collected into tubes containing ethylene diamine tetraacetic acid (EDTA). Samples were centrifuged and stored at -70°C. Specific and sensitive complement C3a, C4a and C5a enzyme-linked immunoassays (ELISA) were performed as previously described [133,141].

Statistical analysis

The Kolmogorov–Smirnov test was used to test for normal distribution of the data. Because the maternal plasma concentrations of C3a, C4a and C5a were not normally distributed, nonparametric tests were used for analyses. Kruskal–Wallis with *post-hoc* Mann–Whitney U tests were performed when indicated to determine the difference of the median among and between groups, and Bonferroni correction was applied to adjust for multiple comparisons. Chi-square test was used for comparison of proportions. The statistical package used was SPSS 12 (SPSS, Chicago, IL). A probability value <0.05 was considered significant.

Results

Table I displays the demographic and clinical characteristics of women in each group. Patients with SGA had the highest proportion of smoking among the study groups. The normal pregnancy group had a significantly lower rate of nulliparous women than the other groups. The median birth weight of patients with preeclampsia with and without SGA, as well as that of those in the SGA group, was lower than that of normal pregnancy (all comparisons $P < 0.05$). The median birth weight of neonates from the SGA group was lower than that of patients with preeclampsia in the absence of an SGA ($P < 0.05$). No significant differences were observed in the median gestational age at the time of blood collection among groups.

Table II displays the median plasma concentrations of complement splits products C3a, C4a and

Figure 1. Median plasma anaphylatoxins concentration of normal pregnant women, women with small for gestational age neonates (SGA) and women with preeclampsia with and without SGA. (A) The median plasma C5a concentration was higher in patients with preeclampsia with SGA (median: 19.7 ng/ml; range: 4.5–119 ng/ml) or without SGA (median: 19.7 ng/ml; range: 4.3–94.1 ng/ml) than normal pregnant women (median: 12.4 ng/ml; range: 1.2–87.1 ng/ml) or women with isolated SGA (median: 14.3 ng/ml; range: 1.1–30.5 ng/ml). (B) In contrast, women with preeclampsia and SGA had a median plasma C4a concentration lower than normal pregnant women (preeclampsia and SGA median: 5696 ng/ml; range: 389.9–35,690 ng/ml vs. normal pregnancy median: 10125.4 ng/ml; range: 850.7–32,640 ng/ml; $P = 0.001$). No differences in plasma C4a concentration were observed among other groups. (C) There were no significant differences in the C3a plasma concentration among the study groups.

C5a among the study groups. Women with preeclampsia, regardless of the presence or absence of an SGA neonate, had a higher median plasma concentration of C5a than normal pregnant women and women with an SGA alone [all $P < 0.01$; Figure 1(A)]. Among patients with preeclampsia, there was no significant difference in the median plasma concentration of C5a between those with and without an SGA neonate ($P = 0.9$). Similarly, there was no difference in the median plasma concentrations of C5a between patients with an SGA neonate and normal pregnant women ($P = 0.1$). In contrast, the median plasma C4a concentration in women with preeclampsia who delivered an SGA neonate was lower than that of normal pregnant women [$P = 0.001$; Figure 1(B)], and no significant differences in the median plasma concentration of C4a were observed among other groups. Also, there was no difference in the median plasma concentration of C3a among the study groups [Figure 1(C)].

A subanalysis conducted in patients with mild and severe preeclampsia demonstrated that there was no difference in the median plasma concentration of the complement splits products C3a, C4a and C5a according to the severity of the disease ($P > 0.05$).

Discussion

Principal findings of the study

This study demonstrates that patients with preeclampsia, with or without SGA neonates, had higher maternal median plasma C5a concentrations than normal pregnant women and those with SGA by itself. In contrast, preeclampsia with SGA is characterized by lower median plasma C4a concentrations than normal pregnancy.

The complement system and preeclampsia

Previous studies of complement system in preeclampsia found no differences [136,142,143] in serum complement hemolytic activity (CH_{50}) in women with preeclampsia. In contrast, Haeger et al. [144] reported in a cross-sectional study that plasma C3a and C5a concentrations in patients with preeclampsia were higher than in normal pregnant women at the time of delivery. The same authors reported in a longitudinal study [139] that patients with preeclampsia had increased plasma C5a concentrations but not C3a at delivery [139]. Moreover, patients with severe preeclampsia or with HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count) were found to have higher plasma C3a and C5a concentrations at delivery than the control group [145,146]. In addition, other complement proteins including C1-INH, C4, C3, C3d, C5 and MAC have been described to be reduced [138,147,148], increased [143] or unchanged [137–139,147,149] in patients with preeclampsia. These results differed probably due to the variety of complement components measured, and the different methods employed in their determination. Recently, Lynch et al. [150] reported in a prospective study that women who had elevated plasma fragment Bb (>90th percentile) before 20 weeks of gestation were more likely to develop preeclampsia than those who had a fragment Bb plasma concentration below the 90th percentile.

Complement C5a and preeclampsia

Preeclampsia is associated with phenotypic and metabolic changes in granulocytes and monocytes that suggest leukocyte activation [72,73,101] The high concentrations of C5a in plasma of patients with preeclampsia could be attributed to leukocyte activation, because proteolytic enzymes (e.g. elastase, serine protease) in leukocytes can cleave complement C5 directly [117–120]. The anaphylatoxin C5a exerts its activities through transmembrane receptor C5aR/CD88 [151]. Several biological and pro-inflammatory effects of C5a on white blood cells have been reported. C5a can induce the release of elastase from neutrophils [146], increase respiratory burst with the generation of reactive oxygen species [152–155], induce the expression of adhesion molecules [156] and delay neutrophil apoptosis [157]. It is noteworthy that these markers of leukocyte activation have been described to be increased in women with preeclampsia [73,146,158–160]. The actions of C5a on neutrophils and the generation of C5a by these cells would constitute a positive feedback loop that potentiates the inflammatory response in patients with preeclampsia. In addition, C5a induces the expression and/or the release of pro-inflammatory cytokines such as Interleukin (IL)- 1 [161–163], IL-6 [164], IL-8 [165] and TNF-alpha [162,163] in mononuclear cells and neutrophils. Moreover, it increases endothelial gene expression of IL-6 [166]. On the other hand, IL-6 and IL-1 increases the expression of C5aR on endothelial cells and monocytes, respectively [167,168]. Interestingly, an increase plasma TNF-alpha, IL-6 and IL-8 concentrations has been reported in patients with preeclampsia [19,169–173].

Recently, Mellembakken et al. [174] demonstrated that C5aR (CD88) was decreased in neutrophils from patients with preeclampsia and suggested that it may reflect an enhanced C5a-C5aR interaction. This proposal would be in accordance to our result because high concentration of C5a would saturate white blood cells C5aR [175].

Moreover, decay accelerating factor (DAF), a complement regulatory protein, was increased in neutrophils from patients with preeclampsia [174]. Furthermore, in women with preeclampsia, membrane cofactor protein (MCP) (CD46), complement protectin (CD59) and CR-1 (CD35) were higher in leukocytes obtained from the uterine vein than those obtained from the antecubital vein [174]. Following these results, the authors proposed that complement activation occurs in the uteroplacental compartment and the upregulation of complement regulatory proteins is a protective mechanism for complement attack.

Collectively, these observations suggest that C5a may play a role in the mechanisms of leukocyte activation and intravascular inflammation underlying the maternal syndrome of preeclampsia. Although SGA is also associated with leukocyte activation and inflammation [72], no changes were observed in the plasma concentration of C5a.

Endothelium, coagulation and complement

The endothelium plays an active role in the inflammatory response. Endothelial activation/dysfunction is considered central to the pathophysiology of preeclampsia [20,67]. In fact, women with preeclampsia have increased circulating markers of endothelial cell activation [67,80,176]. C5a increases the gene expression of E-selectin, V-CAM, I-CAM and upregulates P-selectin adhesion molecules on endothelial cells. Furthermore, elevated concentrations of tissue factor (TF) have been found in patient with preeclampsia [177–179]. Both, *in vitro* and *in vivo* studies have demonstrated that C5a induces TF mRNA expression by endothelial cells [180] and increases the procoagulant activity of alveolar macrophages by 5- to 6-fold through TF activation [181]. Therefore, C5a represents one of the links between inflammation and the coagulation system, both of which are enhanced in preeclampsia.

Complement and SGA

Enhance complement activation was not observed in women with SGA. The causes and the significance of low median plasma C4a concentration found in women with preeclampsia and SGA are unknown. We expected to have elevated complement activation in the SGA group because it has been demonstrated in murine models of pregnancy loss that complement activation is a critical mediator of embryo injury and growth restriction [129]. In a model, where maternal T-cells specific for paternal antigens triggered complement activation, embryo demise and growth restriction was observed [52]. Moreover, extensive deposition of C3 at the maternal–fetal interface and macrophage infiltration was detected despite adequate Crry expression (a murine complement regulatory protein) [52]. Surprisingly, when complement C3 was blocked with Crry-Ig it prevented embryo loss and growth restriction [52]. In addition, in a murine model of antiphospholipid pregnancy loss, the blockage of C5 cleavage with anti-C5 monoclonal antibody and C5 deficient mice (C5 –/–) prevented fetal growth restriction and pregnancy loss [125]. Similarly, fetal resorption and growth restriction were prevented when the C5a receptor was neutralized with an antagonist peptide [125].

Preeclampsia and SGA

Despite that preeclampsia and SGA share many maternal and placental pathological features, these obstetrical syndromes have different phenotypes [160,182]. However, it is not clear why some women will manifest the maternal phenotype of the disease (preeclampsia) with or without fetal involvement, while others will have only the fetal phenotype (SGA). The difference in the complement split products between SGA and preeclampsia observed herein is in agreement with previous studies suggesting that SGA and preeclampsia have different biological profiles [160,179,183,184]. Preeclampsia is primarily a systemic maternal disease that is, in some cases, associated with fetal growth restriction. In contrast, SGA is primarily a fetal disease in which the systemic changes in the maternal compartment may not be as prominent as in preeclampsia.

In summary, the increased plasma concentration of C5a in patients with preeclampsia with or without SGA provides additional evidence supporting the view that preeclampsia is characterized by activation of the innate immune system [73]. Whether the increase in C5a plasma concentration precedes the development of preeclampsia or is a consequence of a systemic intravascular inflammation remains to be determined.

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