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Cytokine 154 (2022) 155894

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Cytokine response over the course of COVID-19 infection in pregnant women

Daniel B. Rosen [a,*,1], Elisabeth A. Murphy [b], Ron S. Gejman [a], Allyson Capili [a], Rachel L. Friedlander [a], Sophie Rand [b], Kristen A. Cagino [c], Shannon M. Glynn [a], Kathy C. Matthews [c], Jeff M. Kubiak [b], Jim Yee [d], Malavika Prabhu [c], Laura E. Riley [c], Yawei J. Yang [b,d,*,1]

[a] Weill Cornell Medicine, New York, NY, USA
[b] Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, USA
[c] Department of Obstetrics & Gynecology, Weill Cornell Medicine, New York, NY, USA
[d] New York-Presbyterian Hospital, Weill Cornell Medicine, New York, NY, USA

ARTICLE INFO

Keywords:
Cytokine profile
SARS-CoV-2
Interferon
Interleukin
Pregnancy

ABSTRACT

Objective: To study how severity and progression of coronavirus disease (COVID-19) affect cytokine profiles in pregnant women.
Materials and methods: 69 third-trimester, pregnant women were tested for COVID-19 infection and SARS-CoV-2 specific IgM and IgG antibodies. Patients were stratified according to SARS-CoV-2 Reverse Transcriptase-PCR (RT-PCR) status and serology (IgM and IgG) status. Cytokines G-CSF, HGF, IL-18, IL-1Ra, IL-2Ra, IL-8, and IP-10 were measured via ELISA. Retrospective chart review for COVID-19 symptoms and patient vitals was conducted, and cytokine levels were compared between SARS-CoV-2 positive and negative cohorts, by seronegative and seropositive infection, by time course since onset of infection, and according to NIH defined clinical severity.
Results: IL-18, IL-1Ra, and IP-10 increased in the 44 RT-PCR positive pregnant women compared to the 25 RT-PCR negative pregnant controls. Elevated cytokine levels were found in early infections, defined by positive RT-PCR and seronegative status, and higher cytokine levels were also associated with more severe disease. By IgM seroconversion, IL-8 and IP-10 returned to levels seen in uninfected patients, while IL-18 levels remained significantly elevated.
Conclusion: Cytokine profiles of third-trimester pregnant women vary with the time course of infection and are correlated with clinical severity.

1. Introduction

Since late 2019, the Coronavirus disease 2019 (COVID-19), caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has inflicted a huge public health burden. Infected (unvaccinated) individuals vary in presentation, ranging from asymptomatic to severe disease, resulting in multi-organ failure and death. Advanced age portends worse outcomes, but large proportions of both young, healthy patients[1] and older, nursing home residents are asymptomatic[2]. Among symptomatic patients, symptoms range from mild, managed with supportive care, to severe and can require prolonged respiratory support. These clinical differences are incompletely explained by underlying medical conditions, such as cardiovascular disease and diabetes[3]. Certain populations are especially vulnerable, such as pregnant women who have a higher risk of severe disease and death than their non-pregnant counterparts even after accounting for co-morbidities[4]. Within the course of a single patient, symptoms change and peak relatively late in infection[5], when compared to other respiratory viruses such as influenza.

The heterogeneity in disease presentation implies great differences in host response. Like SARS-CoV-1[6], cytokine storms are correlated with poor outcomes of COVID-19[7]. The use of steroids in critically ill

* Corresponding author at: Weill Cornell Medicine, 1300 York Avenue, F701 New York, NY 10065, USA.
E-mail address: yang@med.cornell.edu (Y.J. Yang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cyto.2022.155894
Received 22 September 2021; Received in revised form 17 April 2022; Accepted 18 April 2022
Available online 25 April 2022

COVID-19 patients reduced all-cause mortality by about 34% in a meta-analysis [8], further implicating immune overactivation in poor outcomes. Yet early administration of steroids in non-severe cases had no impact or even possibly worse outcomes, further implying differential immune responses associated with disease severity[9]. The cytokine response in COVID-19 is being closely investigated as a mediator or signature of these immunologic processes. Although certain cytokine upregulations like interleukin-6 (IL-6) are associated with higher mortality in severely ill COVID-19 patients[10], the use of IL-6 receptor blockade has produced mixed results. It has reduced the likelihood of progression to composite mechanical ventilation or death in hospitalized, non-ventilated patients[11] but did not prevent intubation or death in hospitalized moderately-ill patients[12]. IL-6 receptor blockade also did not prevent mortality in hospitalized severely-ill patients[13]. Other cytokines have been implicated in mild versus severe disease[14] but results are often contradictory, complicated by confounding variables such as dissimilar sample groups, variable time since exposure, and broad definitions of disease severity. Furthermore, it is unclear if these studies can be extrapolated to the pregnant population who may have different immune responses. A prior study has shown that pregnant patients with COVID-19 disease have mixed cytokine profiles compared to non-pregnant counterparts, but a limited set of cytokines were analyzed and did not include asymptomatic patients[15]. Therefore, the impact of systemic cytokine variation in COVID-19, especially within unique populations like pregnant women, needs further study.

2. Materials & methods

2.1. Study population

To study the cytokine response in COVID-19 infection, we analyzed serum, drawn upon hospital admission, from 44 SARS-CoV-2 positive (Positive) and 25 SARS-CoV-2 negative (Negative) third trimester pregnant women admitted to a New York City hospital between 22 March and 30 April 2020. Although non-severe COVID-19 cases in the general population were triaged and recommended to stay home during the early phases of the pandemic, pregnant women were still advised to present to the hospital if they had severe symptoms of COVID-19 or for birth. Thus, we were able to obtain a broad representation of SARS-CoV-2 infection in pregnant women in New York City during the early phases of the pandemic.

2.2. Clinical information

All women underwent screening for history of and duration of COVID-19 symptoms upon admission to labor and delivery. COVID-19 symptoms included self-reported fever, cough, sore throat, rhinorrhea, shortness of breath, diarrhea, gastrointestinal symptoms, myalgias, loss of sense of taste or smell [16,17]. In addition, demographic information, and clinical information, including oxygen saturation (O2 sat), heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), temperature (T), white blood cell count (WBC), lymphocyte count, hemoglobin level (Hgb), platelet count (Plt), creatine kinase (CK), c-reactive protein (CRP), D-dimer, ferritin, lactate dehydrogenase (LDH), procalcitonin, and erythrocyte sedimentation rate (ESR) were retrospectively abstracted from the hospital electronic medical records.

2.3. Cytokine analysis

Cytokine concentrations were measured by human Quantikine ELISA kits from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions.

2.4. RT-PCR

Women underwent universal SARS-CoV-2 screening upon hospital admission using reverse-transcriptase polymerase chain reaction (RT-PCR) testing on nasopharyngeal swabs[18].

Patients were tested on one of the following SARS-CoV-2 RT-PCR clinical testing platforms, depending on availability, to ensure the fastest turn-around time: Altona (internally developed, US Food and Drug Administration [FDA] emergency use authorization approved assay), Roche Cobas 600 (Roche Diagnostics, Indianapolis, IN, USA; FDA approved), and Cepheid Xpert Xpress (Sunnyvale, CA, USA; FDA approved) following manufacturer instructions. Quality control analyses, positive, and negative patient samples were run on a daily basis as previously described[19].

2.5. Severity

Each patient was assigned a classification of COVID-19 severity based on the NIH defined clinical spectrum of SARS-CoV-2 infection [20]. As there were no critically infected patients and few cases of mild or moderate disease, mild and moderate disease were combined into a single group for further analysis.

2.6. Serology

SARS-CoV-2 serologic testing of immunoglobulin M (IgM) and immunoglobulin G (IgG) was performed on all blood serum samples [21]. Semi-quantitative IgM and IgG were measured against anti-spike using the Pylon COVID-19 IgM and IgG assays on the Pylon 3D analyzer (ET HealthCare, Palo Alto, CA). The Pylon 3D platform utilizes a fluorescence-based reporting system for semiquantitative detection of anti-SARS-CoV-2 IgM and IgG with a specificity of 99.4% and 98.8%, respectively. A relative index value is computed for comparative analysis. This value is defined as the instrument measured fluorescence of the test sample antibody value divided by the instrument cutoff value, which was defined as the average value of noninfected and non-vaccinated, control samples with a six standard deviation margin. IgM and IgG measurements were tested on unitized test strips comprised of antibody coated probe, reagents, buffers, and reading wells. Calibration occurs monthly or when a new lot of test strips or reagents from the manufacturer is accessed. High- and low-quality control materials from the manufacturer are run in parallel with positive and negative patient samples for daily calibration. Further methodology is described here [22]. All Negative patients were IgM and IgG seronegative, which confirmed no undetected prior SARS-CoV-2 infection. Seropositive patients were defined as having any anti-SARS-CoV-2 antibodies (i.e. IgM + IgG−, IgM + IgG+, or IgM-IgG+).

2.7. Timeline of infection

The immune response and clinical severity may progress during SARS-Cov-2 infection. Therefore, we wanted to study how the cytokine response changes over time since initial infection. Due to lack of RT-PCR testing to mark the onset of disease as well as persistently asymptomatic patients who cannot report time since symptom onset, we utilized the combination of RT-PCR and serology testing status to deduce the time course of a patient's infection. Patients generate antibodies against SARS-CoV-2 (seroconvert) after infection[23]. Seroconversion in SARS-CoV-2 infection has been reported at a median of 11 days post positive RT-PCR test[24] and 13 days post symptom-onset[25]. Because IgM seroconversion precedes IgG seroconversion in the humoral immune response, we stratified patients into post-infection timeframes: Early (IgM negative, IgG negative), Middle (IgM positive, IgG present or absent), Late (IgM negative, IgG positive) (Table 1). The median days since symptom onset in Early infection (2.5) is sooner than Middle or Late infections (17) ($p = 0.0104$) by Mann-Whitney Wilcox Rank-sum Test (Table 1), which supports the use of a combination of RT-PCR status and serology status as a surrogate for timeline of infection.

Table 1
Serology Status and Timeline in SARS-CoV-2 Infected Pregnant Women.

Time since infection	RT-PCR status	Serology status	IgM	IgG	Days since symptom onset[a]	N
Not Infected	Negative	Negative	–	–	–	25
Early	Positive	Negative	–	–	2.5 (0.5, 6.0)	14
Middle	Positive	Positive	+	−/+	14.0 (13.0, 27.75)	17
Late	Positive	Positive	–	+	20.5 (11.0, 33.75)	13

[a] In 22 infected women with a reported date of symptom onset, median (IQR).

2.8. Statistical analyses

Baseline patient demographics, clinical and obstetric co-morbidities were examined with descriptive statistics. Inferential statistics of cytokine levels, serologic response, clinical severity, and vital signs between the patients were analyzed by Mann-Whitney Wilcox rank sum test for comparisons of medians, unpaired T-test for comparisons of means, Chi-squared test for comparisons of distributions amongst categorical variables, Fisher's exact test for any comparison with a cell of five or fewer samples, and Pearson correlation analysis and linear regression for cytokine levels within individual patients. Statistical analyses were performed using R 3.6.1 (R Core Team, Austria) and RStudio 1.1.463 (RStudio Team, Boston, MA) software statistics.

2.9. Ethics oversight

This study was approved by the institutional review board. A waiver of consent was granted by the institutional review board.

3. Results

Forty-four SARS-CoV-2 RT-PCR positive (Positive) pregnant patients and 25 SARS-CoV-2 RT-PCR negative (Negative) pregnant controls were analyzed in the current study. All patients presented in the third trimester of gestation and were either admitted for COVID-19, labor, or pregnancy complications. There was no significant difference in the percentage of Negative 48% (12/25) compared to Positive 50% (22/44) ($p = 0.87$) patients presenting for labor. 98.6% (68/69) of patients delivered during the admission. Baseline demographics and clinical characteristics showed Positive and Negative patients were not significantly different with regards to maternal age, gestational age, ethnicity, race, health comorbidities of diabetes, hypertension, asthma, cardiac disease, hematologic disease, or obstetric co-morbidities of fetal anomalies, preterm labor, pre-eclampsia, multiple gestations, or intrauterine growth restriction (Table 2). Positive patients did have statistically higher BMI (median 28 kg/m^2 vs 23 kg/m^2; $p = 0.006$) although a quarter of admitted patients did not have BMI recorded. Negative women had statistically more autoimmune rheumatologic disease (12% vs 0%; $p = 0.044$).

Laboratory markers at admission were abstracted for all patients. Hemoglobin, platelet, white blood cell levels, and RPR were not statistically different between PCR Positive and Negative patients, when stratifying by serology status, when stratifying by severity or when further stratifying by infection timeline (data not shown). Other inflammatory markers in COVID-19, creatine kinase, C-reactive protein, D-dimer, ferritin, lactate dehydrogenase, lymphocyte and neutrophil levels, procalcitonin, and erythrocyte sedimentation rate were also abstracted. However, few patients had these tests performed, likely due to presentation in the very early stages of the pandemic and because most patients presented with asymptomatic or mild/moderate disease.

Comparison of cytokine levels between Positive and Negative patients revealed that IL-18, IL-1Ra, and IP-10 were significantly increased in Positive patients (Fig. 1), while there were no significant differences

Table 2
Demographics and clinical characteristics of SARS-CoV-2 infected patients and uninfected controls.

Variable	SARS-CoV-2 PCR				
	Negative		Positive		
	N = 25 (36%)[a]		N = 44 (64%) [a]		p-value [b]
Maternal Age (years)	35	(34, 36)	32	(28, 39)	0.2
Gestational age (weeks)	39.3	(28.6, 40.0)	39	(37.3, 29.6)	0.11
BMI	23	(22, 25)	28	(24, 32)	0.006
unknown	4		13		
Ethnicity					0.4
Hispanic	2	8%	7	16%	
Non-Hispanic	15	60%	19	43%	
Unknown	8	32%	18	41%	
Race					0.3
Asian	3	12%	4	9.1%	
Black	3	12%	6	14%	
other/declined	3	12%	14	32%	
White	16	64%	20	45%	
Admitted For					
Scheduled birth	12	48%	15	34%	0.3
Covid Symptoms	0	0%	4	9.1%	0.3
Other	1	4%	6	14%	0.4
Rupture of membranes	6	24%	6	14%	0.3
Active Labor	6	24%	16	36%	0.3
Pre-eclampsia	2	8%	5	11%	>0.9
Comorbidities					
None	13	52%	32	73%	0.082
Diabetes	2	8%	4	9.1%	>0.9
Hypertension	2	8%	7	16%	0.5
Autoimmune/ Rheumatologic	3	12%	0	0%	0.044
Asthma	3	12%	4	9.1%	0.7
Cardiac Disease	2	8%	1	2.3%	0.3
Hematologic	0	0%	2	4.5%	0.5
Obstetric Co-morbidities					
None	22	88%	35	80%	0.5
Anomaly	0	0%	1	2.3%	>0.9
Preterm Labor/Birth	0	0%	3	6.8%	0.5
Pre-eclampsia	1	4%	4	9.1%	0.6
Multiple-Gestation	2	8%	2	4.5%	0.6
Intrauterine Growth Restriction	0	0%	1	2.3%	>0.9

[a] N (%)
[b] Fisher's exact test; Wilcoxon rank sum exact test; Wilcoxon rank sum test; Pearson's Chi-squared test

in G-CSF, HGF, IL-2Ra, or IL-8. We also examined cytokine levels between patients in and not in labor and found no significant differences (Data not shown).

To investigate if cytokine levels are linked with clinical presentation, we sub-categorized the Positive patients using the NIH-defined clinical spectrum of SARS-CoV-2 infection[26] and compared cytokine levels between asymptomatic, mild/moderately symptomatic, and severely symptomatic Positive patients. We found that most patients had asymptomatic infections (27/44, 61.4%), with the next largest group, mild/moderate illness (10/44, 22.7%), and the smallest group, severe illness (7/44, 15.9%); notably, no patients were critically ill (Table 3). Analyzing cytokine levels by the NIH clinical spectrum of disease revealed that IL-18, IL-1Ra, and IP-10 levels also trended higher with increased disease severity (Fig. 2). HGF was statistically elevated in severe disease, compared to asymptomatic infection (Fig. 2). Severe disease also revealed statistically higher levels of IL-2Ra compared to asymptomatic infection (Fig. 2).

To study if cytokine levels change with serology status, we compared Negative controls that are RT-PCR negative and seronegative (PCR-SER−) vs RT-PCR positive and seronegative Early infections (PCR + SER−) or RT-PCR positive and seropositive Middle/Late infections (PCR + SER+) (Table 1, Fig. 3). IP-10 levels peaked in PCR + SER− patients

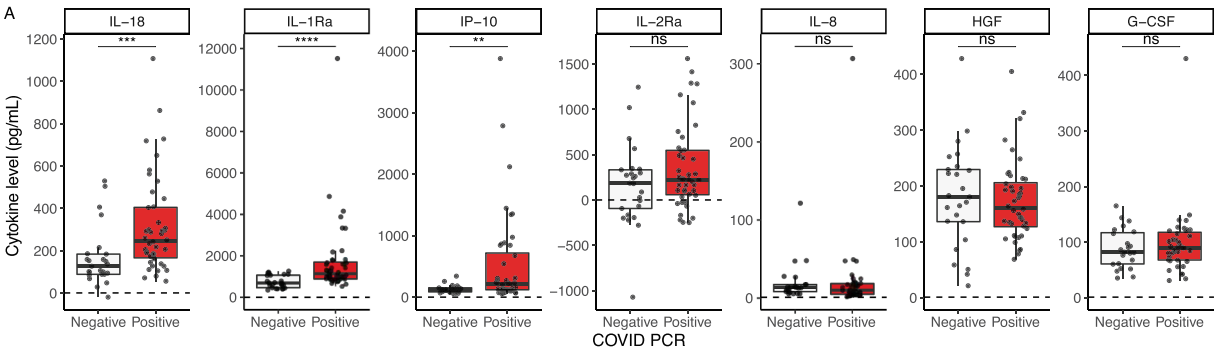

Fig. 1. Cytokine levels in SARS-CoV-2 infected pregnant women. Cytokine values between RT-PCR positive (Positive) and RT-PCR negative (Negative) patients. Each point represents an average of two technical repeats per patient. All positive cytokine cutoffs were 0 (dashed line). Statistical significance was calculated using Mann-Whitney Wilcox rank sum test.

Table 3
Distribution of NIH severity classification and symptom onset by serology status. Statistical significance calculated by 2x3 contingency table with Fisher's exact probability test* and Wilcoxon rank sum test**.

Variable	SARS-CoV-2 Serology				
	Negative (IgM-IgG−)		Positive (IgM + IgG−, IgM + IgG+, IgM-IgG+)		
	N = 14 (32%)[a]		N = 30 (68%)[a]		p-value [b]
NIH Severity					0.3
asymptomatic	7	50%	20	67%	
mild/moderate	3	21%	7	23%	
severe	4	29%	3	10%	

[a] N (%)
[b] Fisher's exact test.

and statistically returned to non-infected baseline by time of seroconversion. Similarly, IL-1Ra levels were significantly elevated in PCR + SER− patients but trended towards normal with seroconversion. IL-18 levels increased in all PCR+ patients but did not significantly differ based on serology status. IL-8 levels, which had not been previously shown to differ with infection, were significantly lower in PCR + SER− patients but returned to non-infected baseline with seroconversion.

To further discriminate cytokines changes with time since infection, we sub-stratified by Early, Middle, and Late infection timing (Table 1). IL-18, IL-1Ra, and IP-10 all peak in Early infection while IL-8 decreases in Early infection (Supplemental Fig. 1). IL-8 and IP-10 also quickly return to baseline by Middle infection, but IL-18 remains elevated and returns to baseline by Late infection. IL-2Ra was significantly lower in Late infection compared to Early infection.

Because time correlates with disease detection and serologic response, and because disease severity may also be associated with time, we tested if serology status was correlated with disease severity. NIH-defined clinical spectrum of disease was not statistically associated with serology status (Table 3). We also did not identify associations between serology status and vital signs (Data not shown) except in PCR

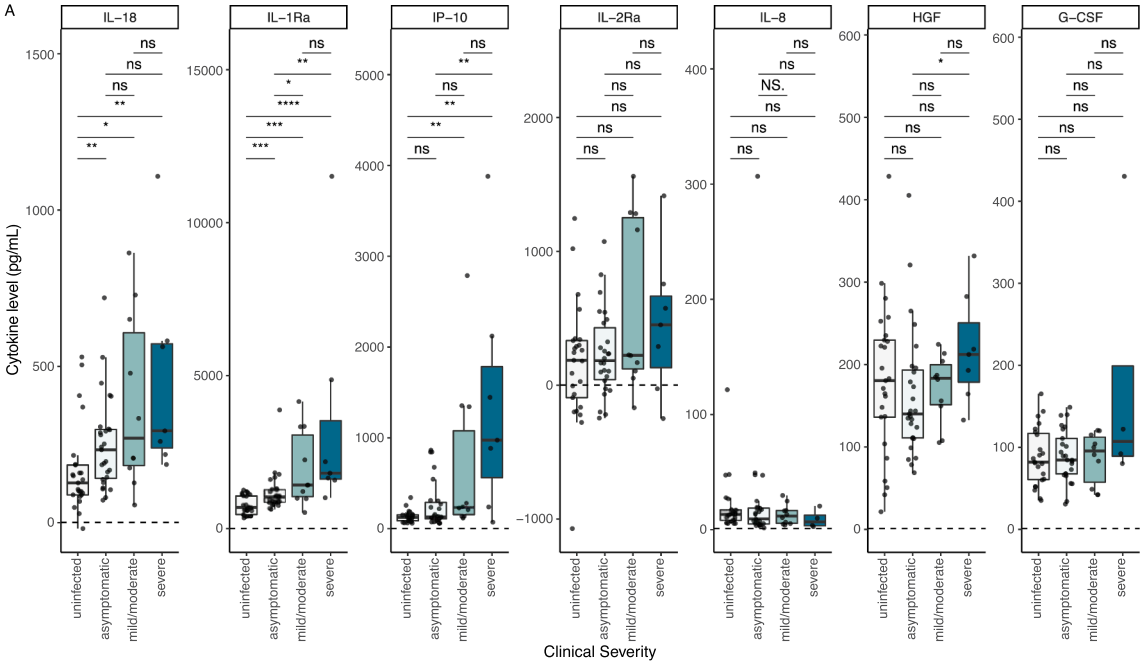

Fig. 2. Cytokine differences between NIH clinical severity groups in COVID-19 patients. Cytokines values between RT-PCR negative (uninfected) patients and RT-PCR positive patients grouped as asymptomatic, mild/moderately symptomatic, and severely symptomatic according to NIH Guidelines. Each point represents an average of two technical repeats per patient. All positive cytokine cutoffs were 0 (dashed line). Statistical significance was calculated using Mann-Whitney Wilcox rank sum test.

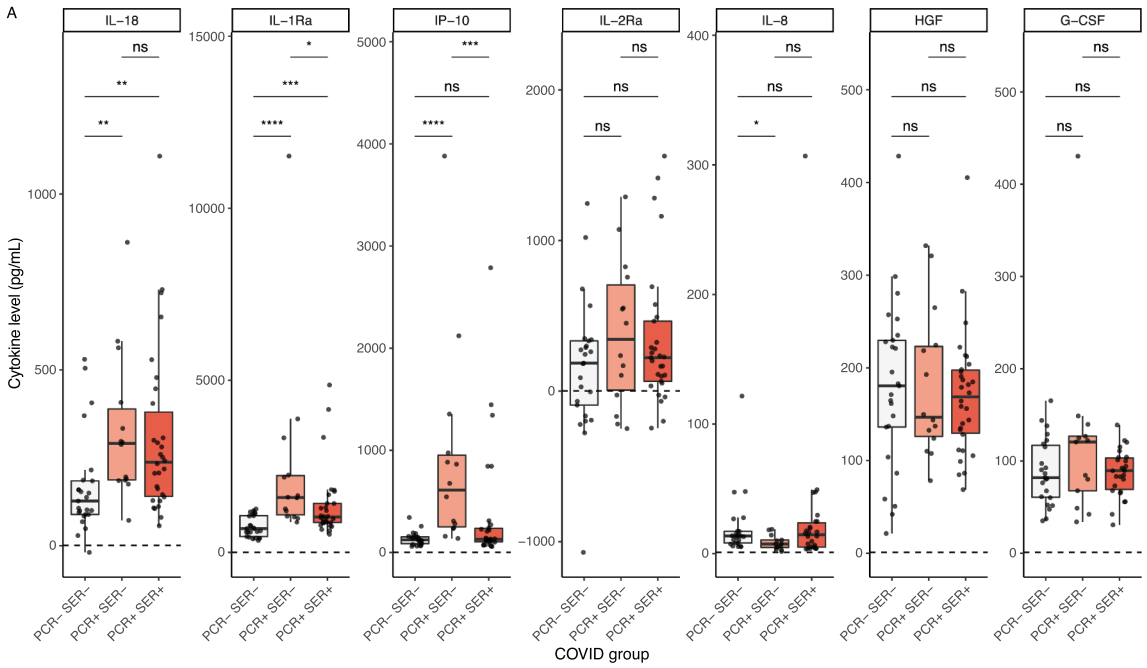

Fig. 3. Cytokine differences and serology status. Cytokines values between uninfected controls (PCR-SER−) vs SARS-CoV-2 RT-PCR+ patients that are seronegative (PCR + SER−) or seropositive for IgM and or IgG (PCR + SER+). Each point represents an average of two technical repeats per patient. All positive cytokine cutoffs were 0 (dashed line). Statistical significance was calculated using Mann-Whitney Wilcox rank sum test.

+ SER− patients who had statistically higher systolic blood pressure as well as higher temperature compared to other patients although only 4/14 (28.6%) PCR + SER− infected patients were febrile (>= 38.0 C).

Given the similar serology and severity-based changes of IL-18, IP-10, IL-1Ra, we investigated if cytokine levels were linearly associated within individual patients (Supplemental Fig. 2). In uninfected controls, these three cytokine levels were not statistically associated. IL-1Ra levels exhibited a large positive association with IP-10 in seronegative and seropositive infected patients. Despite the statistical increase of IL-18 in the PCR + SER− population, similar to IL-1Ra and IP-10, there was no statistical association between IL-18 and either IL-1Ra or IP-10 in individual PCR + SER− patients. However, IL-18 was positively associated with both cytokines in PCR + SER+ patients.

4. Discussion

4.1. Principal findings

In this large study of cytokine levels in third trimester pregnant women infected with SARS-CoV-2, we found significant differences in the levels of cytokines IL-18, IL-1Ra, and IP-10 between infected patients and uninfected controls. IL-18, IL-1Ra, and IP-10 were consistently found to be linked with SARS-CoV-2 infection, while HGF and IL-2Ra were found to be significantly different in subsets of patients with severe disease and those later in infection, respectively. IL-8, which we found not to vary with overall infection status, was depressed in the acute or early phase of infection prior to seroconversion. For patients past the acute stage of infection as measured by seroconversion, cytokines IL-8 and IP-10 returned to uninfected levels, IL-1Ra decreased but not to uninfected levels, and IL-18 remained significantly elevated when compared to uninfected patients but trended lower. In opposition to other studies[27], when we compared the population distribution of disease severity between seronegative and seropositive patients, severity was not correlated with serology status. This suggests that within the patient population seen here cytokine perturbations in acute infection are independent of disease severity. This may be due in part to the relatively homogenous patient population (no critical or severe disease requiring the ICU, and patients were all admitted for birth and not COVID-19). Therefore, although cytokine differences increased with more severe disease, further cytokine differences were revealed when we stratified patients by projected time since infection. Also, despite similar cytokine changes during acute infection, only IL-1Ra and IP-10 were correlated within Positive, seronegative patients and were not associated with IL-18 levels. Conversely, although the cytokines return to baseline or trend to normal at different rates, all three cytokine levels were strongly and positively associated with each other in Positive, seropositive individuals. There were no differences in cytokine levels between patients in and not in labor.

4.2. Clinical and research implications

About 3/5 infected patients in this study were asymptomatic, 1/5 had mild/moderate levels of disease and 1/6 had severe disease. Although the numbers differ in percentage of asymptomatic patients in this study (61.0%) than in a contemporaneous study[28] of pregnant women admitted for delivery at another hospital in New York City (87.9%), both studies confirmed the presence of a large majority of asymptomatic infected patient. These asymptomatic patients also further inhibit our ability to assess the time since infection; however, serology can be used as a symptom-agnostic guide to infection timeline.

This study's findings of the IL-18 response in pregnant patients are consistent with findings in the general SARS-CoV-2 infected population which is that IL-18 increases with SARS-Cov-2 infection and COVID-19 disease severity[29,30], While IL-18 decreases after acute infection, it remains elevated until convalescence[31]. IL-18, alternatively known as IFNγ inducing factor, is a member of the IL-1 family of cytokines and is cleaved into its active form by the inflammasome[32]. IL-18 activates Th1 cells to produce IFNγ in the presence of IL-12[33].

Our results expand upon prior research showing that IP-10 increases with SARS-CoV-2 infection and COVID-19 disease severity[34]. Although IP-10 has been shown to decrease with time from symptom onset[35], this is the first study showing that levels decrease to baseline by the time of seroconversion. IP-10 is induced by interferon gamma (IFNγ) and has functions in T cell generation and trafficking specifically

in the Th1 response[36], which may point towards a function in the resolution of the early immune response.

Despite the IL-18, IFNγ, IP-10, signaling pathway, the levels of IL-18 and IP-10 are not correlated in the acute stage of infection and only positively correlate in the post-acute setting, after seroconversion. This disconnect implies that another factor may be regulating the interaction in acute infection. Levels of IL-18 are tightly and negatively regulated by the naturally occurring IL-18 binding protein (IL-18 BP)[37]. In safety and efficacy studies of recombinant IL-18 (rIL-18), increasing doses of rIL-18 correlated with increased levels of systemic IL-18 BP[38]. It would be interesting to assess if IL-18 related therapeutics could alter the course and progression of COVID-19. Options to test this include a recombinant human IL-18 BP, which has completed a phase II clinical trial[39], which is currently undergoing phase III trial in adult-onset Still's disease. Conversely, a decoy-resistant IL-18 has recently been reported which bypasses the inhibitory effects of IL-18 BP[40].

Our findings also recapitulate that IL-1Ra increases with SARS-CoV-2 infection, COVID-19 disease severity, and early infection[35]. IL-1Ra has been shown to remain elevated up to 15 days beyond the onset of symptoms although it trends lower with time[41]. This decrease is similar to our findings in seropositive patients past the acute phase of infection. IL-1Ra, however, was found to be lower in a small cohort of pregnant patients with increased time to viral clearance[42]. The biopharmaceutical, anakinra, a recombinant, human IL-1Ra, has shown promise in cohort studies to treat hospitalized patients with COVID-19 [43]. After several randomized controlled trials, the data remains mixed. The CORIMUNO-ANA-1 trial in mild/moderate patients did not show improved outcomes[44], and a pre-print of the REMAP-CAP multifactorial, adaptive platform trial showed the inferiority of anakinra to other immune modulating agents in critically ill patients[45]. However, in the SAVE-MORE trial, anakinra reduced the risk of COVID-19 progression as assessed by the WHO-CPS score in severely ill patients with soluble urokinase plasminogen receptor (suPAR) levels >= 6 ng/mL[46]. Due to the conflicting data and lack of commercial suPAR laboratory testing in the United States, the NIH reports that there is insufficient evidence to recommend for or against the use of anakinra[20]. In the European Union, where suPAR laboratory testing is available, anakinra (commercially Kineret) is approved for use in COVID-19 disease requiring supplemental oxygen and with suPAR at least 6 ng/mL[47]. IL-1Ra is a specific receptor antagonist to IL-1, which is itself a mediator of innate inflammation[48].

The decrease in IL-8 in acutely infected pregnant women departs from previously reported increases of IL-8 in SARS-CoV-2 infection of the general (non-pregnant) population. IL-8 levels have variously been shown to increase with COVID-19 disease[10], with some reports of increases with severity[49], decreases with severity[34], no difference with severity[50], only increases with COVID-19 disease requiring ICU-level of care[51], or returns to baseline in convalescent patients[52]. Recently, a small study examined IL-8 in pregnant patients with and without COVID-19 disease and IL-8 trended lower with COVID-19 disease[53]. The patients in this study differ significantly from the aforementioned groups showing increased IL-8 levels with infection; specifically, in this study, patients were younger, exclusively female and pregnant. It should also be noted that the prior study of 10 pregnant patients, COVID-19 disease was defined either via RT-PCR or serology testing—which may have included convalescent patients. As we found that IL-8 regressed to baseline with seroconversion, it is difficult to compare the studies directly. Given that IL-8 increases in certain pathologies of pregnancy such as pre-eclampsia[54], but also reaches a nadir half-way through pregnancy[55], studies of IL-8 in pregnant women should be robustly stratified by gestational age and comorbidities. At least one clinical trial on anti-IL-8 treatment in hospitalized COVID-19 patients is underway as of this writing[56], which may help clarify the variability in cytokine response with age, disease severity, and pregnancy status. Functionally, IL-8 recruits neutrophils to sites of infection and facilitates phagocytosis and were found to be low in patients with acute respiratory distress syndrome[57].

Therefore, cytokine levels in SARS-CoV-2 infection in pregnant women closely reflected levels found in the general population with respect to IP-10, IL-1Ra, and IL-18. Data on the IL-8 response in SARS-CoV-2 infection remain mixed, but the decreased levels with acute infection seen in this study are consistent with other reports that IL-8 does not rise with infection and may fall with acute infection in pregnant patients[53]. IP-10, IL-1Ra, and IL-18 levels in pregnant women followed similar temporal patterns to the general population with regards to symptom onset. Surprisingly, despite these associations at the group level, IL-18 levels were not associated with IP-10 or IL-1Ra levels in specific individuals during acute infection, but after seroconversion all three cytokines were highly positively and significantly correlated—which may hint that the cytokine response converges on a common pathway with the emergence of humoral immunity. This may be related to the link between IL-18 and IP-10 via IFNγ. As mentioned earlier, the majority of young or pregnant patients are asymptomatic, not just pre-symptomatic[56,58], which may affect the generalizability of earlier studies which used symptom onset to define temporal response. By assessing cytokine levels according to serology status, researchers may be able to stratify the immune response to SARS-CoV-2 for all infected patients.

4.3. Strength and limitations

Our study has many strengths. It is a large study analyzing levels of cytokines G-CSF, HGF, IL-18, IL-1Ra, IL-2Ra, IL-8, and IP-10 in third trimester pregnant patients infected with SARS-CoV-2 compared to non-infected pregnant controls. Additionally, our study further divides the cohort according to clinical severity and discriminates changes in cytokine levels based on serologic conversion.

While labor may be a confounding variable that leads to changes in cytokines, we confirmed that there were no statistically significant differences in cytokine levels when comparing patients admitted for labor and those not admitted for labor. This study is limited by the lack of serial cytokine measurements to measure disease progression in individual patients. Due to the limited amount of serum obtained from each patient, only a small number of cytokines were able to be measured. It would be ideal to investigate further cytokines implicated in the temporal response of SARS-CoV-2 infection, such as IL-18 BP. Additionally, we were unable to attempt to profile immune cells within patients due to limited samples and testing capabilities. Despite the large number of patients analyzed, it was still a fraction of all Positive pregnant patients admitted during this time period. Although pregnant women have worse outcomes with COVID-19 than age and gender matched populations, the rate is still low and no critically ill patients or deaths were recorded in this cohort. Additionally, we only included patients in the third trimester of pregnancy and findings may not be generalizable to patients in the earlier stages of pregnancy. Also, as the COVID-19 pandemic has progressed, new therapeutics and new therapeutic indications have become available for patients. As these patients contracted COVID-19 at the beginning of the pandemic, such interventions were not available. There may be cytokine perturbations in patients undergoing COVID-19 therapies that differ from the results found here.

4.4. Conclusions

In conclusion, the cytokine response to SARS-CoV-2 infection in third trimester pregnant women closely resembles that seen in the general population. By stratifying patients by serology status, we were able to objectively measure disease progression. The cytokine changes uncovered here are most pronounced in the acute phase of infection, prior to seroconversion. Furthermore, analysis by serology status revealed new patterns of the cytokine response to SARS-CoV-2 infection, that were not seen based on RT-PCR positivity or severity classifications. Thus, the specific and differing patterns of quickly changing individual cytokine

levels can be uncovered and may prove useful as markers of future disease course, outcomes, and even suggest therapeutic targets or guide timing of interventions.

Funding

Yawei Jenny Yang was funded by the Weill Cornell Medicine COVID-19 Research Grant (Weill Cornell Medicine, New York Presbyterian, New York, NY). Daniel B Rosen and Ron S Gejman were funded by a Medical Scientist Training Program grant from the National Institute of General Medical Sciences of the NIH (T32GM007739) to the Weill Cornell/Rockefeller/Sloan-Kettering Tri-Institutional MD-PhD Program. The content of this study is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

CRediT authorship contribution statement

Daniel B. Rosen: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Supervision, Writing - original draft, Writing - review & editing. **Elisabeth A. Murphy:** Investigation, Methodology, Formal analysis, Writing - review & editing. **Ron S. Gejman:** Conceptualization, Investigation, Methodology, Data curation, Writing - review & editing. **Allyson Capili:** Conceptualization, Investigation, Methodology, Data curation, Writing - review & editing. **Rachel L. Friedlander:** Data curation, Writing - review & editing. **Sophie Rand:** Data curation, Writing - review & editing. **Kristen A. Cagino:** Data curation, Writing - review & editing. **Shannon M. Glynn:** Data curation, Writing - review & editing. **Kathy C. Matthews:** Data curation, Writing - review & editing. **Jeff M. Kubiak:** Methodology, Data curation, Writing - review & editing. **Jim Yee:** Methodology, Data curation, Writing - review & editing. **Malavika Prabhu:** Conceptualization, Writing - review & editing. **Laura E. Riley:** Conceptualization, Writing - review & editing. **Yawei J. Yang:** Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2022.155894.

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