TGF- β Blood Levels Distinguish Between Influenza A (H1N1)pdm09 Virus Sepsis and Sepsis due to Other Forms of Community-Acquired Pneumonia

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Abstract

There is a strong interest in finding adequate biomarkers to aid in the diagnosis and prognosis of influenza A (H1N1)pdm09 virus infection. In this study, serum levels of inflammatory cytokines and laboratory markers were evaluated to assess their usefulness as biomarkers of influenza A (H1N1)pdm09 and their association with fatal cases. Serum samples of consecutive patients with a clinical presentation suggestive of influenza A (H1N1)pdm09 and progression to sepsis were evaluated. Serum inflammatory cytokines and routine laboratory tests were performed and correlated with positivity for influenza A (H1N1)pdm09 influenza by real time reverse transcription polymerase chain reaction and the results of three clinical severity scores (Sequential Organ Failure Assessment [SOFA], CURB-65, and Acute Physiology and Chronic Health Evaluation II [APACHE II]). High SOFA scores and some of its individual components, but not CURB-65 or APACHE II scores, correlate with fatal cases regardless of etiology. Total and unconjugated bilirubin, Ca^{++} , Cl^- , prothrombin times, and partial thromboplastin times discriminate influenza A (H1N1)pdm09 from other causes of community-acquired pneumonia. High levels of IL-8, IL-10, and IL-17 were increased in influenza A (H1N1)pdm09 patients when compared with controls ($p < 0.05$). IL-6 levels were significantly elevated in influenza A (H1N1)pdm09 patients and non-(H1N1)pdm09 patients when compared with controls ($p < 0.05$). TGF- β serum levels discern between healthy controls, influenza A (H1N1)pdm09 patients, and patients with other causes of community-acquired pneumonia. TGF- β levels were negatively correlated with SOFA on admission in influenza A (H1N1)pdm09 patients. TGF- β levels are a useful tool for differentiating influenza A (H1N1)pdm09 from other causes of pneumonia progressing to sepsis.

Introduction

INFLUENZA PANDEMICS HAVE BEEN historically associated with high mortality (30). In 2009, the emergence of a new strain of H1N1 influenza A virus was reported; early in the outbreak it was estimated that 9% of the cases required hospitalization, and 9–31% of hospitalized patients were admitted to an intensive care unit (ICU) (2). The first cases of pandemic influenza A (2009) H1N1 virus were detected in Mexico. Influenza A H1N1 causes fulminant pneumonia and acute respiratory distress syndrome (ARDS). Fatal cases of influenza occur most often in children, individuals older than 65 years of age, and those with chronic diseases. In contrast, pandemic influenza A (2009) H1N1 was more frequent in individuals younger than 60 years of age (17,21).

The development of tools to aid in the prognosis of patients during bouts of influenza has gained worldwide relevance, especially the detection of inflammatory mediators. Activation of the immune system by influenza viruses results in the production of inflammatory mediators, such as cytokines (23,38). Cytokines play an important role in inhibiting viral replication by acting as immune cell chemoattractants, activating cells and regulating both the innate and adaptive immune response. However, dysregulation of cytokine production (hypercytokinemia) may contribute to tissue damage. Hypercytokinemia has been reported to occur in several infectious diseases, such as tuberculosis, tularemia, varicella zoster, Epstein–Barr virus, and influenza (8,18,26,39,40). IL-10 and TGF- β 1 are two immunoregulatory cytokines produced primarily by macrophages and T regulatory cells (4,14). These cytokines are

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associated with immune tolerance and anti-inflammatory processes. However, their role in hypercytokinemia during influenza virus infection has not been studied. Several groups have reported their findings, and the role of IL-6 seems to be the common feature among influenza A (H1N1)pdm09-infected patients (1,11,33), while local variations seem to correlate with higher levels of other cytokines, such as IL-10 or IL-17 (24,43). Because of this, the objective in the present study was to analyze cytokine levels in patients with influenza A (H1N1)pdm09 infection, in addition to clinical severity scores, and blood chemistry.

Methods

Patients and controls

Patients with a clinical presentation suggestive of influenza, that is, a fever $>38^{\circ}\text{C}$, respiratory distress, and an abnormal chest x-ray, with disease progression to sepsis within 24 h of admission, were sequentially recruited from the emergency room of the Hospital Universitario “Dr. José Eleuterio González” from July 2009 to August 2010. Patients with previous hospitalizations lasting more than 48 h within the last 3 months, a history of chronic steroid use, a previous diagnosis of cancer or tuberculosis, or HIV antibody positivity were excluded. A control group of healthy age-matched individuals was recruited from personnel working at the same institution. The study was conducted with approval of the bioethics committee of the School of Medicine of the Universidad Autónoma de Nuevo León. Informed consent was obtained from both patients and controls.

Influenza A (H1N1)pdm09 diagnosis

The diagnosis of influenza A (H1N1)pdm09 was made using real time reverse transcription polymerase chain reaction amplification of viral RNA from pharyngeal epithelial samples collected at the time of recruitment. Processing of the samples

was carried out at a central diagnostic state-sanctioned facility according to protocols established by the CDC (6).

Clinical scores, blood chemistry, and serum cytokine determinations

The severity of community-acquired pneumonia (CAP) was determined using clinical scores, the need for intensive care, mechanical ventilation, and development of severe sepsis or septic shock. In addition, study cases were classified as “fatal” if death occurred within 28 days after recruitment; otherwise, they were classified as “resolved.”

Three clinical scores were applied in this study: Sequential Organ Failure Assessment (SOFA), CURB-65, and Acute Physiology and Chronic Health Evaluation II (APACHE II). The CURB-65 is a severity score based on five parameters (confusion, blood urea nitrogen ≥ 7 mmol/L, respiratory rate $\geq 30/\text{min}$, blood pressure $\leq 90/60$ mm Hg, age ≥ 65 years). It is useful to stratify patients according to mortality risk as low, intermediate, or high, and to define the best management based on the score: 0–1, outpatient treatment, mortality 1.5%; 2–3, requires hospitalization, mortality 9.2%; and 4–5 requires treatment in intensive care, mortality 22% (25). The APACHE II is another severity predictor. It is performed by measuring 12 physiological variables, and has a score of 0–71 points; higher scores indicate more severe disease (22). The SOFA is used to assess a patient’s status during ICU stay. It is a predictor of mortality initially created to assess the degree of organ dysfunction in septic patients. It was subsequently found that it could be applied to nonseptic patients. A score of 1–4 is assigned depending on the dysfunction in each of six evaluated organ systems (28).

Serum samples were collected within the first 24 h after recruitment and again 96 h later. Samples were kept at -80°C until analysis. IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IL-15, IL-17, TNF- α , IFN- α , and IFN- γ serum concentrations were determined using the MILLIPLEX[®]

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS

	Influenza A (H1N1)pdm09	Other pneumonias	Healthy
Total patients	14	15	23
Age, mean ($\pm$ SD)	37.68 ($\pm$ 11.56)	43.1 ($\pm$ 19.1)	35.08 ($\pm$ 12.6)
Gender, M/F	7/7	5/10	6/17
Deaths, <i>n</i> (%)	8 (57.14)	8 (53)	0 (0)
Smoking, <i>n</i> (%)	6 (42.85)	7 (46)	4 (17.4)
Pack-years cigarettes (range)	2.7 (0–15)	4.34 (0–25)	0 (0–2)
Alcoholism, <i>n</i> (%)	6 (42.9)	7 (46)	5 (21.7)
ARDS, <i>n</i> (%)	13 (92.85)	8 (53)	0 (0)
Influenza vaccination, <i>n</i> (%)	0 (0)	0 (0)	22 (95.65)
Diabetes, <i>n</i> (%)	1 (7.14)	1 (6.6)	0 (0)
Hypertension, <i>n</i> (%)	1 (7.14)	1 (6.6)	1 (4.3)
Chronic kidney disease, <i>n</i> (%)	0 (0)	0 (0)	0 (0)
Obesity, <i>n</i> (%)	1 (7.14)	3 (20)	0 (0)
Drug addiction, <i>n</i> (%)	1 (7.14)	1 (6.6)	0 (0)
Obstructive sleep apnea, <i>n</i> (%)	1 (7.14)	1 (6.6)	0 (0)
Asthma, <i>n</i> (%)	0 (0)	1 (6.6)	0 (0)
Biomass smoke exposure, <i>n</i> (%)	0 (0)	1 (6.6)	0 (0)
Heart failure, <i>n</i> (%)	0 (0)	1 (6.6)	0 (0)
Mechanical ventilation, <i>n</i> (%)	11 (78.57)	6 (40)	0 (0)

ARDS, acute respiratory distress syndrome; SD, standard deviation.

TABLE 2. SEVERITY CLINICAL SCORE

	<i>Influenza</i>			<i>Pneumonia</i>			<i>All causes</i>		
	<i>Lived</i>	<i>Died</i>	<i>p</i>	<i>Lived</i>	<i>Died</i>	<i>p</i>	<i>Lived</i>	<i>Died</i>	<i>p</i>
SOFA	3.5 (1.9)	5.8 (2)	2.30E-001	3.3 (1.5)	7 (2.9)	9.00E-003	3.4 (1.6)	6.4 (2.5)	7.50E-004
APACHE II	12 (3.3)	11.3 (3.5)	6.40E-001	9.2 (4.3)	13.7 (6.9)	1.65E-001	10.5 (3.9)	12.5 (5.4)	2.74E-001
CURB-65	1.5 (0.8)	1.71 (0.7)	4.90E-001	1.3 (0.9)	2.25 (1.2)	1.00E-001	1.3 (0.9)	2.0 (0.9)	8.50E-002

Statistically significant values are shown in bold.

SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II.

MAP kit (Millipore Corp., Billerica, MA). Plates were read using a Luminex 100 TM IS analyzer (Luminex Corp., Austin, TX), and standard curves were calculated using five-parameter curve fitting according to the manufacturer's instructions. Results were analyzed using Luminex 100 IS 2.3 software (Luminex Corp.). IFN- β (PBL Interferon Source, Piscataway, NJ), IL-29/IFN- λ 1 (eBioscience, San Diego, CA), and TGF- β 1 (R&D Systems, Minneapolis, MN) serum levels were determined by enzyme-linked immunosorbent assay according to the manufacturer's instructions. Blood chemistry determinations were carried out by the hospital's central laboratory using standard methodologies.

Statistics

Comparisons between groups were done via two-tailed Student's *t*-test or with one-way analysis of variance using LibreOffice software (The Document Foundation, Germany). Spearman correlation analysis was performed using GraphPad Prism v6 for Mac (San Diego, CA). Statistical significance was considered with *p*-values of <0.05.

Results

Patient demographics and clinical features

Twenty-nine patients with a diagnosis of influenza A (H1N1)pdm09 ($M_{\text{age}} = 37.68 \pm 11.56$ years) and non-(H1N1)pdm09 pneumonia ($M_{\text{age}} = 43.1 \pm 19.1$ years) were included in this study. Eight patients in the influenza A (H1N1)pdm09 group (57.14%) and eight in the non-(H1N1)pdm09 pneumonia (53%) group died. In the influenza A

(H1N1)pdm09 group, 92% developed ARDS, and in the non-(H1N1)pdm09 pneumonia group, 53% developed ARDS. Two patients infected with influenza A (H1N1)pdm09 were confirmed to be co-infected with *Staphylococcus aureus*, one with *Streptococcus agalactiae*, and another with *Klebsiella* sp. Two patients with non-(H1N1)pdm09 pneumonia developed a nosocomial infection with *Acinetobacter baumani*. Treatment consisted of oseltamivir and antibiotics (moxifloxacin and ceftriaxone; Table 1).

Clinical severity scores and patient outcomes

On admission to the emergency room, patients who survived H1N1 pneumonia had APACHE II scores of 12 points, CURB65 of 1.5 point, and SOFA of 3.5 points. Patients who died had an APACHE II of 11.3 points, a CURB65 of 1.71 points, and a SOFA of 5.8 points. CURB-65 scores were not associated with increased mortality. Neither CURB-65 nor APACHE II predicted ARDS in any group or subgroup studied. Fatal cases, due to either influenza A (H1N1)pdm09 or non-(H1N1)pdm09 pneumonia, had higher SOFA scores on admission. Additionally, some of the individual components of the SOFA score were significantly different between patients who resolved from those who succumbed. Overall mortality did not differ between influenza A (H1N1)pdm09 and non-(H1N1)pdm09 pneumonia patients (Table 2). Eight of 14 patients with influenza A (H1N1)pdm09 pneumonia died within the 28 days of follow-up, and 8 of 15 non-(H1N1)pdm09 pneumonia also died within 28 days. Neither lethality (influenza A (H1N1)pdm09 57.14% vs. non-(H1N1)pdm09 pneumonia

TABLE 3. LABORATORY FINDINGS, AVERAGE (STANDARD DEVIATION)

<i>SOFA</i>	<i>Healthy</i>	<i>Lived</i>	<i>Died</i>	<i>p</i>
PaO ₂ /FiO ₂ mmHg	ND	206.4 (97.9)	67.4 (39.5)	2.92E-005
Blood pH	ND	7.46 (0.05)	7.33 (0.1)	5.90E-004
[HCO ₃] (mmol/L)	ND	25.9 (4.3)	20.8 (4.9)	8.10E-003
[Creatinine] (mg/dL)	ND	0.82 (0.21)	1.32 (0.76)	3.00E-002
Platelets/ μ L	ND	226,916 (68,009)	173,333 (41,235)	1.80E-002
<i>Blood chemistry</i>	<i>Healthy</i>	<i>Influenza A (H1N1)pdm09</i>	<i>Other pneumonias</i>	<i>p</i>
[Total bilirubin] mg/dL	ND	0.68 (0.33)	1.21 (0.63)	2.40E-002
[Unconjugated bilirubin] mg/dL	ND	0.36 (0.1)	0.76 (0.48)	2.60E-002
[Ca ⁺⁺] mg/dL	ND	7.87 (0.7)	8.7 (0.66)	2.50E-002
[Cl] mmol/L	ND	97.8 (4.8)	104.4 (7.1)	9.00E-003
Prothrombin time (sec)	ND	11.37 (0.95)	13.55 (2.74)	4.10E-002
Partial thromboplastin time (sec)	ND	35.47 (5.74)	28.15 (4.56)	9.00E-003

Italics = $p < 0.05$; bold = $p < 0.01$.

ND, not determined.

53.33%) nor time to death (influenza A (H1N1)pdm09 151.25 ± 115.47 h vs. non-(H1N1)pdm09 pneumonia 103.78 ± 73.76 h, $p=0.34$) were significantly different between groups.

Total bilirubin, unconjugated bilirubin, Ca^{++} , Cl^- , prothrombin times, and partial thromboplastin times distinguish between influenza A (H1N1)pdm09 and non-H1N1pdm09 pneumonia patients

Liver function tests, blood electrolyte levels, coagulation times, blood cell counts, serum glucose, and protein levels were examined. Six of the parameters tested were able to differentiate influenza A (H1N1)pdm09 from non-(H1N1)pdm09 pneumonia (Table 3). Fatal cases had significantly worse pulmonary function, acidosis, renal dysfunction, and fewer platelets (Table 3). Patients with influenza A (H1N1)pdm09 had lower levels of total and unconjugated bilirubin when compared with non-(H1N1)pdm09 pneumonia patients (0.686 ± 0.33 mg/dL vs. 1.21 ± 0.63 mg/dL, $p=0.02$; 0.36 ± 0.10 mg/dL vs. 0.76 ± 0.48 mg/dL, $p=0.02$, respectively). Influenza A (H1N1)pdm09 patients also had lower levels of serum Ca^{++} and Cl^- when compared with non-(H1N1)pdm09 pneumonia patients (7.87 ± 0.7 mg/dL vs. 8.70 ± 0.66 mg/dL, $p=0.02$; 97.87 ± 4.82 mmol/L vs. 104.42 ± 7.16 mmol/L, $p=0.009$, respectively). Finally, prothrombin times were lower in influenza A (H1N1)pdm09 pneumonia compared with non-(H1N1)pdm09 pneumonia patients (11.37 ± 0.95 sec vs. 13.55 ± 2.74 sec, $p=0.04$, respectively), while partial thromboplastin times were higher with influenza A (H1N1)pdm09 pneumonia compared with non-(H1N1)pdm09 pneumonia patients (35.47 ± 5.74 sec vs. 28.15 ± 4.56 sec, $p=0.009$, respectively; Table 3).

IL-8, IL-10, and IL-17 are slightly more elevated in influenza A (H1N1)pdm09 pneumonia patients than in non-(H1N1)pdm09 pneumonia patients

With regard to the large panel of cytokines tested, there were significant differences at 24 h in IL-8 (13.48 ± 31.75 pg/mL vs. $1,144.74 \pm 2,174.19$ pg/mL, $p=0.02$), IL-10 (11.33 ± 38.87 pg/mL vs. 183.88 ± 363.68 pg/mL, $p=0.03$) and IL-17 (124.62 ± 231.21 pg/mL vs. 370.76 ± 396.88 pg/mL, $p=0.04$) levels when healthy controls were compared with influenza A (H1N1)pdm09 pneumonia patients. No differences were found between non-H1N1pdm09 pneumonia patients and healthy controls (Table 4).

IL-6 levels distinguish between healthy controls and septic patients

IL-6 serum levels distinguished healthy controls (0.19 ± 0.3 ng/mL) from influenza A (H1N1)pdm09 and non-(H1N1)pdm09 pneumonia patients (1.36 ± 2.79 ng/mL and 1.2 ± 2.4 ng/mL, respectively; Table 4). However, elevated IL-6 values in both influenza A (H1N1)pdm09 and non-(H1N1)pdm09 pneumonia patients prevents any distinction between those groups.

TGF- β 1 distinguishes between influenza A (H1N1)pdm09 and non-(H1N1)pdm09 pneumonia and correlates negatively with SOFA

Mean TGF- β levels in healthy controls were 11.25 ± 2.14 ng/mL, while those of influenza A (H1N1)pdm09 patients

TABLE 4. SERUM INFLAMMATORY MEDIATORS, AVERAGE (INTERVAL)

Serum inflammatory mediators (pg/mL)	Healthy	Influenza A (H1N1)pdm09	Other pneumonias	p (Influenza A (H1N1)pdm09 vs. Healthy)	p (Other pneumonias vs. Healthy)	p (Influenza A (H1N1)pdm09 vs. Other pneumonias)	ANOVA
IL-8	13.5 (0–120)	1,144 (0–6,399)	684 (0–4,560)	2.00E-002	5.40E-002	6.10E-001	7.44E-002
IL-10	11.3 (0–166)	183 (0–1,212)	317 (0–2,233)	3.00E-002	7.00E-002	6.20E-001	1.49E-001
IL-17	124.6 (0–800)	350 (4.8–1,269)	154 (0–809)	4.90E-002	7.70E-001	2.40E-001	1.21E-001
IL-6	19.1 (0–96)	1,367 (20–7,147)	1,200 (24–6,869)	3.00E-002	2.80E-002	8.90E-001	9.18E-002
TGF- β	11,253 (6195–1,4148)	5,418 (2,868–9,088)	3,025 (1,980–4,346)	1.13E-008	2.00E-011	4.90E-003	1.47E-013

Statistically significant values are shown in bold. ANOVA, analysis of variance.

FIG. 1. TGF- β levels correlate with Sequential Organ Failure Assessment (SOFA) scale in influenza A (H1N1)pdm09 patients on day of admission.

were 5.41 ± 1.95 ng/mL (vs. healthy, $p < 0.001$; vs. non-(H1N1)pdm09, $p = 0.004$). Non-(H1N1)pdm09 pneumonia patients had the lowest TGF- β levels with a mean of only 3.05 ± 0.82 ng/mL (vs. healthy, $p < 0.001$; Table 4). These lower and abnormal levels of TGF- β were also seen when samples taken 96 hours after admission were analyzed. While lower levels of TGF- β could be detected on admission among influenza A (H1N1)pdm09 patients who subsequently died compared with those who recovered (4.71 ± 1.74 ng/mL vs. 6.26 ± 2.04 ng/mL), this tendency was not statistically significant. It has been reported that platelets are responsible for the high levels of TGF- β sometimes seen in serum samples, and there was no correlation of TGF- β with platelet levels in the present series of cases (data not shown). On the other hand, a negative correlation was found between TGF- β and SOFA clinical score on admission in influenza A (H1N1)pdm09 patients ($p < 0.05$; Fig. 1).

Discussion

Little is known about a specific diagnosis or prognosis biomarker of the influenza A (H1N1)pdm09 pandemic. This study evaluated clinical scores, blood chemistry, and cytokines in the sera of patients with a diagnosis of influenza A (H1N1)pdm09 pandemic or non-influenza A (H1N1)pdm09. Several clinical scores are available to clinicians to evaluate critically ill patients. This study compared their usefulness in evaluating patients presenting to the authors' hospital with clinical manifestations suggestive of influenza infection during the influenza A (H1N1)pdm09 pandemic. Three of the most common clinical scores—CURB-65, APACHE II, and SOFA—were evaluated, all of which have been used to evaluate the severity of viral and bacterial CAP (15,19,31). Recently, studies demonstrated that CURB-65 is not a good predictor of the severity of pneumonia caused by influenza A (H1N1)pdm09 (29,36). In a previous study, the authors demonstrated that the SOFA score was the best severity predictor in CAP cases, including those patients infected with influenza A (H1N1)pdm09 (32), similar to this study. The present study reports that not only did SOFA scores predict the mortality of patients with CAP who

progress to sepsis within 24 h, regardless of etiology, but also several individual components of the SOFA score could distinguish between patients who subsequently died from those who survived. It is important to point out that the degree of pulmonary dysfunction had the most significant relationship with progression to death. It is impossible to know if a more aggressive therapy for those patients would have made any difference in their outcome, but the assessment of lung function upon admission should remain a priority during the admission of patients with CAP.

While both APACHE II and CURB-65 assess respiratory failure, SOFA was still the better tool. The reason for these discrepancies is not clear at the moment, but the inclusion of platelet counts and bilirubin levels within the SOFA score could account for its increased correlation with progression to death, since both of these parameters are excluded from APACHE II and CURB-65.

Surprisingly, influenza A (H1N1)pdm09 patients seemed to share several laboratory findings that distinguish them from patients with other causes of pneumonia. It was found that influenza A (H1N1)pdm09 patients presented both hypocalcemia and hypochloremia. Hypocalcemia has been associated with alkalosis (10), but this was not found to be the case, since blood pH was not different between influenza A (H1N1)pdm09 and non-(H1N1)pdm09 pneumonia patients. Influenza A (H1N1)pdm09 has been associated with rhabdomyolysis (34), which can manifest as hypocalcemia. However, this possibility was not explored in patients in the present study. Hypochloremia in turn has been associated with acidosis and hypoventilation (16), but influenza A (H1N1)pdm09 patients were not found to be hypoventilated when compared with non-H1N1pdm09 pneumonia patients. The strength of the association between hypochloremia and influenza A (H1N1)pdm09 suggests that it might be a component of the disease rather than just a random finding. Steps toward illuminating if this is indeed the case are currently being undertaken in the authors' laboratory.

Hypercytokinemia has been described in patients infected with influenza A virus, and may be associated with fatal infections (3,13). One of the pathogenic mechanisms attributed to influenza A (H1N1)pdm09 is its capacity to unleash what has been named a "cytokine storm" (3,9,37). The present findings partially support this hypothesis, since blood cytokine levels were overall higher in influenza A (H1N1)pdm09 than in non-(H1N1)pdm09 pneumonia patients, as was clearly seen for IL-8, IL-10, and IL-17. These high levels may indicate that the immune system is activated during the early stage of viral infection and leads to an inflammatory environment for viral control. A recent study demonstrated that IL-8 is associated with disease progression among hospitalized patients with influenza A (H1N1)pdm09 (12). The present results are in agreement with another group that reported that IL-10 was found to be higher in influenza A (H1N1)pdm09 patients when compared with other influenza A viruses. However, other influenza viruses in the group of non-(H1N1)pdm09 pneumonia patients were not tested (42,43). IL-17 has been associated with acute lung injury in influenza A (H1N1)pdm09 patients (24). High levels were also detected in patients in the present study. It may be a systemic effect that increases chemokine production and neutrophil recruitment in the lung. IL-6, which has been identified as one of the critical mediators of damage due to the influenza A (H1N1)pdm09 virus (1), was also

significantly elevated in non-(H1N1)pdm09 pneumonia patients. To what extent IL-6 levels are exclusively related to the pathogenesis of influenza A (H1N1)pdm09 must be questioned. Contrary to the present results, a different study did not find differences in cytokine levels when studying sera from 16 hospitalized patients with influenza A (H1N1)pdm09 (41). This difference could be attributed to sample collection dates. In the present study, samples were collected on admission and at day 4, whereas in the above-mentioned study, samples were collected on days 7 and 14.

Finally, TGF- β is an immunoregulatory cytokine that plays an important role during inflammatory processes (4). Low levels of IFN- β and TGF- β in a rich pro-inflammatory environment were found in pregnant woman with severe influenza A (H1N1)pdm09 infection (35); these results are accordance with the present findings. Previous reports demonstrate a role for this cytokine in the morbi-mortality and viral load during influenza A virus infection (5). Another study found that TGF- β blood levels correlated with the etiological agent of CAP (20). The finding that low serum levels of TGF- β correlate with influenza A (H1N1)pdm09 infection has been previously reported for another series, but it was, surprisingly, reported as not significant (27). Platelet levels in the blood are known to correlate with TGF- β when serum samples are obtained by venipuncture (7). This was not found to be the case in the present study, suggesting that the findings are biologically relevant. Contrary to the present findings, a different group has reported overproduction of TGF- β in influenza A (H1N1)pdm09 patients. Whether these differences are due to the genetic background of the studied population or due to other causes is an interesting avenue of research. This is the first study that reports a negative correlation between TGF- β and SOFA score in influenza A (H1N1)pdm09 patients. A possible explanation is that organ failure may be due to high pro-inflammatory cytokine production in response to influenza A (H1N1)pdm09 infection and IL-10 is not enough to control the inflammatory response in those patients.

In conclusion, TGF- β levels are a useful tool aiding in the differentiation of influenza A (H1N1)pdm09 from other causes of pneumonia progressing to sepsis, and it correlates negatively with the SOFA score.

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Author Disclosure Statement

No competing financial interests exist.

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