Experimental and Molecular Pathology 94 (2013) 486–492

Contents lists available at SciVerse ScienceDirect

Experimental and Molecular Pathology

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

Angiogenic and inflammatory markers in acute respiratory distress syndrome and renal injury associated to A/H1N1 virus infection

Edgar Bautista [a,*], Magali Arcos [a], Luís Jimenez-Alvarez [a], Ma. Cecilia García-Sancho [a], María E. Vázquez [a], Erika Peña [a], Anjarath Higuera [a], Gustavo Ramírez [a], Rosario Fernández-Plata [a], Alfredo Cruz-Lagunas [a], Sara A. García-Moreno [a], Francisco Urrea [a], Remedios Ramírez [b], Ricardo Correa-Rotter [c], José Rogelio Pérez-Padilla [a], Joaquín Zúñiga [a,**]

[a] Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Tlalpan 4502, Tlalpan, 14080, Mexico City, Mexico
[b] Facultad de Ciencias, Universidad Nacional Autónoma de México, Av. Universidad 3000, Circuito Exterior S/N, Coyoacán, 04510, Mexico City, Mexico
[c] Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Mexico City, Mexico

ARTICLE INFO

Article history:
Received 6 November 2012
Available online 28 March 2013

Keywords:
Acute kidney failure
A/H1N1
ARDS
Chemokines
MCP-1
VEGF

ABSTRACT

Acute kidney injury (AKI) is often associated to acute respiratory distress syndrome (ARDS) due to influenza A/H1N1 virus infection. The profile of angiogenic and inflammatory factors in ARDS patients may be relevant for AKI. We analyzed the serum levels of several angiogenic factors, cytokines, and chemokines in 32 patients with A/H1N1 virus infection (17 with ARDS/AKI and 15 ARDS patients who did not developed AKI) and in 18 healthy controls. Significantly higher levels of VEGF, MCP-1, IL-6, IL-8 and IP-10 in ARDS/AKI patients were detected. Adjusting by confusing variables, levels of MCP-1 ≥150 pg/mL (OR = 12.0, p = 0.04) and VEGF ≥225 pg/mL (OR = 6.4, p = 0.03) were associated with the development of AKI in ARDS patients. Higher levels of MCP-1 and IP-10 were significantly associated with a higher risk of death in patients with ARDS (hazard ratio (HR) = 10.0, p = 0.02; HR = 25.5, p = 0.03, respectively) even taking into account AKI. Patients with influenza A/H1N1 infection and ARDS/AKI have an over-production of MCP-1, VEGF and IP-10 possibly contributing to kidney injury and are associated to a higher risk of death.

Introduction

During March of 2009, an unusual outbreak of influenza-like illness was observed in Mexico later found to be caused by a new strain of the swine-origin influenza virus, now called pandemic A/H1N1 2009 virus (WHO, 2009). The new virus was able to produce acute respiratory distress syndrome (ARDS) requiring mechanical ventilation, acute kidney injury (AKI), and death (Perez-Padilla et al., 2009). AKI is uncommon in patients with seasonal influenza A virus infection, whereas a high incidence of AKI (33.6%–51%) was reported in patients with severe forms of pandemic A/H1N1 2009 virus infection, and presents a mortality rate between 36.6 and 51% (Nin et al., 2011; Pettilä et al., 2011). The AKI associated to influenza virus infection has been mostly described as being due to rhabdomyolisis (Cunningham et al., 1979). The development of AKI in this subset of patients has been linked to ischemic damage, drug toxicity, and interstitial nephritis amongst others (Chertow et al., 2005). However, excessive immune activation and a dysregulated production of inflammatory mediators, typical of severe disease due to pandemic A/H1N1 virus infection, may contribute to the pathogenesis of early AKI (La Gruta et al., 2007). In addition, angiogenic factors may play an important role in the pathogenesis of AKI and/or in the tissue repair mechanisms induced by ischemia associated AKI (Villanueva et al., 2006), but it is unclear if they also contribute to the development of AKI in ARDS patients due to the pandemic A/H1N1 virus infection.

In the present study, we compared the concentration of angiogenic factors as well as pro-inflammatory cytokines and chemokines in blood from patients with ARDS with and without AKI.

Materials and methods

Subjects

A total group of 32 patients with ARDS associated to pandemic A/H1N1 influenza virus infection were studied. The diagnosis of ARDS was based on standard definitions (Bernard et al., 1994) and the patients were classified in those who developed AKI (ARDS/AKI: N = 17), and who did not develop AKI (ARDS: N = 15). We excluded patients who developed renal failure after 3 days of admission to reduce the risk

* Correspondence to: E. Bautista, Intensive Care Unit, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico.
** Correspondence to: J. Zúñiga, Department of Immunology, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Tlalpan 4502, Tlalpan 14080, Mexico City, Mexico.
E-mail addresses: drbautista40@iner.gob.mx (E. Bautista), joazu@yahoo.com (J. Zúñiga).

0014-4800/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yexmp.2013.03.007

of including patients with late renal failure, which could be attributed to other pathogenic mechanisms. The diagnosis of renal failure was established according to the Acute Kidney Injury Network (AKIN) criteria: 1) Rapid time course (less than 48 h), 2) Reduction of kidney function, 3) Rise in serum creatinine, 4) Absolute increase in serum creatinine of ≥0.3 mg/dl (≥26.4 μmol/l), 5) Percentage increase in serum creatinine of ≥50%, and 6) Reduction in urine output, defined as <0.5 ml/kg/h for more than 6 h.

We simultaneously recruited a control group of 18 asymptomatic healthy controls (mean age ± SD 35 ± 13 years, 8 males and 10 females) that were in close contact with A/H1N1-confirmed critical patients but did not developed severe respiratory acute disease. Their serum samples were obtained at the same time as the critical patients' samples.

The Institutional Review Board of the National Institute of Respiratory Diseases (INER) reviewed and approved the protocol for biomarker studies (Protocol number B27-10) under which all subjects were recruited in accordance with the ethical standards of the Declaration of Helsinki in 1964. All subjects provided written informed consent for these studies, and they authorized the storage of their serum and plasma samples at INER repositories for this and future studies. After obtaining the signed informed consent letters from patients and controls, serum samples were obtained.

A/H1N1 virus detection

Nasopharyngeal swab samples were obtained from hospitalized patients and processed at the INER Microbiology Laboratory for RNA isolation using the viral RNA mini kit (Qiagen Westburg, Leusden, The Netherlands). Detection of influenza A/H1N1 virus in respiratory specimens was assessed by real time RT-PCR according to the CDC and World Health Organization guidelines.

Measurement of angiogenic factors, cytokines, and chemokines

A set of various angiogenic factors, vascular endothelial growth factor A (VEGF-A), fibroblast growth factor 2 (FGF-2), platelet derived growth factor (PDGF), monocyte chemotactic protein-1 (MCP-1), and plasminogen activator inhibitor-1 (PAI-1) as well as pro-inflammatory cytokines/chemokines (IL-6, IL-8, IFN-γ, IP-10 and TNF-α) were measured in serum samples obtained from all studied patients and controls by Luminex (*Bio-Rad Laboratories, Inc., CA, USA*). The concentrations of angiogenic factors, cytokines, and chemokines, were calculated using Bio-Plex v 4.1 software (*Bio-Rad Laboratories, Inc., Hercules, CA, USA*).

Muscle biopsies

Muscle samples were obtained from the quadriceps (vastus lateralis) by open muscle biopsy from AKI patients and stained with hematoxylin and eosin (HE) and Masson's trichrome stains, and examined by light microscopy to rule out muscle destruction and rhabdomyolysis.

VEGF determination by immunohistochemistry

Renal sections from deceased patients who developed AKI associated to severe pneumonia by the A/H1N1 infection and from control individuals without renal disease, were obtained from the pathology tissue bank of the INER. Fixed tissue sections were treated as previously described (Selman et al., 2000). Antigen retrieval was carried out in citrate buffer 10 mM, pH 6.0 for 6 min in a microwave. Slides were incubated with anti-VEGF monoclonal antibody (5 μg/ml; ab-46154; Abcam, Cambridge, UK) at 4 °C overnight and then with a secondary biotinylated anti-immunoglobulin antibody followed by horseradish peroxidase-conjugated streptavidin (BioGenex, San Ramon, CA, USA). They were used according to the manufacturer instructions, using 3-amino-9-ethyl-carbazole (AEC, BioGenex) as a substrate in acetate buffer containing 0.05% H_2O_2. The tissue sections were counterstained with hematoxylin. The primary antibody was replaced by non-immune serum for negative controls.

Data collection and statistical analysis

Demographic characteristics, vital signs, clinical outcomes, treatments, mechanical ventilation and gas exchange parameters, laboratory tests, and APACHE II scores were determined at days 1, 2, and 3 after the admission to the ICU. All measures summarized were then expressed as means and standard deviation, or as frequencies and proportions. Comparisons of the continuous variables among groups were performed using the Mann–Whitney–Wilcoxon test, whereas the X^2 test was used to compare frequencies. Cox logistic regression models were constructed to explore the factors associated with death due to ARDS/AKI. The independent variables tested in the models were: demographic and anthropometric data, lung function, laboratory findings, and comorbid conditions. Any variables that proved significant in the univariate analysis were included in the multivariate models. The significance level was set at $p < 0.05$. All analyses were performed using a commercially available statistical package (Stata v. 10.0 StataCorp, College Station, TX, USA). Differences in the serum levels of cytokines/chemokines/growth factors between controls, ARDS/AKI, and ARDS patients were evaluated by the Mann–Whitney–Wilcoxon test, and graphics were generated using the Graph Pad Prism software version 5.04 (Graph Pad Software, La Jolla, CA).

Results

The clinical and demographic characteristics of the ARDS/AKI and ARDS patients are presented in Table 1. All patients required

Table 1
Demographic and clinical characteristics of patients with ARDS-AKI and ARDS associated to A/H1N1 infection.

Variable	ARDS/AKI (N = 17)	ARDS (N = 15)	P[a]
Age years	41.4 (±11.8)	39.6 (±11.5)	0.7
Male (%)	13/17 (76.5)	9/15 (60)	0.3
Obesity (BMI ≥ 30 kg/m^2)	13/17 (76.5)	7/15 (46.7)	0.08
Oseltamivir delay (days)	10.5 (±4.9)	8.8 (±2.5)	0.3
Mechanical ventilation (days)	14.2 (±5.8)	16.1 (±9.1)	0.5
APACHE II score	21.4 (±5.3)	18.1 (±2.8)	0.05
Hospitalization (days)	22 (±8.3)	28.7 (±16.1)	0.1
Co-morbidities (%)	13/17 (76.5)	5/15 (80)	0.8
Current smoking (%)	10/17 (58.8)	6/15 (40)	0.3
High blood pressure (%)	6/17 (35.3)	3/15 (20)	0.3
Prior corticosteroid treatment (%)	1/17 (6)	1/15 (6.7)	0.9
Deaths (%)	6/17 (35.2)	1/15 (0.06)	0.3
Laboratory findings			
Platelets (x10^3)	134 (±470)	169 (±78)	0.1
Glucose (mg/dL)	154.5 (±52.5)	134.5 (±43.19)	0.07
Creatinine (mg/dL)	1.4 (±0.7)	0.8 (±0.2)	0.03
Albumine (gr/dl)	3.4 (±0.4)	2.9 (±0.6)	0.04
Diuresis (L/day)	1.2 (±0.8)	3.5 (±2.5)	0.003
BUN (mg/dl)	19.6 (±12)	15.1 (±4.9)	0.2
LDH (IU/L)	1553 (±2420)	877 (±399)	0.5
CPK (IU/L)	1085 (±1942)	331 (±370)	0.07
Na (mEq/L)	137 (±4.3)	139 (±4)	0.09
PaO2 (Torr)	66.8 (±23.4)	69.1 (±31.3)	0.8
PaCO2 (Torr)	32.1 (±9.7)	34.4 (±12.4)	0.6
PaO2/FiO2 (Torr)	214 (±161)	199 (±135)	0.8
PEEP (cm H20)	14.9 (±5.7)	13.3 (±4.2)	0.3
Angiogenic/inflammatory factors			
VEGF (≥225 pg/mL)	12/17 (70.6)	4/15 (26.7)	0.01
MCP-1 (≥150 pg/mL)	8/17 (47.1)	1/15 (6.7)	0.01
IL-6 (≥50 pg/mL)	11/17 (64.7)	4/15 (26.7)	0.03
IL-8 (≥40 pg/mL)	11/17 (64.7)	3/15 (20)	0.01
IP-10 (≥6000 pg/mL)	13/17 (76.5)	6/15 (40)	0.03
IFN-γ (≥100 pg/mL)	4/17 (23.5)	11/15 (73.3)	0.005

ARDS: Acute respiratory distress syndrome; AKI: acute kidney injury. Data are means ± standard deviation, or number and percentage.

[a] Comparisons of the continuous variables among groups were performed using the Mann–Whitney–Wilcoxon test, whereas the X^2 test was used to compare frequencies.