Cytokine 73 (2015) 163–168

Contents lists available at ScienceDirect

Cytokine

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

Angiopoietin-2 associations with the underlying infection and sepsis severity

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ARTICLE INFO

Article history:
Received 8 June 2014
Received in revised form 17 January 2015
Accepted 23 January 2015
Available online 6 March 2015

Keywords:
Angiopoietin-2
Sepsis
Infection
Organ failure

ABSTRACT

Angiopoietin-2 (Ang-2) is an important mediator in sepsis. We have previously shown that endotoxemia levels are related to the underlying infection and affect septic patients' outcome. Based on this background we now investigated if circulating Ang-2 (cAng-2) and monocyte *Ang-2* expression in septic patients are associated with the underlying infection and organ failure. We measured cAng-2 in 288 septic patients (121 with sepsis, 167 with severe sepsis/septic shock) at less than 24 h post study inclusion (day 1) and on days 3 and 7. Peripheral blood mononuclear cells (PBMCs) were additionally isolated; *Ang-2* gene expression was estimated by means of real-time PCR. Levels of cAng-2 were higher under severe sepsis and septic shock, as compared to uncomplicated sepsis; PBMC *Ang-2* copies were higher in severe sepsis. On day 1, cAng-2 and *Ang-2* gene copies were greater under severe sepsis/septic shock in sufferers from all types of infections with the exception of community-acquired pneumonia and ventilator-associated pneumonia. cAng-2 increased proportionally to the number of failing organs, and was higher under metabolic acidosis and acute coagulopathy as compared to no failing organ. On day 1, copies of *Ang-2* were higher in survivors, whereas cAng-2 was higher in non-survivors. In a large cohort of septic patients, cAng-2 kinetics appears associated with the underlying infection and organ failure type.

1. Introduction

Despite progress in medicine and the use of intensive care units (ICUs), morbidity and mortality of sepsis remain high [1]. Microvascular endothelial dysfunction is central to pathogenesis where numerous endothelial-derived and/or endothelial-affecting mediators contribute to the inflammatory pathophysiology [2]. Among such mediators, angiopoietins (Angs) are recognized to play a substantial role in the inflammatory process [3,4]. Ang-1 maintains an anti-inflammatory role, leading to vessel stabilization whereas Ang-2 promotes inflammation and contributes to vascular integrity loss [5,6].

Various studies by us and others have shown that circulating Ang-2 (cAng-2) is increased in severe sepsis/septic shock [7–12]. In addition our group has recently provided evidence that endothelial cells (EC) are not the only source of Ang-2 during the septic process [7]; its expression was detected in the supernatants of cultured circulating monocytes from septic patients suffering from ventilator-associated pneumonia (VAP), providing thus evidence that Ang-2 is partly secreted by monocytes as well [7]. More recent work has provided evidence for an anti-inflammatory-protective role of Ang-2; in a murine septic model pre-treatment with Ang-2 prolonged survival from lethal infection by *Pseudomonas aeruginosa* [13].

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http://dx.doi.org/10.1016/j.cyto.2015.01.022
1043-4666/

Recent studies of the Hellenic Sepsis Study Group conducted in large cohorts of patients [14–16] provided evidence that early alterations of the innate and adaptive immune status in sepsis are present and associated with the type of underlying infection, underscoring a pivotal role of the type of infection in the personalized nature of sepsis pathogenesis. In this respect Kritselis and coworkers have shown that kinetics of circulating lipopolysaccharide (LPS) differ according to the type of underlying infection and are associated with final outcome [16]. Based on this background, and the fact that Ang-2 is synthesized by monocytes and it is associated to LPS kinetics, we hypothesized that cAng-2 and its monocyte gene expression in sepsis shall differ in relation to the underlying infection and disease severity. To test our hypothesis, we quantified Ang-2 in a cohort of 288 septic patients, and investigated Ang-2 relationships with the underlying type of infection, disease severity, type of organ dysfunction and final outcome.

2. Patients and methods

2.1. Study design

This multi-center study was conducted in nine hospitals participating in the Hellenic Sepsis Study Group. The study protocol was reviewed and approved by the Ethics committees of all hospitals of the participating study sites. Written consent was provided from all patients or next-to-kin for patients unable to consent. Every patient was enrolled once in the study.

Inclusion criteria were: (a) age $\geqslant$ 18 years; (b) diagnosis of one of the following infections: community-acquired pneumonia (CAP), acute pyelonephritis (UTI), intraabdominal infection (IAI), primary Gram-negative bacteremia (BSI) and ventilator-associated pneumonia (VAP) defined by international accepted definitions [17–19]; (c) at least two signs of the systemic inflammatory response syndrome (SIRS) [20]; and (d) first blood sampling within the first 24 h from the first signs of SIRS.

Exclusion criteria were: (a) HIV infection; (b) neutropenia, defined as less than 1000 neutrophils/mm^3; and (c) chronic intake of corticosteroids defined as any daily oral intake of 1 mg/kg or more of equivalent prednisone for more than one month.

Patients were classified according to the criteria of the ACCP/SCCM as suffering from uncomplicated sepsis, severe sepsis or septic shock [20]. Organ failures were defined as follows: (i) Acute Respiratory Distress Syndrome (ARDS), as any value of PO_2/FiO_2 below 200 with the presence of diffuse infiltrates in chest X-ray; (ii) acute renal dysfunction, as the production of less than 0.5 ml/kg body weight/h of urine for at least 2 h provided that the negative fluid balance of the patient was corrected; (iii) metabolic acidosis as any pH < 7.30 or any base deficit greater than 5 mEq/l and serum lactate at least more than 2$\times$ upper normal limit; (iv) acute coagulopathy as any platelet count < 100,000/mm^3 and /or international normalized ratio (INR) > 1.5.

Blood sampling was started on the first day of sepsis i.e. within the first 24 h from start of signs of SIRS and repeated on days 3 and 7 of hospitalization. 14 ml of blood were sampled after venipuncture of one forearm vein under aseptic conditions on days 1 and 3, and 3 ml on day 7. From the amount of blood collected on days 1 and 3 10 ml were collected into one heparin-coated tube for isolation of PBMCs; and (b) 4 ml were collected into one pyrogen-free tube. The tube was centrifuged and serum was stored at −80 °C until assayed for Ang-2. Demographic, clinical and laboratory data were recorded on study enrolment. Severity of illness was assessed by calculating Acute Physiology and Chronic Health Evaluation (APACHE) II score at study enrolment. Outcome was assessed at 28 days.

2.2. Laboratory techniques

Concentrations of Ang-1 of day 1 and of Ang-2 of all days of sampling in sera were estimated in duplicate by an enzyme immunosorbent assay (R&D Minneapolis Mo). The lower limit of detection was 15 pg/ml. For the measurement of LPS of day 1, serum was diluted 1:10 with pyrogen-free water (BioWhittaker, Maryland, USA) and incubated for five minutes at 70 °C. LPS was

Table 1
Demographic and clinical characteristics of patients enrolled in the study.

	Uncomplicated sepsis	Severe sepsis/shock	*p*
Number	121	167	
Male/female	65/56	89/78	0.177
Age (years, mean ± SD)	60.8 ± 22.2	69.7 ± 16.6	<0.0001
White blood cell count (/mm^3, mean ± SD)	13818.5 ± 5959.5	15048.1 ± 7996.1	0.170
APACHE II score (mean ± SD)	11.60 ± 6.15	21.17 ± 9.43	<0.0001
Type of infection (number, %)			
Acute pyelonephritis	31 (25.6)	24 (14.4)	0.035
Intraabdominal infection	29 (24.0)	47 (28.1)	
Primary bacteremia	11 (9.1)	25 (15.0)	
Community-acquired pneumonia	35 (28.9)	35 (21.0)	
Ventilator-associated pneumonia	15 (12.4)	36 (21.6)	
Type of intraabdominal infection			
Acute peritonitis	7	13	
Acute cholangitis	7	13	
Acute cholecystitis	5	14	0.196
Acute diverticulitis	5	4	
Liver abscess	5	3	
Isolated pathogens (number, %)			
Escherichia coli	21 (16.4)	13 (8.2)	
Klebsiella pneumoniae	13 (10.2)	19 (11.8)	
Pseudomonas aeruginosa	5 (3.9)	7 (3.4)	
Proteus mirabilis	0 (0)	3 (1.8)	
Acinetobacter baumannii	5 (3.9)	9 (5.6)	0.130
Other Gram-negatives	4 (3.2)	6 (3.7)	
Staphylococcus aureus	9 (0)	2 (1.2)	
Enterococcus faecalis	1 (0.8)	6 (3.8)	