Usefulness of the endotoxin activity assay to evaluate the degree of lung injury

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Abstract

A major problem of the techniques used for quantifying endotoxin levels has been their low sensitivity. To address this problem, a diagnostic kit called Endotoxin Activity Assay (EAA^{TM}) was developed. The degree of lung injury must be determined when formulating treatment strategies for patients with sepsis and prognosticating the outcome of this condition. Based on the observations described above, we hypothesize that endotoxin can serve as an important biomarker in evaluating lung injury. Currently, lung injuries in patients with septic shock or ARDS can be assessed by cardiorespiratory monitoring. One such monitoring system is called Pulse index Continuous Cardiac Output (PiCCO). It measures cardiac output (CO) using a thermodilution technique that employs a cold thermal indicator. It then calculates CO per beat using pulse contour analysis. Importantly, PiCCO can also measure extravascular lung water (EVLW) and the pulmonary vascular permeability index (PVPI) that reflect the severity of lung injury. PVPI was significantly higher in patients with high EAA levels than in those with normal EAA levels (3.55 ± 0.48 vs. 1.99 ± 0.68, $p = 0.0029$). In addition, the patient group with high PCT levels showed significantly lower cardiac indices than the group with normal PCT levels (3.40 ± 1.05 vs. 4.80 ± 0.39, $p = 0.0325$). The results described above indicate that the EAA level is closely correlated with the degree of lung injury assessed by the PiCCO monitor. This suggests that EAA could also be used as a valuable tool in monitoring lung injury.

Key Words

Endotoxin Activity Assay (EAA^{TM}), Pulse index Continuous Cardiac Output (PiCCO), lung injury

The current concept of acute respiratory distress syndrome (ARDS) is "a pathological response of the lung to an insult". The basis for this concept was first established in a landmark case report published by Ashbaugh et al in 1967 (1). This article recognized that ARDS is a group of related pathological abnormalities in the lung initiated by a wide variety of different insults, such as sepsis, trauma and aspiration of gastric contents. The initial description of ARDS was not specific enough to distinguish it from other lung diseases. Nevertheless, it served as a guiding principle for the diagnosis of this syndrome. In 1988, Murray et al proposed a more precise definition of ARDS using a lung injury scoring system (2). However, this definition was not practical enough to be accepted globally. It was not until 1994 that the American-European Consensus Conference (AECC) on ARDS formulated the first clear definition of the syndrome to be adopted internationally (3). The Committee recommended that ARDS be defined as "a syndrome of inflammation and increased permeability of the pulmonary capillaries caused by an insult(s)". In addition, the severity of hypoxemia necessary to make the diagnosis of ARDS was defined by the ratio of the partial pressure of oxygen in the patient's arterial blood (PaO_2) to the fraction of oxygen in the inspired air (FiO_2). ARDS was defined as a PaO_2/FiO_2 ratio of less than 200. When the impairment of oxygenation was less severe (i.e., PaO_2/FiO_2 201 - 300), the syndrome was classified as acute lung injury (ALI). This definition of ARDS remained unchanged until 2012, when it was further refined and given the new name "Berlin definition." In the new definition, ARDS was classified into the following three categories based on the degree of oxygenation (PaO_2/FiO_2 ratio): mild (PaO_2/FiO_2 201-300), moderate (101-200) and severe (≤100). Another major change in the Berlin definition was that the term "ALI" was abandoned, thus eliminating the confusion derived from the previous AECC criteria (4).

ARDS as pulmonary damage

Pathologically, diffuse alveolar damage (DAD) is the hallmark of ARDS. However, among patients who met the Berlin definition of ARDS, as few as 12% of patients with mild ARDS had DAD, as did only 58% of patients with severe ARDS (5). This indicates the limitations of the Berlin definition in predicting specific pathologic

events in the lung. Irrespective of the definition of ARDS, it is critical for health care professionals to make an accurate diagnosis of the syndrome and initiate treatments that will improve clinical outcomes. One of the most important modalities in diagnosing ARDS is imaging, such as computed tomography (CT) of the chest. On CT scans, asymmetrical consolidation can be observed in the gravity-dependent, high-blood-flow regions of the lung. Importantly, the diffusion level of heterogeneous opacities observed by CT scans is correlated with the severity of ARDS and the volume of the physiologic dead space. Thus, the imaging results are also correlated with the outcome of patients with this syndrome (6). In addition, studies have shown the potential of imaging in identifying pathological features of DAD (7). Therefore, imaging is useful in evaluating the effects of ARDS treatments and prognosticating the outcome of the syndrome.

Objective diagnosis of pulmonary damage

Right ventricular dysfunction and pulmonary hypertension are reportedly correlated with poor outcome of ARDS. These observations suggest that novel treatment strategies (for example, mechanical ventilation and pharmacological interventions) targeting these abnormalities could improve the outcome of ARDS. Thus, these observations can be considered as an objective, though supportive, diagnosis that directly leads to important clinical management decisions (8,9).

Major approaches for objectively investigating the pathogenesis of ARDS include the analysis of blood and bronchoalveolar lavage fluid samples. Studies utilizing these approaches have reported that the pathogenesis is associated with alterations in surfactant function, increases in proinflammatory cytokine levels, and damage to the endothelial surfaces of the lung. It should be noted that some researchers analyzed multiple biomarkers simultaneously to improve the accuracy of their diagnostic capabilities (10,11,12). In this article, we will discuss the usefulness of endotoxin activity assays in evaluating lung injury such as sepsis. Analysis of the levels of two biomarkers, i.e., endotoxin and neutrophil gelatinase-associated lipocalin, has been reported to increase the sensitivity and specificity for the diagnosis of sepsis-induced acute kidney injury (13).

Sepsis and endotoxin

A recent study reported that the annual incidence of sepsis in Western countries is 377 cases per 100,000 population. This is much higher than the annual rates of 223 cases for stroke and 208 cases for heart attacks (14). In the United States, about 200,000 people die of sepsis every year. Over the past decade, the number of patients with sepsis increased by 8 - 13% each year (15). Possible reasons for this increase include population aging, multidrug-resistant organisms, malnutrition, poverty and shortage of vaccines. The increase in health care costs is another major factor.

Despite enormous efforts in developing novel compounds for the treatment of sepsis, no specific therapeutic agent is currently approved for this medical condition in Japan. Activated protein C was once widely accepted as a therapy for sepsis. However, the use of this agent is not recommended in the present guidelines for the management of sepsis. In a review article, leading intensive-care physicians from many different Western countries recommended a select number of therapeutic agents and strategies for the treatment of sepsis. These included statins, low-dose steroids and genetically- engineered thrombomodulin. Another promising treatment modality described in this article was an endotoxin adsorption column called PMX (16). Endotoxin is one of the principal components of the outer membrane of Gram-negative bacteria. It is the most prominent alarm molecule sensed by the host's innate immune system following invasion by the bacteria. PMX removes endotoxin by adsorption from the circulating blood, thus preventing the onset and progression of sepsis (17).

Endotoxin and clinical treatment

Toraymyxin^{TM}, a PMX cartridge for hemoperfusion, was developed in Japan. It is comprised of polymyxin B covalently linked to the surface of chemically modified sea-island-type composite fibers. Polymyxin B is an antibiotic that binds endotoxin with high affinity. The use of fibrous materials made it possible to increase the adsorbing surface area. It also allowed for the construction of a column that exhibits a low blood pressure drop in the blood flow compartment. Animal studies showed favorable biocompatibility of Toraymyxin. They also demonstrated improved survival in animals treated with this device. These results suggested the potential of Toraymyxin for clinical application (18).

In Japan, PMX has been clinically used in patients since August 1994. PMX was used in approximately 1,000 cases during the first year, but two decades later, in 2013, the use of PMX has increased ten-fold. The cartridge is mainly used in intensive care units (ICUs), emergency medical centers and dialysis centers. PMX is most effective in treating septic shock from abdominal infections, such as diffuse peritonitis resulting from lower gastrointestinal tract perforation. However, it is also used for the treatment of severe sepsis and septic shock from respiratory tract infections (such as pneumonia), urinary tract infections and soft tissue infections. Toraymyxin was certified as being in compliance with the requirements of the European Medical Device Directives and obtained a CE mark in 1998. In 2003, clinical application of this cartridge began in Italy. Since then, its clinical use has expanded to several other European (such as Spain, Russia and Switzerland) and Asian (such as India and Taiwan) countries. In countries outside of Japan, similar endotoxin adsorption columns have been developed and marketed. Furthermore, different types of endotoxin removal devices have also been invented. Thus, endotoxin is now universally accepted as a promising target for the treatment of sepsis (19).

Measurement of endotoxin levels

A major problem of the techniques used for quantifying endotoxin levels has been their low sensitivity. To address this problem, a diagnostic kit called Endotoxin Activity Assay (EAA^{TM}) was developed. In EAA, a monoclonal antibody against lipopolysaccharide (LPS) makes immune complexes with endotoxin in the whole blood. Upon opsonization of the complexes with complement, they are phagocytosed by neutrophils in the blood, leading to the production of reactive oxygen species (ROS). The production of ROS is enhanced by internalization of zymosan, which is included in the reagents. The ROS then oxidize luminol in the reagents, resulting in light emission. Because of this chemiluminescence-based immunoassay platform, EAA shows a higher sensitivity than other endotoxin assay protocols (20).

EAA and pulmonary damage (21)

The degree of lung injury must be determined when formulating treatment strategies for patients with sepsis and prognosticating the outcome of this condition. Based on the observations

described above, we hypothesize that endotoxin can serve as an important biomarker in evaluating lung injury. (We expect that the roles of endotoxin in the pathogenesis of sepsis will be the focus of future studies, as LPS has been reported to stimulate the innate immune response even without binding to toll-like receptor (4). Currently, lung injuries in patients with septic shock or ARDS can be assessed by cardiorespiratory monitoring. One such monitoring system is called Pulse index Continuous Cardiac Output (PiCCO). It measures cardiac output (CO) using a thermodilution technique that employs a cold thermal indicator. It then calculates CO per beat using pulse contour analysis. Importantly, PiCCO can also measure extravascular lung water (EVLW) and the pulmonary vascular permeability index (PVPI) that reflect the severity of lung injury (22). Using this system, we monitored cardiorespiratory dynamics in 11 critically ill patients who required a respirator in our ICU. In the same patients, we also measured the levels of endotoxin (EAA units) and the inflammatory markers C-reactive protein (CRP) and procalcitonin (PCT). Here, we analyzed the potential correlation between cardiorespiratory dynamics and these biomarkers. The results indicated that patients with high EVLW values might exhibit higher levels of EAA than those with normal EVLW values (0.46 ± 0.20 vs. 0.21 ± 0.19), although the difference was not statistically significant ($p = 0.0664$). Similarly, the PCT level might be higher in patients with high PVPI compared with those with normal PVPI (18.9 ± 21.8 vs. 2.4 ± 2.2, $p = 0.0676$). In contrast with these observations, PVPI was significantly higher in patients with high EAA levels than in those with normal EAA levels (3.55 ± 0.48 vs. 1.99 ± 0.68, $p = 0.0029$). In addition, the patient group with high PCT levels showed significantly lower cardiac indices than the group with normal PCT levels (3.40 ± 1.05 vs. 4.80 ± 0.39, $p = 0.0325$).

EAA is designed to measure the systemic level of endotoxin using whole blood. Although there is a possibility that activated neutrophils in the blood may reduce the sensitivity and specificity of the assay, clinical studies have so far demonstrated that it can be effectively used for evaluating the medical condition of patients with sepsis. The results described above indicate that the EAA level is closely correlated with the degree of lung injury assessed by the PiCCO monitor. This suggests that EAA could also be used as a valuable tool in monitoring lung injury.

Further studies will be necessary to determine whether EAA can provide useful information in diagnosing the severity of ARDS and tailoring treatment strategies for ARDS.

We investigated the effects of direct hemoperfusion with polymyxin Bimmobilized fibers (PMX-DHP) on respiratory impairment in endotoxemic pigs. The pigs received intravenous infusions of live Escherichia coli (LD50). Thirteen pigs were assigned to either the PMXDHP group (n=6) or the control group (n=7). In addition, all pigs underwent hemodynamic monitoring with the PiCCO system. The PiCCO system is a less invasive type of advanced hemodynamic monitoring that employs transpulmonary thermodilution and an arterial pulse contour analysis. By using the PiCCO system, it is easy to evaluate lung edema, as well as the cardiac output, cardiac function, and cardiac load. In the PMX-DHP group EAA increased 30 minutes after endotoxin administration, gradually decreased, and was significantly reduced after 210 minutes. Examination with CT showed that pulmonary edema gradually worsened after the administration of endotoxin and Pulmonary edema showed improvements only in the PMX-DHP group and progressed more in the control group than in the PMX-DHP group (23). This animal examination date suggests that EAA could also be used as a valuable tool in monitoring lung injury too.

References

(1) Ashbaugh DG, Bigelow DB, Petty TL, et al. Acute respiratory distress in adults. Lancet 1967; 2: 319-23.

(2) Murray JF, Matthay MA, Luce JM, et al. An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 1988; 138: 720-3

(3) Bernard GR, Artigas A, Brigham KL, et al. Report of the American-European Consensus conference on acute respiratory distress syndrome: definitions, mechanisms, relevant outcomes, and clinical trial coordination. Consensus Committee. J Crit Care 1994; 9: 72-81

(4) ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012; 307: 2526-33.

(5) Thille AW, Esteban A, Fernandez-Segoviano P, et al. Comparison of the Berlin definition for acute respiratory distress syndrome with autopsy. Am J Respir Crit Care Med 2013; 187: 761-7

(6) Cressoni M, Cadringher P, Chiurazzi C, et al. Lung inhomogeneity in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 2014; 189: 149-58.

(7) Ichikado K. High-resolution computed tomography findings of acute respiratory distress syndrome, acute interstitial pneumonia, and acute exacerbation of idiopathic pulmonary fibrosis. Semin Ultrasound CT MR 2014; 35: 39-46

(8) Boissier F, Katsahian S, Razazi K, et al. Prevalence and prognosis of corpulmonale during protective ventilation for acute respiratory distress syndrome. Intensive Care Med 2013; 39: 1725-33.

(9) National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network, Wiedemann HP, Wheeler AP, et al. Comparison of two fluid-management strategies in acute lung injury. N Engl J Med 2006; 354: 2564-75.

(10) Binnie A, Tsang JL, dos Santos CC. Biomarkers in acute respiratory distress syndrome. Curr Opin Crit Care 2014; 20: 47-55.

(11) Bhargava M, Wendt CH. Biomarkers in acute lung injury. Transl Res 2012; 159: 205-17.

(12) Barnett N, Ware LB. Biomarkers in acute lung injury-marking forward progress. Crit Care Clin 2011; 27: 661-83.

(13) Katagiri D, Doi K, Matsubara T, et al. New biomarker panel of plasma neutrophil gelatinase-associated lipocalin and endotoxin activity assay for detecting sepsis in acute kidney injury. J Crit Care 2013; 28: 564-70.

(14) World Sepsis Day Head Office Global Sepsis Alliance Center for Sepsis Control & Care Erlanger Allee 101 07747 Jena Germany T +49 3641 9323101 F +49 3641 9323102 E office@world-sepsis-day.org www.world-sepsis-day.org

(15) Vin Vincent JL, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006; 34: 344-53.

(16) Opal SM, Dellinger RP, Vincent JL, et al. The next generation of sepsis clinical trial designs: what is next after the demise of recombinant human activated protein C?*. Crit Care Med. 2014; 42: 1714-21.

(17) Cutuli SL, Artigas A, Fumagalli R, et al. Polymyxin-B hemoperfusion in septic patients: analysis of a multicenter registry. Ann Intensive Care. 2016: 77. doi: 10.1186/s13613-016-0178-9. Epub 2016 Aug 8.

(18) Hanasawa K1, Aoki H, Yoshioka T, et al. Novel mechanical assistance in the treatment of endotoxic and septicemic shock. ASAIO Trans. 1989; 35: 341-3.

(19) Ronco C, Klein DJ. Polymyxin B hemoperfusion: a mechanistic perspective. Crit Care. 2014; 18: 309. doi: 10.1186/cc13912.

(20) Romaschin AD, Klein DJ, Marshall JC. Bench-to-bedside review: Clinical experience with the endotoxin activity assay. Crit Care. 2012; 16: 248. doi: 10.1186/cc11495.

(21) Sakamoto Y, Inoue S, Iwamura T, et al. Usefulness of the endotoxin activity assay to evaluate the degree of lung injury. Yonsei Med J. 2014 ;55 :975-9.

(22) Ritter S, Rudiger A, Maggiorini M. Transpulmonary thermodilution-derived cardiac function index identifies cardiac dysfunction in acute heart failure and septic patients: an observational study. Crit Care 2009;13:R133.

(23) Koami H, Sakamoto Y, Miyasho T, et al. The effects of polymyxin B-immobilized fiber hemoperfusion on respiratory impairment in endotoxemic pigs. J Nippon Med Sch 2014; 81: 130-8.

