Sepsis Markers

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A novel method for the rapid detection of human endotoxaemia

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Sepsis is the most common cause of death in non-cardiac intensive care units (ICUs), and is the 12th leading cause of death overall. Progression of sepsis can lead to severe sepsis characterised as sepsis with organ dysfunction and shock. Endotoxin is the primary Gram-negative bacterial product responsible for sepsis. The methodology of an Endotoxin Activity Assay (EAA) used to measure endotoxin in whole blood is described. In addition, with the help of results from a multi-national, multi-centre clinical study, this article shows the clinical utility of the EAA in the risk assessment for sepsis. Accurate and timely diagnosis is essential for the clinician to initiate appropriate therapy and improve patient outcomes.

Endotoxin or lipopolysaccharide (LPS) is a major component of the cell wall of Gram-negative bacteria and is a potent trigger for the release of host-derived inflammatory mediators including proteins, free radicals and lipids that drive the systemic inflammatory response [1 -3]. Endotoxin causes a shock-like state with hypotension and organ dysfunction when injected into animals [4] and has predictable effects in healthy humans [5]. A single controlled intravenous bolus of *E. coli* endotoxin evokes many of the same responses (malaise, headaches, nausea, and vomiting) and clinical signs such as tachycardia, tachypnea, leukocytosis and hypotension) that characterise septic shock. In a report of a patient who self-administered a single large dose of endotoxin (1 mg *Salmonella minnesota* endotoxin) the full clinical manifestation of septic shock syndrome developed. Moreover, it was endotoxin alone, in the absence of an ongoing infection that produced an inflammatory response that lasted for days [6].

A considerable body of evidence has accumulated that implicates endotoxin as a principal initiator in the pathogenesis of sepsis. This has raised interest in the possibility of measuring endotoxin as a risk marker of sepsis. Endotoxin assays in the past have not been optimal for the application to clinical testing and thus assessments of the association between endotoxemia and sepsis have yielded conflicting results.

Methods to measure endotoxin have evolved over the last 50 years from a rabbit pyrogen test (1940s) to the Limulus Amebocyte Assay (LAL) (1970s). For measuring endotoxin, standardised Endotoxin Units (EU), rather than units of weight were developed by the United States Food and Drug Administration (FDA). The FDA determined that for measurements of endotoxin, EU/ml should be used to standardise the differences observed in the potency of endotoxin for causing pyrogenic effects. This variation in potency is caused by differences in endotoxin purity, polysaccharide side chain length, minor structural variations in the lipid A moiety and the physical state of endotoxin in biological fluids. The effectiveness of the LAL assay, however, in human blood and blood plasma has been controversial and problematic due to numerous interfering reactions [7] and variations in methodology and reagent source. To date, no FDA approved LAL assay exists for human diagnostic applications.

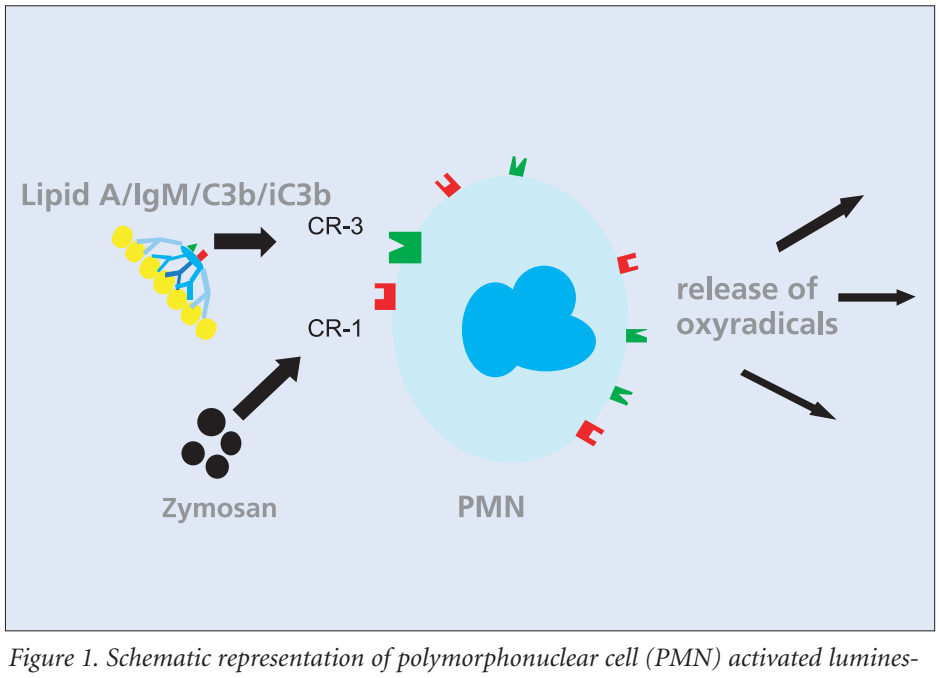

Figure 1. Schematic representation of polymorphonuclear cell (PMN) activated luminescent response to antigen (Lipid A) antibody (IgM) complex in whole blood.

Spectral Diagnostics Inc. has developed a rapid, homogenous assay for the detection of endotoxin activity (EA) in whole blood based on *in vitro* neutrophil activation [8]. This novel type of assay employs the priming effects of complement opsonised immune complexes on the respiratory burst activity of neutrophils as an analytical platform. [Figure 1]

Analytical sensitivity of the EAA assay

The EA assay has been optimised to reproducibly measure the endogenous endotoxin present in the patient sample over an EA range of 0.0 to 1.0 which corresponds to whole blood levels of 0-3 EU/ml as confirmed in recovery studies [8]. The EAA is optimised to be sensitive at the low range of endotoxin concentration as depicted in a typical dose curve shown in Figure 2. When patient samples contain low levels of endotoxin, i.e. EAA < 0.4 (less than 25 pg/ml, 0.02 EU/ml), small increases in endotoxin will result in significant increases in EA values. The steepest part of the dose response curve at low endotoxin concentrations has been engineered to detect levels of endotoxin, which are likely to trigger a significant biological response indicative of invasive gram-negative infection. At a threshold of about 50 pg/ml (0.04 EU/ml) an EA value above 0.4 is achieved indicating a potentially pathological level of endotoxin. The assay is therefore designed to be most sensitive at endotoxin concentrations which are likely to be released from early invasive gram-negative infections or other reservoirs of toxin such as the gastrointestinal tract.

Clinical relevance

The results from a multinational multi-centre clinical trial (MEDIC) evaluating the EAA in over 1000 critically ill patients demonstrated that increasing levels of endotoxaemia as measured by the EAA are associated with significantly worse outcomes such as mortality ($p=0.04$) and length of ICU stay ($p=0.04$). In addition, endotoxaemic patients showed significantly greater evidence of organ dysfunction with respect to shock ($p<0.001$), hypoxaemia ($p=0.005$) and abnormal white blood cell count ($p<0.001$).

Based on results from the MEDIC trial [9], a patient admitted to the ICU with an EAA greater than or equal to 0.60 EAA units is approximately 3 times more likely to develop severe sepsis within the first 24 hours ($p=0.007$) or 3 days ($p=0.004$) of their ICU stay than a patient who presents with a low level of endotoxaemia (EA < 0.04). The test is not affected by common interferents such as lipaemia, haemolysis and icterus, is simple to perform, effective, rapid and can

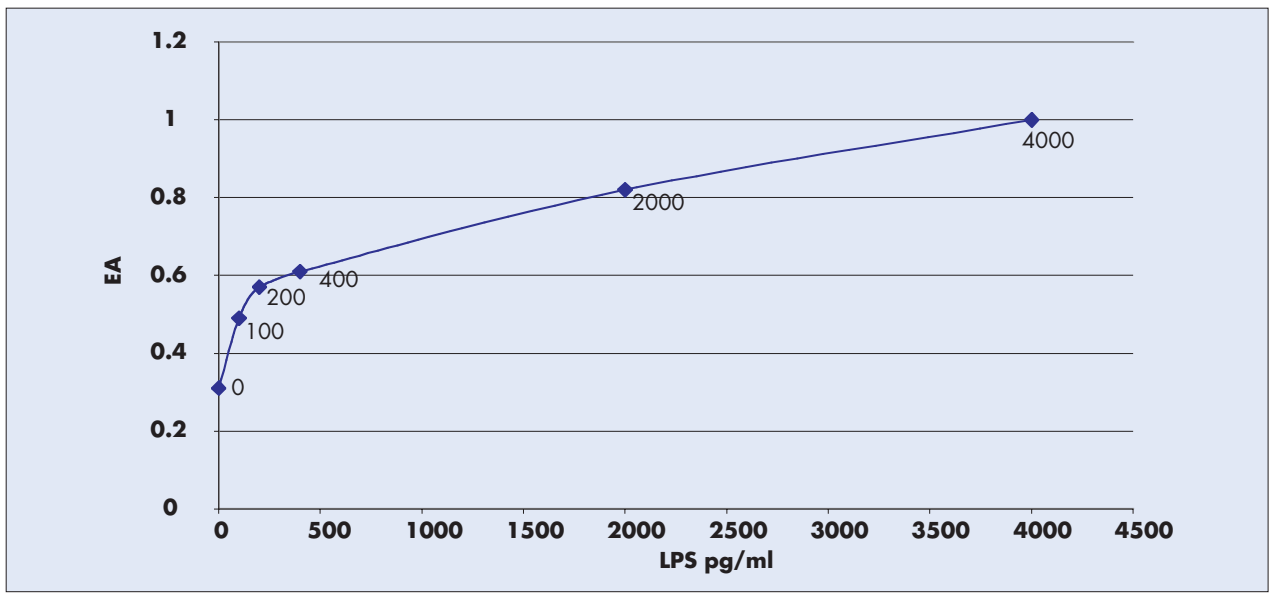

Figure 2. Typical EAA Dose Response Curve

be used in the general ICU population.

How the assay works

The EAA test kit is comprised of sets of three colour-coded glass tubes [Figure 3] each containing lyophilized beaded reagents, an aliquot tube, an LPS Max tube and a bottle of EAA reagent. The EAA kit is designed for duplicate EAA determinations. In addition, quality control tubes containing beaded reagents are provided with each kit and should be used according to each laboratory's standard practice for quality control testing. The quality control assay should be performed with the patient assay.

A basal activity measurement (Tube 1) in the absence of the specific anti-endotoxin antibody measures the non-specific oxidative burst of the patient's neutrophils. An additional measurement including specific anti-endotoxin antibody (Tube 2) and an excess of exogenous endotoxin (Tube 3) measures the maximum oxidative burst of the patient's neutrophils stimulated by endotoxin-antibody complexes. The test measurement (Tube 2) includes the specific antibody to measure the endogenous level of endotoxin activity. The EAA level is calculated by normalising the chemiluminescence in the test sample (Tube 2) against the maximum chemiluminescence (Tube 3), correcting both measurements for the basal activity chemiluminescence (Tube 1).

Blood samples for EAA testing must be collected using EDTA anti-coagulant blood collection tubes. A minimum of 2.5 mL of whole blood is required for the EAA assay. Collected samples may be stored refrigerated or on ice for up to 3 hours without compromising the assay results. A maximum of 5 patient assays and 1 QC test may be analysed at one time. The total run time for a batch of 5 assays is 20 minutes. The complete EAA turnaround time is 60 minutes.

Figure 3. Colour coded tubes containing lyophilised reagents for EAA test kit. Tube 1(red label): measures baseline neutrophil activity; Tube 2 (blue label): contains reagents to detect circulating LPS; Tube 3 (black label): contains reagents and excess LPS to detect maximum oxidative burst of a patients' neutrophils.

Summary

Advances in the understanding and management of patients with sepsis will allow more rigorous approaches to disease definition and stratification. With the development of this new, rapid and reliable whole blood endotoxin assay a better estimate of the risk for sepsis in a heterogeneous group of critically ill patients can now be made. Severe sepsis becomes less deadly as doctors learn to recognize it early and initiate treatment [10].

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