

Fig. 1

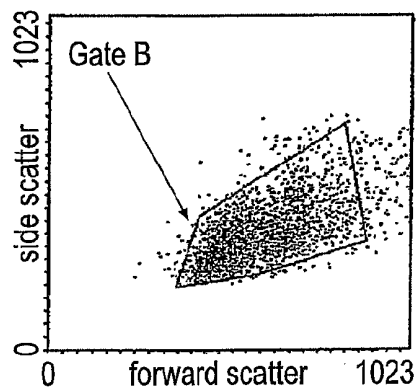


Fig. 2

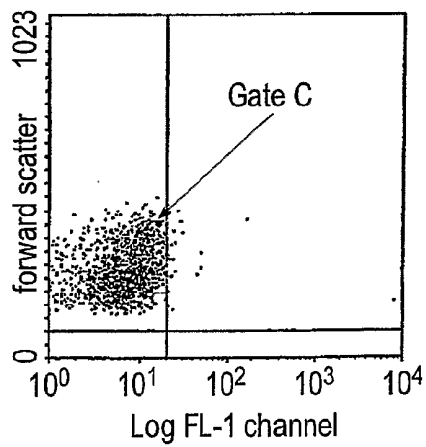


Fig. 3

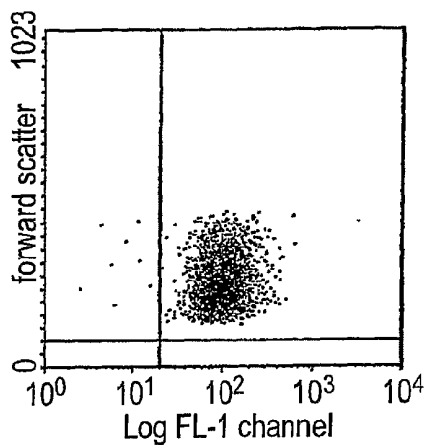


Fig. 4

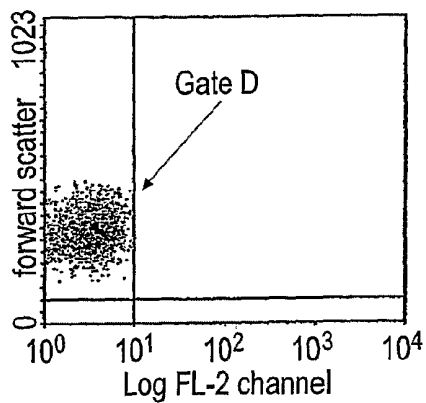


Fig. 5

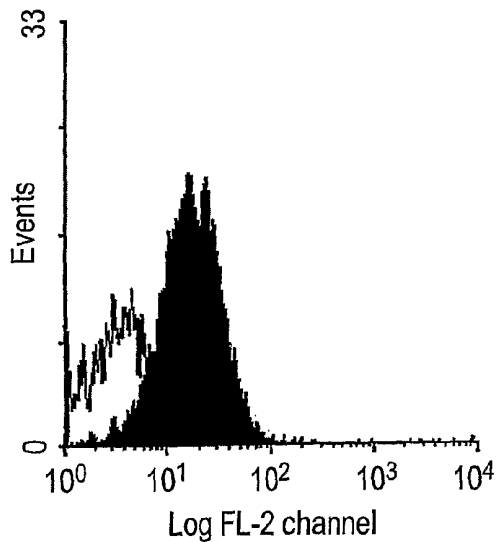


Fig. 6

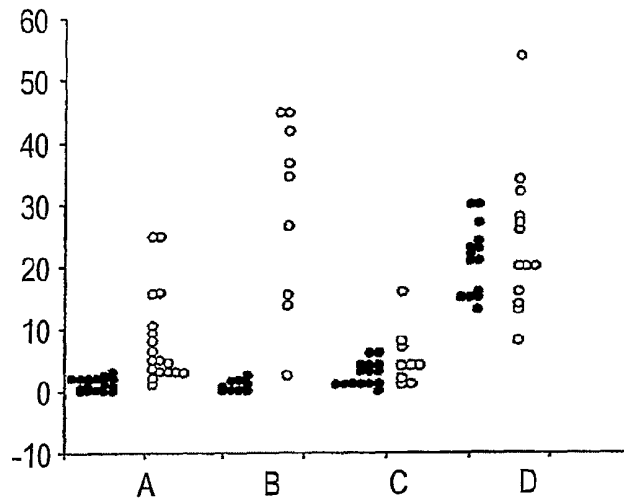


Fig. 7

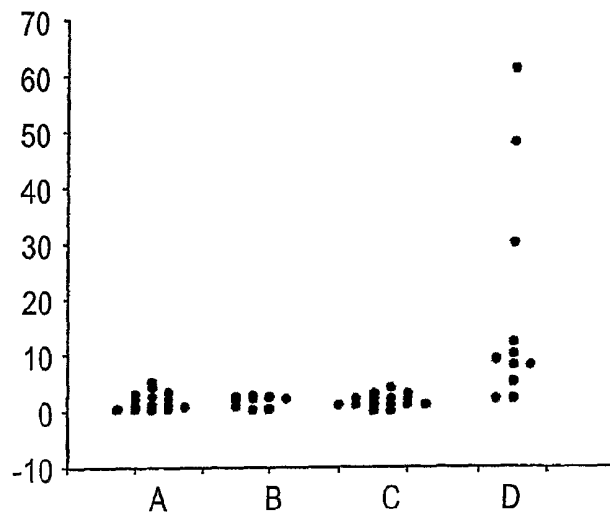


Fig. 8

SEPSIS TEST

The invention relates to a test for determining whether a subject has a bacterial infection, especially bacterial sepsis.

Organ failure arising from sepsis is one of the major causes of patient mortality within the Intensive Care Unit (ICU). Despite the technological support of organ function, a mortality of approximately 30% is reported in patients with sepsis and should multiple organ failure develop then the mortality may exceed 80%. Current guidelines recommend early antibiotic treatment in response to definitive evidence that bacterial infection is the cause of organ failure. Confirmation of bacterial infection in blood and other sites is only available in a proportion of patients and takes at least 24-48 hours. Rapid indirect markers of bacterial infection include increases in peripheral blood leucocyte counts and in serum levels of acute phase proteins, C-reactive protein and pro-calcitonin. Since these markers are also elevated in ICU patients with systemic inflammation, who have no evidence of bacterial infection, they cannot be used to guide therapy. Thus, definitive information necessary for clinical diagnosis is unavailable at a critical stage of patient management. Consequently, broad spectrum antibiotics are often started early, in the hope that they will arrest patient deterioration. Alternatively, in some situations no antibiotics are prescribed in the first 48 hours in the mistaken belief that the cause of the organ failure is non-bacterial. This may result in a potentially life-threatening delay in starting antibiotics.

Antibiotics are often administered inappropriately for several days to patients in the ICU who do not have bacterial infections, because it is considered unethical to withhold antibiotics if bacterial infection cannot be confidently excluded. The bacteria implicated in ICU-acquired sepsis are endogenous, commensal or environmental. The over use of antibiotics, when unnecessary, is adding to increasingly unpredictable antibiotic resistance. The consequence of this therapeutic approach has been a steady but inexorable rise in antibiotic resistance of hospital-acquired bacteria, as illustrated by methicillin resistant *Staphylococcus aureus* (MRSA) and multi-resistant Gram-negative bacteria such as *Acinetobacter* and *Pseudomonas* species. With new international ICU guidelines recommending that treatment of sepsis patients with broad-spectrum antibiotics be started within a few hours of diagnosis as a standard of care, it seems that antibiotic resistance will increase. Against this background there is a need to develop an assay for the rapid detection of bacterial infections in critically ill patients.

The organ failure associated with sepsis is precipitated by the systemic inflammation that is produced in response to invasive bacteria. The main function of neutrophils is to destroy pathogenic bacteria but evidence is emerging that these cells are also implicated in the initiation of organ dysfunction in patients with sepsis. In recent years the inventors have been addressing the working hypothesis that an untoward interaction of blood neutrophils with endothelium produces organ dysfunction, either by vascular occlusion that leads to hypoxia and tissue hyperperfusion, or by disruption of the endothelium and an increase in vascular permeability. Accordingly, the inventors' studies of neutrophils from patients with sepsis have focused on the expression of adhesion molecules, which are known to promote adhesion to blood vessel walls, and on cellular receptors that contribute to bacterial recognition. During the course of this work the inventors noted that neutrophils expressing seven distinct molecules were more prevalent in the blood of patients with sepsis than in patients with systemic inflammation initiated by non-infective stimuli such as surgery, trauma and pancre-

atitis and in healthy control subjects (see Tables 1, 2, 3 & 4). These molecules were CD49e, CD14, CD11c, CD49f, CD29; TLR2 and TLR4.

The inventors have developed a rapid and accurate test for assessing whether a subject has a bacterial infection. This test is particularly useful for assessing whether a patient with systemic inflammation has a bacterial infection:

According to the invention, there is provided a method for determining whether a subject has a bacterial infection comprising:

a) identifying an abnormal expression of one or more of CD49e, CD14, CD11c, CD49f, and CD29 on leucocytes in a sample obtained from the subject; wherein an abnormal expression of CD49e, CD14, CD11c, CD49f or CD29 is indicative of the subject having a bacterial infection.

The method is particularly useful because it may be carried out quickly, within several hours of sample collection, allowing a rapid decision to be taken as to whether to administer antibiotics to the subject.

The terms "sepsis" and "systemic inflammatory response syndrome" (SIRS) are well known in the art and mean a systemic inflammatory response to infection, usually bacterial infection. In its most severe form, sepsis results in organ dysfunction and failure.

The term "bacterial infection" means the presence in a subject of bacteria not normally found in the subject or an increased number or change of location of bacteria normally found in the subject such as endogenous or commensal bacteria. For example, the infection could be due to an invasion of the blood by bacteria normally found in the gut. Alternatively the infection could be due to bacteria not normally found within the body.

The infection may be an infection of Gram positive or Gram negative bacteria. Both terms are well known in the art.

As indicated above, it would be very useful to be able to determine whether organ failure or dysfunction in a patient arises from sepsis caused by a bacterial infection and hence whether to treat the patient with antibiotics.

The subject may be any animal, preferably a mammal. It is more preferred that the subject is a primate, especially a human.

The subject may be showing signs of systemic inflammation, organ dysfunction or failure or may not be exhibiting any such symptoms.

The leucocytes may be any type of leucocytes, but are preferably monocytes or neutrophils. In particular, the leucocytes may be neutrophils.

CD49e & CD49f belong to a family of β 1 integrin adhesion molecules. Integrins are heterodimers that consist of different α chains and a common β chain. It is the β chain that defines the family and for the β 1 integrins this molecule is known as CD29. There are six members (CD49a/CD29 \rightarrow CD49f/CD29) and their α chains are, in addition to their CD nomenclature, referred to as very late antigen (VLA-1 to VLA-6). Hence, CD49e/CD29 is also known as VLA-5, and CD49f/CD29 is also known as VLA-6. The ligands for the β 1 integrins are components of the extracellular matrix and CD49e binds to fibronectin. In general, expression of the β 1 integrins is confined to the surface of lymphocytes and monocytes but not resting blood neutrophils. CD11c is a member of the β 2 integral family whereas CD14 is a receptor for lipopolysaccharide (LPS).

An abnormal expression of CD49e, CD14, CD49f, CD11c or CD29 means that the expression of the molecule is significantly higher, as assessed by statistical analysis, on some leucocytes or that many leucocytes show a significantly

increased expression compared with levels on normal resting blood leucocytes. That is to say, some leucocytes may express an increased number of CD49e, CD14, CD49f, CD11c or CD29 molecules, or an increased number of leucocytes may express CD49e, CD14, CD49f, CD11c or CD29 molecules when compared with normal resting blood leucocytes.

In particular, an abnormal expression of CD49e preferably means that the mean distribution of CD49e positive leucocytes is at least 50% more than the mean distribution of CD49e positive leucocytes in normal resting blood leucocytes. Preferably the mean distribution is increased by at least 75%, more preferably by at least 100%, even more preferably by at least 150% and most preferably by at least 200%.

An abnormal expression of CD14 preferably means that the mean distribution of CD14 positive leucocytes is at least 50% more than the mean distribution of CD14 positive leucocytes in normal resting blood leucocytes. Preferably the mean distribution is increased by at least 75%, more preferably by at least 100%, even more preferably by at least 150% and most preferably by at least 200%.

An abnormal expression of CD49f preferably means that the mean distribution of CD49f positive leucocytes is at least 50% more than the mean distribution of CD49f positive leucocytes in normal resting blood leucocytes. Preferably the mean distribution is increased by at least 75%, more preferably by at least 100%, even more preferably by at least 150% and most preferably by at least 200%.

An abnormal expression of CD11c preferably means that the Mean distribution of CD11c positive leucocytes is at least 50% more than the mean distribution of CD11c positive leucocytes in normal resting blood leucocytes. Preferably the mean distribution is increased by at least 75%, more preferably by at least 100%, even more preferably by at least 150% and most preferably by at least 200%.

An abnormal expression of CD29 preferably, means that the mean distribution of CD29 positive leucocytes is at least 50% more than the mean distribution of CD29 positive leucocytes in normal resting blood leucocytes. Preferably the mean distribution is increased by at least 75%, more preferably by at least 100%, even more preferably by at least 150% and most preferably by at least 200%.

An abnormal expression of CD49e on neutrophils preferably means that the mean distribution of CD49e positive neutrophils in a sample is at least 20% of neutrophils, more preferably at least 25%, even more preferably at least 30%, most preferably at least 40%.

An abnormal expression of CD14 on neutrophils preferably means that the mean distribution of CD14 positive neutrophils in a sample is at least 10% of neutrophils, more preferably at, least 12%, even more preferably at least 15%, most preferably at least 20%.

An abnormal expression of CD49f on neutrophils preferably means that the mean distribution of CD49f positive neutrophils in a sample is at least 20% of neutrophils, more preferably at least 25%, even more preferably at least 30%, most preferably at least 40%.

An abnormal expression of CD11c on neutrophils preferably means that the mean distribution of CD11c positive neutrophils in a sample is at least 40% of neutrophils, more preferably at least 50%, even more preferably at least 60%, most preferably at least 70%.

An abnormal expression of CD29 on neutrophils preferably means that the mean distribution of CD29 positive neutrophils in a sample is at least 40% of neutrophils, more preferably at least 50%, even more preferably at least 60%, most preferably at least 70%.

The sample may be any sample in which leucocytes may be found e.g. bronchioalveolar lavage, urine. Preferably the sample is a blood sample.

It is generally thought by those skilled in the art that in response to bacterial infection, the phenotype of neutrophils changes only when the cells enter an infective lesion. There is no evidence to suggest that neutrophils re-enter the circulation from sites of infection. Those skilled in the art were not interested in examining the surface of neutrophils for signs of bacterial induced changes because it is often very difficult to obtain a sample from the site of the infection. In cases of suspected sepsis, there is a doubt as to whether the patient has a bacterial infection, and, hence, the site of any such infection may be unknown. This reinforced the lack of interest in neutrophils. Surprisingly the inventors have found evidence of bacterial infection on the surface of neutrophils circulating in the blood, thus negating the need to obtain neutrophils from sites of bacterial infection.

The method includes identifying an abnormal expression of any one or more of CD49e, CD14, CD11c, CD49f and CD29. The method preferably comprises identifying an abnormal expression of more than one of these molecules.

The method preferably additionally comprises identifying the abnormal expression of one or both of TLR2 and TLR4 on the leucocytes in the sample, wherein the abnormal expression of one or both of TLR2 and TLR4 is further indication of a bacterial infection.

TLR2 and TLR4 are toll-like receptors. Such receptors are pattern recognition molecules that control innate immune responses to various microbial ligands. These terms are well known in the art.

Both TLR2 and TLR4 may be expressed by leucocytes, especially macrophages at a site of bacterial infection but it is much more likely that one or the other of them is expressed abnormally. TLR2 has been found to be associated with Gram positive bacterial infection, so its abnormal expression on leucocytes is preferably indicative thereof. TLR4 has been found to be associated with Gram negative bacterial infection so its abnormal expression on leucocytes is preferably indicative of such an infection.

Normal blood neutrophils express little TLR2 or TLR4. Generally less than 5% of neutrophils in a sample express TLR2 or TLR4. During the course of their work, the inventors noticed that neutrophils expressing TLR2 were more prevalent in the blood of patients with sepsis who had Gram+ve infections when compared with neutrophils from control subjects (Table 2). The inventors also noted that neutrophils expressing TLR4 and CD14 were increased significantly in the blood of patients with sepsis who had Gram+ve infections when compared with control subjects (Table 2).

An abnormal expression of TLR2 or TLR4 preferably means that the mean distribution of TLR2 or TLR4 positive leucocytes is at least 50% more than the mean distribution of TLR2 or TLR4 positive leucocytes in normal resting blood leucocytes. Preferably the mean distribution is increased by at least 75%, more preferably by at least 100%, even more preferably by at least 150% and most preferably by at least 200%.

Abnormal expression of TLR2 on neutrophils preferably means that the mean distribution of TLR2 positive neutrophils in a sample is at least 7% of neutrophils, more preferably at least 10%, even more preferably at least 15%, most preferably at least 20%.

Abnormal expression of TLR4 on neutrophils preferably means that the mean distribution of TLR4 positive neutro-

MEDIAN VALUES					
MOLECULE	SIRS + SEPSIS	SIRS - SEPSIS	NORMALS		
CD11C	71% (n = 35)	34% (n = 20)	37% (n = 45)	p < 0.001	SIRS + SEPSIS versus SIRS - SEPSIS/NORMAL
CD49F	19% (n = 24)	10% (n = 17)	8% (n = 11)	p < 0.05	SIRS + SEPSIS versus SIRS - SEPSIS/NORMAL

MOLECULE	SEPSIS (Gram + ve)	SEPSIS (Gram - ve)	NORMAL		
CD29	73% (n = 20)	35% (n = 35)	33% (n = 21)	p < 0.001	SEPSIS (Gram + ve) versus SEPSIS (Gram - ve)/NORMAL

We claim:

1. A method for determining whether a subject has a bacterial infection comprising:
 contacting detectably labeled antibodies specific for CD49e, TLR2, and TLR4 with blood leucocytes in a sample obtained from the subject;
 forming a measurable amount of CD49e-antibody complex, TLR2-antibody complex, and TLR4-antibody complex;
 identifying abnormal expression of CD49e on blood leucocytes in a sample obtained from the subject from the amount of CD49e-antibody complex; and
 identifying abnormal expression of one or both of TLR2 and TLR4 on the blood leucocytes in the sample from the amount of TLR2-antibody complex and TLR4-antibody complex, wherein abnormal expression of CD49e

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and of one or both of TLR2 and TLR4 is indicative of the subject having a bacterial infection.
 2. The method of claim 1 wherein the sample is whole blood.
 3. A method according to claim 1, wherein the leucocytes are neutrophils.
 4. A method according to claim 3, wherein at least 20% of neutrophils in the sample express CD49e.
 5. A method according to claim 1, wherein abnormal expression of TLR2 is identified which is indicative of a Gram positive bacterial infection.
 6. A method according to claim 1, wherein abnormal expression of TLR4 is identified which is indicative of a Gram negative bacterial infection.
 7. A method according to claim 1, wherein at least 10% of leucocytes in a sample express TLR2 or TLR4.

* * * * *

专利名称(译)	脓毒症试验		
公开(公告)号	US8476028	公开(公告)日	2013-07-02
申请号	US13/342669	申请日	2012-01-03
[标]申请(专利权)人(译)	伦敦国王学院		
申请(专利权)人(译)	伦敦大学国王学院		
当前申请(专利权)人(译)	伦敦大学国王学院		
[标]发明人	BROWN KENNETH ALUN LEWIS SION MARC		
发明人	BROWN, KENNETH ALUN LEWIS, SION MARC		
IPC分类号	G01N33/00 G01N33/554 G01N33/569 G01N33/53		
CPC分类号	G01N33/56911 G01N2333/7055 G01N2333/70553 G01N2333/70596 G01N2800/26		
代理机构(译)	BANNER & WITCOFF有限公司		
审查员(译)	纳瓦罗, ALBERT		
优先权	2006001959 2006-01-31 GB 12/162820 2008-12-16 US PCT/GB2007/000325 2007-01-31 WO		
其他公开文献	US20120315653A1		
外部链接	Espacenet USPTO		

摘要(译)

提供了一种用于确定受试者是否患有细菌感染的方法，包括：鉴定从受试者获得的样品中白细胞上的CD49e，CD14，CD11c，CD49f和CD29中的一种或多种的异常表达；其中CD49e，CD14，CD11c，CD49f或CD29的异常表达指示受试者患有细菌感染。

