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INVITED REVIEW ARTICLE

Clinical application of sepsis biomarkers

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Abstract Sepsis is one of the leading causes of death in the critically ill. Early diagnosis is important to avoid delay in instituting appropriate treatment. However, diagnosis can be delayed because of difficulty in interpreting clinical features. Sepsis biomarkers can aid early diagnosis. This article reviews the application of readily available biomarkers for diagnosis of sepsis, for predicting prognosis, and for antibiotic stewardship. 178 biomarkers are described in the literature—ranging from specimen cultures, which lack sensitivity and specificity for early diagnosis of sepsis, to biomarkers such as C-reactive protein, procalcitonin, and genetic biomarkers, which have their own limitations. Future research will mainly focus on use of more than one biomarker, but the main problem in sepsis biomarker research seems to be a lack of a recommended biomarker.

Keywords Sepsis · Biomarker · Procalcitonin · C-reactive protein

Introduction

Sepsis is the 10th leading cause of death in industrialized countries; its incidence is continuously increasing at approximately 5–10 % per year [1, 2]. It is also the leading cause of death in critical care practice, despite advances in resuscitation therapy and use of modern antibiotics [2]. Early accurate diagnosis is important, because every hour of delay of appropriate antibiotic therapy increases mortality by 5–10 % [1]. Diagnosis is not always simple, especially in critically ill patients for whom clinical signs and symptoms are not always present and are difficult to interpret. Microbiological cultures are frequently negative because of previous or concomitant antibiotic therapy [3]. Diagnostic uncertainty is compensated for by liberal use of broad-spectrum antibiotics, with inherent antibiotic resistance as an increasing problem [1].

Biomarkers have been suggested as means of aiding early diagnosis and, therefore, early initiation of appropriate therapy in patients with sepsis in intensive care units (ICU). They may also, in conjunction with other techniques, be useful for antibiotic stewardship and for predicting prognosis [3]. A biomarker is defined as a characteristic that is objectively measured as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [4]. An ideal sepsis biomarker should be easy to measure, readily available, and inexpensive, and enable highly specific and sensitive diagnosis of sepsis, quantification of severity in the absence of clinical signs, and monitoring of disease course and response to therapy. Although 178 biomarkers are described in literature, many are not used in clinical practice because they lack sensitivity and specificity. Also, studies conducted with those biomarkers are small and lack external validation of results (Table 1) [5]. Therefore, the search for an ideal biomarker continues.

This article reviews the application of readily available biomarkers of sepsis in critical care practice. The key question is—what is the role of these markers in diagnosis

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Table 1 Biomarkers described in the literature [5]

SI no.	Biomarkers	Comment
Cytokine/chemokine biomarkers		
1	GRO-alpha	Higher in septic shock than in sepsis
2	High mobility group-box 1protein (HMGB-1)	No difference between survivors and non-survivors at 28 days
3	IL-1 receptor antagonist	Correlation with SOFA score
4	IL-1b	Increased in septic compared with non-septic individuals
5	IL-2	Increased in parallel with disease severity
6	IL-4	Increased levels associated with development of sepsis
7	IL-6	Distinguished between survivors and non-survivors at 28 days
8	IL-8	Prediction of MOF, DIC
9	IL-10	Higher in septic shock than sepsis, distinguished between survivors and non-survivors at 28 days
10	IL-12	Predictive of lethal outcome from postoperative sepsis
11	IL-13	Higher in septic shock than sepsis
12	IL-18	Distinguished between survivors and non-survivors at 28 days
13	Macrophage inflammatory protein (MIP)-1 and -2	Increased in sepsis compared with healthy controls
14	Macrophage migration inhibitory factor (MIF)	Distinguished between survivors and non-survivors at 28 days
15	Monocyte chemotactic protein (MCP)-1 and -2	Distinguished between survivors and non-survivors at 28 days
16	Osteopontin	Increased in sepsis compared with healthy controls
17	RANTES	Increased in sepsis compared with healthy controls
18	TNF	Distinguished between survivors and non-survivors at 28 days
Cell marker biomarkers		
19	CD10	Decreased in septic shock compared with healthy controls
20	CD11b	Correlation with SOFA score
21	CD11c	Decreased in septic shock compared with healthy controls
22	CD14 (cellular and soluble)	Distinguished between survivors and non-survivors at 28 days
23	CD18	
24	CD25 (cellular and soluble	Distinguished between survivors and non-survivors at 28 days
25	CD28 (soluble)	Distinguished between survivors and non-survivors at 28 days
26	CD40 (cellular and soluble)	Distinguished between survivors and non-survivors at 28 days
27	CD48	Increased in sepsis compared with healthy controls
28	CD64	Correlation with APACHE II and SOFA scores
29	CD69	Increased in sepsis compared with healthy controls
30	CD80	Predicted development of septic shock
31	CD163	Distinguished between survivors and non-survivors at 28 days
32	mHLA-DR (soluble)	Distinguished between survivors and non-survivors at 28 days
Receptor biomarkers		
33	CC chemokine receptor (CCR)	
34	CCR 3	Distinguished between survivors and non-survivors at 28 days
35	C5L2	Predicted development of MOF
36	CRTh2	Distinguished between survivors and non-survivors at 28 days
37	Fas receptor (soluble)	Predicted development of MOF
38	Fc-gamma RIII	Increased in sepsis compared with healthy controls, correlated with APACHE II score
39	FLT-1 (soluble)	Correlated with APACHE II score
40	GP130	Increased in sepsis compared with healthy controls
41	IL-2 receptor (soluble)	Predicted development of septic shock
42	Group II phospholipase A2 (PLA2-II) (soluble)	Distinguished between survivors and non-survivors at 28 days
43	RAGE (soluble)	Distinguished between survivors and non-survivors at 28 days

Table 1 continued

SI no.	Biomarkers	Comment
44	ST2 (soluble, IL-1 receptor)	Increased in sepsis compared with healthy controls
45	Toll-like receptor (TLR) 2 and 4	Increased in septic compared with non-septic critically ill patients
46	Transient receptor potential vanillin (TRPV)1	
47	TREM-1 (soluble)	Distinguished between survivors and non-survivors at 28 days
48	TNF-receptor (soluble)	Predicted development of MOF
49	Urokinase type plasminogen activator receptor (uPAR) (soluble)	Distinguished between survivors and non-survivors at 28 days
Coagulation biomarkers		
50	Antithrombin	Distinguished between survivors and non-survivors at 28 days
51	Activated partial thromboplastin time (aPTT)	Correlated with MOF score in patients with sepsis and DIC, high negative predictive value
52	D-dimers, thrombin–antithrombin complex, prothrombin time	Distinguished between survivors and non-survivors at 28 days
53	Fibrin	Increased in patients with Gram-negative bacteremia
54	Platelet factor-4	Predicted response to therapy
55	Plasminogen activator inhibitor (PAI)	Distinguished between survivors and non-survivors at 28 days
56	Protein C and S	Distinguished between survivors and non-survivors at 28 days
57	Thrombomodulin	Predicted development of MOF, DIC, and response to therapy
Biomarkers related to vascular endothelial damage		
58	ADAMTS-13	Decreased in septic patients with DIC compared with no DIC
59	Angiopoietin (1 and 2)	Distinguished between survivors and non-survivors at 28 days
60	Endocan	Predicted development of septic shock
61	Endothelial leukocyte adhesion molecule (ELAM)-1 (cellular and soluble)	Distinguished between survivors and non-survivors at 28 days
62	Endothelial progenitor cells (EPC)	Distinguished between survivors and non-survivors at 28 days
63	Intracellular adhesion molecule (ICAM)-1 (soluble)	
64	Laminin	Increased in sepsis compared with non-infected controls
65	Neopterin	Distinguished between survivors and non-survivors at 28 days
66	Platelet-derived growth factor (PDGF)-BB	Distinguished between survivors and non-survivors at 28 days
67	E-selectin (cellular and soluble)	Predicted development of MOF, correlated with SAPS score
68	L-selectin (soluble)	Distinguished between survivors and non-survivors at 28 days
69	P-selectin	
70	Vascular cell adhesion molecule (VCAM)-1	Predicted development of MOF
71	Vascular endothelial growth factor (VEGF)	Distinguished between survivors and non-survivors at 28 days, predicted development of MOF
72	Von Willebrand factor and antigen	Distinguished between survivors and non-survivors at 28 days, predicted development of acute lung injury
Biomarkers related to vasodilatation		
73	Adrenomedullin and proadrenomedullin	Predicted development of septic shock
74	Anandamide	Increased in sepsis compared with healthy controls
75	Angiotensin converting enzyme (ACE) (activity and serum)	Increased in sepsis compared with healthy controls
76	2-Arachidonoylglycerol	Increased in sepsis compared with healthy controls
77	Copeptin	Distinguished between survivors and non-survivors at 28 days, correlated with APACHE II score
78	C-type natriuretic peptide (CNP)	Increased in patients with septic shock compared with healthy controls
79	Cycling nucleotides	Distinguished between survivors and non-survivors at 28 days
80	Elastin	Decreased in sepsis compared with healthy controls
81	cGRP	Distinguished between survivors and non-survivors at 28 days, correlated with APACHE II score

Table 1 continued

SI no.	Biomarkers	Comment
82	47 kD HK	Correlated with severity of sepsis
83	Neuropeptide Y	Increased in sepsis compared with healthy controls
84	Nitric oxide (NO), nitrate, nitrite	Predicted development of septic shock
85	Substance P	Distinguished between survivors and non-survivors at 28 days (predictive only in the late phase of sepsis, 2 days before death)
86	Tetrahydrobiopterin	Increased in sepsis compared with non-septic critically ill patients
87	Vasoactive intestinal peptide	Increased in tissue samples from patients with peritonitis compared with no peritonitis
Biomarkers of organ dysfunction		
88	Atrial natriuretic peptide (ANP)	Distinguished between survivors and non-survivors at 28 days
89	Brain natriuretic peptide (BNP)	Distinguished between survivors and non-survivors at 28 days, correlated to APACHE II score
90	Carbomyl phosphate synthase (CPS)-1	
91	Endothelin-1 and pro-endothelin-1	Distinguished between survivors and non-survivors at 28 days, correlated to SOFA score
92	Filterable cardiodepressant substance (FCS)	
93	Gc-globulin	Predicted development of MOF
94	Glial fibrillary acidic protein (GFAP)	Increased in septic shock compared with healthy controls
95	Alpha glutathione *S*-transferase (GST)	
96	Hepatocyte growth factor (HGF) (cellular and soluble)	Predicted response to therapy
97	Mono ethyl glycine xylidide test	Correlated with SAPS II score
98	Myocardial angiotensin II	
99	Neuron specific enolase	Correlated with SOFA scores
100	Pancreatitis-associated protein-I	
101	Pre B cell colony-enhancing factor (PBEF)	Increased in sepsis compared with healthy controls
102	Protein S-100b	Distinguished between survivors and non-survivors at 28 days, correlated with SOFA score
103	Surfactant protein (A, B, C, D)	Increased in sepsis compared with healthy controls
104	Troponin	Distinguished between survivors and non-survivors at 28 days, correlated with APACHE II score
Acute phase protein biomarkers		
105	Serum amyloid A (SAA)	Correlated with CRP in patients with septic shock
106	Cerruloplasmin	Predicted liver dysfunction in patients with sepsis
107	C-reactive protein (CRP)	Predicted response to therapy
108	Ferritin	Distinguished between survivors and non-survivors at 28 days, correlated with SOFA score
109	Alpha1-acid glycoprotein	Distinguished between survivors and non-survivors at 28 days, correlated with SOFA score
110	Hepcidin	Increased in sepsis compared with healthy controls and patients with chronic renal failure
111	Lipopolysaccharide-binding protein (LBP)	Higher in sepsis compared with no sepsis, no prognostic value
112	Procalcitonin	Increased in infected compared with non-infected patients
113	Pentraxin 3	Distinguished between survivors and non-survivors at 28 days, correlated with APACHE II score
Other biomarkers		
114	Alpha2 macroglobulin	
115	Albumin	
116	Anti-endotoxin core antibodies (EndoCab)	Distinguished between survivors and non-survivors at 28 days
117	Apolipoprotein C	Distinguished between survivors and non-survivors at 28 days
118	Bcl-2	Distinguished between survivors and non-survivors at 28 days

Table 1 continued

SI no.	Biomarkers	Comment
119	Beta-thromboglobulin	Predicted response to therapy
120	Caspase-1	Increased in septic shock compared with healthy controls
121	Ceramide	Predicted development of MOF
122	Cholesterol	Distinguished between survivors and non-survivors at 28 days in patients with severe sepsis
123	Complement (C3, C4, C5a levels)	Distinguished between survivors and non-survivors at 28 days
124	Terminal complement complex	
125	Dendritic cell	Distinguished between survivors and non-survivors at 28 days, correlated with SOFA score
126	Dipeptidylpeptidase	Decreased in sepsis compared with healthy controls
127	Diiodotyrosine (DIT)	Increased in sepsis compared with non-septic critically ill
128	Eicosanoid	Correlated with SAPS score, predicted response to therapy
129	Elastase	Predicted response to therapy in patients with joint infections
130	Elastase-a1-antitrypsin complex	Predicted response to therapy
131	Erythropoietin	Distinguished between survivors and non-survivors at 28 days in patients with septic shock, correlated with lactate levels
132	F2 isoprostanes	Increased in infected diabetic patients compared with non-infected diabetics
133	Fatty acid amide hydrolase	Decreased in sepsis compared with healthy controls
134	Free DNA	Distinguished between survivors and non-survivors at 28 days
135	G-CSF and GM-CSF	Distinguished between survivors and non-survivors at 28 days
136	Gelsolin	
137	Ghrelin	Distinguished between survivors and non-survivors at 28 days
138	Growth arrest specific protein (Gas)	Correlated with APACHE II score in patients with severe sepsis
139	Heat shock protein (HSP) 70, 72, 73, 90 and 32	Increased in sepsis compared with healthy controls
140	HDL cholesterol	Distinguished between survivors and non-survivors at 28 days, predicted prolonged ICU length of stay
141	HLA-G5 protein (soluble)	Distinguished between survivors and non-survivors at 28 days in patients with septic shock
142	H2S	
143	Hyaluronan	Distinguished between survivors and non-survivors at 28 days in patients with septic shock
144	Hydrolytic IgG antibodies	Distinguished between survivors and non-survivors at 28 days, correlation with SAPS II score
145	Inter-alpha inhibitor proteins (IalphaIp)	Predicted development of MOF
146	Intracellular nitric oxide in leukocyte	Negatively correlated with SOFA score
147	IP-10	Increased in sepsis compared with healthy controls
148	Lactate	Distinguished between survivors and non-survivors at 28 days, predicted response to therapy
149	Lactoferrin	Predicted response to therapy
150	Leptin	No prognostic value, higher in septic than in non-septic ICU patients
151	Serum lysozyme (enzyme activity)	Increased in sepsis compared with healthy controls
152	Matrix-metalloproteinase (MMP)-9	Increased in severe sepsis compared with healthy controls
153	Micro particles (cell derived)	Distinguished between survivors and non-survivors at 28 days, correlation with SOFA score
154	Neurotensin	
155	Nitrate excretion (urinary and expired)	
156	Nociceptin/orphanin FQ (N/OFQ)	Distinguished between survivors and non-survivors at 28 days
157	Nuclear factor-B (activity and expression)	Distinguished between survivors and non-survivors at 28 days in patients with severe sepsis, correlation with APACHE II score

Table 1 continued

SI no.	Biomarkers	Comment
158	Nucleosomes	Distinguished between survivors and non-survivors at 28 days
159	Peptidoglycan	Increased in sepsis compared with healthy controls
160	Placental growth factor	
161	Plasma amino acids	Distinguished between survivors and non-survivors at 28 days, predicted development of MOF
162	Plasma fibronectin	Increased in sepsis compared with healthy controls
163	Plasmin alpha2-antiplasmin complex	Predicted development of MOF
164	Renin	Correlation with lactate levels in patients with septic shock
165	Resistin	Correlation with APACHE II score in patients with severe sepsis
166	Selenium	Correlation with APACHE II in patients with severe sepsis
167	Selenoprotein P	Decreased in sepsis compared with healthy controls
168	Serum bicarbonate	Predicted development of septic shock in neutropenic patients
169	Sphingomyelinase (enzyme activity)	Distinguished between survivors and non-survivors at 28 days in patients with severe sepsis
170	Sulfite	Predicted response to therapy
171	Transforming growth factor (TGF)-b1	Distinguished between survivors and non-survivors at 28 days
172	Tissue Inhibitor of Metalloproteinase (TIMP-1 and -2)	Distinguished between survivors and non-survivors at 28 days
173	TIMP-3	
174	Uric acid	Decreased in postoperative patients with sepsis compared with those with no sepsis
175	Urinary 8-OhdG	Distinguished between survivors and non-survivors at 28 days
176	Urinary bilirubin oxidative metabolites	Correlation with APACHE II score
177	Annexin V binding	Increased in sepsis compared with healthy controls
178	Xanthine oxidase (activity)	Distinguished between survivors and non-survivors at 28 days

of sepsis, predicting outcome, and guiding antimicrobial therapy?

Methods

A detailed description of the methods of literature search is depicted in the flowchart (Fig. 1). The search was conducted in 2 stages using PubMed. Filters were used in both stages to include only the adult critical care population, human studies, and articles published in English. After title and abstract review, articles relevant to answering the key questions were selected.

Results

A total of 65 articles were reviewed; these identified 178 biomarkers in use. Readily available biomarkers that are useful or potentially useful in clinical practice are discussed in detail. Other biomarkers are listed in Table 1. They are not described in detail, because most of these are still used for experimental purposes and not validated for clinical use.

Discussion

Microbiological biomarkers

Specimen cultures

Identifying organisms by culturing appropriate specimens in the context of clinical suspicion remains the recommended method for diagnosing infection. They identify the causative organism and also provide in-vitro antibiotic sensitivity. Administering appropriate antimicrobial agents early in the disease course can affect patient outcome, development of antimicrobial-resistant strains, and costs related to management of sepsis. Mere presence of bacteremia is associated with mortality of 26 % of critically ill patients [6].

However, specimen cultures have several limitations. First, the yield of these tests is low and depends on several factors detailed in Table 2. In one study microbiological infection was documented in only 71 % of patients with suspected severe sepsis, and bacteremia was documented in only 53 % [6]. Furthermore, detection of a low abundance of anaerobic organisms can be difficult [7]. Second, false-positive results are common. Up to 50 % of positive blood

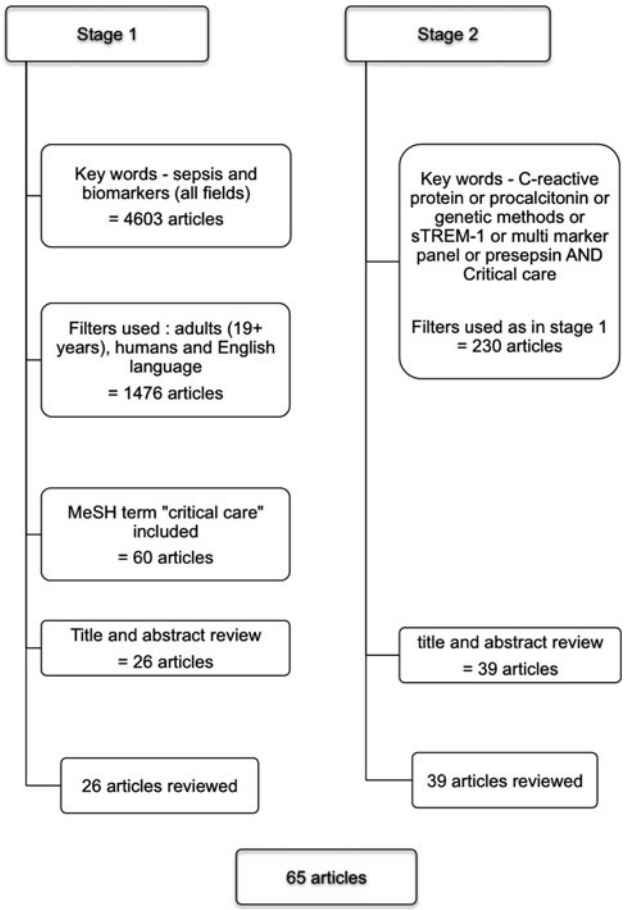

Fig. 1 Materials and methods

Table 2 Factors affecting yield of microbiological cultures

Intermittent bacteremia
Small number of detectable colony-forming units
Bactericidal properties of blood components
Volume of blood drawn (optimum 20 ml)
Concomitant antibiotic use
Number of blood samples cultured
Timing of blood cultures
Length of incubation time
Culture media and collecting system used

cultures are contaminated rather than indicative of true bacteremia [6]. Third, there is a delay in the availability of results, which can compromise initial management of patients. As a result focus has now changed to the development of techniques to shorten the time required for pathogen detection.

Gram stain

Gram stain may provide early evidence of the presence of specific pathogens but may not enable accurate detection of all infectious agents. The presence of bacteria in Gram stains of broncho-alveolar lavage (BAL) specimens had sensitivity of 44–90 % and specificity of 49–100 % in identifying patients with ventilator associated pneumonia (VAP) [8]. Results from Gram stains correlate with those from quantitative cultures on 79–86 % of occasions [2]. Accuracy is slightly better for Gram-positive than Gram-negative microbes.

Nucleic acid-based techniques

Nucleic acid based techniques have been applied to the direct detection of pathogens in blood and tissue samples over the last 20 years. They may shorten the time to pathogen detection and subsequently affect early therapeutic decisions. The techniques include polymerase chain reaction (PCR), microarrays, and hybridization. The major limitations of these methods are the relatively high cost and lack of provision of antimicrobial susceptibility patterns. They are summarised in Table 3.

Detection of pathogen-related antigens

Detection of pathogen related antigens is another useful approach to early diagnosis of infections. Helbig et al. assessed the clinical utility of *Legionella* urinary antigen assay for diagnosis of Legionnaires disease in 317 culture proven cases using the Binax enzyme immuno assay (EIA) or Bio test EIA or both. The sensitivity of these assays were, respectively, 93.7 and 94.4 % for travel-associated disease, 86.5 and 76 % for community-acquired infection, and 44.2 and 45.7 % for nosocomially acquired infection [9]. Testing of urinary samples for detection of the C-polysaccharide antigen in *Pneumococcal* pneumonia has also been investigated in a variety of studies, and variable sensitivity (70–100 %) and specificity (82–96 %) have been reported [10, 11].

Antigen detection may also be useful for diagnosis of fungal infections. Galactomannan (GM) is an *Aspergillus* species cell-wall component, detected by enzyme linked immunosorbent assay (ELISA). In a meta-analysis, the overall sensitivity and specificity of GM assays were 71 and 89 %, respectively, for proved cases of invasive Aspergillosis. [1→3]-β-D-Glucan (BG) is another cell-wall component present in *Candida* cell walls. These tests are particularly useful for detection of these infections in immuno-compromised hosts [2].

Haematological biomarkers

Raised white cell count and neutrophilia with a shift to left, and, less commonly, neutropenia are well known responses to sepsis. However, they are non-specific and can occur as

Table 3 Nucleic acid-based techniques [62–65]

Type	Comments	Example
Pathogen specific PCR	Useful when a specific infectious agent is strongly suspected	Rickettsiosis, brucellosis, or Q fever
Broad-range PCR	Detection of any cultivatable or non-cultivatable bacterial or fungal pathogens	16S rRNA for bacterial, 18S rRNA for fungal
Multiplex PCR	Provides a rapid means of detecting several bacterial and fungal pathogens in one test	Septifast, Roche Molecular Systems
Microarray technology	Enables the use of primers capable of amplifying all the genetic variants of the target genes	Can identify a large number of common pathogens
Fluorescence in-situ hybridization	Uses fluorescent labelled oligonucleotide probes complementary to unique target sites on the rRNA	Can identify most of the common pathogens. Can differentiate between different staphylococcal species

a result of any systemic insult, including non-infective causes. Eosinopenia is a simple and readily available means of differentiating between infectious and non-infectious causes of inflammation. The mechanism underlying eosinopenia is believed to be chemotactic factors that draw eosinophils to the site of infection [12]. Abidi et al. found that eosinopenia was a good diagnostic marker for distinguishing between the presence and absence of infection with the area under the receiver operating characteristic curve (ROC) of 0.89. In discriminating between SIRS (systemic inflammatory response syndrome) and infection, the area under ROC was 0.84 [13]. This was superior to C-reactive protein (CRP) at a cut-off value of 70 but inferior to procalcitonin [12].

Biochemical biomarkers

C-reactive protein

C-reactive protein is a member of the pentraxin family of calcium-dependent ligand-binding plasma proteins. CRP was first described in 1930 and was isolated from the plasma of acutely ill patients with *Streptococcal* pneumonia. It is synthesized principally by hepatocytes in response to stimulation by cytokines, for example interleukin-6 (IL-6), and has a plasma half-life of approximately 19 h. The rate of synthesis is the only significant determinant of its plasma level and, hence, CRP level is a useful objective index of acute phase response. The normal concentration is approximately 0.8 mg/L but may be elevated in the elderly. The level is increased in most forms of acute and chronic inflammatory states including sepsis syndromes and CRP is, perhaps, the most widely used biomarker of infection in critically ill patients [3].

CRP as a diagnostic biomarker

The sensitivity and specificity of CRP for diagnosis of infection varies in the literature and trends of CRP values seem to be more useful than absolute CRP values. Using a cut-off value of approximately 80 mg/L, the sensitivity varies from 67.6 to 93.4 % and specificity from 61.3 to 86.3 %. When combined with a temperature >38.2 °C, the specificity for diagnosis increased to 100 % [3]. Peres Bota et al. derived an "infection probability score" for prediction of the presence of infection by use of 6 different variables: temperature (0–2 points), heart rate (0–12 points), respiratory rate (0–1 point), white blood cell count (0–3 points), CRP (0–6 points), and SOFA (sequential organ failure assessment score) score (0–2 points). When the cut-off value was 14, the infection probability score had a positive predictive value of 53.6 and a negative predictive value of 89.5 %. The area under ROC curve was 0.82. The probability of infection in patients with a score of <14 was <10 % [14]. The score was higher in patients with the highest clinical probability of infection and decreased after effective antibiotic therapy; it was below this cut-off value in non-infected patients [15].

CRP as a prognostic biomarker

Again, absolute CRP values are less useful than trends in predicting prognosis and disease severity in sepsis. Lobo et al. showed that patients admitted with CRP levels >100 mg/L had a significantly higher incidence of organ failure and mortality than patients admitted with levels <10 mg/L. In patients with higher CRP levels, mortality decreased to 15 % if CRP levels decreased after 48 h whereas mortality was 61 % in patients whose CRP did not decrease [16].

Higher CRP levels on ICU discharge are associated with higher ICU readmission and higher hospital mortality [17].

Daily measurement of CRP after antibiotic prescription seems useful in predicting outcomes. Patients with an average fall of 10 % per day had 32 % more chance of surviving. A patient with a rapid response (described as CRP day 5/CRP day 1 <0.4) was 3 times more likely to

survive than patients with no response (described as CRP day 5/CRP day 1 >0.8) [18].

CRP for antibiotic stewardship

Reducing unnecessary antibiotic usage has advantages of reducing healthcare costs and adverse events, and reduces the development of antimicrobial resistance. CRP levels can be used for guiding duration of antibiotic therapy, because the decrease in CRP levels correlates with resolution of sepsis. In contrast, persistently high CRP levels 48 h after initiation of antibiotic therapy was suggestive of ineffective initial antibiotic therapy [19].

Limitations of CRP measurement

CRP lacks specificity, and concentrations may be increased in other inflammatory and all infectious disorders. Concentrations may be affected in patients taking steroid therapy. Low levels of CRP should be interpreted with caution in patients with fulminant hepatic failure, because these may reflect severity of hepatic synthetic function rather than absence of infection [20].

Procalcitonin (PCT)

Procalcitonin is the prohormone of the hormone calcitonin which is normally produced in the neuroendocrine C-cells of the thyroid gland in a regulated manner. In the absence of infection extra thyroidal production of PCT is suppressed. In the presence of a microbial infection pro-inflammatory mediators and bacterial toxins induce increased production and an unregulated and constitutive release of PCT from all parenchymal tissues and differentiated cell types, for example liver, adipose tissue, kidney, and muscle [21].

In healthy individuals, levels of PCT are <0.1 ng/ml [2]. Levels start to rise 4 h after the onset of systemic infection and peak at between 8 and 24 h. Levels can be elevated in renal failure in the absence of infection [22]. PCT levels are increased severalfold in sepsis. Its increase in bacterial infection is more prominent, especially in Gram-negative bacteremia; however, the rise is negligible in viral and mycoplasma infections. Levels >0.25 ng/mL may be associated with infection, but infection is less likely below these levels. However, the cut-off levels for diagnosing infection have not been clearly established. PCT has superior diagnostic accuracy compared with other markers in sepsis, and this remains unaffected even in the presence of concomitant immunosuppressive therapy in which the response of CRP is attenuated.

Tang et al. performed a meta-analysis of 2,000 patients in 18 studies of the accuracy of PCT for diagnosis of sepsis in critically ill patients. The performance of PCT was low, with sensitivity and specificity of 71 % and area under ROC curve of 0.78. They questioned widespread use of the PCT test in critical care for differentiating sepsis as a cause of SIRS [23]. In another meta-analysis of 33 studies with 3,943 patients, PCT was a superior diagnostic marker compared with CRP with an odds ratio of 15.7 for PCT and 5.4 for CRP [24]. These two studies compared the diagnostic efficacy of PCT and CRP and, obviously, PCT outperformed CRP. However, the diagnostic odds ratio was <25, making it a test of little use on its own. It should be noted that PCT levels were measured on admission in only 9 studies, thus introducing a bias. Also there was significant heterogeneity amongst the population studied. PCT on its own is a poor diagnostic marker of sepsis; it is, however, better than CRP; also sequential measurement of PCT levels may be more useful than single measurements.

PCT as a prognostic biomarker

PCT increases with increasing severity of sepsis and organ dysfunction [22]. Absolute values and trends of PCT levels are predictors of 90-day mortality [25]. PCT levels >1 ng/ml on day 1, and >0.5 ng/ml on day 7, in patients with VAP were predictors of unfavourable outcome which were defined as death or VAP recurrence or extra pulmonary infection requiring antibiotic therapy in 28 days [26]. However, another study showed that CRP, SOFA scores, age, and gender predicted mortality whereas PCT did not and, in particular, PCT levels on the day of admission often failed to predict prognosis [27].

PCT in antibiotic stewardship

The use of PCT as a marker for antimicrobial stewardship is extremely attractive in the current climate of increasing antibiotic resistance. In the "ProResp" study, antibiotic prescription was reduced by 46.6 % in a group of patients with lower respiratory tract infections without change in outcome. In the "ProCAP" study the same authors showed reduction in duration of antibiotic therapy from a median of 12–5 days in patients with community-acquired pneumonia [21].

The PRORATA trial compared PCT-guided antibiotic therapy with usual care for predominantly non-surgical patients with suspected bacterial sepsis. PCT-guided patients were exposed to significantly fewer days of antibiotics and stopping antibiotics early did not increase any of the sepsis-related worst outcomes. The limitations of the study were that only <50 % of the eligible patients entered the trial, the recommendations were not followed for 71 % of patients, and the prescribing algorithm was overruled for 20 % of the PCT-guided group. Patients with neutropenia

were excluded. It is also important to note that the patients in the control arm received nearly 10 days of antibiotic therapy, which is longer than the usual practice in the UK [28]. A systematic review addressing the safety of using serum PCT levels to reduce antibiotic use in ICU patients was conducted by Agarwal et al. [29]; again PCT guidance was associated with significantly reduced antibiotic exposure. Neither infection relapse nor mortality varied significantly in any of these studies. Not all studies report adherence to PCT guidance and in studies that report guidance adherence, a high incidence of non-adherence was found [30, 31].

In the Procalcitonin and Survival Study (PASS) Jensen et al. [32] attempted to determine whether a strategy of antimicrobial escalation, guided by daily measurements of PCT, could reduce the time to appropriate therapy, thus improving survival. Clinicians were prompted, on the basis of an "alert PCT" level (1 ng/ml), to investigate more thoroughly for a source of sepsis and to escalate the antibiotic regime on the basis of a protocol. This trial did not improve survival but led to increased use of broad-spectrum antibiotic therapy. Deleterious effects on organ function, increased mechanical ventilator days, and increased ICU length of stay were observed in the PCT group. These were primarily because of the toxicity of broad-spectrum antibiotics but could also have been because of the reluctance of clinicians to discharge patients with higher PCT levels [33].

It seems PCT-guided antibiotic therapy is not very useful for escalating antibiotic therapy in ICUs but may help to de-escalate such therapy. Interestingly, several studies have been published on antibiotic de-escalation or discontinuation policies based on clinical protocols in ICU and non-ICU settings [34–38]. In the UK, because of the strong drive to control healthcare-associated methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile*-associated diarrhoea, the duration of antibiotic courses has generally been reduced to 5–7 days even without PCT levels [22].

Limitations of PCT

There are several limitations to the use of PCT. First, there is no single cut-off range of PCT levels for defining sepsis. The optimum cut-off ranges for PCT depend on:

- the clinical setting—e.g. primary care, emergency room, ICU, post-operative, or trauma patients;
- the site and extent of infection—useful in systemic infections and respiratory tract infection, endocarditis, meningitis, etc.;
- co-morbidities—e.g. impaired pulmonary reserve in chronic obstructive lung disease patients, immunosuppression, etc., and
- the clinical implications drawn (e.g. diagnosis, prognosis, and antibiotic stewardship).

Optimum cut-off ranges must still be determined for most infections.

Second, false-positive and false-negative results can occur (Table 4). Last, PCT assays are more expensive than those for other commonly available biomarkers such as CRP—one tenth the cost [39].

Table 4 False-positive and false-negative results of PCT assay

False-positive	False-negative
Acute respiratory distress syndromes	Early infections
Acute graft-versus-host disease	Localised infections
Falciparum malariae infections	Sub-acute bacterial endocarditis
Systemic fungal infections	
Mechanical and surgical trauma	
Chemical pneumonitis	
Severe burns and heat strokes, pancreatitis	
Familial Mediterranean fever	
Malignancies—medullary thyroid cancer, small-cell-cancer of the lung, liver metastasis, carcinoid tumours, and paraneoplastic syndromes,	
Treatment with T cell antibodies, granulocyte transfusions, anti-thymocyte globulin administration, therapeutic TNFα administration for melanoma, etc.	
Newborns	

Biomarkers with potential to be used in clinical practice

Triggering receptor expressed on myeloid cells-1 (TREM-1)

Triggering receptor expressed on myeloid cells is a new family of receptors expressed on myeloid cells. Among this family, TREM-1 has been identified on human and murine polymorphonuclear cells and mature monocytes. Its expression is dramatically increased in tissues infected with bacteria and fungi. It has been confirmed that activation of TREM-1 amplifies the inflammatory response in mouse models of septic shock. Blocking these signals through TREM-1 in experimental sepsis animal models resulted in reduced mortality [40].

In addition to the membrane-bound form, a soluble form of TREM-1 (sTREM-1) is also found in septic patients. By binding to the TREM-1 receptor, it dampens TREM-1 activation and the inflammatory response. Considering the pitfalls of traditional biomarkers and the a-priori

involvement of TREM-1 during infections, the usefulness of sTREM-1 in the diagnosis of sepsis has been the focus of several studies in the last 5 years.

sTREM as a diagnostic biomarker in systemic infections

Gibot et al. found that plasma concentration of sTREM-1 in infected patients performed better than concentrations of CRP and PCT [41]—sensitivity, specificity, positive predictive value, and negative predictive values were 96, 89, 94 and 93 %, respectively. These results were not confirmed in other studies, however, [42, 43]. Therefore, measurement of plasma sTREM-1 does not seem to maintain its initial promise for diagnosis of systemic infections, and the different results could be because of the use of different laboratory techniques, cut-off values, patient cohorts, and study designs. However, combining sTREM-1 with other biomarker levels in a multimarker panel has performed far better than each marker alone in systemic infections [44].

There is evidence to show that local measurement of sTREM-1 concentrations may be useful for a variety of localised infections. For example, BAL sTREM-1 is a better marker of infection in bacterial pneumonia. Huh et al. examined diagnostic use of BAL sTREM-1 in 80 patients with bilateral lung infiltrates; concentrations were significantly greater in the extracellular bacteria and fungal group (521.2 ± 94.7 pg/ml) than in the viral/mycobacterial/atypical pathogen group (92.9 ± 20 pg/ml) and the non-infected group (92.8 ± 10.7 pg/ml). At a cut-off level of 184 pg/ml, sTREM-1 had a sensitivity and specificity of 86 and 90 %, respectively, and sTREM-1 had the highest area under ROC curve at 0.91 and remained the only statistically significant variable on multiple logistic regression analysis compared with the clinical pneumonia infection severity score and BAL neutrophil percentage [45].

In 5 different studies including 350 patients with pleural effusions, pleural fluid sTREM-1 concentrations enabled discrimination between infective and non-infective causes of pleural effusion. Similar results have been shown for meningitis, peritonitis, and septic arthritis [41]. In a recent meta-analysis to evaluate the accuracy of sTREM-1 as a diagnostic test for bacterial infection sensitivity and specificity were 82 and 84 %, respectively, and the area under the ROC curve was 0.86 [46].

sTREM-1 as a prognostic biomarker

Gibot et al. found baseline plasma sTREM-1 concentrations in septic patients were able to discriminate between survivors and non-survivors. Levels were higher and declined progressively in survivors [47]. Further studies have confirmed the prognostic value in patients with pneumonia [41].

Limitations of sTREM-1

Although earlier studies identified involvement of the TREM-1 pathway in sepsis only, recent studies have identified the involvement of this pathway in sterile inflammatory disorders, for example vasculitis, psoriasis, and inflammatory bowel disease, thus reducing the specificity of this test. There is huge heterogeneity between studies conducted in this field making it difficult to interpret results. The case mix is variable and so were the cut-off limits, and techniques used to measure sTREM-1 were also different.

Multimarker panels as sepsis biomarker

Because of the limitations of individual biomarkers, focus has now changed to combining several biomarkers (multimarker panels). Because sepsis and the host immune response to infection are complex, it is unlikely any single biomarker can be used to precisely classify and risk-stratify this syndrome. Combining several biomarkers has the theoretical advantage of improving diagnostic accuracy and their clinical usefulness. If each tested biomarker behaves independently as a predictor of disease, testing many biomarkers simultaneously provides the opportunity to improve both sensitivity and specificity.

Use of a multimarker panel entails using each biomarker to provide input to a multivariable, computational prediction or classification model. It can be homogeneous or heterogeneous, depending on the diversity of source data. For example, the homogeneous panel will consist exclusively of gene expression microarray analysis and a heterogeneous panel might include clinical variables also (e.g. lung injury score) [7].

Multimarker panel in diagnosis of sepsis

Kofoed et al. developed and validated a multiplex add-on assay as a sepsis biomarker, using a technique that compared a 6-marker panel (V-suPAR-soluble urokinase type plasminogen activator receptor, sTREM-1, macrophage inhibiting factor (MIF), CRP, PCT, and neutrophil count) with a 3-marker panel (neutrophil count, CRP, and PCT) and with each component of the 6-marker panel individually. The primary end-point was to assess which marker could identify bacterial infection in patients with SIRS. The 6-marker panel outperformed others [44].

Selberg et al. compared combination of PCT and complement 3a (C3a) in a "sepsis score" model with the performance of PCT, IL-6, C3a, elastase, and CRP

individually in identifying sepsis. The sepsis score was derived using a complicated logistic regression equation that identified 28.6 as optimum cut-off between the patients with SIRS and sepsis. The sepsis score with the combination of PCT and C3a outperformed individual biomarkers [48].

Shapiro et al. designed a large multicentre study with 971 patients for developing a sepsis score with a biomarker panel that consisted of neutrophil gelatinase-associated lipocalcin, protein-C, and IL-1 receptor antagonist. Initially 9 biomarkers were used but based on their performance in a multivariate logistic regression model only 3 biomarkers were finally used to derive the score. Models with more than 3 biomarkers were not shown to improve diagnostic accuracy. The area under the ROC curve for the sepsis score was 0.80 for severe sepsis, 0.77 for septic shock, and 0.79 for death [49].

Multimarker panel as a prognostic biomarker

Some studies have looked into the use of multimarker panels to predict mortality in sepsis. Kofoed et al. showed the ability of 4 composite biomarkers including a 3-marker panel (V-suPAR, sTREM-1, MIF) and age to predict 30 and 180-day mortality for patients presenting with community-acquired infection. The 3-marker panel plus age outperformed others with area under the ROC curve of 0.93 for 30-day mortality and 0.87 for 180-day mortality [50].

Dhainaut et al. created a composite coagulopathy score using data such as antithrombin measurement at baseline, decrease in antithrombin measurement in time, lack of decrease in antithrombin measurement in time, and a lack of a decrease in D-dimer measurement in time. This, in combination with the APACHE II score, was able to predict 28-day mortality and organ failure better than APACHE II score alone [51].

Challenges and limitations

The real challenge will be optimum selection and validation of a subgroup of clinically useful markers from a large pool of biomarkers. Searching for optimum combinations of biomarkers will significantly increase the complexity of the statistical modelling and increase computational demands. The process of converting multiple reliable biomarkers to a practical clinical test may be difficult. Appropriate methodology for clinical and statistical evaluation of multimarker panels has not yet been systematically defined. As a result most of the studies are not well designed and are single-centred with limited numbers of patients. Most study results to-date require external validation [7].

Presepsin

CD14 is a glycoprotein expressed on the surface membrane of monocytes, macrophages, and other cells. It is a high-affinity receptor for lipopolysaccharides (LPS) and lipopolysaccharide binding protein (LBP). The complex LPS-LBP-CD14 is released into the circulation in high levels in response to sepsis. This soluble form is called presepsin or sCD14 subtype (sCD14-ST) [52].

Presepsin has been shown by some studies to be a specific and sensitive marker for diagnosis of sepsis. In a preliminary study with 221 subjects involving healthy volunteers, and patients with sepsis and aseptic SIRS, Yaegashi et al. showed that presepsin levels were significantly higher in patients with sepsis than in those with aseptic SIRS or in healthy volunteers. The author also showed that the area under ROC was higher for presepsin than for other biomarkers, for example IL-6 and PCT [53].

Subsequently, Endo et al. demonstrated that presepsin was comparable with PCT as a diagnostic biomarker for sepsis in a group of 185 patients with SIRS. The area under ROC for presepsin and PCT for diagnosing sepsis was 0.908 and 0.905, respectively. At a cut off value of 600 pg/ml, presepsin had a sensitivity, specificity, PPV, and NPV of 87.8, 81.4, 88.6, and 80.3 %, respectively. The sensitivity of presepsin was higher for Gram-positive bacterial infection (95.5 %) than for Gram-negative infections (77.8 %) [54].

Presepsin has also been shown to be helpful for assessing the severity of sepsis and for monitoring therapeutic responses [55]. Results from a newly developed assay called PATHFAST, a presepsin assay system based on chemiluminescent enzyme immune assay, have correlated well with those from ELISA. Results are revealed in 17 min, which makes it very attractive for point-of-care assays in intensive care or emergency departments. Although widely used in some centres for diagnostic purposes, further multi-centre studies are required before drawing firm conclusions.

Other biomarkers

Other biomarkers in this field are listed in Table 1. It is worth mentioning IL-6 that has been part of several research studies. It is a non-specific diagnostic marker of systemic inflammation and hence not a good diagnostic marker of sepsis on its own; it may, however, be useful as a part of a multimarker panel as discussed above. Also it can be a very useful prognostic tool and high IL-6 levels correlate with mortality and organ failure. Studies have demonstrated that IL-6 levels were <1,000 pg/ml in survivors of sepsis and higher in non-survivors [56].

Future biomarkers

Genome-based sepsis biomarkers

Genome-based biomarkers are expected to expand rapidly in near future—largely as a result of improvements in assay techniques. Data mining and interrogation of the DNA sequences for large number of septic patients should yield a large number of novel genes that alter the outcome of sepsis [57]. Genes regulating the immune response are heavily affected during systemic inflammation and sepsis. The alterations in gene transcriptions that affect cytokine synthesis, cytokine receptor expression, T cell differentiation, protein synthesis regulation, and regulators of apoptosis are readily observed in severely septic patients. This has led to the development of gene expression assays as biomarkers for sepsis [58]. If this type of analysis is confirmed, this technique could provide a novel biomarker for differentiating sterile inflammation from sepsis.

Proteomics and sepsis markers

Proteomics is the global assessment of the nature of synthesized proteins. The advantage of studying proteins in sepsis is that secreted proteins are the signalling systems that convert genetic signals into enzymatic activity. Advances in systems biology and technical aspects of proteomics will provide a new level of understanding of sepsis in the future—rather than measuring individual proteins, a whole battery of proteins can be studied simultaneously. Early experience with proteomics and systems biology in sepsis has already revealed several interesting findings in sepsis in experimental models and septic patients [57].

Angiopoietin 1 and 2

Angiopoietins are potential endothelial biomarkers of sepsis. By binding to the TIE2 (tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domain) receptor, angiopoietin-1 stabilises the endothelium, inhibits vascular leakage and inflammatory gene expression, and prevents recruitment and transmigration of leucocytes. Angiopoietin-2 competes with angiopoietin-1 and antagonises its effects. Angiopoietin-2 levels <11 ng/ml have been associated with good prognosis for critically ill patients, and higher levels are associated with poor outcomes [59].

Endotoxin activity assay (EAA)

The USA Food and Drug Administration has recently approved an endotoxin assay for assessing the risk of developing severe sepsis in ICUs. Klein et al. found positive correlation with mortality and EAA levels—mortality in patients with septic shock was 14 % when EAA levels were <0.4, but nearly double that when levels were >0.6 [57].

Activated partial thromboplastin time biphasic waveform analysis (BPW)

Downey et al. first discovered an abnormality of the optical transmission waveform obtained during the measurement of activated partial thromboplastin time. This is caused by in-vitro formation of calcium-induced complexes between very low-density lipoproteins and CRP. Chopin et al. studied use of this technology in 187 septic patients and demonstrated sensitivity and specificity of 92 and 67 %, respectively. It performs better when combined with PCT, which increases specificity. Delannoy et al. were able to discriminate sepsis and SIRS, in a group of post-cardiac surgery patients, with a sensitivity and specificity of 100 and 93 %, respectively [60, 61]. Further studies are required to corroborate the use of BPW analysis and, if precise diagnostic significance is established, BPW analysis may become an important component in the panel of sepsis markers currently used routinely.

Problems with sepsis biomarker research

First, we lack a recommended biomarker to define sepsis. We need to accept the reality that "no current biomarker will answer the question—is this patient infected?". Microbiological cultures continue to be described as the recommended method for diagnosis of sepsis. However, it is a far from being an ideal method, for the reasons discussed above. Second, we lack appropriate methods for clinical and statistical evaluation of sepsis biomarkers. Third, although sepsis is a common phenomenon we lack understanding of its biology.

A consensus conference on sepsis biomarkers involving experts in clinical medicine, microbiology, biochemistry, and biostatistics to address the problems in biomarker research may be useful. They should agree on a recommended biomarker. This biomarker may be a combination or a multimarker panel taking together the clinical predictive scores and readily available biomarkers. This may not be an ideal biomarker but may be better than the currently thought-to-recommended method. The expert panel should, where appropriate, also propose a specific cut-off range for biomarkers.

The panel should decide on standards of methodology and publication. For example emphasis should be laid on publishing likelihood ratios. By defining standards in biomarker research, it is quite possible for research in this field to progress seamlessly. Heterogeneity, inadequately powered studies, and duplication of small studies may be avoided. Development of sepsis biomarkers will help us

understand sepsis biology better, and the vice versa. Therapeutic research also may advance.

Conclusion

None of the currently available biomarkers can be used to definitely determine whether or not a patient is infected, and the search for an ideal biomarker continues. Current problems with biomarker research must be addressed by an expert panel to make adequate progress in this field. Until then, diagnosis of bacterial infections will continue to require critical clinical awareness, careful patient history, dedicated physical examination, and judicious use of currently available biomarkers or combination biomarkers. Some of the biomarkers currently available may aid assessment of disease severity or the appropriate use of antimicrobial therapy.

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