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Procalcitonin, C-reactive protein, and presepsin for the diagnosis of sepsis in adults and children.

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Procalcitonin, C-reactive protein, and presepsin for the diagnosis of sepsis in adults and children

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ABSTRACT

This is a protocol for a Cochrane Review (Diagnostic test accuracy). The objectives are as follows:

The objectives of this review are:

- To assess the diagnostic accuracy of PCT, CRP and presepsin for sepsis in adults and children.
- To investigate sources of heterogeneity in the estimates of diagnostic accuracy
- To compare the performance of the above tests.

BACKGROUND

This is the protocol for the three diagnostic test accuracy reviews:

1. Procalcitonin for the diagnosis of sepsis in adults and children;
2. C-reactive protein for the diagnosis of sepsis in adults and children; and

A comparison of the three diagnostic tests will be included in the review: Presepsin for the diagnosis of sepsis in adults and children.

Target condition being diagnosed

Sepsis, which consists of systemic inflammatory response to an infection, is increasing in incidence (Bone 2009; Martin 2012). There was more than a double-fold rise in the hospitalization rate, from 11.6 to 24.0 per 10,000 population between 2000 and 2008, for people primarily diagnosed with sepsis. This increase may be due to increasing antimicrobial resistance, greater use of invasive medical procedures and immunosuppressive drugs, and the increasing elderly population (Martin 2012). The sepsis spectrum: sepsis, severe sepsis and septic shock, is a leading cause of mortality in critically ill people (Angus 2001). The number of deaths from severe sepsis may be equivalent to, or surpass, those from cancer, stroke or acute myocardial infarction (Angus 2001). The likelihood of in-hospital mortality is at least eight times higher in
people with sepsis than in people with other medical conditions (Hall 2011). The average length of stay in hospital for people who are hospitalized for sepsis is 75% longer than for those hospitalized for other diagnoses (Hall 2011).

Early signs of sepsis are quite variable and non-specific, making the routine diagnosis of sepsis challenging. Delay in diagnosing sepsis further worsens the outcome in people with sepsis. The challenge lies in the immediate and accurate distinction of sepsis from the non-infectious systemic inflammatory response syndrome (SIRS) which may occur in critically ill people. In adults, SIRS consists of a core temperature over 38°C or lower than 36°C, a respiratory rate over 20 breaths per minute or PCO₂ (partial pressure of carbon dioxide in the blood) below 32 mmHg, a pulse rate over 90 beats per minute and a leucocyte count less than 4000/mm³ or more than 12,000/mm³ or more than 10% band cells (Bone 2009). In children, the cut-off values for the four criteria of temperature, pulse rate/heart rate, respiratory rate and leucocyte count vary for different age groups (Goldstein 2005). Additionally, the confirmation of bloodstream infections (which may arise from bacterial, fungal, viral or parasitic origins) by culture, is positive in only about 30% to 50% of sepsis cases (Murray 2012). In 1991, a consensus panel of the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) provided a practical framework for the definition of sepsis (Bone 2009). The panel defined sepsis as the presence of two or more SIRS criteria, with documented or suspected infection. However, in 2003, an international consensus panel of the SCCM, the European Society of Intensive Care Medicine (ESICM), the ACCP, the American Thoracic Society (ATS) and the Surgical Infection Society (SIS), having noted that the SIRS criteria published in 1992 were unduly sensitive and non-specific, provided an expanded list of variables for the diagnosis of sepsis, severe sepsis and septic shock (Levy 2003). Despite the revised definition for sepsis, the ACCP/SCCM definition which entails SIRS criteria may be preferred by most clinicians, because of its concise nature.

Reports from a recent study among people with severe sepsis also question the sensitivity of the SIRS criteria for sepsis diagnosis. The use of the SIRS criteria for the diagnosis of sepsis would miss out one in eight people with infection and organ failure. These people with SIRS-negative severe sepsis have similar epidemiologic trends to people who have the typical SIRS criteria (Kaukonen 2015).

Early in 2016, another set of updated criteria for sepsis was proposed by a consensus task force of the ESCIM and the SCCM defining sepsis as a life-threatening organ dysfunction resulting from a dysregulated host response to inflammation. The new definition recommended discarding the term severe sepsis and focused on organ dysfunction as a distinguishing point between an infection and sepsis. It proposed a tool - quick SOFA (qSOFA)-Sequential (sepsis-related) Organ Failure Assessment. The qSOFA is based on the presence of two of three warning signs for the rapid identification of organ dysfunction and people at increased risk of mortality or prolonged stay in the intensive care unit. The three warning signs include an altered mental status, a systolic blood pressure of 100 mmHg or less and a respiratory rate of 22 breaths per minute or more (Singer 2016).

These continuous changes in the definition of sepsis reflect the challenges experienced in the diagnosis of sepsis. In order to aid the rapid distinction of sepsis from SIRS, the use of various biochemical tests has been proposed and some are in clinical use for this purpose (Dellinger 2013).

Index test(s)

We will evaluate the diagnostic performance of three different biochemical blood tests: Procalcitonin (PCT), C-reactive protein (CRP), and presepsin which are used as biomarkers for sepsis. Because we anticipate many primary studies evaluating these biomarkers, we will present the results of these tests as three separate reviews. Presenting these test results as three separate reviews will also enable us to interpret and discuss the findings with sufficient detail. In light of the need relevant for clinical practice, we shall conduct and present these reviews in the following order: PCT, CRP and presepsin.

Procalcitonin (PCT) is a precursor of the hormone calcitonin produced by the parafollicular cells of the thyroid and the neuroendocrine cells of the lung and the intestine. Due to its being released in response to sepsis, the PCT assay is presently in use as a diagnostic tool for sepsis, in the USA, Europe, Australia, Asia and in some parts of Africa (Lloyd 2012; Schneider 2007). The PCT test quantifies PCT in serum or plasma and results are usually available within one hour. Assays can be performed at point of care or in the routine laboratory. Commonly-observed PCT values in healthy individuals who are aged three days old and above, are less than 0.05 ng/mL. While PCT levels 0.5 ng/mL to 2.0 ng/mL may indicate sepsis, levels above 2.0 ng/mL but less than 10 ng/mL indicate a high risk for progression to organ dysfunction (Meisner 2014; Nargis 2014). In the absence of sepsis, a rise in the levels of PCT has been reported in people with Addisonian crises (Schumm 2010), people undergoing conditioning treatment for stem cell transplantation using anti-thymocyte globulin (Brodsk 2009) and in people undergoing transplants administered with pan T-cell antibody (Satar 2001).

CRP is a plasma protein synthesized by the hepatocytes. It rises in response to inflammation (cell injury) and various pathogens (infection) because of its cell-membrane-binding capability which occurs through its attachment to the phosphocholine in exposed cell membranes during cell injury, and the phosphocholine in the polysaccharides of pathogens present in infections (Volanakis 2001). In humans, plasma CRP levels are typically below 5 mg/L but may rise exponentially, within a few hours, in response to an acute inflammatory stimulus (Black 2004). The typical cut off for CRP test is 10 mg/L and most assays have a lower limit of sensitivity of 5 mg/L. However, plasma CRP levels may not rise
to 10 mg/L until after 24 hours, consequently, two values more than 10 mg/L taken 24 hours apart contend the diagnosis of sepsis (Zecca 2009). The CRP test results are available within minutes for the point-of-care testing (POCT) assays or within an hour for the laboratory-based assays. The POCT assays require very minute volumes of blood and may be semi-quantitative, such that values less than 10 mg/L give a negative result, ruling out sepsis. The laboratory assays are quantitative hence, they are ideal for the serial monitoring of patients (Vallance 1991; Zecca 2009). Presepsin is also known as soluble CD14 subtype (sCD14-ST). It is a glycoprotein-fragment derived from monocytes and macrophages and produced in association with infections. Though its diagnostic accuracy has not been as extensively studied as that of CRP and PCT; a few reports have found that it may have a better prognostic value in sepsis than PCT (Masson 2014; Ulla 2013). Presepsin assays are available as POCT or routine laboratory tests and are based on immunochemical methods (Okamura 2011; Shirakawa 2011). Results are available as early as 20 minutes after sample collection. Reported levels of presepsin in plasma of healthy individuals, people with SIRS, local infection or sepsis are several folds higher than the assay limit of quantitation (Masson 2014; Ulla 2013).

Clinical pathway

When a person has signs of systemic inflammation and a suspected infection, blood samples and relevant body fluid samples are taken for culture to confirm the presence of an infection. The results become available in 24 hours to 48 hours after sample collection. In some cases, the source of infection may be obvious, such as some respiratory tract infections in which there may be signs of a pneumonia on a chest radiograph; urinary tract infection where the person may present with symptoms and signs like dysuria and urinary frequency respectively. In other cases, the presence of predisposing factors in an individual raises the suspicion for sepsis. These factors include the presence of in-dwelling catheters or medical devices; elderly people; immunosuppression such as seen in severe burns, transplant recipients, people receiving chemotherapy, people with poorly-controlled diabetes mellitus; cellulitis; recent surgery or invasive procedure, perforated vescic and syndromes associated with high risk of infection such as ascending cholangitis (Wacker 2013).

Sepsis is classified as severe when there is cardiovascular dysfunction or acute respiratory distress syndrome (ARDS) or two or more other acute organ dysfunctions manifested as acute kidney injury, or acute liver failure, thrombocytopenia or coagulopathy. Hypoperfusion from cardiovascular dysfunction may manifest as oliguria, lactic acidosis or an acute mental status alteration (Dellinger 2008).

Septic shock is severe sepsis with arterial hypotension, in spite of adequate fluid resuscitation of 20 mL/kg crystalloid, in the presence of perfusion abnormalities. Hypotension occurs when the mean arterial pressure is less than 70 mmHg or the systolic blood pressure (SBP) is less than 90 mmHg or falls by more than 40 mmHg from the baseline SBP. People receiving inotropics may not be hypotensive (Bone 2009).

Current management guidelines recommend immediate institution of therapy once sepsis is suspected. Some of the therapeutic measures include the administration of antimicrobials within one hour of recognition of septic shock, the quantitative resuscitation of patients within six hours of recognition of sepsis, and the collection of samples for culture before administration of antibiotics (Dellinger 2013).

Role of index test(s)

The diagnosis of sepsis is largely dependent on clinical judgement. Early diagnosis and treatment influence the course of the illness and patient outcome. Accurate distinction between sepsis and SIRS is challenging, however, it is required for decision-making as regards prompt institution of antimicrobial therapy and subsequent continuation of such therapy. Microbiological culture, which remains the reference laboratory test for sepsis, is rife with shortfalls, some of which include a long turnaround time of up to 48 hours for test results and false negative results in prior antimicrobial therapy and with fastidious organisms. Biomarkers such as CRP, PCT, and presepsin with rapid turnaround time, have been proposed as adjuncts in the early diagnosis of sepsis while awaiting culture results (Sierra 2004; Wacker 2013; Zhang 2015). They do not serve as substitutes for microbiological culture. Although CRP, PCT and presepsin have been studied for diagnosis of sepsis, they are not recommended for use as independent diagnostic tools for sepsis (Chan 2011). These biomarkers, when used in conjunction with clinical assessment, may also guide antimicrobial therapy and reduce unnecessary antimicrobial exposure. (Schuetz 2012).

Alternative test(s)

There are a number of other biomarkers, especially inflammatory cytokines like interleukin 6 (IL-6), IL-8, which have been proposed as diagnostic tools for sepsis. These other biomarkers will not be considered for this review. There is an on-going Cochrane Review for the role of IL-6 in the diagnosis of sepsis (Molano Franco 2015).

Rationale

In recent years, various biomarkers such as CRP, PCT and presepsin, have been studied and are proposed as helpful diagnostic tools for sepsis (Harbarth 2001; Rothenburger 1999; Ulla 2013). The early distinction of sepsis from non-infectious conditions with similar clinical signs, is required for instituting a prompt and appropriate intervention. This ensures a favourable patient outcome and prevents unnecessary antibiotic usage, which is a driving force

Procalcitonin, C-reactive protein, and presepsin for the diagnosis of sepsis in adults and children (Protocol)
for antibiotic resistance. The results of CRP, PCT or presepsin as biomarkers for sepsis are usually obtained within a shorter time compared to the traditional laboratory test for sepsis, the blood culture or culture of other relevant body fluids. Assaying these biomarkers may be expensive and there is a need to objectively review the diagnostic performance of these biomarkers in order to determine what role each may play in the prompt and accurate distinction of sepsis from SIRS. There is currently no Cochrane systematic review that has evaluated the test accuracy of these biomarkers for diagnosis of sepsis in adults and children.

**OBJECTIVES**

The objectives of this review are:

- To assess the diagnostic accuracy of PCT, CRP and presepsin for sepsis in adults and children.
- To investigate sources of heterogeneity in the estimates of diagnostic accuracy
- To compare the performance of the above tests.

**METHODS**

**Criteria for considering studies for this review**

**Types of studies**

We will consider any study that compared PCT, CRP or presepsin levels in participants aged 29 days and older with suspected sepsis, in whom the confirmation of sepsis was by clinical diagnosis or microbiological confirmation of infection in cultures, or both. We will exclude case histories and case control studies. We will also exclude any cross-sectional study in which data for true positives, true negatives, false positives and false negatives cannot be extracted.

**Participants**

We will include adults and children admitted to wards, intensive care units or emergency departments with suspected sepsis, or confirmed sepsis, or both, severe sepsis or septic shock. We will exclude neonates as there is another ongoing Cochrane Review specifically evaluating the use of CRP and PCT for sepsis in neonates (Seliga-Siwecka 2015).

We will also include reports of studies with only a subgroup of participants eligible for inclusion.

**Index tests**

The index tests will be CRP, PCT and presepsin.

**Target conditions**

The target condition is sepsis (including severe sepsis and septic shock) as defined by the SCCM/ESICM/ACCP/ATS/SIS criteria (Levy 2003). These criteria are listed in Appendix 1.

**Reference standards**

The reference standard for the diagnosis of sepsis is the criteria developed by the International Sepsis Definitions Conference (Levy 2003), and these are listed in Appendix 1. These criteria include the presence of an infection in association with other variables. The clinical suspicion of infection is as a result of certain characteristics: perforated viscus, white blood cells in a normally sterile body fluid, radiographic features of pneumonia with production of purulent sputum and syndromes associated with a high risk of infection such as ascending cholangitis (Wacker 2013). Microbiological confirmation of infection involves the use of blood culture or culture of other body fluids, it is strictly a laboratory-based test to detect the presence of micro-organisms in blood or body fluids. We anticipate a lot of differences in the definition used for sepsis in the primary studies. We would include all studies that meet any of the criteria or definitions for sepsis and perform a subgroup analysis based on the reference standard definition.

**Search methods for identification of studies**

**Electronic searches**

We will search MEDLINE (OvidSP, 1946 to Present); EMBASE (OvidSP, 1974 to Present); LILACS (1982 to Present); CINAHL (1981 to Present); BIOSIS Previews (1969 to Present).

We will use an electronic search strategy that combines indexing terms and text words to capture the index tests and the target condition.

The current version of our search strategy for MEDLINE is shown in Appendix 2 and was developed by Cochrane Anaesthesia, Critical and Emergency Care’s Information Specialist. We shall adapt this strategy to the other listed databases and we will consider articles in all languages.

We will scan The World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) and ClinicalTrials.gov for ongoing and unpublished studies.
Data collection and analysis

Two review authors (CPO and CIO) will independently apply the selection criteria to all titles and abstracts.

Selection of studies

We will consider studies published in all languages. We (CPO and CIO) will retrieve the full text of all relevant articles and independently assess for inclusion. One review author (EAO) will adjudicate any case of discrepancy between CPO and CIO, concerning the inclusion of a report. When there are multiple reports or there is a possibility of overlapping study populations, we will select the most recent and complete report for a study.

Data extraction and management

We will use a separate data extraction form for each index test (PCT, CRP and Presepsin; results of each test will be presented as a separate systematic review). Two review authors (CPO and CIO) will use a standardized form to independently abstract information from each study meeting the inclusion criteria. For any study in which a subgroup of participants meets the inclusion criteria for the review, we will extract and analyse data for this subgroup only. If data are available, we will construct two-by-two tables for each index test evaluated in the study at all the reported test thresholds. Otherwise, we will compute the number of true positives, true negatives, false positives and false negatives using the summary estimates of sensitivity and specificity of the index test, if available. Where reported, we will exclude any infinite or undetermined index test results from the analyses.

Table 1 presents the data to be extracted from each study. We will resolve any disparities in the data extraction by consensus. Where this fails, we will consult the third review author (EAO) for adjudication.

Assessment of methodological quality

Two review authors (CPO and CIO) will independently assess the methodological quality of all studies based on the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool (Whiting 2011). In order to ensure consistent assessments, we have developed a rating guideline with criteria for answering signalling questions and assessing risk of bias and concerns regarding applicability. This is presented in Appendix 3. We (CPO and CIO) will pilot our review-tailored QUADAS-2 tool against 10 primary studies to assess how consistent it is and to detect any possible areas of discrepancy between review authors. If necessary, we will make amendments to the tool to ensure consistency. We will resolve all discordant assessments by discussion or, where necessary, adjudication by a third review author (EAO). We will present the outcome of the methodological quality assessment in tabular form, summarizing the number of studies with low, high or unclear risk of bias for each of the four domains in our QUADAS-2 rating guideline. We will present concerns regarding applicability in a similar tabular form. We will explore the influence of risk of bias on accuracy in sensitivity analyses by excluding studies with a high risk of bias.

Statistical analysis and data synthesis

We will first descriptively present the results of sensitivity and specificity (and their 95% confidence intervals (CI) graphically in both forest plots and receiver-operating characteristics (ROC) space using the software Review Manager 5 (RevMan 5) (RevMan 2014)). For each test, we will identify the most commonly reported test threshold in all included studies and use the bivariate random-effects model to perform the overall meta-analysis at that threshold (Macaskill 2010). For example, commonly used thresholds include: for CRP (10 mg/L for most assays or a lower limit of sensitivity of 5 mg/L and above) and for PCT (general cut-off for sepsis is 0.5 ng/mL, levels as low as 0.05 ng/mL may occur in viral infections (Lloyd 2012; Zecca 2009). Should multiple thresholds be reported by each study, we will perform meta-analyses at the commonly used thresholds for each test using the bivariate random-effects model.

Where data are sufficient we shall compare the results of the tests directly (tests applied to the same individual) and indirectly. To facilitate these comparisons, we shall include the covariate test type as a covariate in the bivariate model and check the effects on sensitivity and specificity. We will conduct and present direct and indirect comparisons in the last systematic review we shall present, that is, on presepsin.

We shall conduct these analyses with the statistical software SAS, version 9.4 (sas.com/en_gb/software/sas9.html). The unit of analysis will be individual participants.

Investigations of heterogeneity

If sufficient data are available we will investigate the following sources of heterogeneity in the diagnostic performance across studies: severity of illness (e.g. severe sepsis versus septic shock), age (children versus adults), admission category (medical versus surgical patients), test manufacturer, type of assay and reference standard by fitting the covariates in the bivariate model. Studies that do not present criteria or stratified results for the covariates age (children versus adults) and severity of illness (severe sepsis versus...
Sensitivity analyses
We will perform a sensitivity analysis to check robustness of the results by excluding studies with high risk of bias for each QUADAS-2 domain: patient selection, index test, reference standard and patient flow (Whiting 2011).

Assessment of reporting bias
We will not attempt to carry out any formal assessment of reporting bias because methods for diagnostic test accuracy (DTA) reviews have still not been conclusively recommended (Macaskill 2010).

Acknowledgements
We would like to thank the Cochrane Anaesthesia, Critical and Emergency Care (ACE) editorial team: Harald Herkner (content editor), Peter Schlattmann, David T Huang, Martin Hoenigl (peer reviewers) and the Cochrane Diagnostic Test Accuracy editorial team for their help and editorial advice during the preparation of this protocol for the systematic review. We are also grateful to the Anaesthesia, Critical and Emergency Care Group’s Information Specialist, Janne Vendt, for developing the search strategy.

References

Additional references

Angus 2001

Black 2004

Bone 2009

Brodska 2009

Chan 2011

Dellinger 2008

Dellinger 2013

Goldstein 2005

Hall 2011

Harbarth 2001

Kaukonen 2015

Levy 2003
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**Ulla 2013**  

**Vallance 1991**  

**Volanakis 2001**  

**Wacker 2013**  

**Whiting 2011**  

**Zecca 2009**  

**Zhang 2015**  

* Indicates the major publication for the study

## ADDITIONAL TABLES

### Table 1. Data from each study

<table>
<thead>
<tr>
<th>Study ID</th>
<th>First author, year of publication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Journal article or unpublished study</td>
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<tr>
<td></td>
<td>Type of study</td>
</tr>
<tr>
<td></td>
<td>Clinical features and settings</td>
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<tr>
<td></td>
<td>Participants</td>
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<tr>
<td></td>
<td>Study design</td>
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<tr>
<td></td>
<td>Reference standard</td>
</tr>
<tr>
<td></td>
<td>Index tests</td>
</tr>
</tbody>
</table>

- Presenting signs and symptoms
- Medical or surgical
- Age range for inclusion
- Intensive care unit, wards or emergency department
- Single or multi-centre study

- Sample size (n)
- Country of study
- Age distribution
- Empirical antibiotics usage

- Retrospective or prospective design
- Sample (consecutive, random or unclear)

- Clinical diagnosis
- Culture - blood, body fluid
- Culture and clinical diagnosis
- Interval between index test and reference standard

- Name of assays
- Manufacturer
- Analyser/device

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Procalcitonin, C-reactive protein, and presepsin for the diagnosis of sepsis in adults and children (Protocol)  
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Table 1. Data from each study  

<table>
<thead>
<tr>
<th>Target Condition</th>
<th>Sepsis spectrum - sepsis, severe sepsis, septic shock</th>
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<tbody>
<tr>
<td>Specimen type (venous/capillary; whole blood, serum or plasma)</td>
<td>Sepsis spectrum - sepsis, severe sepsis, septic shock</td>
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<tr>
<td>Specimen tube (for laboratory-based assay)</td>
<td>Sepsis spectrum - sepsis, severe sepsis, septic shock</td>
</tr>
<tr>
<td>Cut-off values</td>
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<td>Number of true positives, false positives, true negatives, false negatives and undetermined/uninterpretable results</td>
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<tr>
<td>Missing results for reference standard</td>
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<td>Notes</td>
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<td>Source of funding (whether any author is affiliated with the manufacturer of the index test; the study was directly funded by the manufacturer; study authors reported conflicts of interests related to the manufacturer or other funding sources)</td>
<td>Sepsis spectrum - sepsis, severe sepsis, septic shock</td>
</tr>
<tr>
<td>Anything else of relevance</td>
<td>Sepsis spectrum - sepsis, severe sepsis, septic shock</td>
</tr>
</tbody>
</table>

CRP: C-reactive protein  
n: sample size  
PCT: Procalcitonin

A P P E N D I C E S

Appendix 1. Criteria for sepsis diagnosis

Diagnostic criteria for sepsis

Sepsis - documented or suspected infection in association with some of the following parameters

General parameters
- Fever (core temperature, > 38.3°C)
- Hypothermia (core temperature, < 36°C)
- Elevated heart rate (> 90 beats per min or > 2 SD above the upper limit of the normal range for age)
- Tachypnoea
- Altered mental status
- Substantial oedema or positive fluid balance (> 20 mL/kg of body weight over 24 h)
Inflammatory parameters
- Leukocytosis (white-cell count, > 12,000/mm³)
- Leukopaenia (white-cell count, < 4000/mm³)
- Normal white-cell count with > 10% immature forms

Haemodynamic parameters
- Arterial hypotension (systolic pressure, < 90 mmHg; mean arterial pressure, < 70 mm Hg; or decrease in systolic pressure of > 40 mmHg in adults or to >2 SD below the lower limit of the normal range for age)
- Elevated mixed venous oxygen saturation (> 70%)\(^c\)
- Elevated cardiac index (> 3.5 L/min/m² of body surface area)\(^d\)

Organ dysfunction parameters
- Arterial hypoxaemia (ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, < 300)
- Acute oliguria (urine output, < 0.5 mL/kg/h or 45 mL/h for at least 2 h)
- Increase in creatinine level of > 0.5 mg/dL (> 44 µmol/L)
- Coagulation abnormalities (international normalised ratio, > 1.5; or activated partial-thromboplastin time, > 60 s)
- Paralytic ileus (absence of bowel sounds)
- Thrombocytopaenia (platelet count, < 100,000/mm³)
- Hyperbilirubinaemia (plasma total bilirubin, > 4 mg/dL (68 µmol/L))

Tissue-perfusion parameters
- Hyperlactataemia (> 3 mmoL/L)
- Decreased capillary refill or mottling

\(^a\)Adapted from Levy 2003.
\(^b\)In children, diagnostic criteria for sepsis are signs and symptoms of inflammation plus infection with hyperthermia or hypothermia (rectal temperature, > 38.5°C or < 35°C, respectively), tachycardia (may be absent with hypothermia), and at least one of the following indications of altered organ function: altered mental status, hypoxaemia, increased serum lactate level, or bounding pulses.
\(^c\)In children, normal values are 75%-80%, therefore values above 70% should not be used as a sign of sepsis in children.
\(^d\)Normal paediatric values are 3.5-5.5 hence values above 3.5 should not be used as a sign of sepsis in children.

Appendix 2. MEDLINE search strategy via Ovid platform
1 exp Bacteremia/ or exp Sepsis/ or exp Shock, Septic/ or exp Systemic Inflammatory Response Syndrome/ or Critical Illness/ or (sepsis or septic* or bacter?em* or septic/em* or SIRS or Inflammatory Response Syndrome* or ((critical* or severe) adj3 (ill* or disease*))) or (bacteria* adj6 infect* adj6 (blood* or serum or invas* or severe or systemic))).ti,ab.
2 exp C-Reactive Protein/ or (C reactive protein* or CRP or procalcitonin or PCT or presepsin or Soluble CD14 or sCD14 or sCD 14).af. (76457)
3 1 and 2 (6609)
4 3 not (exp animals/ not humans.sh.) (6387)
Appendix 3. QUADAS-2 rating guideline

Domain 1. Patient selection

Signaling questions and answering guidelines

1. Was a consecutive or a random sample of patients enrolled?
   Answer 'yes' if one of the following conditions are met:
   a) It is explicitly stated in the study that enrolment was consecutive (or random)
   b) It is reported that all eligible, potential study participant were included and enrolment took place at all hours in any day during the enrolment period.
   Answer 'no' if neither of the conditions is met.
   Answer 'unclear' if insufficient information is available to answer 'yes' or 'no'.

2. Was a case-control design avoided?
   This question is irrelevant because the answer will always be 'yes', since case-control studies are excluded from the review. It raises no concern for bias.

3. Did the study avoid inappropriate exclusions?
   Answer 'yes' if the stated inclusion and exclusion criteria are clear and appropriate
   Answer 'no' if the stated inclusion and exclusion criteria include inappropriate subjects.
   Answer 'unclear' if insufficient information is available to answer 'yes' or 'no'.

Guidelines for assessing risk of bias
Risk of bias from patient selection will be assessed as 'low' when signalling questions 1 and 3 are answered 'yes'. Risk will be assessed as 'high' when signalling questions 1, or 3 are answered 'no'. Risk will be assessed as 'unclear' when insufficient information is reported to answer signalling question 1 or 3.

Guidelines for assessing concern regarding applicability

Are there concerns that the included participants do not match the review question?
Answer 'low concern' if the included participants in the study match our study question.
Answer 'high concern' if the included participants do not match our study question.
Answer 'unclear' if there is insufficient information to make a judgement.

Domain 2. Index test

Signaling question and answering guidelines

1. Were the index test results interpreted without knowledge of the results of the reference standard?
   Answer 'yes' if it is stated in the study report that the index test was interpreted by an individual who was kept unaware of the result(s) of the reference standard.
2. If a threshold was used, was it pre-specified?
Answer 'yes' if a pre-specified positivity threshold is stated for the index test. Answer 'no' if a threshold was not pre-specified. Answer 'unclear' if there is insufficient information available to answer 'yes' or 'no'.

Guidelines for assessing risk of bias
Risk of bias from index test execution will be assessed as 'low' when signalling questions 1 and 2 are answered 'yes'. Risk will be assessed as 'high' when signalling question 1 or 2 is answered 'no'. Risk will be assessed as 'unclear' if there is insufficient information to answer signalling questions 1 or 2.

Guidelines for assessing concern regarding applicability
The index test should be described in sufficient detail to allow for replication. Concern regarding applicability in relation to the execution of the index test will be assessed as 'low' if one of the following two conditions is in place:
1. The assay for the index test is consistent with the most widely performed assays for the index test, as determined from included studies.
2. The following details are provided for any non-standardised assay for an index test:
   a) Name of manufacturer of index test
   b) Instrument/analyser utilised for the assay
   c) Type of specimen used for testing (venous/capillary, whole blood/plasma or serum)
   d) Type of test (laboratory-based or point-of-care)
   e) Type of specimen tube (for a laboratory-based assay)
Concern will be assessed as 'high' if none of the two conditions listed above is met. Concern will be assessed as 'unclear' if insufficient information is available to make a judgement.

Domain 3. Reference standard

Signaling questions and answering guidelines

1. Is the reference standard likely to correctly classify the target condition?
Answer 'yes' if the diagnosis of sepsis is based on the SCCM/ESICM/ACCP/ATS/SIS criteria for sepsis. Answer 'no' if the diagnosis of sepsis is not based on the SCCM/ESICM/ACCP/ATS/SIS criteria for sepsis. Answer 'unclear' if there is insufficient information to answer 'yes' or 'no'.

2. Were the reference standard results interpreted without knowledge of the results of the index test?
Answer 'yes' if the following relevant conditions are met:
a) The clinical staff making the diagnosis are kept unaware of the results of the index test
b) The radiologist interpreting the chest radiographs (in the case of a respiratory infection) was kept unaware of the results of the index test.
c) The microbiologist interpreting culture results (in case of blood/body fluid culture) was kept unaware of the results of the index test.
Answer 'no' if one of the relevant conditions stated above are not met. Answer 'unclear' if insufficient information is available to answer 'yes' or 'no'.
Guidelines for assessing risk of bias
Risk of bias related to the reference standard will be assessed as ‘low’ when signalling questions 1 and 2 are answered ‘yes’. Risk will be assessed as ‘high’ when signalling question 1 or 2 is answered ‘no’. Risk will be assessed as ‘unclear’ when insufficient information is available to answer signalling questions 1 or 2.

Guidelines for assessing concern regarding applicability
Concern that the target condition as defined by the reference standard does not match the review question, will be assessed as ‘low’ if the target condition is sepsis diagnosed by the SCCM/ESICM/ACCP/ATS/SIS criteria. Concern will be assessed as ‘high’ if it is not clearly stated that the target condition is sepsis diagnosed by the SCCM/ESICM/ACCP/ATS/SIS criteria. Concern will be assessed as ‘unclear’ if insufficient information is available to make a judgement.

Domain 4. Flow and timing
Signaling questions and answering guidelines

1. Did all participants receive a reference standard?
   Answer ‘yes’ if at least 95% of included participants diagnosed using the SCCM/ESICM/ACCP/ATS/SIS criteria for sepsis. Answer ‘no’ if less than 95% of participants were diagnosed using the SCCM/ESICM/ACCP/ATS/SIS criteria for sepsis. Answer ‘unclear’ if insufficient information is available to answer ‘yes’ or ‘no’.

2. Did all the participants receive the same reference standard?
   Answer ‘yes’ if at least 95% of included participants diagnosed using the SCCM/ESICM/ACCP/ATS/SIS criteria for sepsis. Answer ‘no’ if less than 95% of participants were diagnosed using the SCCM/ESICM/ACCP/ATS/SIS criteria for sepsis. Answer ‘unclear’ if insufficient information is available to answer ‘yes’ or ‘no’.

3. Were all participants included in the analysis?
   Answer ‘yes’ if the analysis encompassed all included participants; or if 5% or less are excluded from the analysis due to unavailability of reference standard assessment. Answer ‘no’ if the above requirement is unmet. Answer ‘unclear’ if insufficient information is available to answer ‘yes’ or ‘no’.

4. Was there an appropriate interval between index test and reference standard?
   Answer ‘yes’ if the index tests are performed on samples collected at the same time SCCM/ESICM/ACCP/ATS/SIS criteria is applied on the participant. Answer ‘no’ if the above requirement is unmet. Answer ‘unclear’ if insufficient information is available to answer ‘yes’ or ‘no’.

Guidelines for assessing risk of bias
Risk of bias related to participant flow and timing will be assessed as ‘low’ when the four signalling questions are answered ‘yes’. Risk will be assessed as ‘high’ when any of the questions is answered ‘no’. Risk will be assessed as ‘unclear’ when insufficient information is reported to answer any one of the four signalling questions.

Footnotes
ACCP: American College of Chest Physicians
ATS: American Thoracic Society
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CIO conceived the review.

CPO wrote the first draft of the protocol.

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Co-ordinating the review: CIO

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Organizing retrieval of papers: CPO and CIO

Screening retrieved papers against inclusion criteria: CPO and CIO

Appraising quality of papers: CPO, CIO and EAO

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RevMan 5 statistical data: EAO

Interpretation of data: EAO

Statistical inferences: EAO

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DECLARATIONS OF INTEREST

Chinelo P Onyenekwu: none known
Charles I Okwundu: none known
Eleanor A Ochodo: none known