

Supplement 1: Calprotectin as a sepsis diagnostic marker in critical care: a retrospective observational study

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1 Methods supplement

1.1 Definition of clinically relevant pathogens in microbiological samples

Microbiological cultures were defined as clinically relevant if there was growth or detection of a pathogen from a culture taken within the stipulated time frame, regardless of the anatomical site of culturing. The following culture results were considered clinically irrelevant:

1. Yeast fungi from non-sterile anatomical sites (e.g. airways, lower urinary tract, skin lesions)
2. Potentially colonising bacteria of the upper or lower respiratory tract, if found in only one airway culture: Moraxella sp., coagulase-negative Staphylococci, viridans (alpha hemolytic) Streptococci
3. Potential skin contaminants found in only one blood culture: coagulase-negative Staphylococci, viridans (alpha hemolytic) Streptococci, micrococcus sp., Propionibacterium Acnes, Corynebacterium sp., Bacillus sp. [1]
4. Unspecific culture results (e.g. "gram-positive mixed flora", "vaginal flora", "skin flora", "anaerobic mixed flora") from non-sterile anatomical sites
5. Bacterial growth of Clostridium difficile, without detection of toxin[2]
6. Pneumococcus antigen tests from urinary samples

1.2 Classification of pathogens

Pathogens were categorized into gram-positive bacteria, gram-negative bacteria, viruses, and fungi. Bacteria were further categorised by family or species. Atypical bacteria were considered gram-negative bacteria in this study. Viruses and fungi were categorized as seen below. *Sp; species.*

Gram-negative bacteria

- Acinetobacter sp.
- Bacteroides sp.
- Escherichia coli
- Fusobacterium sp.
- Haemophilus influenzae
- Klebsiella sp.
- Moraxella sp.
- Neisseria meningitidis
- Other Enterobacter sp.
- Pseudomonas aeruginosa
- Stenotrophomonas maltophilia
- Other gram-negative bacteria
- Unspecified gram-negative bacteria
- Atypical bacteria

Fungi

- Candida sp.
- Pneumocystis sp.
- Other fungi

Viruses

- Influenza type A and B
- Other viruses

Gram-positive bacteria

- Bacillus sp.
- Clostridium difficile
- Coagulase-negative staphylococci
- Corynebacterium sp.
- Cutibacterium acnes
- Enterococcus faecalis
- Enterococcus faecium
- Lactobacillus sp.
- Mycobacterium sp.
- Staphylococcus aureus
- Streptococcus pneumoniae
- Streptococcus pyogenes
- Viridans streptococci
- Other Clostridium
- Other Enterococci
- Other Betastreptococci
- Other Streptococci
- Other gram-positive bacteria
- Unspecified gram-positive bacteria

1.3 Stratification of patients with culture-positive sepsis into one of five subgroups

Microbiological cultures and samples were filtered by if they were taken within ± 48 of ICU admission and if they were considered clinically relevant.

Based on the culture results, all sepsis patients were sorted into one of five categories. The five categories were gram-positive bacteria, gram-negative bacteria, fungal, viral and polymicrobial. The polymicrobial patient could have the following combinations of pathogens:

- Mixed bacteria (meaning cultures contain both gram-positive bacteria and gram-negative bacteria)
- Gram-negative bacteria + fungi or virus
- Gram-positive bacteria + fungi or virus
- Mixed bacteria + fungi or virus
- Fungi + virus

Most of the patients had cultures containing only one group of pathogens (i.e. gram-positive bacteria, gram-negative bacteria, fungi or viruses). However, about a third of the patients had cultures belonging to more than one of these groups. These were sorted as follows:

1. If the patient had a positive blood culture, the type of pathogen growing in the blood culture decided which group the patient belonged to. If more than one type of pathogen was found in blood cultures, the patient was allocated to the polymicrobial cohort. If the patient, in addition to positive blood cultures, tested positive for viruses (with any method), they were also categorised as polymicrobial. A few exceptions were made when there was a significantly higher occurrence of one type of pathogen type among the cultures from one patient.
2. If there were no blood cultures, the type of pathogen and the anatomic location it was sampled from were considered next. With the help of Infectious Diseases specialist LM (co-author), the following hierarchy was decided upon:
 - (a) If primary pathogens (see below) were found in their respective anatomic location, the type of that pathogen decided which group the patient belonged to. If more than one type of primary pathogen was found, the patient was assigned to the polymicrobial group.
 - (b) Other significant pathogens were considered if no primary pathogens were found.
 - (c) Viruses were always considered relevant.
 - (d) Fungi (other than candida sp. in upper respiratory specimen) were considered relevant when found alone in the lungs or the abdomen without other relevant pathogens.

Primary pathogens:

- Urinary tract: *Escherichia coli*, *Proteus* sp, *Klebsiella* and other enterobacter sp.
- Respiratory tract: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Legionella* sp. *Mycoplasma pneumoniae*.
- All cultures from the central nervous system, bones, and joints
- Group A streptococcus and meningococcus sp: always significant regardless of anatomical site.

Significant pathogens:

- *Enterococcus faecium* in abdomen.
- *Enterococcus faecalis* in abdomen. When found in urine without other significant pathogens elsewhere.

- Pseudomonas sp. in airways or urine
- Staphylococcus Aureus in any other location besides the urinary tract unless it coexists with the primary pathogen in the respiratory tract.
- Group C and G streptococcus in airways, skin, and soft tissue.

Non-significant pathogens (unless no other significant pathogens were found)

- Enterococcus faecium in urine. Enterococcus faecium and faecalis in the respiratory tract.
- Moraxella, Coagulase negative staphylococcus, corynebacteria, acinetobacter, propionibacterium, stenotrophomonas sp.
- Staphylococcus Aureus in urine
- Group B streptococcus
- Viridans streptococcus

1.4 All pathogens found in microbiological cultures/tests

S 1: All pathogens found in cultures and other microbiological tests sorted by gram stain (bacteria) and family or species (all pathogens). In some cases, the laboratory was unable to identify bacteria further than by general characteristics.

Family or species	Pathogen
<i>Gram-negative bacteria</i>	
Acinetobacter sp.	Acinetobacter baumannii
	Acinetobacter baumannii-calcoaceticus complex
	Acinetobacter pittii
	Acinetobacter sp.
	Acinetobacter ursingii
Bacteroides sp.	Bacteroides caccae
	Bacteroides fragilis
	Bacteroides ovatus
	Bacteroides sp.
	Bacteroides thetaiota omicron
	Bacteroides uniformis
	Bacteroides vulgatus
Escherichia Coli	Escherichia coli
Fusobacterium sp.	Fusobacterium gonidia formans
	Fusobacterium necrophorum
	Fusobacterium nucleatum
	Fusobacterium sp.
Haemophilus	Haemophilus influenzae
	Haemophilus parainfluenzae
	Haemophilus sp.
Klebsiella sp.	Klebsiella-Enterobacter sp.
	Klebsiella aerogenes
	Klebsiella oxytoca

	Klebsiella oxytoca group
	Klebsiella pneumoniae
	Klebsiella sp.
Moraxella sp.	Moraxella catarrhalis
	Moraxellanon liquefaciens
Neisseria meningitidis	Neisseria meningitidis
Other enterobacter	Citrobacter freundii
	Citrobacter koseri
	Citrobacter sp.
	Enterobacter asburiae
	Enterobacter cloacae
	Enterobacter cloacae complex
	Enterobacter sp.
	Enterobacterales
	Hafnia alvei
	Morganella morganii
	Pantoea agglomerans
	Pantoea sp.
	Proteus mirabilis
	Proteus penneri
	Proteus sp.
	Proteus vulgaris
	Proteus vulgaris group
	Providencia rettgeri
	Salmonella dublin
	Salmonella enteritidis
	Salmonella florida
	Salmonella group O10
	Salmonella sp.
	Salmonella subsp.
	Salmonella weltevreden
	Serratia liquefaciens
	Serratia marcescens
	Serratia rubidaea
	Serratia sp.
Pseudomonas aeruginosa	Pseudomonas aeruginosa
Stenotrophomonas maltophilia	Stenotrophomonas maltophilia
Other gram-negative bacteria	Achromobacter sp.
	Achromobacter xylosoxidans
	Aeromonas hydrophila
	Aeromonas sp.
	Alcaligenes faecalis
	Alloscardovia omnicoles
	Bordetella bronchiseptica
	Burkholderia cenocepacia
	Burkholderia cepacia
	Burkholderia cepacia complex
	Burkholderia gladioli

	Campylobacter jejuni/coli
	Campylobacter ureolyticus
	Capnocytophaga sp.
	Chryseobacterium gleum
	Chryseobacterium indologenes
	Chryseobacterium sp.
	Comamonas testosteroni
	Cronobacter sakazakii
	Cyberlindner afabianii
	Delftia acidovorans
	Dermabacter hominis
	Dialister pneumosintes
	Eikenella corrodens
	Elizabethkingia meningoseptica
	Elizabethkingia miricola
	Elizabethkingia sp.
	Hungatella hathewayi
	Leclercia adecarboxylata
	Lichtheimia corymbifera
	Neisseria bacilliformis
	Neisseria sp.
	Neisseria subflava
	Ochrobactrum anthropi
	Ochrobactrum sp.
	Pasteurella multocida
	Porphyromonas endodontalis
	Porphyromonas somerae
	Porphyromonas sp.
	Prevotella bivia
	Prevotella denticola
	Prevotella melaninogenica
	Prevotella nigrescens
	Prevotella oralis
	Prevotella oris
	Prevotella sp.
	Pseudomonas sp.
	Sphingomonas paucimobilis
	Sphingomonas sp.
	Sutterella wadsworthensis
	Veillonella parvula
	Veillonella sp.
	Vibrio sp.
	Vibrio vulnificus
Atypical bacteria	Legionella longbeachae
	Legionella pneumophila
	Legionella sp.
	Mycoplasma hominis
	Chlamydophila psittaci (DNA)

Unspecified gram-negative bacteria	Anaerobe gram-negative rods
	Gram-negative mixed flora
	Gram-negative mixed flora, e.g proteus
	Gram-negative mixed flora, e.g pseudomonas
	Gram-negative mixed environmental bacteria
<i>Gram-positive bacteria</i>	
Bacillus sp.	Bacillus cereus
	Bacillus cereus complex
	Bacillus sp.
Clostridium Difficile	Clostridium difficile toxin Vidas
	Clostridium difficile bacteria
Coagulase-negative staphylococci	Staphylococcus capitis
	Staphylococcus epidermidis
	Staphylococcus haemolyticus
	Staphylococcus hominis
	Staphylococcus lugdunensis
	Staphylococcus pasteurii
	Staphylococcus pettenkoferi
	Staphylococcus pseudintermedius
	Staphylococcus saprophyticus
	Staphylococcus schleiferi
	Staphylococcus simulans
	Staphylococcus sp. (coagulase-negative)
Corynebacterium sp.	Corynebacterium pseudodiphtheriticum
	Corynebacterium sp.
	Corynebacterium striatum
	Corynebacterium tuberculostrictum
Cutibacterium acnes	Cutibacterium acnes
Enterococcus faecalis	Enterococcus faecalis
Enterococcus faecium	Enterococcus faecium
Lactobacillus sp.	Lactobacillus gasseri
	Lactobacillus plantarum
	Lactobacillus rhamnosus
	Lactobacillus sp.
	Lactococcus sp.
Mycobacteria	Mycobacteria seen in microscope
	Mycobacterium tuberculosis
Staphylococcus aureus	Staphylococcus aureus
Streptococcus pneumoniae	Streptococcus pneumoniae
	Likely pneumococci
Streptococcus pyogenes	Streptococcus pyogenes(GAS)
Viridans streptococci	Streptococcus anginosus
	Streptococcus anginosus complex
	Streptococcus massiliensis
	Streptococcus mitis
	Streptococcus mitis complex
	Streptococcus mutans complex
	Streptococcus oralis

	<i>Streptococcus parasanguinis</i>
	<i>Streptococcus salivarius</i> complex
	<i>Streptococcus sanguinis</i> complex
	<i>Streptococcus vestibularis</i>
Other Clostridium	<i>Clostridium aldenense</i>
	<i>Clostridium butyricum</i>
	<i>Clostridium cadaveris</i>
	<i>Clostridium innocuum</i>
	<i>Clostridium paraputrificum</i>
	<i>Clostridium perfringens</i>
	<i>Clostridium ramosum</i>
	<i>Clostridium septicum</i>
	<i>Clostridium</i> sp.
	<i>Clostridium symbiosum</i>
	<i>Clostridium tertium</i>
Other enterococci	<i>Enterococcus avium</i>
	<i>Enterococcus casseliflavus</i>
	<i>Enterococcus durans</i>
	<i>Enterococcus gallinarum</i>
	<i>Enterococcus gallinarum</i> group
	<i>Enterococcus hirae</i>
	<i>Enterococcus raffinosus</i>
	<i>Enterococcus</i> sp.
Other beta streptococci	Beta streptococci group C
	Beta streptococci group G
	<i>Streptococcus agalactiae</i> (group B)
	<i>Streptococcus dysgalactiae</i>
	<i>Streptococcus dysgalactiae</i> (group C)
	<i>Streptococcus dysgalactiae</i> (group G)
Other streptococci	<i>Streptococcus bovis</i>
	<i>Streptococcus bovis</i> complex
	<i>Streptococcus</i> sp.
Other gram-positive bacteria	<i>Actinomyces odontolyticus</i>
	<i>Actinomyces</i> sp.
	<i>Actinomyces turicensis</i>
	<i>Actinotignum sanguinis</i>
	<i>Actinotignum schaalii</i>
	<i>Actinotignum</i> sp.
	<i>Aerococcus sanguinicola</i>
	<i>Aerococcus</i> sp.
	<i>Aerococcus urinae</i>
	<i>Aggregatibacteria phrophilus</i>
	<i>Aggregatibacter</i> sp.
	<i>Alistipes onder donki</i>
	<i>Anaerococcus</i> sp.
	<i>Aureobasidium pullulans</i>
	<i>Bifidobacterium breve</i>
	<i>Bifidobacterium</i> sp.

Brevundimonas aurantiaca
Brevundimonas sp.
Cutibacterium avidum
Cutibacterium granulosum
Cutibacterium sp.
Desulfovibrio desulfuricans
Dialister sp.
Difteroida stavar
Eggerthella lenta
Eggerthella sp.
Eggerthiaca tenaformis
Enterocloster clostridioformis
Eubacterium sp.
Facklamia hominis
Finegoldi amagna
Galactomyces candidus
Gemella haemolysans
Gemella morbillorum
Gemella sp.
Globicatella sp.
Grampositiva kocker
Granulicatella adiacens
Granulicatella sp.
Listeria innocua
Listeria monocytogenes
Massilia sp.
Micrococcus luteus
Micrococcus sp.
Nocardia sp.
Odoribacter splanchnicus
Paenibacillus sp.
Paeniclostridium sordellii
Parabacteroides distasonis
Parabacteroides sp.
Paracoccus sp.
Parvimonas micra
Peptoniphilus harei
Peptoniphilus sp.
Peptostreptococcus anaerobius
Peptostreptococcuss tomatidis
Pichia cactophila
Pichia norvegensis
Rothia mucilaginosa
Rothia sp.
Ruminococcus gnavus
Solobacterium moorei
Staphylococcus warneri
Weissella sp.

Unspecified gram-positive bacteria	Anaerobe gram-positive rods
	Gram-positive cocci, likely streptococci or enterococci
	Gram-positive rods
	Gram-positive mixed flora
<i>Unspecified gram stain</i>	
Unspecified	Anaerobe bacteria
	Anaerobe mixed flora
	Bacteria
	Mixed flora
	Mixed flora, e.g proteus
	Mixed flora, e.g pseudomonas
	Fecal flora
	Skin flora
	Oral flora
	Oropharyngeal flora
	Vaginal flora
<i>Fungi</i>	
Candida	Candida albicans
	Candida dubliniensis
	Candida glabrata
	Candida inconspicua
	Candida krusei
	Candida parapsilosis
	Candida sp.
	Candida tropicalis
	Candida utilis
Pneumocystis	Pneumocystis
Other fungi	Aspergillus fumigatus
	Aspergillus nidulans
	Aspergillus sp.
	Aspergillus terreus
	Clavispora lusitaniae
	Diutinaea tenulata
	Diutinaea tenulata
	Fusarium solani
	Fusarium sp.
	Geotrichum sp.
	Yeast fungi
	Yeast fungi, not C. albicans
	Kluyveromyces marxianus
	Magnusiomyces capitatus
	Malassezia sp.
	Meyerozyma guilliermondii
	Mold fungi
	Mucor circinelloides
	Rhizopus microsporus
	Saccharomyces cerevisiae
	Thread fungus

	Trichosporona sahii
	Wickerhamomyces anomalus
<i>Viruses</i>	
Influenza	Influenzae A
	Influenzae B
Other viruses	Varicella zoster virus
	RS-virus type B
	RS-virus type A
	Rhinovirus
	Parainfluenzae
	Norovirus I
	Norovirus II
	Metapneumovirus
	Herpes simplex virus 1
	Enterovirus
	Coronavirus
	Cytomegalovirus
	Adenovirus

2 Results supplement

2.1 Droput analysis

Table of dropout analysis of included and excluded patients. The excluded patients did not have blood samples in the biobank. CRP in this table is from routine laboratory analysis. All variables, except outcomes, are at ICU admission. CRRT and mechanical ventilatorion are during the ICU stay. *ICU*; intensive care unit. *LOS*; length of stay. *ER*; emergency room. *IQR*; interquartile range. *SD*; standard deviation. *CRP*; C-reactive protein. *WBC*; white blood cell count. *SAPS-3*; simplified acute physiology score-3. *SOFA*; sequential organ failure assessment. *CRRT*; continuous renal replacement therapy.

	Included	Excluded	p-value
n	4732	804	
Age, years (median [IQR])	68 [56-75]	66 [50-74]	<0.001
Male sex (%)	2856 (60)	467 (58)	0.24
ICU admission >48h from hospital admission (%)	1196 (25)	264 (33)	<0.001
Admitted from ER (%)	1956 (41)	294 (37)	0.012
Biomarkers			
C-reactive protein, mg/L (median [IQR])	43 [7-144]	42 [7-128]	0.28
Lactate, mmol/L (median [IQR])	2.0 [1.2-4.1]	1.8 [1-4.4]	0.031
WBC, 10 ⁹ /L (median [IQR])	12.9 [8.7-18]	12.6 [9.1-18.7]	0.4
Physiological parameters			
Sepsis (%)	2071 (44)	260 (32)	<0.001
Shock (%)	1520 (32)	207 (27)	0.004
SAPS-3 and SOFA score at ICU admission			
SAPS-3 score (median [IQR])	64 [52-75]	62 [49-74]	0.008
SOFA score (mean (SD))	7.2 (4)	6.4 (4.2)	<0.001
Outcomes			
ICU LOS (median [IQR])	2.5 [1.5-4.8]	2.2 [1.2-4.4]	<0.001
ICU mortality (%)	871 (18.4)	175 (21.8)	0.028
30-day mortality (%)	1435 (30)	252 (35)	0.008
CRRT (%)	627 (13)	87 (11)	0.065
Invasive mechanical ventilation (%)	3029 (64)	479 (60)	0.018

2.2 Sample handling times

The median time from ICU admission to freezing of the biobank sample was 1.6 hours (IQR 1.3-2.1 hours). Among the samples with missing data at the time of freezing (n=45), the median time from ICU admission to the refrigerator was 2.2 hours (IQR 1.6-3.0).

2.3 Calprotectin levels in subgroups of ICU patients.

Calprotectin levels are displayed as median (IQR) for the non-sepsis and sepsis groups within the subgroups of ICU patients. Community or hospital-acquired disease was defined as a hospital LOS under respective over 48 hours before ICU admission. Shock was defined as vasopressor need and a lactate >2 mmol/L. Suspected infection was defined as an obtained blood culture and administered antibiotics around the time of ICU admission. *ICU*; intensive care unit. *LOS*; length of stay. *n*; number. *IQR*; interquartile range.

	n	Sepsis (%)	Non-sepsis median (IQR)	Sepsis median (IQR)	p-value
<i>Full ICU cohort</i>	<i>4732</i>	<i>44%</i>	<i>1.3 (0.6-2.8)</i>	<i>2.1 (1.0-4.3)</i>	<0.001
Community-acquired	3536	43%	1.1 (0.5-2.4)	1.9 (0.9-3.9)	<0.001
Hospital-acquired	1196	45%	2.1 (1.0-4.5)	2.6 (1.2-5.6)	0.003
Neutropenic	83	82%	0.6 (0.3-3)	1.0 (0.2-3.5)	0.91
Age<65 years	1899	46%	1.3 (0.6-2.6)	2.1 (1-4.1)	<0.001
Age≥65 years	2833	41%	1.4 (0.5-3.1)	2 (0.9-4.6)	<0.001
Shock	1520	51%	1.6 (0.8-3.1)	2.3 (1.1-4.4)	<0.001
SOFA≥2	4329	49%	1.4 (0.6-2.9)	2.1 (1-4.3)	<0.001
Suspected infection	2008	77%	1.4 (0.6-3.2)	2.2 (1-4.7)	<0.001

2.4 Calprotectin and CRP levels in microbiological subgroups of sepsis patients.

The microbiological subgroups of sepsis patients were based on relevant culture results in ±48 hours from ICU admission. Patients were assigned to only one of the six microbiological sepsis subgroups (gram-positive, gram-negative, fungal, virus, polymicrobial and culture-negative). All microbiological sepsis subgroups had higher calprotectin and CRP levels than the non-sepsis group (p<0.05). When the sepsis group was divided into bacteriemia and non-bacteriemia, the difference in calprotectin and CRP levels was significant with a p-value<0.001. *n*; number. *IQR*; interquartile range.

Microbiological subgroup	n	Calprotectin, median (IQR)	CRP, median (IQR)
<i>Non-sepsis</i>	<i>2661</i>	<i>1.3 (0.6-2.8)</i>	<i>17 (3-69)</i>
Gram-positive	613	1.9 (1.0-4.5)	88 (18-188)
Gram-negative	697	1.9 (0.8-3.7)	81 (19-174)
Fungal	44	3.0 (1-5.6)	108 (40-218)
Virus	92	2.3 (1.1-4.5)	69 (31-153)
Polymicrobial	236	2.8 (1.3-6.2)	119 (39-205)
Culture-negative	389	2.1 (1-4.3)	118 (51-196)
Bacteriemic	539	2.6 (1.2-5.7)	143 (74-240)
Non-bacteriemic	1532	1.9 (0.9-3.8)	75 (18-164)

2.5 Association between calprotectin and CRP in microbiological subgroups of sepsis patients.

The Spearman's r is displayed for the non-sepsis patients and for each microbiological subgroup of sepsis patients. All correlations were statistically significant with $p < 0.05$. The number (n) of patients in each subgroup is the same as in table 2.4 above. *CRP*; *C-reactive protein*.

Microbiological subgroup	Spearman's r
<i>Full ICU cohort</i>	0.47
<i>Non-sepsis</i>	0.41
Gram-positive	0.51
Gram-negative	0.40
Fungal	0.41
Virus	0.57
Polymicrobial	0.54
Culture-negative	0.41

References

- [1] Alcorn K, Meier F, Schiffman R. Blood Culture Contamination: Q-tracks 2013. College of American Pathologists. 2013.
- [2] Crobach M, Planche T, Eckert C, Barbut F, Terveer E, Dekkers O, et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. *Clinical microbiology and infection*. 2016;22:S63-81.