

Life-threatening infections in children in Europe – a prospective cohort study (The EUCLIDS Project)

Prof. F. Martinon-Torres^{1,2*}, Prof. A. Salas^{2,3*}, M.D. I. Rivero^{1,2*}, PhD. M. Cebey-López^{2*}, PhD. J. Pardo-Seco^{2*}, PhD. J. Herberg^{4*}, M.D. N.P. Boeddha⁵, M.D. D.S. Klobassa⁶, MMED. F. Secka⁷, M.D. S. Paulus⁸, Prof. R. de Groot⁹, M.D. L.J. Schlapbach^{10,11,12, 13}, PhD. G.J. Driessen⁵, PhD. S.T. Anderson⁷, PhD. M. Emonts^{14,15}, Prof. W. Zenz⁶, Prof. E.D. Carrol⁸⁺, PhD. M. Van der Flier⁹, Prof. M. Levin⁴⁺ on behalf of EUCLIDS consortium[&].

* contributed equally, + contributed equally

& EUCLIDS consortium members are detailed in Appendix, page 35

Affiliations:

1. Translational Pediatrics and Infectious Diseases Section- Pediatrics Department, Santiago de Compostela, Spain.
2. Instituto de Investigación Sanitaria de Santiago (IDIS), Genetics- Vaccines- Infectious Diseases and Pediatrics research group GENVIP, Santiago de Compostela, Spain.
3. Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, Instituto de Ciencias Forenses, Facultade de Medicina, Universidade de Santiago de Compostela, and GenPoB Research Group, Instituto de Investigaciones Sanitarias (IDIS), Hospital Clínico Universitario de Santiago (SERGAS), Galicia, Spain
4. Section of Paediatrics Imperial College London, London, United Kingdom.
5. Erasmus MC-Sophia Children's Hospital, University Medical Center Rotterdam, Intensive Care and Department of Pediatric Surgery, Rotterdam, The Netherlands.
6. Medical University of Graz, Department of General Pediatrics, Graz, Austria.
7. Medical Research Council Unit The Gambia, Fajara, The Gambia.
8. University of Liverpool Institute of Infection and Global Health, Department of Clinical Infection Microbiology and Immunology, Liverpool, United Kingdom.
9. Department of Pediatrics, division of Pediatric Infectious Diseases and Immunology and Laboratory of Infectious Diseases, Radboud Institute of Molecular Life Sciences, Radboudumc Nijmegen, the Netherlands
10. Faculty of Medicine, The University of Queensland, Brisbane, Australia
11. Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland
12. Paediatric Intensive Care Unit, Lady Cilento Children's Hospital, Brisbane, Australia
13. Paediatric Critical Care Research Group, Mater Research, University of Queensland, Brisbane, Australia
14. Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom
15. Paediatric Infectious Diseases and Immunology Department, Newcastle upon Tyne Hospitals Foundation Trust, Great North Children's Hospital, Newcastle upon Tyne, United Kingdom

Author for correspondence: Federico Martín-Torres, Hospital Clínico Universitario de Santiago de Compostela, Choupana s/n, 15706, Santiago de Compostela, A Coruña, Spain. E-mail: federico.martinon.torres@sergas.es, tel. +34981955093, fax +34981950596.

Research in context

Evidence before this study

The burden of life-threatening infections on childhood morbidity and mortality persists in spite of the substantial reduction in vaccine-preventable invasive bacterial infections since the introduction of conjugate vaccines in childhood, and the availability of antimicrobial agents.

We carried out comprehensive and focused reviews of the scientific literature published between 2000 and July 2017, on severe childhood infections. For this purpose, we searched the PubMed and Medline databases for articles published in English, Spanish and French up to July 31, 2017. Our search terms included a combination of the following terms “meningococcal disease”, “bacterial infection”, “sepsis”, “septic shock”, “children”, “severe focal infection”, “paediatric intensive care”, “microorganism” and “diagnosis”.

We found that information on the global epidemiology of severe infections in the paediatric population is scarce and most published studies on sepsis and severe focal infection are biased towards the paediatric intensive care population (Schlapbach LJ et al. and Weiss et al).

Added value of this study

Our study highlights the burden of severe childhood infections, drawing on detailed clinical information from the largest prospective cohort of children with severe infection in Europe published to date. We demonstrate the continued impact of severe bacterial infection and mortality caused by vaccine-preventable infections (*N. meningitidis* and *S. pneumoniae*), and by pathogens for which vaccines are urgently required (*S. aureus* and *Group A streptococcus*).

Implications of all the available evidence

Data collection was made possible by a diverse and widely representative EU funded European network (EUCLIDS Project GA: 279185): 194 hospitals in 9 European countries, with information on 98 hospitals of 6 of these countries included in this report. Conclusions from our data are likely to reflect generalised patterns of illness and to be widely relevant across Europe. Our findings emphasize the current burden of infection and the need for on going studies of the prevalence and characteristics of serious infections in childhood, to guide prioritization of therapeutic, diagnostic and preventive measures.

This project has received funding from the European Union’s seventh Framework program under EC-GA no. 279185 (EUCLIDS).

Abstract

Background: Sepsis and severe focal infections (SFI) represent a significant burden of disease in hospitalized children. To understand the burden of disease and outcome of childhood infection in Europe, children with life-threatening bacterial infections were studied in a multi-centre study in six countries in Europe.

Methods: Children aged 1 month-to-18 years old with sepsis or SFI, admitted to 98 European EUCLIDS network hospitals were prospectively recruited during July 2012-December 2016. Demographic, clinical, microbiological data and outcomes were collected.

Findings: A total of 2,844 patients were included (53.2% male; median age: 39.1 months). 43.2% of patients ($n=1229$) had sepsis and 56.8% ($n=1615$) SFI. Sepsis was diagnosed predominantly in younger children and SFI in older ones (P -value <0.0001). Main SFI were pneumonia ($n=511$, 18%), central nervous system infection ($n=469$, 16.5%) and skin and soft tissue infection ($n=247$, 8.7%). Causal microorganism was identified in 47.8% of children ($n=1,359$). Most prevalent causative agent was *Neisseria meningitidis* (9.1%, $n=259$) followed by *Staphylococcus aureus* (7.8%, $n=222$), *Streptococcus pneumoniae* (7.7%, $n=219$) and *Group A streptococcus* (5.7%, $n=162$). Mortality rate was 2.2% ($n=57$); and 37.6% of patients ($n=1,070$) required intensive care.

Interpretation: Mortality rate in European children hospitalised due to sepsis or SFI is low. Burden of disease lies predominantly in children under 5 years and is largely due to vaccine-preventable infections by meningococcus and pneumococcus. More than a third of children required intensive care. Despite availability and application of current clinical

methods for microbiological diagnosis, the causative organism remained unidentified in approximately 50% of the patients.

This project has received funding from the European Union's seventh Framework program under EC-GA no. 279185 (EUCLIDS).

Introduction

The Confidential Enquiry into Maternal and Child Health (CEMACH) report ‘Why Children Die’ demonstrated that infectious illness was ‘the single largest cause of death in children dying of an acute physical illness’, constituting ‘20% of the deaths overall’ with the 1-4 year old group the most affected [1]. Amongst all the infectious agents, bacteria represent the principal cause of death in young children, accounting for over a third of all child deaths globally [2].

The World Health Organization (WHO) recently issued a resolution on sepsis in all age groups, recognizing deaths by severe infection as a main target for global and national prioritization in healthcare delivery [3]. This burden on childhood morbidity and mortality persists despite of the substantial reduction in vaccine-preventable invasive bacterial infections after the introduction of conjugate vaccines in childhood and the availability of antimicrobial agents [4-6], highlighting the need for a better understanding of the host response to infection, novel treatments of acute infection, new methods to identify those at risk, and better preventative strategies.

Currently, information regarding the global epidemiology of severe infections in the paediatric population is scarce. Most published studies on sepsis and severe focal infection (SFI) are biased towards a predominantly paediatric intensive care unit (PICU) population. Reported mortality and morbidity from recent large paediatric sepsis and septic shock studies ranged from 17% to 25% [7, 8].

In this paper, we present data from the European Union Childhood Life-threatening Infectious Disease Study (EUCLIDS), which aimed to describe the current burden of severe paediatric infectious diseases, with respect to demographic, clinical, microbiological data and outcomes, across Europe.

Materials and methods

Study design and recruitment criteria

This prospective, multicenter, observational study of children with life-threatening bacterial infection presenting to hospital was conducted between July 2012-December 2016 by the EUCLIDS Consortium (<http://www.euclids-project.eu/>). This network included 194 hospitals in Europe (in 9 countries) and one hospital in Africa (The Gambia). Data from Switzerland were not included in the analysis because they used different inclusion criteria. The African partner was also excluded because the present study focuses on the European burden of disease.

Eligible participants were children from 1 month to 18 years of age admitted to hospital with sepsis (or suspected sepsis) and/or severe focal infection including but not limited to pneumonia, soft tissue infection, meningitis, encephalitis, osteomyelitis, and septic arthritis (Appendix: Full definitions document, page 43). In order to enrol children as early as possible during the infection, potential recruits were identified from their clinical characteristics on presentation often before the results from confirmatory microbiology tests were available. Additionally, children admitted with proven infections due to *N. meningitidis*, *S. pneumoniae*, *S. aureus* and *Group A streptococcus* (GAS) who had not been included in the study on initial presentation to hospital were specifically targeted for recruitment. For this reason our findings cannot be used to accurately establish the relative prevalence of other potentially causative pathogens. although recruitment mostly took place before any causal pathogen was identified. Patients with hospital-acquired infections were not included.

The study used harmonised procedures for patient recruitment, sample processing and sample storage. A common clinical protocol agreed by EUCLIDS Clinical Network and approved by the Ethics Committee was implemented at all hospitals. All clinical staff were trained in the projects procedures, and specified criteria were used for clinical definitions and assignment of patients to diagnostic categories. Written informed consent was obtained from a parent or legal guardian for each subject before study inclusion.

Among 7,276 eligible patients included in the EUCLIDS database, we excluded 2,012 patients labelled as controls, 706 patients recruited retrospectively, 1,479 patients from the Swiss and Gambian Cohorts, and 235 that did not meet eligibility criteria or were incomplete (Figure 1). Analysis was limited to the remaining 2,844 subjects with a complete minimal dataset including patient age and discharge diagnosis.

Clinical data collection

The clinical information for each patient was collected using a secured web-based platform, including data on demographics, comorbidity, immunisation status, selected laboratory results, and past medical and family history of severe infectious diseases defined as: (a) any infection requiring hospitalization, if outpatient at onset; (b) any infection requiring oxygen, pressors or fluids to support blood pressure, or intubation; or (c) deep tissue (invasive) infection requiring intravenous or oral antibiotics to treat infection. Discharge diagnosis, clinical course, treatments and specific procedures during admission and outcomes (such as death or sequelae) were recorded.

Patients were categorised into two main groups according to the clinical characteristics during the hospital admission: sepsis or SFI. Sepsis was defined as suspected or confirmed infection (infectious organisms or toxins) plus systemic inflammatory response syndrome (SIRS) [9], and SFI included those illnesses with a suspected or

confirmed infection but without SIRS. Patients were assigned one or more pre-defined clinical syndromes. (Appendix: Full definitions document, page 43).

Laboratory methods

Microbiological diagnosis was undertaken as part of clinical care using locally available clinical diagnostic procedures, including, as appropriate, bacterial culture from normally sterile sites (blood, cerebrospinal fluid, urine and invasive diagnostic samples), and from non-sterile sites (throat and wound swabs); bacterial and viral molecular diagnostics were applied to blood, cerebrospinal fluid and respiratory secretions, according to local availability.

In order to assign microbiological aetiology of infection in prospective patients recruited to the study, each patient was phenotyped according to their likelihood of bacterial infection, using an agreed algorithm, when all the results of investigations were available (Figure 2).

Specific inflammatory parameters: maximum levels of serum C-reactive protein (CRP) and neutrophil counts were compared to further assess their utility and sensitivity in discriminating focal *vs.* sepsis, PICU *vs.* non-PICU admission, and prognosis (survivors *vs.* death). For CRP values, all cohort values were used; while for neutrophil counts only UK values were available. Sensitivity and specificity was assessed using pre-agreed cut offs and numeric values were used to obtain receiver operating characteristic curves (ROC) Figure 2 [10].

Statistical analysis

General data are presented as percentages and odds ratios (OR) computed from contingency tables, and medians and interquartile ranges (IQR). Analysis was performed

using R version 3.3.1 (www.r-project.org). The level of statistical significance was set at 0.05. Bonferroni correction was used in order to reduce the likelihood of false positive results caused by multiple testing. Associations were assessed using non-parametric tests: Fisher's exact test for discrete variables and Wilcoxon test for continuous variables (package *stats*). ROC curves and areas under curve (AUC) were calculated with *P*-values to test the null hypothesis that the AUC equals 0.50 (package *pROC*).

Role of the funding source

This project has received funding from the European Union's seventh Framework program under EC-GA no.279185 (EUCLIDS). The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Characteristics of the EUCLIDS cohort

A total of 2,844 subjects were analysed. 53.2% (1512/2841) were male and the median age was 39.1 months (IQR=12.4-93.9). Characteristics of the patients are summarised in Table 1.

A history of previous severe infection was found in 432 (16.9%) cases, whilst 240 cases had 1st or 2nd order family members with a history of serious infection (11.0%, 240/2174). Previous infections included meningitis (32.9%, 79/240), pneumonia (20.4%, 49/240), severe sepsis (11.3%, 27/240) and meningococemia (7.5%, 18/240). 2.4% of cases (51/2127) had parental consanguinity and 2.1% (45/2150) had first- or second-degree

relatives with an immunodeficiency. Prematurity was present in 9.8% (230/2343) of the cases. 30.1% (497/1652) of the patients lived with smokers at home (Table 1).

Immunisations were up-to-date according to the local schedules in 93.0% (2240/2409) of the patients. Nevertheless, we found that 89.5% (204/228) of the meningococcus isolated and serotyped could be eventually covered by vaccines that were not available or not included in the immunization calendars implemented in Europe at that time.

Sepsis was diagnosed predominantly in younger children and SFI in older ones (Figure 3A), with significant statistical differences in the age distribution between those in whom a causative organism was identified and those with no organism identified (Figure 3B, Table 1).

Most of patients (93.4%, 2282/2444) had a favourable clinical course (no death, skin grafts, amputations, hearing loss >40dB) with complete recovery from the illness. The mortality rate was 2.2% (57/2569) in the entire cohort, 0.5% (7/1549) in SFI vs. 4.9% (50/1020) for sepsis. The cause of death for patients included in the SFI sub-cohort is specified in Appendix: Cause of death for patients with SFI, page 63.

A total of 37.6% (1070/2844) patients were admitted to PICU of which 62.1% (763/1229) admissions presented with sepsis.

Microbiological and clinical diagnosis

A total of 44.8% of children (1155/2581) had definite bacterial infection; 5.9% (152/2581) had definite viral; and 47.9% (1202/2509) suffered from uncertain type of infection (454 probable bacterial, 65 probable viral and 683 unknown) (Figure 2).

A causative microorganism was identified in 47.8% (1359/2844) of the cases. The most prevalent bacterial causative agent was *Neisseria meningitidis* in 9.1% (259/2844) followed by *Staphylococcus aureus* (7.8%, 222/2844), *Streptococcus pneumoniae* (7.7%, 219/2844) and *GAS* (5.7%, 162/2844) (Figure 4). Viruses were identified as causative agents in 6.5% (185/2844) of the patients with the most common ones being: enterovirus, rhinovirus and respiratory syncytial virus.

In patients admitted to PICU, the main identified bacteria were: *N. meningitidis* (16.5%, 162/981), *S. pneumoniae*, (9.9%, 97/981), *GAS* (8.1%, 79/981) and *S. aureus* (5.5%, 54/981). Viruses were the causative pathogen in the 8.1% (79/981) of the cases, and there was no organism identified in 41.6% (408/981) of the patients. Ward and PICU clinical syndromes, and causal agents are shown in Appendix Figure 1, page 64.

Significant differences were found in *N. meningitidis* rates in patients with a family history of severe bacterial infection [OR: 2.02 (95%CI: 1.31-3.04), *P*-value=0.0011], and in patients exposed to tobacco [OR: 3.21 (95%CI: 2.19-4.74), *P*-value<0.0001]. In premature patients there is a significant difference for viral infection rates [OR: 2.13 (95%CI: 1.38-3.22), *P*-value=0.0005].

Those patients in whom a causative organism was identified were more likely to have severe disease: a higher proportion was admitted to PICU (*P*-value<0.0001) and had a prolonged hospital length of stay (LOS) (*P*-value<0.0001), furthermore, they required more respiratory support (*P*-value<0.0001), and supplemental oxygen (*P*-value<0.0001). Additionally, inotropes (*P*-value=0.0122) and mortality were higher in patients with an identified causative organism (*P*-value=0.0045) although this was not statistically significant after Bonferroni adjustment (Table 1A).

Among patients with bacterial SFI, the most prevalent clinical syndromes were pneumonia (20.4%, 329/1615), central nervous system (CNS) infection (12.1%, 196/1615), skin and soft tissue infection (11.5%, 185/1615) and osteomyelitis (9.6%, 155/1615).

No correlation was found between administration of antimicrobial agents before cultures and organism identification (P -value=0.7813).

Children whose immunisations were not up to date (7.0%, 169/2409) were admitted mainly due to pneumonia (18.9%, 32/169), CNS infections (15.4%, 26/169) and urinary tract infections–pyelonephritis (11.8%, 20/169); with *S. pneumoniae* and *Escherichia coli* being the main causative microorganisms (6.5%, 11/169; and 5.9%, 10/169, respectively).

We further analysed the main presenting clinical syndromes according to the presence of a microorganism. For the main pathologies studied we found that CNS infections were caused mainly by *N. meningitidis* (29.9%, 140/469) and *S. pneumoniae* (19.0%, 89/469); soft tissue infection, osteomyelitis, toxic shock syndrome and septic arthritis by *S. aureus* and GAS, and abdominal conditions and urinary tract infections-pyelonephritis by *E. coli*. (Figure 4A)

Infection with *N. meningitidis* (22.8%, 13/57) was the most prevalent among the fatal cases, mainly associated with severe sepsis, followed by *S. pneumoniae* (19.3%, 11/57) and *S. aureus* (10.5%, 6/57). In 33.3% (19/57) of the non-survivors no causative pathogen was identified (Figure 4B).

Sepsis vs. SFI

The main differences observed between patients with sepsis or SFI were that septic patients had a more severe course, with significant differences for all parameters

including full recovery at discharge (P -value<0.0001), need for supplemental oxygen (P -value<0.0001), respiratory support requirement (P -value<0.0001), inotropes (P -value<0.0001), PICU admission (P -value<0.0091) and death outcome (P -value<0.0001) (Table 1B).

Antibiotics had been administrated before blood cultures were taken in 40.0% (355/887) of septic patients and in 29.8% (359/1204) patients with SFI (P -value<0.0001).

Utility of inflammatory markers

We compared maximum CRP and neutrophil counts levels between different groups (Table 2). Patients with sepsis and those requiring intensive care, had an increased serum CRP (≥ 60 mg/L) compared to those with focal infection and non-PICU admission (P -value<0.0001). (Appendix Figure 2, page 65). No differences were found when comparing survivors vs. non-survivors.

ROC analysis for CRP to discriminate sepsis vs. SFI showed an AUC of 0.655 (95%CI 0.616-0.694, P -value<0.0001) and 0.661 (95%CI 0.621-0.701, P -value<0.0001) for distinguishing between PICU vs. non-PICU admission. The CRP AUC for discriminating between survivors and death was also significant (0.655, 95%CI 0.535-0.776, P -value=0.0153) (Appendix Figure 3, page 66).

ROC analysis for neutrophil count to discriminate sepsis vs. SFI showed an AUC of 0.553 (95%CI 0.523-0.583, P -value<0.0001) and 0.550 (95%CI 0.518-0.582, P -value=0.0015) for discriminating between PICU vs. non-PICU admission. The neutrophil AUC for discriminating between survivors and death was not significant (0.522, 95%CI 0.390-0.655, P -value=0.7158) (Appendix Figure 3, page 66).

Discussion

Our study highlights the burden of severe childhood infections, drawing on detailed clinical information from the largest prospective cohort of children with severe infection in Europe, recruited at 98 hospitals in 6 European countries. We demonstrate the continued importance of severe illness and mortality caused by vaccine-preventable infections (*N. meningitidis* and *S. pneumoniae*), and by pathogens for which vaccines are urgently required (*S. aureus* and GAS).

Laboratory tests failed to identify a causative pathogen in over half of children with severe illness, in line with data from the previous two decades [8, 11], despite the introduction of more sensitive and precise techniques in diagnostics in recent years. In over 50% of paediatric patients admitted with suspected life-threatening infections, decisions on need, type and duration of antimicrobial therapy thus have to be made with no clear guidance from the microbiological findings, indicating an urgent need for improved diagnostics. Patients with an identified microorganism suffered from more severe disease, which may suggest a higher pathogen load and more successful detection in these patients, but may be associated as well to increased diagnostic effort in the sickest patients.

Mortality

In our study, the case fatality ratio was 2.2%, significantly lower than that recently reported by two recent large studies [7, 8], although it should be noted that these studies were restricted to PICU patients with a more severe population (sepsis/septic shock). Mortality was highest in children with sepsis as defined by the International Paediatric Sepsis consensus conference [9]. The new sepsis definitions from 2016 [12] were not established for children, hence were not used in our study. Delay in timely treatment has been considered to increase the mortality risk in sepsis [6, 13]. Esteban et al. [14] reported a trend towards reduction in mortality after implementing an educational intervention for

appropriate empiric antibiotic administration within the first hour of admission in children with sepsis. However, we were not able to assess this in our data. Our results are consistent with the reported mortality rates of patients with sepsis after the introduction of adequate treatment guidelines (hospital mortality 1%–3% in previously healthy, and 7%–10% in chronically ill children) [15], and with a recent population-based study on blood culture-proven bacterial sepsis [16]. As previously described [15], we found that mortality in community-acquired severe infections [6] was associated with the identification of the causative organism, the presence of sepsis, higher PICU admission rates, oxygen and/or respiratory support requirement, inotrope administration and prolonged LOS.

Severity and pathogen type

Though our study was not designed to reliably establish the relative prevalence of potentially causative pathogens; our results show the relative frequency of *N. meningitidis*, *S. pneumoniae*, *S. aureus* and GAS are roughly equal. Overall, the most frequent clinical syndromes were meningitis and pneumonia. Almost half of the patients admitted to hospital with a bacterial infection required intensive care admission. These findings are consistent with the reported leading causes of morbidity and mortality in children worldwide [1, 2]. The causative pathogens in our study differed from findings in Asia: were *Salmonella enterica* serotype Typhi was the most common bacterial pathogen, followed by *S. pneumoniae* and *Haemophilus influenzae* [17] and Africa: were *S. pneumoniae* is the most common isolate in children, followed by *S. aureus* and *E. coli* [18]. We also observed differences from studies in the United States where *S. aureus*, *Pseudomonas* species and Enterobacteriaceae (mainly *E. coli*) were the main pathogens isolated. [19]

Vaccinations are an essential tool in our fight against infectious disease [4, 20, 21], and they have greatly reduced the global burden of infectious disease [21]. Although most patients were up-to-date according to their local immunisation schedule, we found that there was a considerable burden of mortality and morbidity caused by vaccine preventable infections, particularly meningococcal and pneumococcal disease. Vaccines for meningococcal serogroup B, Y, W and for a major proportion of pneumococcal serotypes are not available or implemented in Europe. Thus, improved vaccines and implementation of current vaccines may yield further health gain. Wider implementation of existing vaccines and development of vaccines for *S. aureus* or GAS could contribute to a further decline in the burden of paediatric infectious diseases.

Tobacco smoke exposure

We found an increased risk of meningococcal infections in children exposed to tobacco (P -value<0.0001). In previous studies tobacco smoke exposure was associated with increased susceptibility to infections including tuberculosis, pneumonia, meningitis or otitis media. This could be explained by increased nasopharyngeal colonization with pathogens including *S. pneumoniae*, *H. influenzae* (non-type b), *M. catarrhalis*, GAS and *S. aureus* [22]. Furthermore, increased infection risk can also be explained by the reported interference of tobacco smoke with the antibacterial function of leukocytes (e.g. neutrophils, monocytes, T cells and B cells) [23].

Family history

The huge variation in clinical response to identical infecting pathogens is most likely the result of the combined effects of genetic variation in both the infecting pathogen and the infected host [24]. There is now strong evidence that host genetic factors influence occurrence of meningococcal disease, and a number of genes controlling susceptibility

and severity of meningococcal disease have been identified in candidate gene association studies [25-28]. We found a significant association between family history of severe infectious diseases and meningococcal infection (P -value=0.0011),

Inflammatory biomarkers

In our study, higher CRP levels were associated with an increased risk of severe outcomes. Biomarkers may contribute to outcome prediction in life threatening infections [29]. However, there is still a need for improved host biomarker and pathogen diagnostics that can establish the clinical diagnosis, direct appropriate therapy and enable prediction of outcome [10]. Improved diagnostic discrimination in this group could have major implications for tackling rising antimicrobial resistance.

Sepsis outcomes for children in high-income countries have not changed dramatically over the past decade [6-8, 13]. Additional diagnostic approaches may help to establish the clinical diagnosis, direct early and adequate therapy and enable a more reliable outcome prognosis. It has been proposed that an approach combining sensitive pathogen diagnostics and novel host response biomarkers may improve treatment and clinical outcomes for children with serious infection [10]

PIRO concept

We identified specific variables associated with each of the components of PIRO concept [30] (predisposition, infection characteristics, host response and organ dysfunction): including age, gender, family history of severe infection, tobacco exposure, type of microorganism, infection focus, inflammatory biomarkers and a dynamic view of the patient's clinical course and outcomes. All of these variables described, contribute as a

proof of concept of this novel approach and as a predictor of mortality for patients with community-acquired sepsis.

Limitations

Although children were recruited early in their clinical course, before a pathogen diagnosis was known, a limitation of our study is that children with known infection due to *N. meningitidis*, *S. pneumoniae*, *S. aureus*, and *GAS* were targeted for recruitment. The reason behind this targeted recruitment was to study the genetic basis of these pathogens as one of the main objectives of EUCLIDS Project (GA 279185). Overall the majority of patients were recruited unbiased, providing a cross section of different etiologies in children presenting with severe infection. But specific targeting of the four core pathogens will have caused bias towards these infections. The burden of disease from these selectively-target pathogens is within this study cohort but our study design limits the ability to generalize this to the broader population. Our findings thus cannot be used to establish population prevalence of each organism. Of note, one of EUCLIDS centres (Switzerland), where the recruitment strategy was to enroll children at a later time point, solely after confirmation of positive blood culture, were not included in this paper. In order to determine the disease burden and to elucidate the contributing factors to severe infection outcome, large epidemiological studies are needed. Recruitment was restricted to the hospital setting and did not capture out-of-hospital deaths, or severe focal infections managed as outpatients; our data therefore under-represent less severe infections. Eventhough the study used harmonised procedures for patient recruitment, sample processing, and sample storage, microbiological diagnosis was undertaken as part of clinical care using locally available clinical diagnostic procedures which could have limited in some way the assignment of patients as having viral infection, bacterial

infection or co infection. We will report separately on additional viral studies undertaken as part of EUCLIDS using molecular diagnosis for a wide range of viruses.

Conclusions

This is the largest reported prospective study of severe childhood infections in Europe. Data collection was made possible by a diverse and widely representative European network. Recommendations or interventions based on our data are likely to reflect generalised patterns of illness and to be widely relevant across Europe. Although the mortality rate due to sepsis or SFI was low, we found considerable morbidity associated with severe childhood infection and more than a third of children required PICU admission. The burden of disease lies predominantly in children under 5 years and was predominantly caused by infections where vaccines are available: pneumococcus and meningococcus. We found that children had infections by common pathogens such as *S. aureus* (7.8%) and *GAS* (5.7%) for which there are no effective vaccines, and that 11.0% of the bacterial microorganisms were Gram-negative bacilli. Both of which, should have important implications for vaccine development and for empirical antimicrobial strategies implementation in Europe.

Contributions

FMT, JH, EDC, MVdF, ME, RdG, WZ and ML designed the study.

IRC, MCL, JH, NPB, DK, FS, SP, MVdF, LJS, GJD, STA, ME, EDC assisted in patient recruitment, data- and sample collection

FMT, IRC, MCL, JPS, AS performed statistical analysis.

PA, LC, SG provided database and informatics support.

FMT, IRC, MCL, EDC and JH wrote the first draft of the manuscript.

FMT, IRC, MCL, JPS, AS, JH, NPB, DK, FS, SP, MVdF, LJS, ME, WZ, EDC, RdG and ML contributed to writing the manuscript.

All authors approved the final manuscript.

Conflicts of interest

Other than the grants, we declare that we have no conflicts of interest regarding this paper.

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Table 1: Description of the main characteristics of the EUCLIDS study cohort.

Comparison between (A) no organism and organism identified, and (B) focal infection and sepsis. Data are expressed as % (*n*) or median (IQR). * *P*-values lower than Bonferroni correction threshold (0.05/37=0.0014).

A)

Variables	All patients	No organism identified	Organism identified	<i>P</i> -value
Total cohort	2844	52.2% (1485/2484)	47.8% (1359/2844)	
Demographic characteristics				
Sex (male)	53.2% (1512/2841)	53.9% (800/1484)	52.5% (712/1357)	0.4517
Age	39.1 (12.4-93.9)	42.8 (14.9-95.5)	33.2 (10.25-91.05)	0.0007*
0-12 months	24.3% (691/2844)	21.1% (313/1485)	27.8% (378/1359)	0.0005*
12-24 months	14.8% (421/2844)	14.5% (215/1485)	15.2% (206/1359)	—
24-48 months	17.1% (487/2844)	18.3% (272/1485)	15.8% (215/1359)	—
>48 months	43.8% (1245/2844)	46.1% (685/1485)	41.2% (560/1359)	—
Weight (kg)	14.8 (9.9-25.8)	15.4 (10.3-26.5)	14.0 (9.2-25.5)	0.0005*
Family history				
Severe infections	11.0% (240/2174)	10.1% (115/1143)	12.1% (125/1031)	0.1319
Immunodeficiency	2.1% (45/2150)	2.1% (24/1133)	2.1% (21/1017)	1.0000
Consanguinity	2.4% (51/2127)	2.6% (29/1122)	2.2% (22/1005)	0.5734
Smoker in the household	30.1% (497/1652)	28.3% (250/883)	32.1% (247/769)	0.0957
Patient medical history				
Premature birth	9.8% (230/2343)	9.9% (123/1244)	9.7% (107/1099)	0.9446
Past severe infections	16.9% (432/2563)	18.9% (252/1336)	14.7% (180/1227)	0.0051
Immunisations up-to-date	93.0% (2240/2409)	93.5% (1194/1277)	92.4% (1046/1132)	0.2998
Clinical data				
Antibiotics before culture	34.1% (714/2091)	34.4% (393/1142)	33.8% (321/949)	0.7813
PRISM Score	11 (5-20)	10.5 (4-16)	12.0 (5.0-21)	0.1097
Full recovery expected	93.4% (2282/2444)	95.7% (1219/1274)	90.9% (1063/1170)	<0.0001*
PICU admission	37.6% (1070/2844)	30.0% (445/1485)	46.0% (625/1359)	<0.0001*
Oxygen needed	36.3% (923/2546)	32.0% (426/1333)	41.0% (497/1213)	<0.0001*
Respiratory support	28.1% (720/2564)	23.3% (313/1345)	33.4% (407/1219)	<0.001*
Inotropes	11.8% (304/2578)	10.3% (138/1346)	13.5% (166/1232)	0.0122
Hospital LOS	7 (4-13)	6 (3-10)	10 (6-16)	<0.0001*
Death	2.2% (57/2569)	1.4% (19/1345)	3.1% (38/1224)	0.0045
Clinical syndrome				
CLABSI	0.5% (13/2844)	0.1% (2/1485)	0.8% (11/1359)	0.0099
CNS infection	16.5% (469/2844)	8.8% (130/1485)	24.9% (339/1359)	<0.0001*
Bronchiolitis	2.7% (78/2844)	2.1% (31/1485)	3.5% (47/1359)	0.0287
Pneumonia	18.0% (511/2844)	22.5% (334/1485)	13.0% (177/1359)	<0.0001*
LRTI	3.5% (100/2844)	4.7% (70/1485)	2.2% (30/1359)	0.0003*
Lung effusion or empyema	7.4% (210/2844)	6.3% (94/1485)	8.5% (116/1359)	0.0261
Soft tissue infection	8.7% (247/2844)	9.2% (136/1485)	8.2% (111/1359)	0.3518
Toxic shock syndrome	2.3% (64/2844)	1.1% (16/1485)	3.5% (48/1359)	<0.0001*
Endocarditis	0.7% (20/2844)	0.1% (2/1485)	1.3% (18/1359)	0.0001*
Osteomyelitis	6.7% (191/2844)	5.2% (77/1485)	8.4% (114/1359)	0.0010
Scarlet fever	0.3% (9/2844)	0.3% (5/1485)	0.3% (4/1359)	1.0000
Septic arthritis	5.2% (149/2844)	3.4% (50/1485)	7.3% (99/1359)	<0.0001*
Gastroenteritis	1.6% (45/2844)	1.3% (19/1485)	1.9% (26/1359)	0.1800
UTI-pyelonephritis	3.8% (109/2844)	2.6% (39/1485)	5.2% (70/1359)	0.0006*
ENT	6.3% (178/2844)	7.8% (116/1485)	4.6% (62/1359)	0.0003*
Abdominal condition	1.3% (38/2844)	1.5% (22/1485)	1.2% (16/1359)	0.5166
Severe sepsis	5.5% (157/2844)	3.6% (54/1485)	7.6% (103/1359)	<0.0001*
Septic shock	9.3% (264/2844)	6.2% (92/1485)	12.7% (172/1359)	<0.0001*

B)

Variables	Focal infection	Sepsis	P-value
Total cohort	56.8% (1615/2844)	43.2% (1229/2844)	
Demographic characteristics			
Sex (male)	53.5% (863/1612)	52.8% (649/1229)	0.7045
Age	46.5 (15.8-100.4)	27.6 (9.0-80.2)	<0.0001*
0-12 months	19.8% (319/1615)	30.3% (372/1229)	0.0005*
12-24 months	13.6% (220/1615)	16.4% (201/1229)	—
24-48 months	18.1% (293/1615)	15.8% (194/1229)	—
>48 months	48.5% (783/1615)	37.6% (462/1229)	—
Weight (kg)	15.8 (10.7-28.0)	13.0 (8.7-23.1)	<0.0001*
Family history			
Severe infections	11.2% (137/1220)	10.8% (103/954)	0.7828
Immunodeficiency	2.1% (25/1211)	2.1% (20/939)	1.0000
Consanguinity	1.9% (22/1186)	3.1% (29/941)	0.0859
Smoker in the household	31.7% (301/951)	28% (196/701)	0.1155
Patient medical history			
Premature birth	8.5% (112/1316)	11.5% (118/1027)	0.0173
Past severe infections	18.7% (270/1441)	14.4% (162/1122)	0.0041
Immunisations up-to-date	93.2% (1282/1375)	92.6% (958/1034)	0.5740
Clinical data			
Antibiotics before culture	29.8% (359/1204)	40% (355/887)	<0.0001*
PRISM Score	5 (4-11.75)	14 (6-22)	<0.0001*
Full recovery expected	97.2% (1369/1409)	88.2% (913/1035)	<0.0001*
PICU admission	19.0% (307/1615)	62.1% (763/1229)	<0.0001*
Oxygen needed	22.1% (323/1463)	55.4% (600/1083)	<0.0001*
Respiratory support	11.8% (172/1454)	49.4% (548/1110)	<0.0001*
Inotropes	10.3% (151/1472)	44.4% (498/1121)	<0.0001*
Hospital LOS	6 (3-12)	9 (5-15)	<0.0001*
Death	0.5% (7/1549)	4.9% (50/1020)	<0.0001*
Clinical syndrome			
CLABSI	0.1% (1/1615)	1.0% (12/1229)	0.0001*
CNS infection	12.1% (196/1615)	22.2% (273/1229)	<0.0001*
Bronchiolitis	2.7% (44/1615)	2.8% (34/1229)	1.0000
Pneumonia	20.4% (329/1615)	14.8% (182/1229)	<0.0001*
LRTI	4.3% (69/1615)	2.5% (31/1229)	0.0134
Lung effusion or empyema	8.4% (136/1615)	6.0% (74/1229)	0.0168
Soft tissue infection	11.5% (185/1615)	5.0% (62/1229)	<0.0001*
Toxic shock syndrome	0.3% (5/1615)	4.8% (59/1229)	<0.0001*
Endocarditis	0.2% (4/1615)	1.3% (16/1229)	0.0011
Osteomyelitis	9.6% (155/1615)	2.9% (36/1229)	<0.0001*
Scarlet fever	0.4% (7/1615)	0.2% (2/1229)	0.3150
Septic arthritis	7.5% (121/1615)	2.3% (28/1229)	<0.0001*
Gastroenteritis	1.9% (31/1615)	1.1% (14/1229)	0.1285
UTI-pyelonephritis	4.0% (64/1615)	3.7% (45/1229)	0.6947
ENT	9.0% (145/1615)	2.7% (33/1229)	<0.0001*
Abdominal condition	1.4% (22/1615)	1.3% (16/1229)	1.0000
Severe sepsis	0% (0/1615)	12.8% (157/1229)	<0.0001*
Septic shock	0% (0/1615)	21.5% (264/1229)	<0.0001*

Table 2: Description of serum levels of C-reactive protein (CRP) and neutrophil counts in different group of patients. Data are expressed as % (n). * *P*-values lower than Bonferroni correction threshold (0.05/4=0.0125). SFI: Severe focal infection; PICU: paediatric intensive care unit.

	CRP≥60 mg/L	CRP<60 mg/L	<i>P</i> -value	Neutrophils≥12×10 ⁹ /L	Neutrophils<12×10 ⁹ /L	<i>P</i> -value
Total	39.7 (966/2432)	60.3 (1466/2432)		68.2 (977/1432)	31.8 (455/1432)	
Sepsis vs. focal						
Sepsis	71.6 (755/1054)	28.4 (299/1054)	<0.0001*	35.8 (226/631)	64.2 (405/631)	0.0042*
SFI	51.6 (711/1378)	48.4 (667/1378)		28.6 (229/801)	71.4 (572/801)	
PICU vs. not PICU						
PICU	70.9 (654/922)	29.1 (268/922)	<0.0001*	36.3 (190/524)	63.7 (334/524)	0.0067*
Non-PICU	53.8 (812/1510)	46.2 (698/1510)		29.2 (265/908)	70.8 (643/908)	
Survivors vs. death						
Survivors	59.5 (1273/2139)	40.5 (866/2139)	0.0878	32.3 (397/1230)	67.7 (833/1230)	0.5039
Death	72.7 (32/44)	27.3 (12/44)		39.1 (9/23)	60.9 (14/23)	

Figure 1: Consort Flow Diagram

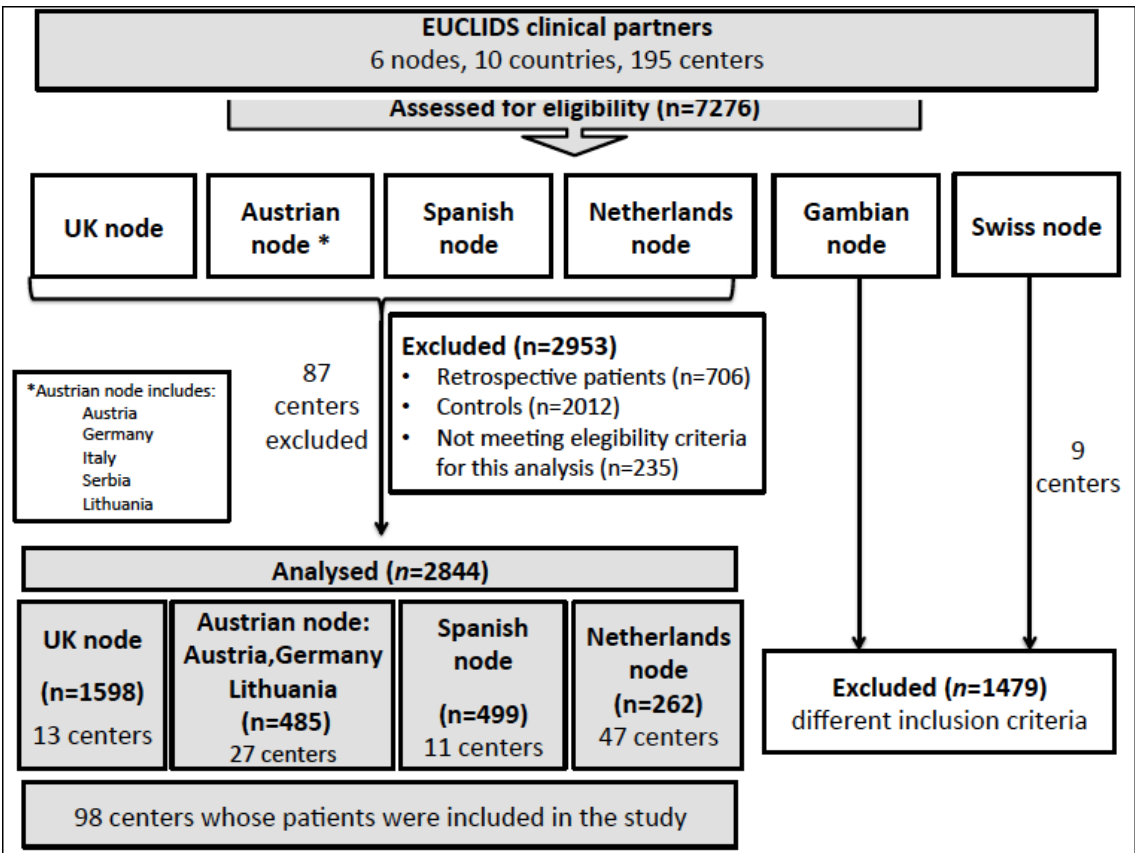


Figure 2: Phenotyping algorithm. Figure adapted from Herberg et al. [10]

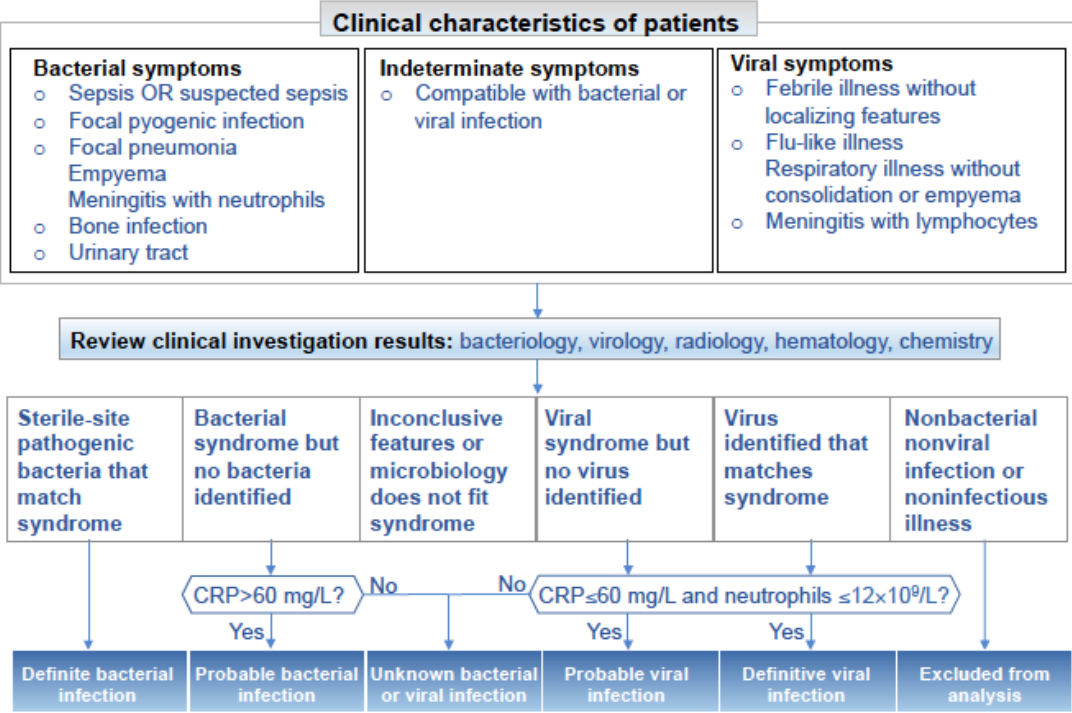


Figure 3: A) Age distribution in the EUCLIDS cohort and in those diagnosed with sepsis or a focal illness. B) Age distribution by causative organism. GPC: gram positive cocci, GAS: *Group A Streptococcus*, GNR: gram negative rods, CoNS: *Coagulase Negative Staphylococci*.

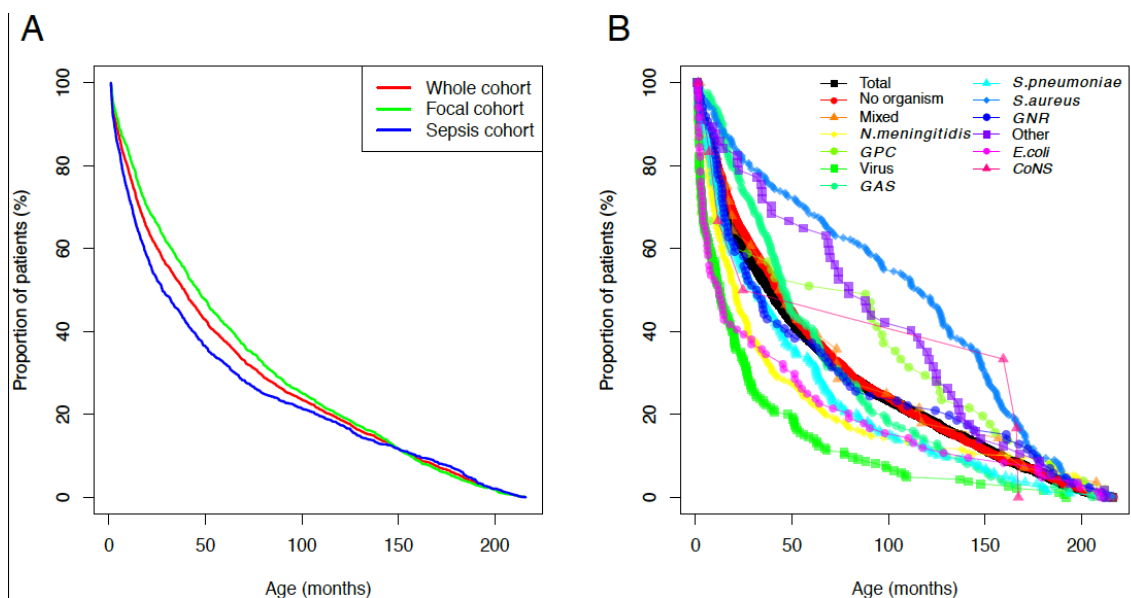
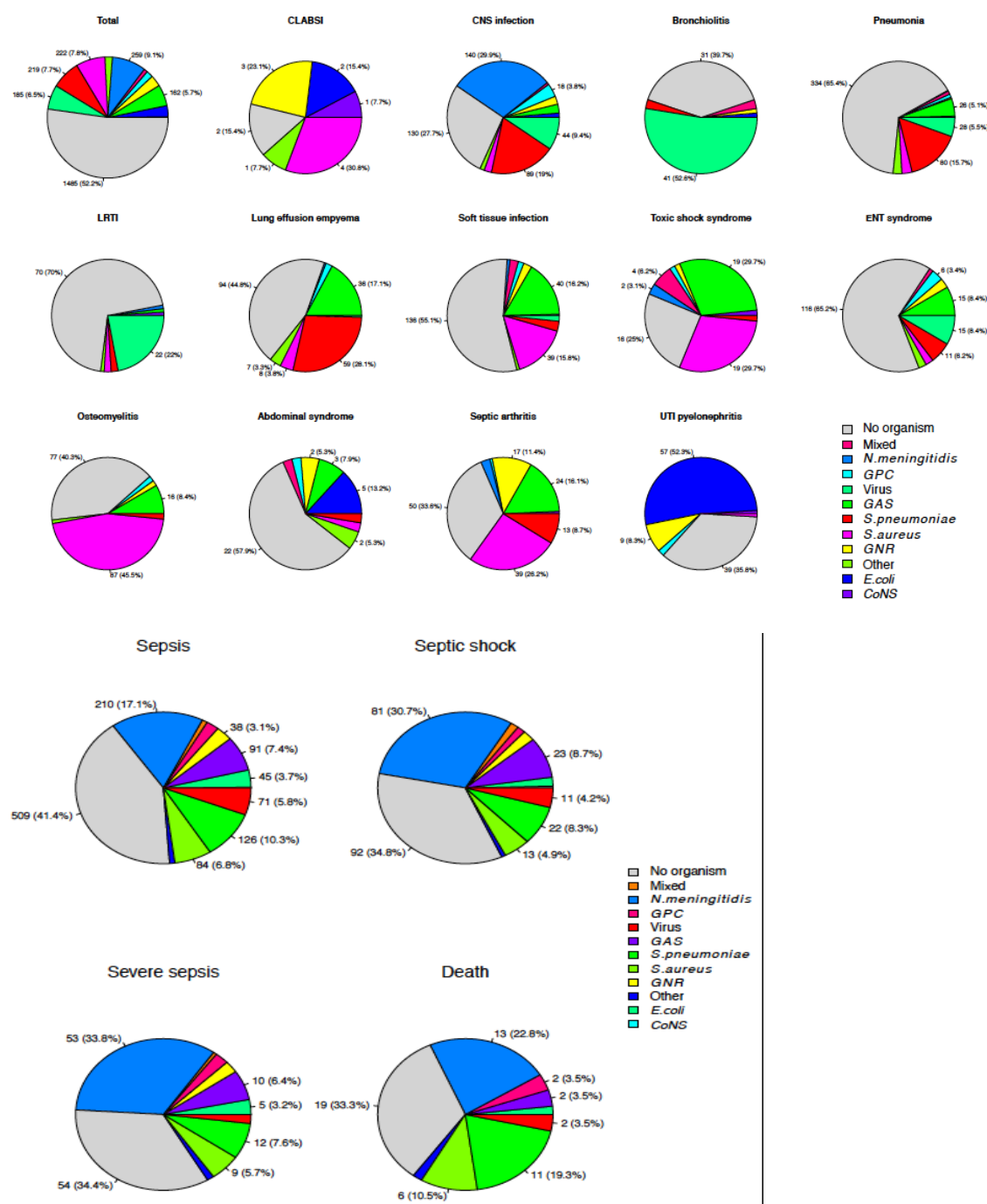


Figure 4. Causative microorganisms identified in EUCLIDS by syndrome. (A) patients with severe focal infections and (B) sepsis. Data are expressed as (n) %. CNS infection: central nervous system infection, LRTI: lower respiratory tract infection, ENT syndrome: ear, nose, throat syndrome, UTI-pyelonephritis: urinary tract infection with pyelonephritis, GPC: gram positive cocci, GAS: *Group A Streptococcus*; GNR: gram negative rods, CoNS: *Coagulase Negative Staphylococci*.



Appendix: EUCLIDS CONSORTIUM MEMBERS

EUCLIDS consortium (www.euclids-project.eu) is composed by:

Imperial College partner (UK)

Members of the EUCLIDS Consortium at Imperial College London (UK)

Principal and co-investigators

Michael Levin (grant application, EUCLIDS Coordinator)

Dr. Lachlan Coin (bioinformatics)

Stuart Gormley (clinical coordination)

Shea Hamilton (proteomics)

Jethro Herberg (grant application, PI)

Bernardo Hourmat (project management)

Clive Hoggart (statistical genomics)

Myrsini Kaforou (bioinformatics)

Vanessa Sancho-Shimizu (genetics)

Victoria Wright (grant application, scientific coordination)

Consortium members at Imperial College

Amina Abdulla

Paul Agapow

Maeve Bartlett

Evangelos Bellos

Hariklia Eleftherohorinou

Rachel Galassini

David Inwald

Meg Mashbat

Stefanie Menikou

Sobia Mustafa

Simon Nadel

Rahmeen Rahman

Clare Thakker

EUCLIDS UK Clinical Network

Poole Hospital NHS Foundation Trust, Poole: Dr S Bokhandi (PI), Sue Power, Heather Barham

Cambridge University Hospitals NHS Trust, Cambridge: Dr N Pathan (PI), Jenna Ridout, Deborah White, Sarah Thurston

University Hospital Southampton, Southampton: Prof S Faust (PI), Dr S Patel (co-investigator), Jenni McCorkell.

Nottingham University Hospital NHS Trust: Dr P Davies (PI), Lindsey Crate, Helen Navarra, Stephanie Carter

University Hospitals of Leicester NHS Trust, Leicester: Dr R Ramaiah (PI), Rekha Patel

Portsmouth Hospitals NHS Trust, London: Dr Catherine Tuffrey (PI), Andrew Gribbin, Sharon McCready

Great Ormond Street Hospital, London: Dr Mark Peters (PI), Katie Hardy, Fran Standing, Lauren O'Neill, Eugenia Abelake

King's College Hospital NHS Foundation Trust, London; Dr Akash Deep (PI), Eniola Nsirim

Oxford University Hospitals NHS Foundation Trust, Oxford Prof A Pollard (PI), Louise Willis, Zoe Young

Kettering General Hospital NHS Foundation Trust, Kettering: Dr C Royad (PI), Sonia White

Central Manchester NHS Trust, Manchester: Dr PM Fortune (PI), Phil Hudnott

SERGAS Partner (Spain)

Principal Investigators

Federico Martinón-Torres¹

Antonio Salas^{1,2}

GENVIP RESEARCH GROUP (in alphabetical order):

Fernando Álvez González¹, Ruth Barral-Arca^{1,2}, Miriam Cebey-López¹, María José Curras-Tuala^{1,2}, Natalia García¹, Luisa García Vicente¹, Alberto Gómez-Carballa^{1,2}, Jose Gómez Rial¹, Andrea Grela Beiroa¹, Antonio Justicia Grande¹, Pilar Leboráns Iglesias¹, Alba Elena Martínez Santos¹, Federico Martinón-Torres¹, Nazareth Martinón-Torres¹, José María Martinón Sánchez¹, Beatriz Morillo Gutiérrez¹, Belén Mosquera Pérez¹, Pablo Obando Pacheco¹, Jacobo Pardo-Seco^{1,2}, Sara Pischedda^{1,2}, Irene Rivero Calle¹, Carmen Rodríguez-Tenreiro¹, Lorenzo Redondo-Collazo¹, Antonio Salas Ellacuriaga^{1,2}, Sonia Serén Fernández¹, María del Sol Porto Silva¹, Ana Vega^{1,3}, Lucía Vilanova Trillo¹.

¹ Translational Pediatrics and Infectious Diseases, Pediatrics Department, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain, and GENVIP Research Group (www.genvip.org), Instituto de Investigación Sanitaria de Santiago, Galicia, Spain.

² Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, Instituto de Ciencias Forenses, Facultade de Medicina, Universidade de Santiago de Compostela, and GenPop Research Group, Instituto de Investigaciones Sanitarias (IDIS), Hospital Clínico Universitario de Santiago, Galicia, Spain

³ Fundación Pública Galega de Medicina Xenómica, Servizo Galego de Saúde (SERGAS), Instituto de Investigaciones Sanitarias (IDIS), and Grupo de Medicina Xenómica, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Universidade de Santiago de Compostela (USC), Santiago de Compostela, Spain

EUCLIDS SPANISH CLINICAL NETWORK:

Susana Beatriz Reyes¹, María Cruz León León¹, Álvaro Navarro Mingorance¹, Xavier Gabaldó Barrios¹, Eider Oñate Vergara², Andrés Concha Torre³, Ana Vivanco³, Reyes Fernández³, Francisco Giménez Sánchez⁴, Miguel Sánchez Forte⁴, Pablo Rojo⁵, J.Ruiz

Contreras⁵, Alba Palacios⁵, Cristina Epalza Ibarrondo⁵, Elizabeth Fernández Cooke⁵, Marisa Navarro⁶, Cristina Álvarez Álvarez⁶, María José Lozano⁶, Eduardo Carreras⁷, Sonia Brió Sanagustín⁷, Olaf Neth⁸, M^a del Carmen Martínez Padilla⁹, Luis Manuel Prieto Tato¹⁰, Sara Guillén¹⁰, Laura Fernández Silveira¹¹, David Moreno¹².

¹ Hospital Clínico Universitario Virgen de la Arrixaca; Murcia, Spain.

² Hospital de Donostia; San Sebastián, Spain.

³ Hospital Universitario Central de Asturias; Asturias, Spain.

⁴ Complejo Hospitalario Torrecárdenas; Almería, Spain.

⁵ Hospital Universitario 12 de Octubre; Madrid, Spain.

⁶ Hospital General Universitario Gregorio Marañón; Madrid, Spain.

⁷ Hospital de la Santa Creu i Sant Pau; Barcelona, Spain.

⁸ Hospital Universitario Virgen del Rocío; Sevilla, Spain.

⁹ Complejo Hospitalario de Jaén; Jaén, Spain.

¹⁰ Hospital Universitario de Getafe; Madrid, Spain.

¹¹ Hospital Universitario y Politécnico de La Fe; Valencia, Spain.

¹² Hospital Regional Universitario Carlos Haya; Málaga, Spain.

Members of the Pediatric Dutch Bacterial Infection Genetics (PeD-BIG) network (the Netherlands)

Steering committee:

Coordination: R. de Groot ¹, A.M. Tutu van Furth ², M. van der Flier ¹

Coordination Intensive Care: N.P. Boeddha ³, G.J.A. Driessen ³, M. Emonts ^{3, 4, 5}, J.A. Hazelzet ³

Other members: T.W. Kuijpers ⁷, D. Pajkrt ⁷, E.A.M. Sanders ⁶, D. van de Beek ⁸, A. van der Ende ⁸

Trial coordinator: H.L.A. Philipsen ¹

Local investigators (in alphabetical order)

A.O.A. Adeel ⁹, M.A. Breukels ¹⁰, D.M.C. Brinkman ¹¹, C.C.M.M. de Korte ¹², E. de Vries ¹³, W.J. de Waal ¹⁵, R. Dekkers ¹⁵, A. Dings-Lammertink ¹⁶, R.A. Doedens ¹⁷, A.E. Donker ¹⁸, M. Dousma ¹⁹, T.E. Faber ²⁰, G.P.J.M. Gerrits ²¹, J.A.M. Gerver ²², J. Heidema ²³, J. Homan-van der Veen ²⁴, M.A.M. Jacobs ²⁵, N.J.G. Jansen ⁶, P. Kawczynski ²⁶, K. Klucovska ²⁷, M.C.J. Kneyber ²⁸, Y. Koopman-Keemink ²⁹, V.J. Langenhorst ³⁰, J. Leusink ³¹, B.F. Loza ³², I.T. Merth ³³, C.J. Miedema ³⁴, C. Neeleman ¹, J.G. Noordzij ³⁵, C.C. Obihara ³⁶, A.L.T. van Overbeek – van Gils ³⁷, G.H. Poortman ³⁸, S.T. Potgieter ³⁹, J. Potjewijd ⁴⁰, P.P.R. Rosias ⁴¹, T. Sprong ²¹, G.W. ten Tusscher ⁴², B.J. Thio ⁴³, G.A. Tramper-Stranders ⁴⁴, M. van Deuren ¹, H. van der Meer ², A.J.M. van Kuppevelt ⁴⁵, A.M. van Wermeskerken ⁴⁶, W.A. Verwijs ⁴⁷, T.F.W. Wolfs ⁴.

- ^{1.} Radboud University Medical Center – Amalia Children’s Hospital, Nijmegen, The Netherlands
- ^{2.} Vrije Universiteit University Medical Center, Amsterdam, The Netherlands
- ^{3.} Erasmus Medical Center – Sophia Children’s Hospital, Rotterdam, The Netherlands
- ^{4.} Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

5. Paediatric Infectious Diseases and Immunology Department, Newcastle upon Tyne Hospitals Foundation Trust, Great North Children's Hospital, Newcastle upon Tyne, United Kingdom
6. University Medical Center Utrecht – Wilhelmina Children's Hospital, Utrecht, The Netherlands
7. Academic Medical Center – Emma Children's Hospital, University of Amsterdam, Amsterdam, The Netherlands
8. Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
9. Kennemer Gasthuis, Haarlem, The Netherlands
10. Elkerliek Hospital, Helmond, The Netherlands
11. Alrijne Hospital, Leiderdorp, The Netherlands
12. Beatrix Hospital, Gorinchem, The Netherlands
13. Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands
14. Diaconessenhuis, Utrecht, The Netherlands
15. Maasziekenhuis Pantein, Boxmeer, The Netherlands
16. Gelre Hospitals, Zutphen, The Netherlands
17. Martini Hospital, Groningen, The Netherlands
18. Maxima Medical Center, Veldhoven, The Netherlands
19. Gemini Hospital, Den Helder, The Netherlands
20. Medical Center Leeuwarden, Leeuwarden, The Netherlands
21. Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands
22. Rode Kruis Hospital, Beverwijk, The Netherlands
23. St. Antonius Hospital, Nieuwegein, The Netherlands
24. Deventer Hospital, Deventer, The Netherlands
25. Slingeland Hospital, Doetinchem, The Netherlands
26. Refaja Hospital, Stadskanaal, The Netherlands
27. Bethesda Hospital, Hoogeveen, The Netherlands
28. University Medical Center Groningen, Beatrix Children's hospital, Groningen, The Netherlands
29. Haga Hospital – Juliana Children's Hospital, Den Haag, The Netherlands
30. Isala Hospital, Zwolle, The Netherlands
31. Bernhoven Hospital, Uden, The Netherlands
32. VieCuri Medical Center, Venlo, The Netherlands
33. Ziekenhuisgroep Twente, Almelo-Hengelo, The Netherlands
34. Catharina Hospital, Eindhoven, The Netherlands
35. Reinier de Graaf Gasthuis, Delft, The Netherlands
36. ETZ Elisabeth, Tilburg, The Netherlands
37. Scheper Hospital, Emmen, The Netherlands
38. St. Jansdal Hospital, Hardewijk, The Netherlands
39. Laurentius Hospital, Roermond, The Netherlands
40. Isala Diaconessenhuis, Meppel, The Netherlands
41. Zuyderland Medical Center, Sittard-Geleen, The Netherlands
42. Westfriesgasthuis, Hoorn, The Netherlands
43. Medisch Spectrum Twente, Enschede, The Netherlands
44. St. Franciscus Gasthuis, Rotterdam, The Netherlands
45. Streekziekenhuis Koningin Beatrix, Winterswijk, The Netherlands
46. Flevo Hospital, Almere, The Netherlands
47. Zuwe Hofpoort Hospital, Woerden, The Netherlands

Swiss Pediatric Sepsis Study

Steering Committee: Luregn J Schlapbach, MD, FCICM^{1,2,3}, Philipp Agyeman, MD¹, Christoph Aebi, MD¹, Christoph Berger, MD¹
Luregn J Schlapbach, MD, FCICM^{1,2,3}, Philipp Agyeman, MD¹, Christoph Aebi, MD¹, Eric Giannoni, MD^{4,5}, Martin Stocker, MD⁶, Klara M Posfay-Barbe, MD⁷, Ulrich Heininger, MD⁸, Sara Bernhard-Stirnemann, MD⁹, Anita Niederer-Loher, MD¹⁰, Christian Kahlert, MD¹⁰, Paul Hasters, MD¹¹, Christa Relly, MD¹², Walter Baer, MD¹³, Christoph Berger, MD¹² **for the Swiss Pediatric Sepsis Study**

- ¹. Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland
- ². Paediatric Critical Care Research Group, Mater Research Institute, University of Queensland, Brisbane, Australia
- ³. Paediatric Intensive Care Unit, Lady Cilento Children's Hospital, Children's Health Queensland, Brisbane, Australia
- ⁴. Service of Neonatology, Lausanne University Hospital, Lausanne, Switzerland
- ⁵. Infectious Diseases Service, Lausanne University Hospital, Lausanne, Switzerland
- ⁶. Department of Pediatrics, Children's Hospital Lucerne, Lucerne, Switzerland
- ⁷. Pediatric Infectious Diseases Unit, Children's Hospital of Geneva, University Hospitals of Geneva, Geneva, Switzerland
- ⁸. Infectious Diseases and Vaccinology, University of Basel Children's Hospital, Basel, Switzerland
- ⁹. Children's Hospital Aarau, Aarau, Switzerland
- ¹⁰. Division of Infectious Diseases and Hospital Epidemiology, Children's Hospital of Eastern Switzerland St. Gallen, St. Gallen, Switzerland
- ¹¹. Department of Neonatology, University Hospital Zurich, Zurich, Switzerland
- ¹². Division of Infectious Diseases and Hospital Epidemiology, and Children's Research Center, University Children's Hospital Zurich, Switzerland
- ¹³. Children's Hospital Chur, Chur, Switzerland

Liverpool Partner

Principal Investigators

Enitan Carroll¹
Stéphane Paulus^{1,2}

ALDER HEY SERIOUS PAEDIATRIC INFECTION RESEARCH GROUP (ASPIRE)
(in alphabetical order):
Hannah Frederick³, Rebecca Jennings³, Joanne Johnston³, Rhian Kenwright³

- ¹ Department of Clinical Infection, Microbiology and Immunology, University of Liverpool Institute of Infection and Global Health, Liverpool, England
- ² Alder Hey Children's Hospital, Department of Infectious Diseases, Eaton Road, Liverpool, L12 2AP

³ Alder Hey Children's Hospital, Clinical Research Business Unit, Eaton Road, Liverpool, L12 2AP

Micropathology Ltd

Colin G Fink^{1,2}, Elli Pinnock¹

¹Micropathology Ltd Research and Diagnosis

²University of Warwick

Newcastle partner

Principle Investigator

Marieke Emonts^{1,2}

Co-Investigator

Rachel Agbeko^{1,3}

¹ Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

² Paediatric Infectious Diseases and Immunology Department, Newcastle upon Tyne Hospitals Foundation Trust, Great North Children's Hospital, Newcastle upon Tyne, United Kingdom

³ Paediatric Intensive Care Unit, Newcastle upon Tyne Hospitals Foundation Trust, Great North Children's Hospital, Newcastle upon Tyne, United Kingdom

Gambia partner

Suzanne Anderson: Principal Investigator and West African study oversight:

Fatou Secka: Clinical research fellow and study co-ordinator

Additional Gambia site team (consortium members):

Kalifa Bojang: co-PI

Isatou Sarr: Senior laboratory technician

Ngane Kebbeh: Junior laboratory technician

Gibbi Sey: lead research nurse Medical Research Council Clinic

Momodou Saidykhan: lead research nurse Edward Francis Small Teaching Hospital

Fatoumatta Cole: Data manager

Gilleh Thomas: Data manager

Martin Antonio: Local collaborator

Medical Research Council Unit Gambia

PO Box 273

Banjul

The Gambia

Austrian partner

PI: Werner Zenz¹

Co-Investigators/Steering committee:

Daniela S. Klobassa¹, Alexander Binder¹, Nina A. Schweintzger¹, Manfred Sagmeister¹

¹University Clinic of Paediatrics and Adolescent Medicine, Department of General Paediatrics, Medical University Graz, Austria

Austrian network, participating centres in Austria, Germany, Italy, Serbia, Lithuania, patient recruitment (in alphabetical order):

Hinrich Baumgart¹, Markus Baumgartner², Uta Behrends³, Ariane Biebl⁴, Robert Birnbacher⁵, Jan-Gerd Blanke⁶, Carsten Boelke⁷, Kai Breuling³, Jürgen Brunner⁸, Maria Buller⁹, Peter Dahlem¹⁰, Beate Dietrich¹¹, Ernst Eber¹², Johannes Elias¹³, Josef Emhofer², Rosa Etschmaier¹⁴, Sebastian Farr¹⁵, Ylenia Girtler¹⁶, Irina Grigorow¹⁷, Konrad Heimann¹⁸, Ulrike Ihm¹⁹, Zdenek Jaros²⁰, Hermann Kalhoff²¹, Wilhelm Kaulfersch²², Christoph Kemen²³, Nina Klocker²⁴, Bernhard Köster²⁵, Benno Kohlmaier²⁶, Eleni Komini²⁷, Lydia Kramer³, Antje Neubert²⁸, Daniel Ortner²⁹, Lydia Pescolliderung¹⁶, Klaus Pfurtscheller³⁰, Karl Reiter³¹, Goran Ristic³², Siegfried Rödl³⁰, Andrea Sellner²⁶, Astrid Sonnleitner²⁶, Matthias Sperl³³, Wolfgang Stelzl³⁴, Holger Till¹, Andreas Trobisch²⁶, Anne Vierzig³⁵, Ulrich Vogel¹², Christina Weingarten³⁶, Stefanie Welke³⁷, Andreas Wimmer³⁸, Uwe Wintergerst³⁹, Daniel Wüller⁴⁰, Andrew Zaunschirm⁴¹, Ieva Ziuraite⁴², Veslava Žukovskaja⁴²

¹Department of Pediatric and Adolescence Surgery, Division of General Pediatric Surgery, Medical University Graz, Austria

²Department of Pediatrics, General Hospital of Steyr, Austria

³Department of Pediatrics/Department of Pediatric Surgery, Technische Universität München (TUM), Munich, Germany

⁴Department of Pediatrics, Kepler University Clinic, Medical Faculty of the Johannes Kepler University, Linz, Austria

⁵Department of Pediatrics and Adolescent Medicine LKH Villach, Austria

⁶Department of Pediatrics and Adolescent Medicine and Neonatology, Hospital Ludmillenstift, Meppen, Germany

⁷Hospital for Children's and Youth Medicine, Oberschwabenklinik, Ravensburg, Germany

⁸Department of Pediatrics, Medical University Innsbruck, Austria

⁹Clinic for Paediatrics and Adolescents Medicine, Sana Hanse-Klinikum Wismar, Germany

¹⁰Department of Pediatrics, Medical Center Coburg, Germany

¹¹University Medicine Rostock, Department of Pediatrics (UKJ), Rostock, Germany

¹²Department of Pulmonology, Medical University Graz, Austria

¹³Institute for Hygiene and Microbiology, University of Würzburg, Germany

¹⁴Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz, Austria

¹⁵Department of Pediatric Orthopedics and Adult Foot and Ankle Surgery, Orthopedic Hospital Speising, Vienna, Austria

¹⁶Department of Paediatrics, Regional Hospital Bolzano, Italy

¹⁷Department of Pediatrics and Adolescent Medicine, General Hospital Hochsteiermark/Leoben, Austria

- ¹⁸Department of Neonatology and Paediatric Intensive Care, Children's University Hospital, RWTH Aachen, Germany
- ¹⁹Paediatric Intensive Care Unit, Department of Paediatric Surgery, Donaushpital Vienna, Austria
- ²⁰Department of Pediatrics, General Public Hospital, Zwettl, Austria
- ²¹Pediatric Clinic Dortmund, Germany
- ²²Department of Pediatrics and Adolescent Medicine, Klinikum Klagenfurt am Wörthersee, Klagenfurt, Austria
- ²³Catholic Children's Hospital Wilhelmstift, Department of Pediatrics, Hamburg, Germany
- ²⁴Department of Pediatrics, Krankenhaus Dornbirn, Austria
- ²⁵Children's Hospital Luedenscheid, Maerkische Kliniken, Luedenscheid, Germany
- ²⁶Department of General Paediatrics, Medical University Graz, Austria
- ²⁷Department of Paediatrics, Schwarzwald-Baar-Hospital, Villingen-Schwenningen, Germany
- ²⁸Department of Paediatrics and Adolescents Medicine, University Hospital Erlangen, Germany
- ²⁹Department of Pediatrics and Adolescent Medicine, Medical University of Salzburg, Austria
- ³⁰Paediatric Intensive Care Unit, Medical University Graz, Austria
- ³¹Dr. von Hauner Children's Hospital, Ludwig-Maximilians- Universitaet, Munich, Germany
- ³²Mother and Child Health Care Institute of Serbia, Belgrade, Serbia
- ³³Department of Pediatric and Adolescence Surgery, Division of Pediatric Orthopedics, Medical University Graz, Austria
- ³⁴Department of Pediatrics, Academic Teaching Hospital, Landeskrankenhaus Feldkirch, Austria
- ³⁵University Children's Hospital, University of Cologne, Germany
- ³⁶Department of Pediatrics and Adolescent Medicine Wilheminspital, Vienna, Austria
- ³⁷Department of Pediatric Surgery, Municipal Hospital Karlsruhe, Germany
- ³⁸Hospital of the Sisters of Mercy Ried, Department of Pediatrics and Adolescent Medicine, Ried, Austria
- ³⁹Hospital St. Josef, Braunau, Austria
- ⁴⁰Christophorus Kliniken Coesfeld Clinic for Pediatrics, Coesfeld, Germany
- ⁴¹Department of Paediatrics, University Hospital Krems, Karl Landsteiner University of Health Sciences, Krems, Austria
- ⁴²Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos, Lithuania

Appendix: Full definitions document

1. Focal infections

1.1. Lung infections

1.1.1. Pneumonia

It is an inflammation of one or both lungs: Lobar or segmental or multilobar collapse/consolidation on CXR. Do not include perihilar consolidation or patchy consolidation.

Clinical symptoms compatible with acute respiratory infection and following radiological findings of consolidation/pleural effusion: alveolar consolidation (defined as a dense or fluffy opacity that occupies a portion or whole of a lobe or of the entire lung that may or may not contain air-bronchograms) or pleural effusion (defined as fluid in the lateral pleural space and not just in the minor or oblique fissure) that was spatially associated with a pulmonary parenchymal infiltrate (including other infiltrate) or obliterated enough of the hemithorax to obscure an opacity.

1.1.2. Pleural Effusion / Empyema

Simple parapneumonic effusion is defined as pleural effusion associated with lung infection (ie, pneumonia). These effusions result from the spread of inflammation and infection to the pleura. Much less commonly, infections in other adjacent areas (eg, retropharyngeal, vertebral, abdominal, and retroperitoneal spaces) may spread to the pleura resulting in the development of effusion.

Empyema is defined as the presence of grossly purulent fluid in the pleural cavity. In practice:

- Thoracentesis with microbial growth from pleural fluid or

- Thoracentesis with no growth on culture of pleural fluid but elevated protein, or cell count (normal and abnormal reference values as determined by clinical laboratory at each center)
- Ultrasound or other diagnostic imaging evidence of pleural fluid assessed by the radiologist as empyema or
- Diagnosis at time of thoracic surgery.

1.1.3. Whooping cough (*Bordetella pertussis*)

Clinical diagnosis — For endemic or sporadic cases, a clinical case of pertussis is defined as an acute cough illness lasting at least 14 days accompanied by one of the following:

- Paroxysms of coughing
- Inspiratory whoop
- Post-tussive vomiting

In an outbreak or following household contact to a known case, a clinical case is defined as a cough illness for at least 14 days; presence of the typical pertussis- associated features is not required.

Definite diagnosis — Clinical diagnosis confirmed by bacterial culture, polymerase chain reaction (PCR) or serology. Note that direct fluorescent antibody should not be considered due to variable specificity.

1.1.4. Bronchiolitis

Bronchiolitis is diagnosed clinically by the presence of viral upper respiratory prodromes followed by increased respiratory effort (eg, tachypnea, nasal flaring, chest retractions) and wheezing and/or rales in children younger than two years of age.

1.2. Neurological infections:

1.2.1. Meningitis

Meningitis is an infection of the membranes covering the brain and spinal cord (leptomeninges).

Compatible clinical syndrome: Any child (0-18 years) with clinical symptoms compatible with meningitis (a severe headache, fever, nausea, vomiting and feeling generally unwell).

The symptoms in babies and young children are: becoming floppy and unresponsive, or stiff with jerky movements, becoming irritable and not wanting to be held, unusual crying, pale and blotchy skin, refusing feeds, loss of appetite, a staring expression, very sleepy and reluctant to wake up.

Definite bacterial meningitis

Compatible clinical syndrome, plus

- All ages: fever, 94%
- 1–5 mos: irritability, 85%
- 6–11 mos: impaired consciousness, 79%
- >12 mos: vomiting, 82%; neck rigidity, 78%
- (note: many other compatible signs and symptoms) plus

Positive culture of cerebrospinal fluid (CSF), or positive CSF Gram stain, PCR or bacterial antigen.

Probable bacterial meningitis

Compatible clinical syndrome, plus

Positive culture of blood, plus

One of the following CSF changes

- >5 leukocytes
- Protein of >100 mg/dL
- Glucose of <40mg/dl (<2.2mmol/l) or 0.5 CSF/serum ratio

Possible bacterial meningitis

Compatible clinical syndrome, plus

- One of the following CSF changes
 - >100 leukocytes
 - Glucose of < 40 mg/dl (<2.2mmol/l) or CSF/serum glucose ratio 0.5
 - Protein of > 100 mg/dL plus
- Negative cultures or antigen for bacteria, viral, fungal, or mycobacteria

Confirmed: A case that is laboratory-confirmed by growing (i.e. culturing) or identifying (i.e. by PCR or Gram stain or antigen detection methods) a bacterial pathogen (Hib, pneumococcus or meningococcus) in the CSF or from the blood in a child with a clinical syndrome consistent with bacterial meningitis

Note: Any persons with *H. influenzae*, meningococcus or pneumococcus isolated from CSF or blood may be considered as confirmed cases of meningitis if their clinical syndrome was meningitis (i.e. culture from normally sterile fluids is the gold standard). Culture of Hib, pneumococcus or meningococcus from a non-sterile site, such as the throat, does not confirm a case of disease, since the bacteria can grow in these other areas without causing disease.

1.2.2. Bacterial brain abscess

Brain abscess is a focal collection within the brain parenchyma caused by a bacterial infection, which can arise as a complication of a variety of infections, trauma, or surgery.

The diagnosis of focal collection is confirmed by CT scan with contrast or MRI.

The diagnosis of bacterial brain abscess is confirmed by a positive culture or positive Gram stain or positive 16s or bacterial antigen in specimen obtained from stereotactic CT-guided aspiration or surgery.

For EUCLIDS purposes, possible bacterial brain abscess can be included only on clinical and radiological findings.

1.3. Bone and Joint infection

1.3.1. Osteomyelitis:

It is defined as an inflammation or an infection in the bone marrow and surrounding bone.

In the EUCLIDS study both acute, subacute and chronic bacterial osteomyelitis are of interest and to be included:

- Acute osteomyelitis is defined by a duration of symptoms < 14 days.
- Subacute osteomyelitis is defined by a duration of symptoms between 14 days and 1 month.
- Chronic osteomyelitis is defined by a duration of symptoms more than 1 month.

The diagnosis of acute/subacute/chronic osteomyelitis is based on the following criteria:

- Presence of localized pain/tenderness and other typical features of osteomyelitis (warmth and/or swelling of the affected region)

AND/OR

- Imaging findings consistent with osteomyelitis (typical MRI findings and/or a positive bone scan)

AND/OR

- Bacteriologic evidence of infection (positive blood and/or bone culture).

AND/OR

- Histopathological finding consistent with osteomyelitis (intraoperative specimen)

For diagnosis of osteomyelitis at least two criteria must be positive.

1.3.2. Septic arthritis:

It is diagnosed when a microorganism is isolated from blood with clinical arthritis, from the synovial fluid and/or purulent fluid is aspirated from the joint. Synovial fluid with white blood cell count (WBC) 50,000/mm³ is considered purulent.

MRI findings consistent with acute/subacute/chronic osteomyelitis:

- On unenhanced images, osteomyelitis is characterized by focally decreased marrow signal intensity on T1-weighted images AND focally increased marrow signal intensity on fluid-sensitive images (fat-suppressed T2-weighted and STIR sequences).

OR

- After contrast administration, osteomyelitis is described as focal abnormal bone marrow enhancement on fat-suppressed T1-weighted images.

AND/OR

- Complications of osteomyelitis can include abscesses: Intraosseous, subperiosteal, and soft-tissue abscesses are defined as well circumscribed areas of focally decreased signal intensity on T1-weighted images with increased signal intensity equal to that of fluid on fluid-sensitive sequences and/or rim enhancement on contrast-enhanced fat-suppressed T1-weighted images.
- Subacute osteomyelitis can manifest as Brodie abscess characterized by a central abscess cavity filled with fluid, an inner ring of enhancing high signal intensity granulation tissue on T1-weighted sequences, an outer ring of very low signal intensity sclerosis, and a peripheral halo of edema.
- In chronic osteomyelitis, imaging might reveal an involucrum (thick periosteal new bone), sequestrum (necrotic bone fragment), or cloaca (draining tract through a defect in the cortex and involucrum).

Bone scan consistent with osteomyelitis (Technetium-99m bone scan):

- The most definitive phase is the delayed phase: There is no osteomyelitis without abnormal radionuclide uptake on the images obtained during the delayed phase, even if there is increased activity on blood flow or blood pool images.

AND/OR

- The hallmark feature of osteomyelitis at ^{99m}Tc scintigraphy is increased activity in all three phases (1. angiographic or blood flow phase, 2. blood pool or tissue phase and 3. delayed phase).

Histopathological finding consistent with acute/subacute/chronic osteomyelitis:

- Inflammatory cells
 - In acute osteomyelitis: predominately polymorphonuclear leucocytes
 - In chronic osteomyelitis: mononuclear cells including plasma cells and macrophage/monocyte cells

AND/OR

- Destruction/necrosis of bone (necrotic marrow and bone, osteoclastic activity)

AND/OR

- Granulation tissue (hemorrhage, polymorphonuclear leucocytes, lymphocytes, and macrophages)

AND/OR

- In implant-associated infections, tissue specimens obtained for histopathology either by biopsy or during surgery as frozen section are important because the presence of neutrophils in significant amounts is indicative of infection. More than five neutrophils per high-power field indicates infection, with sensitivity of 43–84% and specificity of 93–97%. These infections will be considered as “community acquired” depending on the onset of symptoms after implantation: if >24 months have elapsed it is considered a community acquired infection.

1.3.3. Diskitis/spondylodiskitis

Diskitis is an inflammatory process involving the intervertebral disks and the endplates of the vertebral bodies, and associated with characteristic clinical and radiologic findings.

1.3.4. Mastoiditis

Mastoiditis is a suppurative infection of the mastoid air cells, and the most common suppurative complication of acute otitis media. In acute mastoiditis, symptoms are of less than 1 month's duration.

There is a lack of consensus regarding the criteria and strategies for diagnosing acute mastoiditis in the paediatric population. The diagnosis is usually made clinically, without need for imaging studies.

- Clinical features:
 - Fever
 - Otalgia
 - Post-auricular erythema, tenderness, swelling, fluctuance or mass
 - Displacement of the auricle (down and out: children <2years); up and out in children ≥ 2 years
- Imaging: CT, MRI: Haziness or destruction of the mastoid outline; and loss of or decrease in the sharpness of the bony septa that define the mastoid air cells.
- Microbiology: Positive culture or gram stain of a specimen obtained from the middle ear either by tympanocentesis through an intact eardrum or by aspiration through a tympanostomy tube or perforation.

1.4. Soft tissue infections:

1.4.1. Cellulitis

Acute, diffuse, spreading infection of the skin, involving the deeper layers of the skin and the subcutaneous tissue.

1.4.2. Ecthyma /erysipelas

Ecthyma is a bacterial infection of the dermis and epidermis characterized by a vesicle or vesico-pustule with an erythematous base that erodes through the epidermis into the dermis to form a crusted ulcer with elevated margins up to 4 cm in diameter.

Clinical diagnosis is confirmed by a positive culture or Gram stain of the lesion.

Erysipelas is a superficial form of cellulitis with lymphatic involvement.

1.4.3. Necrotizing fasciitis

Necrotizing fasciitis is an infection of the deeper tissues that results in rapidly progressive destruction of the muscle fascia, overlying subcutaneous fat and epidermis. The definite diagnosis is surgical.

1.4.4. Myositis / pyomyositis

Myositis is an inflammation of the skeletal muscles, often caused by infection or autoimmune disease. Pyomyositis is a bacterial infection of the skeletal muscle that is usually caused by *Staphylococcus aureus*.

Pyomyositis is suspected by the clinical presentation (fever and pain with cramping usually localized to a single muscle group) and compatible findings in image techniques (Rx, CT, US, MRI). Definite diagnosis is made by culture and gram stain of drainage specimen.

1.4.5. Deep neck infections

Suppurative infection of the neck, including:

- Peritonsillar abscess: Collection of pus located between the capsule of the palatine tonsil and the pharyngeal muscles.
- Retropharyngeal abscess: Collection of pus located in the retropharyngeal space (extending from the base of the skull to the posterior mediastinum, between the middle layer and the deep layer of the deep cervical fascia).

- Lateral pharyngeal space infection: Collection of pus located in the lateral pharyngeal space (bounded laterally by the carotid sheath).

Suppurative cervical lymphadenitis: Enlarged, inflamed and tender lymph node with or without fluctuance, usually unilateral. Clinical diagnosis is confirmed by positive culture or Gram stain of specimen obtained by needle aspiration or incision and drainage.

1.5. Intra-abdominal infections:

1.5.1. Acute appendicitis

Acute inflammation of the appendix, usually resulting from bacterial infection.

Clinical presentation is variable, often consisting of abdominal pain and tenderness in periumbilical region (early) migrating to the right lower quadrant of the abdomen, vomiting, fever and signs of localized or generalized peritoneal irritation.

Definite diagnosis is made by demonstration of an inflamed or perforated appendix on pathology after surgical removal.

1.5.2. Infectious peritonitis

Infection of the peritoneum, usually secondary to inoculation of the peritoneal cavity with bacteria and other inflammatory debris following intestinal perforation or postoperative anastomotic leak. Acute appendicitis is the most commonly associated condition leading to secondary peritonitis in older children.

Clinical diagnosis is confirmed by positive culture or Gram stain of peritoneal fluid.

1.5.3. Pyelonephritis

Urinary tract infection affecting the renal parenchyma and pelvis. In a patient with fever in absence of another source of infection:

- Possible pyelonephritis: presence of positive leukocyte esterase test results or nitrite test or microscopic analysis results positive for leukocytes or bacteria in a

urine specimen collected by the most convenient means and compatible findings in renal ultrasonography, voiding cystourethrography or nuclear scanning with technetium- labeled dimercaptosuccinic acid.

- Definite pyelonephritis:
 - Presence of both pyuria and at least 50,000 colonies per mL of a single uropathogenic organism in an appropriately collected specimen of urine (by urethral catheterization or suprapubic aspiration)
 - and compatible findings in renal ultrasonography, voiding cystourethrography or nuclear scanning with technetium-labeled dimercaptosuccinic acid.

2. Toxic shock definition:

2.1. Staphylococcal toxic shock syndrome clinical case definition

1. Fever $\geq 38,9^{\circ}\text{C}$
2. Rash—diffuse macular erythroderma
3. Desquamation—1–2 weeks after onset of illness, especially of palms and soles
4. Hypotension—systolic blood pressure ≤ 90 mm Hg for adults
5. Multi-system involvement—3 or more of the following:
 - a) Gastrointestinal—vomiting or diarrhoea at the onset of illness
 - b) Muscular—severe myalgia or elevated creatine phosphokinase
 - c) Mucous membranes—vaginal, oropharyngeal, conjunctival hyperaemia
 - d) Renal—blood urea nitrogen or creatinine twice-upper limit of normal
 - e) Hepatic—total bilirubin twice-upper limit of normal
 - f) Haematological—platelets $\leq 100 \times 10^9/\text{L}$
 - g) CNS—disorientation or alterations in consciousness without focal neurological signs
6. Negative results on the following tests:

- a) Blood, throat, or cerebrospinal fluid culture for another pathogen (blood culture may be positive for *Staphylococcus aureus*)
- b) Rise in titre to Rocky Mountain spotted fever, leptospirosis, or measles

Case classification:

- Probable: case with five of the six clinical findings described
- Confirmed: case with all six of the clinical findings described

2.2. Streptococcal toxic shock syndrome clinical case definition

1. Isolation of group A β -haemolytic streptococci:

- a) From a normally sterile site—blood, CSF, peritoneal fluid, tissue biopsy
- b) From a non-sterile site—throat, vagina, sputum

2. Clinical signs of severity:

- a) Hypotension—systolic blood pressure ≤ 90 mm Hg in adults or below normal age adjusted levels in children
- b) Two or more of the following signs:
 - i) Renal impairment—creatinine > 2 mg/dL (> 177 μ mol/L)
 - ii) Coagulopathy—platelets $\leq 100 \times 10^9$ /L or disseminated intravascular coagulation
 - iii) Hepatic involvement—alanine aminotransferase, aspartate aminotransferase, or total bilirubin twice the upper limit of normal
 - iv) Adult respiratory distress syndrome
 - v) Generalised, erythematous, macular rash that may desquamate
 - vi) Soft-tissue necrosis, including necrotising fasciitis, myositis, or gangrene

Case classification:

- Probable: case fulfils 1b and 2 (a and b) if no other cause for the illness is found –
- Confirmed: case fulfils 1a and 2 (a and b)

3. Clinical syndromes

3.1. Bacteraemia/septicaemia:

3.1.1. Systemic Inflammatory Response Syndrome (SIRS)

As per clinical criteria established by Goldstein et al, SIRS is defined by at least two of the following four criteria:

- 1.- Core (rectal, bladder, oral or central catheter probe) temperature of $> 38.5^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$.
- 2.- Tachycardia, defined as a mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli; or otherwise unexplained persistent elevation over a 0.5- to 4-hr time period or OR for children <1 yr old: bradycardia, defined as a mean heart rate <10 th percentile for age in the absence of external vagal stimulus, B-blocker drugs, or congenital heart disease; or otherwise unexplained persistent depression over a 0.5-hr time period.
- 3.- Mean respiratory rate >2 SD above normal for age or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia.
- 4.- Leukocyte count elevated or depressed for age (not secondary to chemotherapy-induced leukopenia) or $> 10\%$ immature neutrophils.

3.1.2. Sepsis

Defined as suspected infection plus SIRS, as per clinical criteria established by Goldstein et al, as long as temperature or leukocyte count is abnormal.

3.1.3. Severe sepsis

Sepsis plus one of the following:

- Acute respiratory distress syndrome OR
- Two or more other organ dysfunctions.

- NOTE: severe sepsis + cardiovascular organ dysfunction =septic shock (see below)

Respiratory dysfunction

- $\text{PaO}_2/\text{FIO}_2 < 300$ in absence of cyanotic heart disease or preexisting lung disease, OR
- $\text{PaCO}_2 > 65$ torr or 20 mm Hg over baseline PaCO_2 , OR
- Proven need or $>50\%$ FIO_2 to maintain saturation $>92\%$, OR
- Need for nonelective invasive or noninvasive mechanical ventilation Neurologic dysfunction
- Glasgow Coma Score <11 , OR
- Acute change in mental status with a decrease in Glasgow Coma Score >3 points from abnormal baseline

Hematologic dysfunction

- Platelet count $<80,000/\text{mm}^3$ or a decline of 50% in platelet count from highest value recorded over the past 3 days (for chronic hematology/oncology patients), OR
- International normalized ratio >2

Renal dysfunction

- Serum creatinine >2 times upper limit of normal for age or 2-fold increase in baseline creatinine

Hepatic dysfunction

- Total bilirubin >4 mg/dL (not applicable for newborn) OR
- ALT 2 times upper limit of normal for age

It is the presence of bacteria, other infectious organisms, or toxins created by infectious organisms in the bloodstream with spread throughout the body.

3.1.4. Septic shock

Sepsis and cardiovascular organ dysfunction.

Cardiovascular dysfunction

Despite administration of isotonic intravenous fluid bolus >40 mL/kg in 1 hr

- Decrease in BP (hypotension) <5th percentile for age or systolic BP <2 SD below normal for age, OR
- Need for vasoactive drug to maintain BP in normal range (dopamine >5 microg/kg/min or dobutamine, epinephrine, or norepinephrine at any dose), OR
- Two of the following
 - Unexplained metabolic acidosis: basedeficit >5.0mEq/L
 - Increasedarteriallactate >2timesupperlimitofnormal
 - Oliguria: urineoutput <0.5mL/kg/hr
 - Prolongedcapillaryrefill:>5secs
 - Coreto peripheraltemperaturegap>3°C

3.2. CLABSI (CDC definition)

Central line-associated BSI (CLABSI): A laboratory-confirmed bloodstream infection (LCBI) where central line (CL) or umbilical catheter (UC) was in place for >2 calendar days when all elements of the LCBI infection criterion were first present together, with day of device placement being Day 1,

AND

CL or UC was in place on the date of event or the day before. If the patient is admitted or transferred into a facility with a central line in place (e.g., tunnelled or implanted central line), day of first access is considered Day1.

Must meet one of the following criteria:

1. Patient has a recognized pathogen cultured from one or more blood cultures and organism cultured from blood is not related to an infection at another site.
2. Patient has at least one of the following signs or symptoms: fever ($>38^{\circ}\text{C}$), chills, or hypotension

AND

positive laboratory results are not related to an infection at another site

AND

common commensal (i.e., diphtheroids [*Corynebacterium spp.* not *C. diphtheriae*], *Bacillus spp.* [not *B. anthracis*], *Propionibacterium spp.*, coagulase-negative staphylococci [including *S. epidermidis*], viridans group streptococci, *Aerococcus spp.*, and *Micrococcus spp.*) is cultured from two or more blood cultures drawn on separate occasions. Criterion elements must occur within a timeframe that does not exceed a gap of 1 calendar day.

(See complete list of common commensals at <http://www.cdc.gov/nhsn/XLS/master-organism-Com-Commensals-Lists.xls>)

3. Patient ≤ 1 year of age has at least one of the following signs or symptoms: fever ($>38^{\circ}\text{C}$ core) hypothermia ($<36^{\circ}\text{C}$ core), apnea, or bradycardia

AND

positive laboratory results are not related to an infection at another site

AND

common skin commensal (i.e., diphtheroids [*Corynebacterium spp.* not *C. diphtheriae*], *Bacillus spp.* [not *B. anthracis*], *Propionibacterium spp.*, coagulase-negative staphylococci [including *S. epidermidis*], viridans group streptococci, *Aerococcus spp.*, *Micrococcus spp.*) is cultured from two or more blood cultures

drawn on separate occasions. Criterion elements must occur within a timeframe that does not exceed a gap of 1 calendar day.

(See complete list of common commensals at <http://www.cdc.gov/nhsn/XLS/master-organism-Com-Commensals-Lists.xlsx>)

3.3. Scarlet Fever (positive throat swab, admitted)

Scarlet fever is an infection that is caused by *Group A streptococcal* bacteria (*S. pyogenes*). The disease is characterized by a sore throat, fever, and a sandpaper-like rash on reddened skin.

In the EUCLIDS study, scarlet fever (positive throat swab) is of interest and to be included.

3.4. Gastroenteritis by salmonella (salmonellosis)

Salmonellosis is a disease caused by the bacteria salmonella. It is usually characterized by acute onset of fever, abdominal pain, diarrhoea, nausea and sometimes vomiting.

In the EUCLIDS study, gastroenteritis for salmonella (positive culture stool or blood?) is of interest and to be included.

3.5. Endocarditis:

It's an inflammation of one or more of the heart valves and lining tissues of the heart.

Symptoms are nonspecific and include fever, chills, and weakness

In the EUCLIDS study, bacterial endocarditis (positive culture) is of interest and to be included.

Dukes Clinical Criteria for Diagnosis of Infective Endocarditis

DEFINITE INFECTIVE ENDOCARDITIS

Pathologic Criteria

- Microorganisms: demonstrated by culture or histology in a vegetation, in a vegetation that has embolized or in an intracardiac abscess

- Pathologic lesions: vegetation or intracardiac abscess present, confirmed by histology showing active endocarditis

Clinical Criteria (see below)

- Two major criteria, OR
- One major and three minor criteria, OR
- Five minor criteria

POSSIBLE INFECTIVE ENDOCARDITIS

- One major criterion and one minor criterion OR
- Three minor criteria

REJECTED

- Firm alternative diagnosis for manifestations of endocarditis, OR
- Resolution of manifestations of endocarditis with antibiotic therapy for ≤ 4 days, OR
- No pathologic evidence of infective endocarditis at surgery or autopsy, after antibiotic therapy for < 4 days, OR
- Does not fulfill criteria above

Definitions of Major and Minor Criteria Used in the Duke Schema for the Diagnosis of Infective Endocarditis (IE)

MAJOR CRITERIA

1. Positive blood culture for IE

a. Typical microorganism consistent with IE from two separate blood cultures:

- *Viridans streptococci*
- *Streptococcus bovis*
- HACEK group [a]
- *Staphylococcus aureus*

- Community-acquired enterococci (without a primary focus)
- Single positive blood culture for *Coxiella burnetii* or IgG antibody titer > 1:800

2. Evidence of endocardial involvement.

a. Positive echocardiogram for IE, defined as:

- Oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation
- Abscess
- New partial dehiscence of prosthetic valve

b. New valvular regurgitation (worsening or changing of pre-existing murmur not sufficient)

MINOR CRITERIA

1. Predisposition: predisposing heart condition or intravenous drug use
2. Fever: temperature $\geq 38.0^{\circ}\text{C}$
3. Vascular phenomena: major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages, and Janeway lesions
4. Immunologic phenomena: glomerulonephritis, Osler nodes, Roth spots, and rheumatoid factor
5. Microbiologic evidence: positive blood culture but does not meet a major criterion as noted above [b] or serologic evidence of active infection with organism consistent with IE

[a] HACEK: *Haemophilus aphrophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella kingae*.

[b] Excludes single positive cultures for coagulase-negative staphylococci and organisms that do not cause endocarditis.

3.6. Influenza-like illness

Sudden-onset fever ($>38^{\circ}\text{C}$) with headache, myalgia, malaise and manifestation of URTI, such as cough, sore throat or rhinitis, in the absence of other diagnoses.

3.7. Fever without source (FWS)

Children with fever lasting for one week or less without adequate explanation after a careful history and thorough physical examination.

It is also known as fever without localizing signs or fever without focus.

3.8. Fever of unknown origin (FUO)

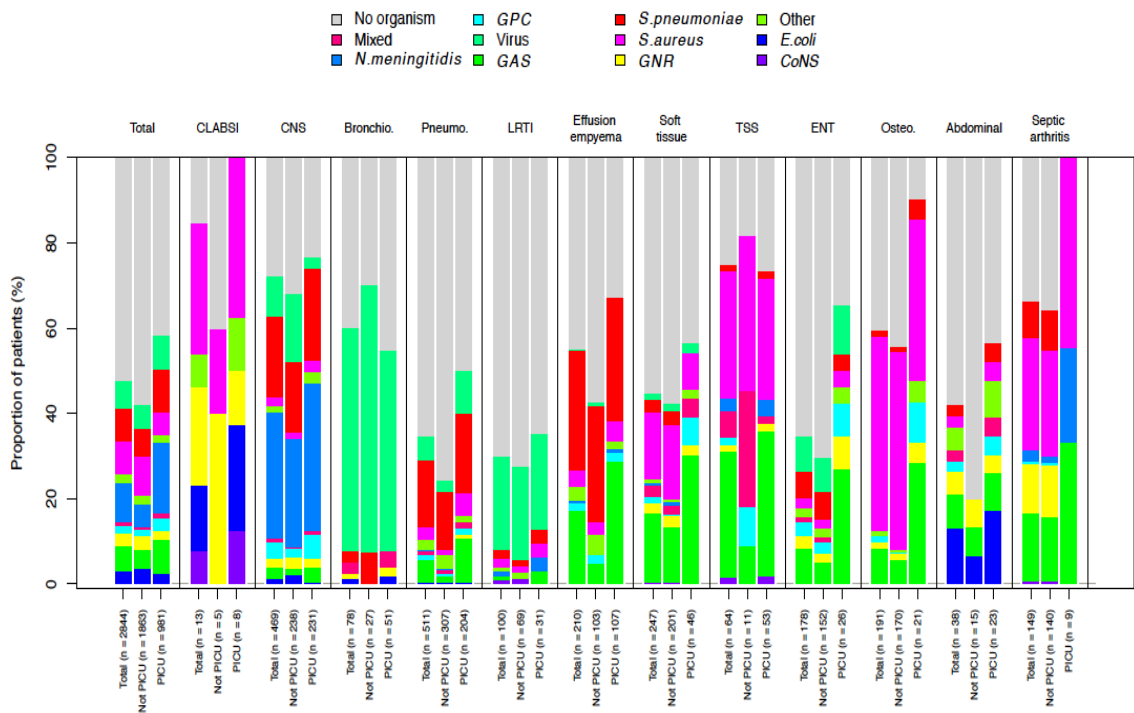
Children with fever $>38.3^{\circ}\text{C}$ of at least 8 days' duration, in whom no diagnosis is apparent after initial outpatient or hospital evaluation that includes a careful history and physical examination and initial laboratory assessment.

Appendix: Causes of death for patients with SFI.

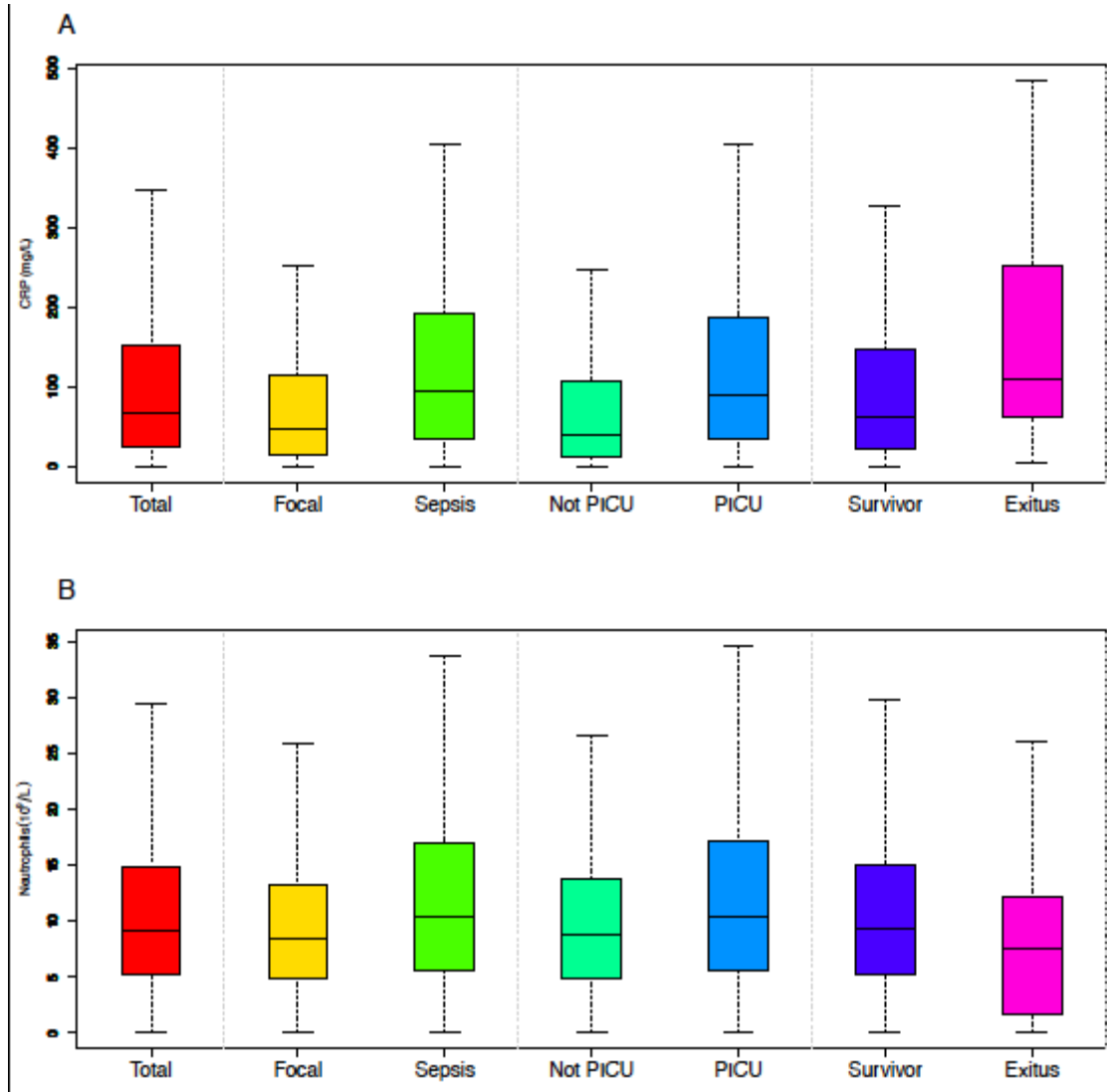
The 7 patients with SFI who died were due to a decompensation of a chronic disease or a complication from the initial infection as follows:

- Patient 1: acute necrotizing encephalopathy
- Patient 2: pneumonia in the context of a complex congenital heart disease
- Patient 3: lower respiratory tract infection on background of chronic lung disease
- Patient 4: acute respiratory distress syndrome and pulmonary haemorrhage in the context of an RSV infection
- Patient 5: cerebral infarction and cardiac failure in the context of a complex congenital disease operated
- Patients 6: respiratory failure in a patient with Leigh's disease
- Patient 7: pulmonary haemorrhage in a patient with epilepsy, scoliosis, deformity of spine and lissencephaly

Appendix Figure 1: Differences between the identified organisms in whole cohort, those admitted to PICU and those admitted to wards by syndrome. GPC: gram positive cocci, GAS: *Group A Streptococcus*, GNR: gram negative rods, CoNS: *Coagulase Negative Staphylococci*.



Appendix Figure 2: Serum levels of (A) C-Reactive protein (CRP) and (B) neutrophils counts in different group of patients on admission. Data are expressed as mg/L for CRP and $\times 10^9/L$ for neutrophils count.



Appendix Figure 3: Receiver operating characteristic curve of CRP, and neutrophil count in different settings.

