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

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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 priorization of therapeutic, diagnostic and preventive measures.

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.

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 Comitte was implemented at all hospitals. All clinical staff were trained in the projects proceedures, 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 retrospecively, 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 contigency 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

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 meningococcemia (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 ($\geq$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 form studies in the United States were *S. aureus*, Pseudomonas species and Enterobacteriacae (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, tabacco 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.

Conflicts of interest

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

meningococcal disease at the Department of General Pediatrics of the Medical University of Graz".

Bibliography

1. Pearson, G.A., M. Ward-Platt, and D. Kelly, *How children die: classifying child deaths.* Arch Dis Child, 2011. **96**(10): p. 922-6.

2. Liu, L., et al., *Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis.* The Lancet, 2015. **385**(9966): p. 430-440.

3. WHO, World Health Organization, *Improving the prevention, diagnosis and clinical management of sepsis.* Report by the Secretariat. Available at: http://apps.who.int/gb/ebwha/pdf_files/EB140/B140_12-en.pdf, 9 January 2017.

4. Martin, N.G., et al., *Hospital admission rates for meningitis and septicaemia caused by Haemophilus influenzae, Neisseria meningitidis, and Streptococcus pneumoniae in children in England over five decades: a population-based observational study.* The Lancet Infectious Diseases, 2014. **14**(5): p. 397-405.

5. Irwin, A.D., et al., *Etiology of childhood bacteremia and timely antibiotics administration in the emergency department.* Pediatrics, 2015. **135**(4): p. 635-42.

6. Maat, M., et al., *Improved survival of children with sepsis and purpura: effects of age, gender, and era.* Crit Care, 2007. **11**(5): p. R112.

7. Schlapbach, L.J., et al., *Mortality related to invasive infections, sepsis, and septic shock in critically ill children in Australia and New Zealand, 2002–13: a multicentre retrospective cohort study.* The Lancet Infectious Diseases, 2015. **15**(1): p. 46-54.

8.   Weiss, S.L., et al., *Global epidemiology of pediatric severe sepsis: the sepsis prevalence, outcomes, and therapies study.* Am J Respir Crit Care Med, 2015. **191**(10): p. 1147-57.

9.   Goldstein, B., et al., *International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics.* Pediatr Crit Care Med, 2005. **6**(1): p. 2-8.

10.   Herberg, J.A., et al., *Diagnostic Test Accuracy of a 2-Transcript Host RNA Signature for Discriminating Bacterial vs Viral Infection in Febrile Children.* JAMA, 2016. **316**(8): p. 835-45.

11.   Bleeker-Rovers, C.P., et al., *A prospective multicenter study on fever of unknown origin: the yield of a structured diagnostic protocol.* Medicine (Baltimore), 2007. **86**(1): p. 26-38.

12.   Singer, M., et al., *The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).* JAMA, 2016. **315**(8): p. 801-10.

13.   Ruth, A., et al., *Pediatric severe sepsis: current trends and outcomes from the Pediatric Health Information Systems database.* Pediatr Crit Care Med, 2014. **15**(9): p. 828-38.

14.   Esteban, E., et al., *A multifaceted educational intervention shortened time to antibiotic administration in children with severe sepsis and septic shock: ABISS Edusepsis pediatric study.* Intensive Care Med, 2017. **43**(12): p. 1916-1918.

15.   Brierley, J., et al., *Clinical practice parameters for hemodynamic support of pediatric and neonatal septic shock: 2007 update from the American College of Critical Care Medicine.* Crit Care Med, 2009. **37**(2): p. 666-88.

16.   Agyeman, P.K.A., et al., *Epidemiology of blood culture-proven bacterial sepsis in children in Switzerland: a population-based cohort study.* The Lancet Child & Adolescent Health, 2017. **1**(2): p. 124-133.

17.   Deen, J., et al., *Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: a systematic review.* The Lancet Infectious Diseases, 2012. **12**(6): p. 480-487.

18.   Reddy, E.A., A.V. Shaw, and J.A. Crump, *Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis.* The Lancet Infectious Diseases, 2010. **10**(6): p. 417-432.

19.   Mayr, F.B., S. Yende, and D.C. Angus, *Epidemiology of severe sepsis.* Virulence, 2014. **5**(1): p. 4-11.

20.   Andre, F.E., et al., *Vaccination greatly reduces disease, disability, death and inequity worldwide.* Bulletin of the World Health Organization, 2008. **86**(2): p. 140-146.

21.   Rivero-Calle, I., et al., *The Burden of Pediatric Invasive Meningococcal Disease in Spain (2008-2013).* Pediatr Infect Dis J, 2016. **35**(4): p. 407-13.

22.   Brook, I. and A.E. Gober, *Recovery of potential pathogens and interfering bacteria in the nasopharynx of smokers and nonsmokers.* Chest, 2005. **127**(6): p. 2072-5.

23. Bagaitkar, J., D.R. Demuth, and D.A. Scott, *Tobacco use increases susceptibility to bacterial infection.* Tob Induc Dis, 2008. **4**: p. 12.

24. Burgner, D., S.E. Jamieson, and J.M. Blackwell, *Genetic susceptibility to infectious diseases: big is beautiful, but will bigger be even better?* The Lancet Infectious Diseases, 2006. **6**(10): p. 653-663.

25. Martinon-Torres, F., et al., *Natural resistance to Meningococcal Disease related to CFH loci: Meta-analysis of genome-wide association studies.* Sci Rep, 2016. **6**: p. 35842.

26. Wright, V., M. Hibberd, and M. Levin, *Genetic polymorphisms in host response to meningococcal infection: the role of susceptibility and severity genes.* Vaccine, 2009. **27 Suppl 2**: p. B90-102.

27. Emonts, M., et al., *Host genetic determinants of Neisseria meningitidis infections.* The Lancet Infectious Diseases, 2003. **3**(9): p. 565-577.

28. Emonts, M., et al., *Polymorphisms in PARP, IL1B, IL4, IL10, C1INH, DEFB1, and DEFA4 in meningococcal disease in three populations.* Shock, 2010. **34**(1): p. 17-22.

29. Van den Bruel, A., et al., *Diagnostic value of laboratory tests in identifying serious infections in febrile children: systematic review.* BMJ, 2011. **342**: p. d3082.

30. Vila Perez, D., et al., *Prognostic factors in pediatric sepsis study, from the Spanish Society of Pediatric Intensive Care.* Pediatr Infect Dis J, 2014. **33**(2): p. 152-7.

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

Comparision 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	*P*-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	*P*-value	Neutrophilis≥12×10^9/L	Neutrophils<12×10^9/L	*P*-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
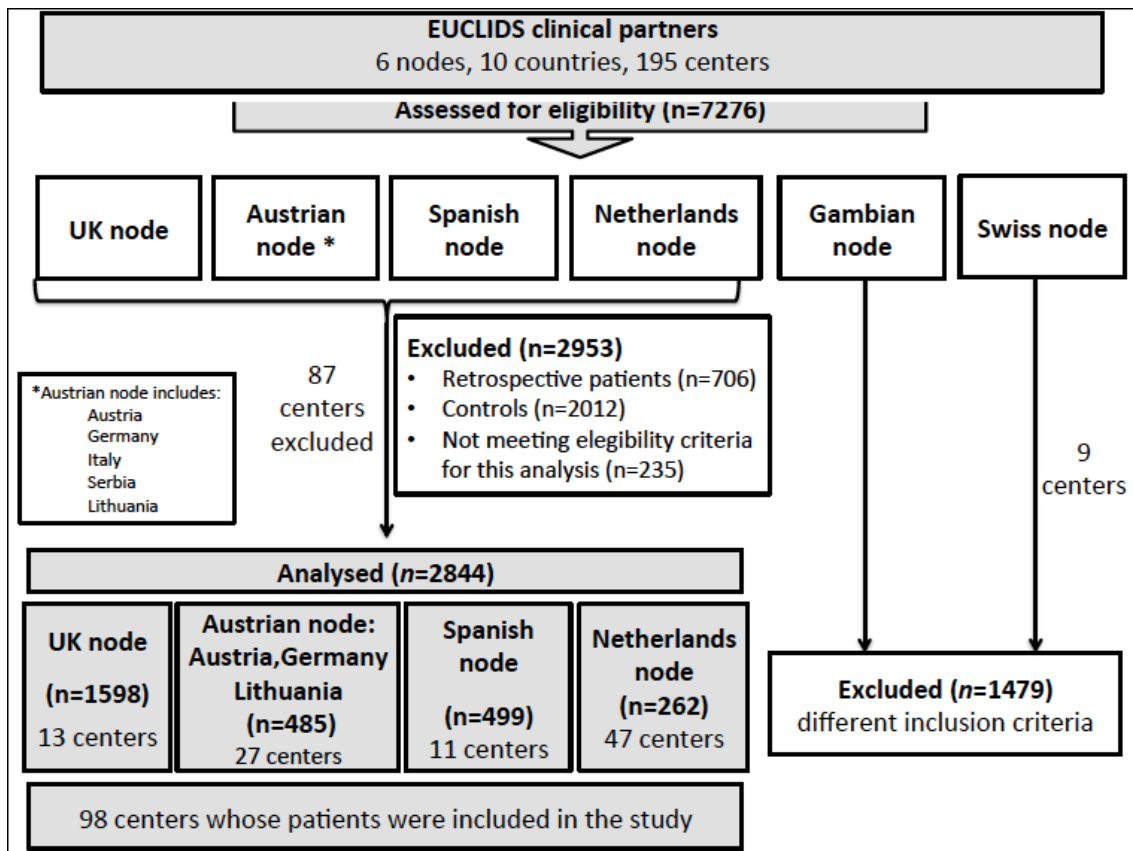
Figure 2: Phenotyping algorithm. Figure adapted from Herberg et al. [10]
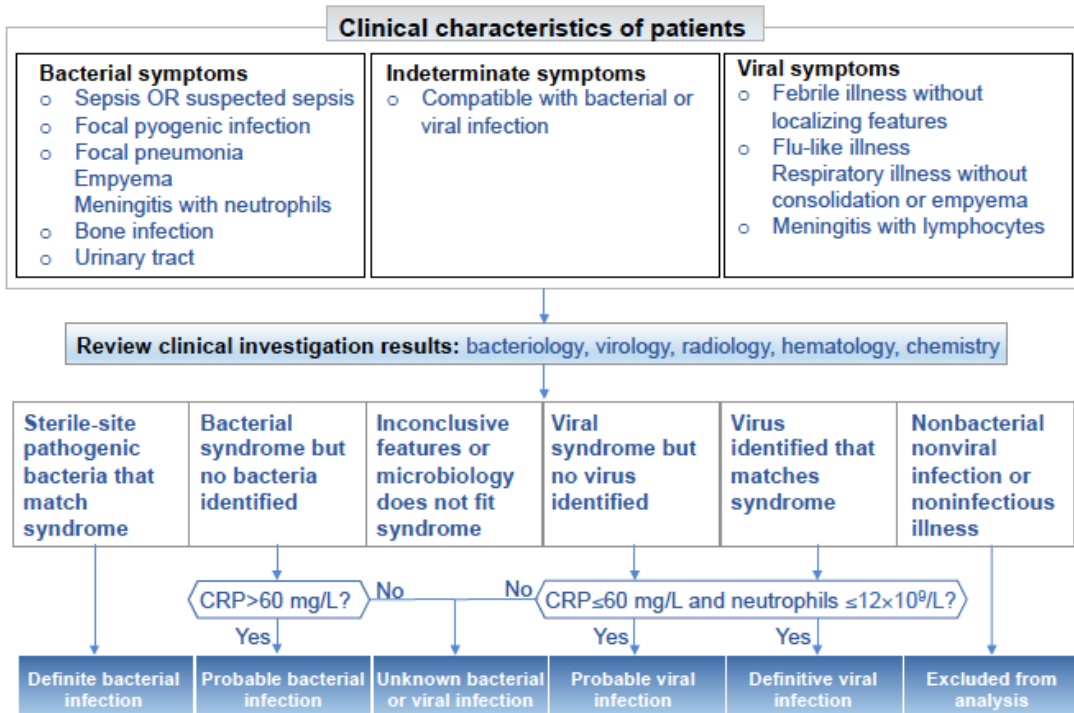
Figure 3: A) Age distribution in the EUCLIDS cohort and in those diagnosed with sepsis or a focal illness. B) Age distribution by causative organism. GPC: gram positive cocci, GAS: *Group A Streptococcus*, GNR: gram negative rods, CoNS: *Coagulase Negative Staphylococci*.
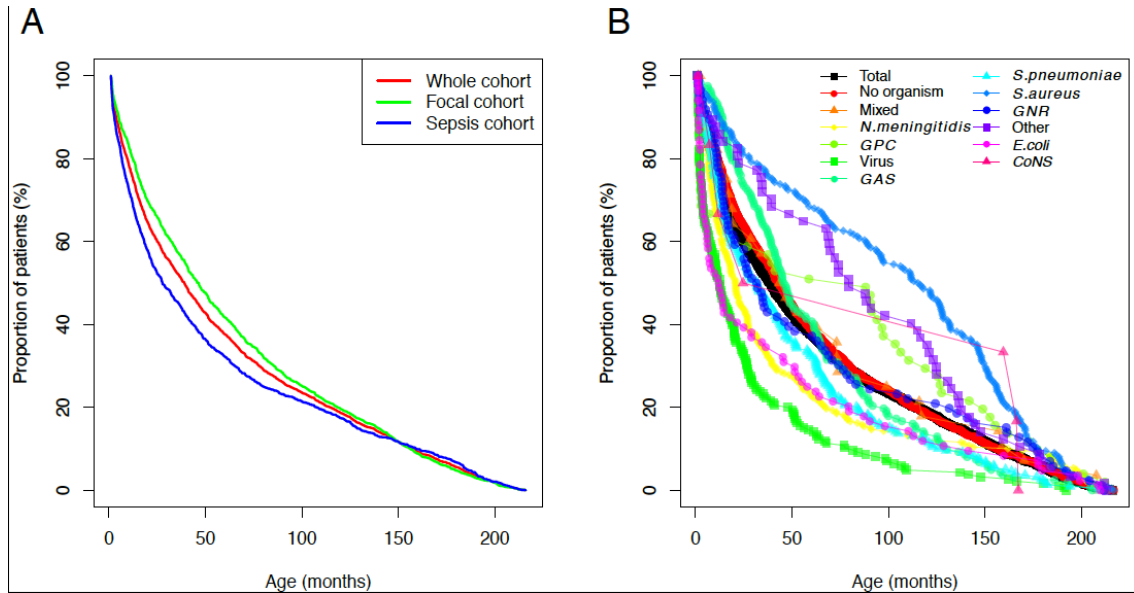
Figure 4. Causative microorganisms identified in EUCLIDS by syndrome. (A) patients with severe focal infections and (B) sepsis. Data are expressed as (*n*) %. CNS infection: central nervous system infection, LRTI: lower respiratory tract infection, ENT syndrome: ear, nose, throat syndrome, UTI-pyelonephritis: 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

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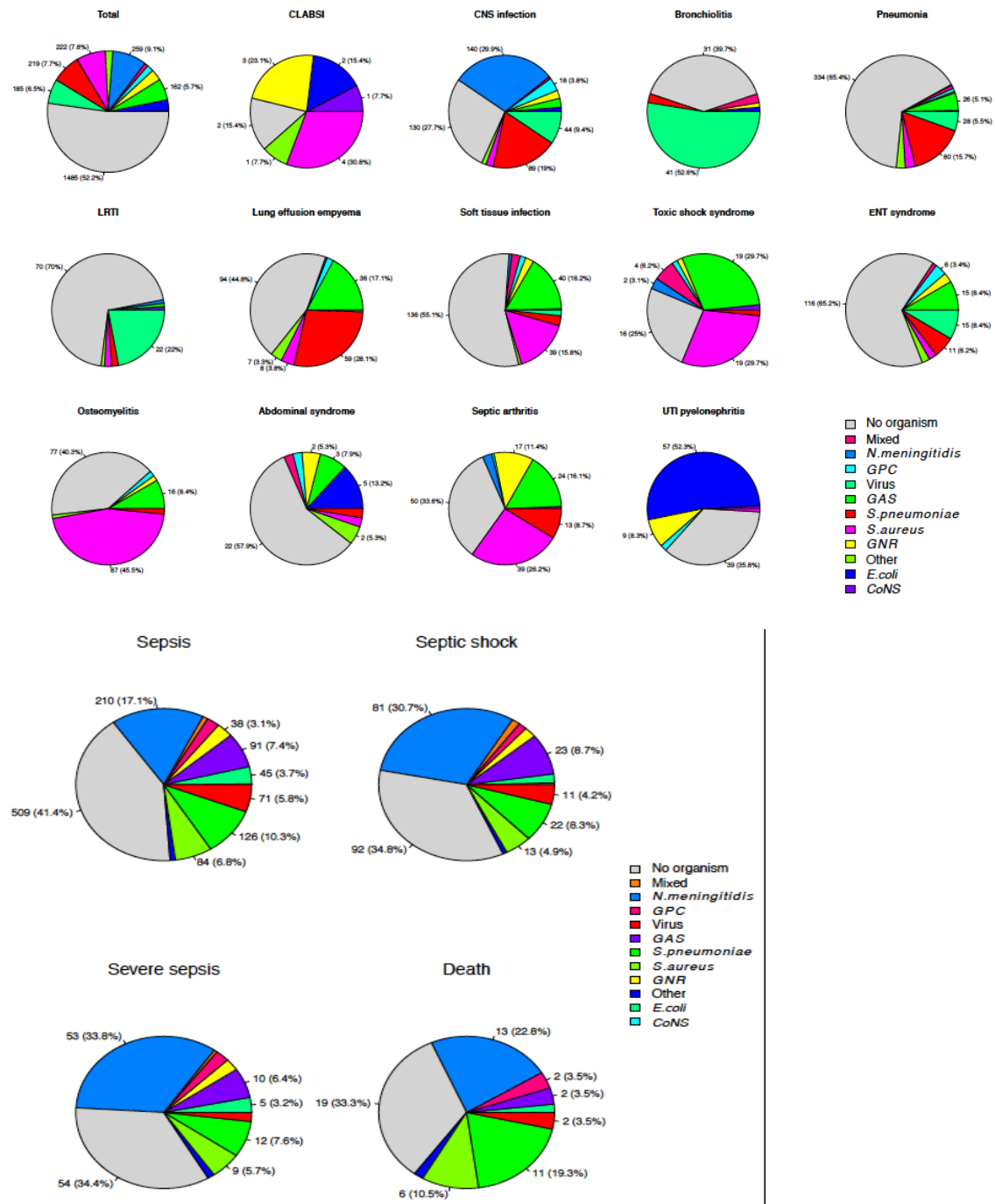Consortium members at Imperial College
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Appendix: Full definitions document

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 osteomylitis is defined by a duration of symptoms < 14 days.

- Subacute osteomyelitis is definedy by a duration of symptoms between 14 days and 1 month.

- Chronic osteomyelits 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 conistent 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 99mTc 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
  - Inacute 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 >24months have elapsed it is considered a community acquired infection.

1.3.3. Diskitis/spondilodiskitis

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 ≥2years
- 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:

  o 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)

  o 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 ≥38,9°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 ≤100×109/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 µmol/L)

ii) Coagulopathy—platelets ≤100×109/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 catether probe) temperature of $> 38.5^{o}$C or $< 36^{o}$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 <10th 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

- $PaO_2/FIO_2 < 300$ in absence of cyanotic heart disease or preexisting lung disease, OR

- $PaCO_2$ >65 torr or 20 mm Hg over baseline $PaCO_2$, OR

- Proven need or >50% FIO2 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/mm3 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

  o Unexplained metabolicacidosis:basedeficit >5.0mEq/L

  o Increasedarteriallactate >2timesupperlimitofnormal

  o Oliguria: urineoutput <0.5mL/kg/hr

  o Prolongedcapillaryrefill:>5secs

  o Coretoperipheraltemperaturegap>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 (>38oC), 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 (>38ºC core) hypothermia (<36ºC 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 $\geq 38.0°C$

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°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°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*.
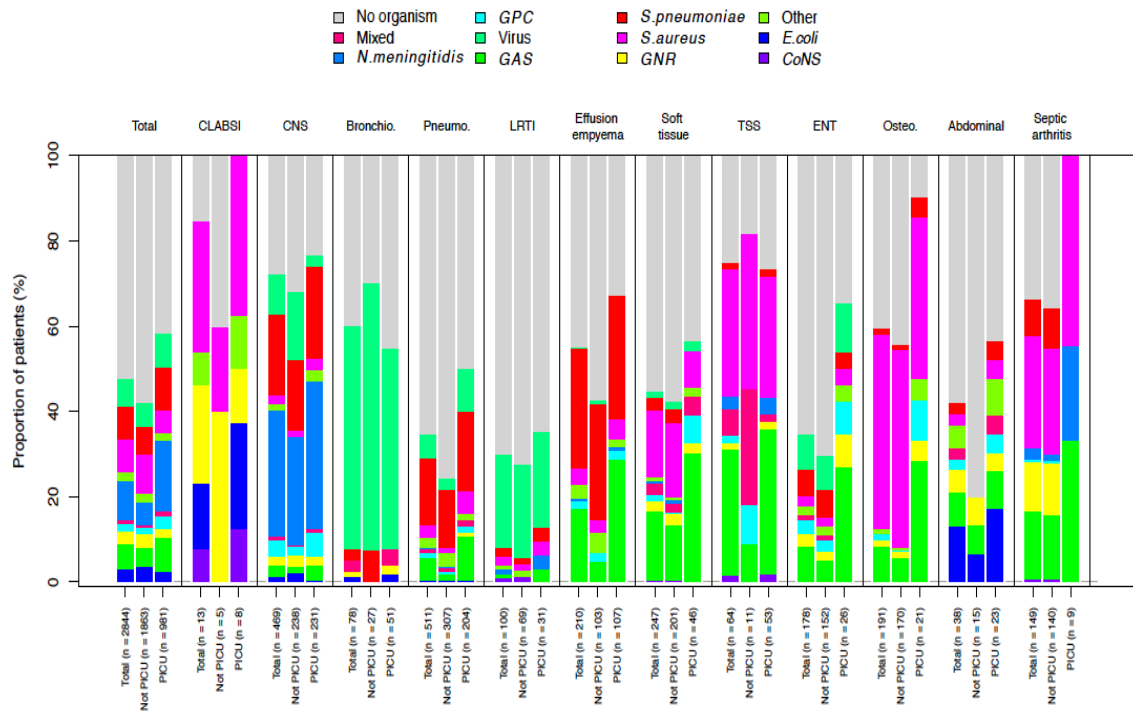
Appendix Figure 2: Serum levels of (A) C-Reactive protein (CRP) and (B) neutrophils counts in different group of patients on admission. Data are expressed as mg/L for CRP and x10^9/L for neutrophils count.
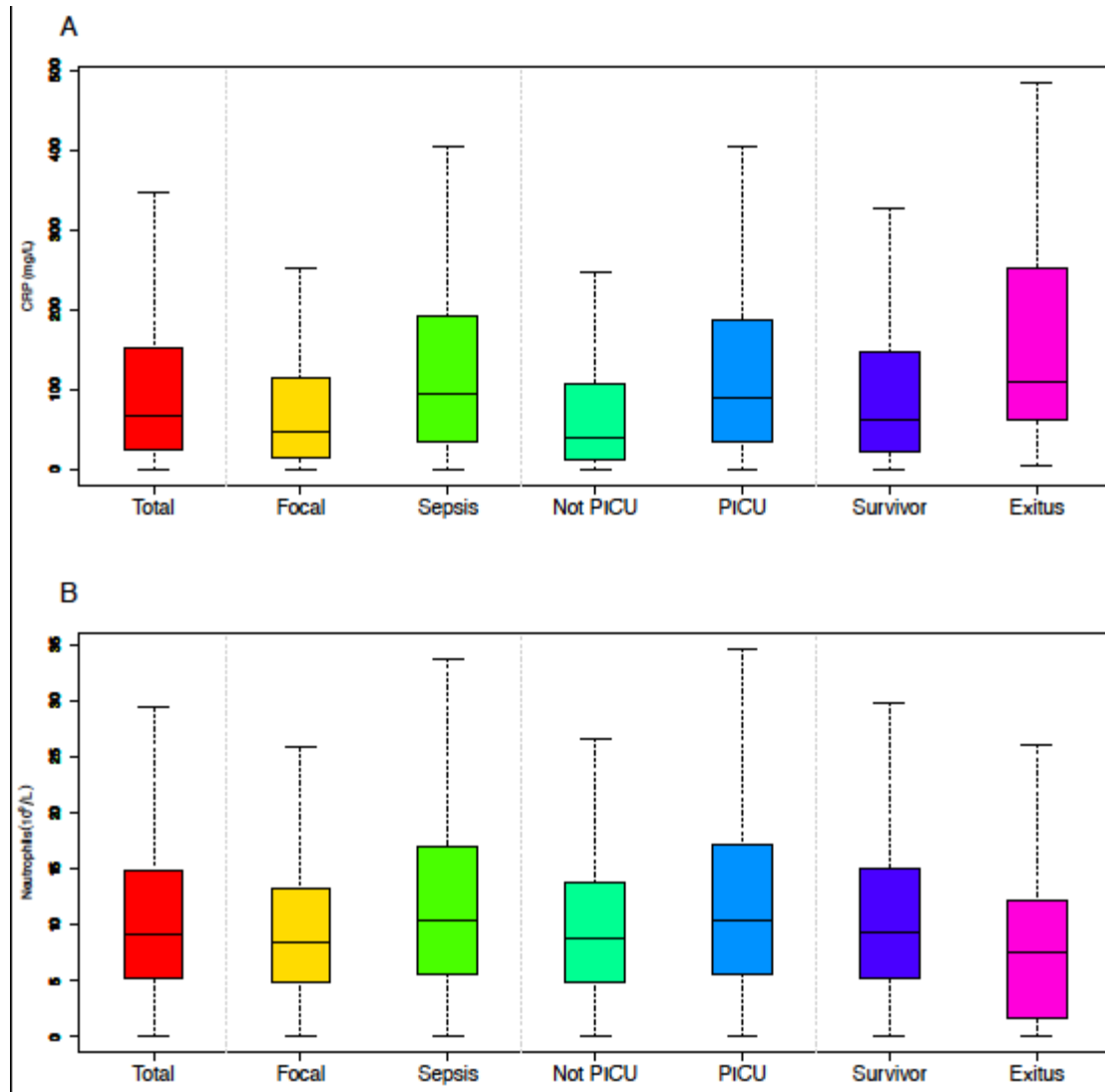
Appendix Figure 3: Receiver operating characteristic curve of CRP, and neutrophil count in different settings.