Improving Emergency Care for Febrile Children Across Europe

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Colofon

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Improving Emergency Care for Febrile Children Across Europe

Het verbeteren van spoedeisende zorg voor kinderen met koorts in Europa

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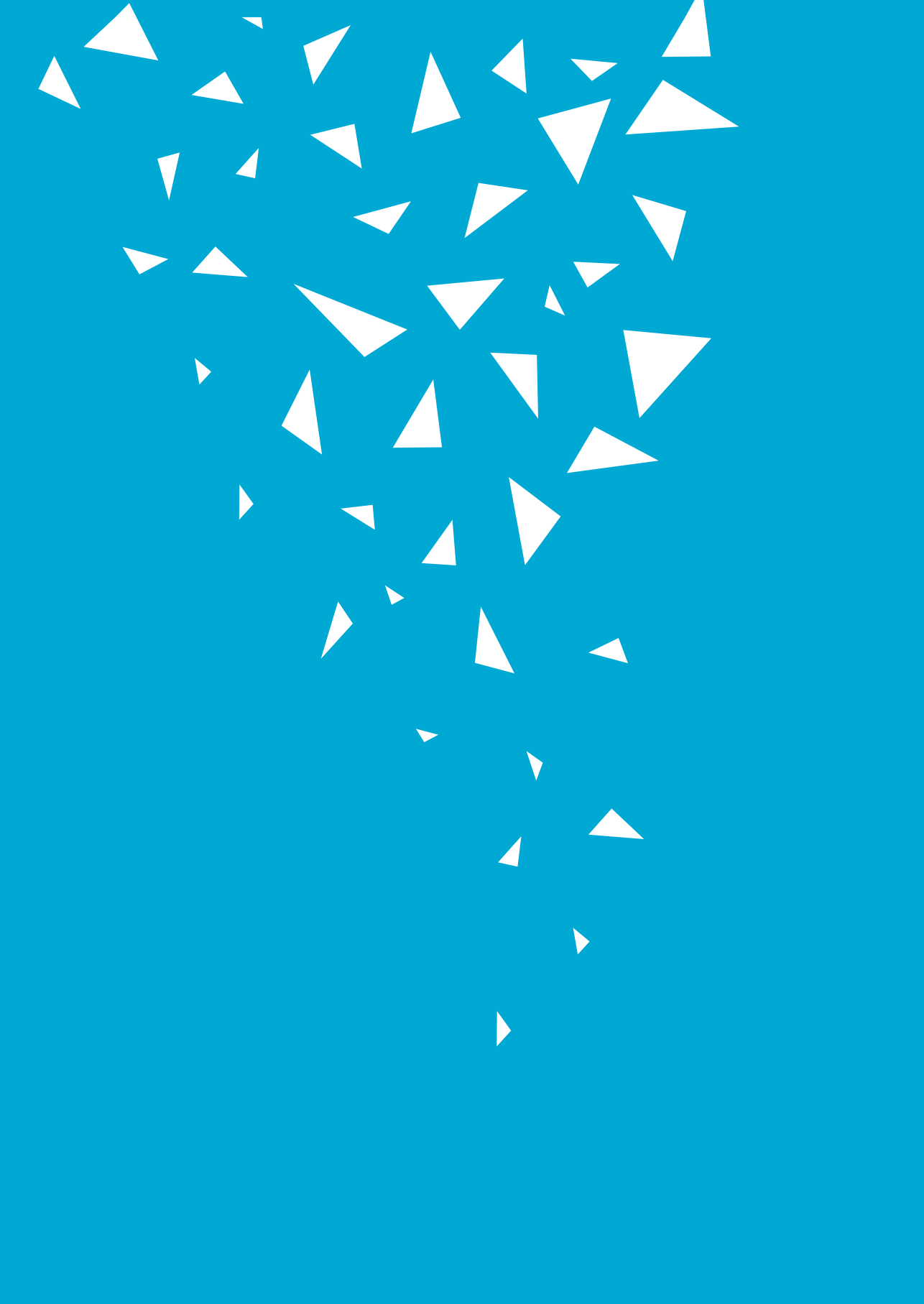
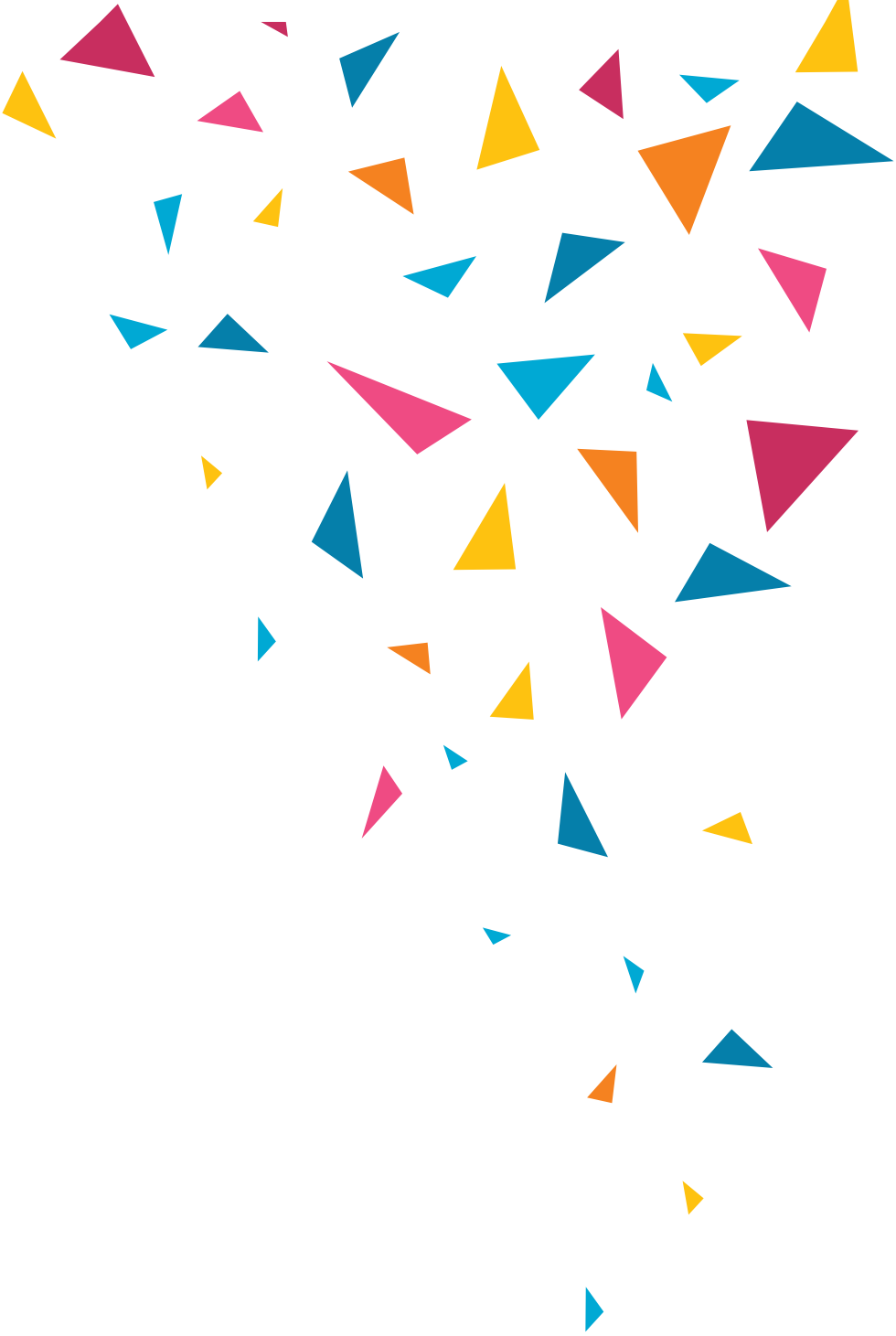


Part I

Introduction



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General Introduction

Spectrum of disease in the febrile child

Fever in children is a frequent reason for healthcare consultation. Healthcare systems for acute care differ between countries in Europe where healthcare can be delivered by general practitioners (GPs), primary care paediatricians, and general or paediatric-focused emergency departments (EDs). Febrile children can be treated by their GPs, but GPs can also decide to refer children to the ED. In addition, when no primary care is available, patients can attend directly the ED.

Viruses or bacteria can cause febrile illness, and the range of disease severity varies widely: some infections have a mild disease course and will recover spontaneously whereas others have severe disease needing treatment with antibiotics and/or other medical interventions like oxygen, ventilation, fluid and specific supportive medication. The most severe infectious diseases could eventually lead to admission to the hospital or the paediatric intensive care unit (PICU), or in rare cases result in death (adapted from Borensztajn *et al.*).[1 2]

The majority of the infections in children are upper and lower respiratory tract infections which are mainly caused by viruses.[3] Incidence of serious bacterial infections, which besides invasive bacterial infections (IBI) (sepsis, bacteraemia, meningitis) also includes pneumonia and bacterial infections of the urinary tract, gastrointestinal tract and soft tissue[4], varies from 7-13% in febrile children attending the ED.[3-5] In primary care, incidence of serious bacterial infections is below 1%, which makes the identification of serious bacterial infections in the early presentation even more difficult.[6,7]

In 2019, overall childhood mortality rate was about 3.4 per 1000 live births in European countries ranging from 0.0 – 11.0 per country. [8] In Figure 1, we observe a decreasing trend in childhood mortality for European countries over the last three decades.[9] Infection-related mortality in childhood has declined due to better prevention by implementation of immunisation programmes, improvement of healthcare systems and supportive care.[9-14] Nevertheless, infectious diseases still account for about 10-20% of childhood mortality in developed countries (Figure 2).[13,15-17] For instance, infection-related morality rate was 3.3/100,000 in the UK in 2013-2015. Half of the deaths occurred in previously healthy children, of which about 60% were caused by bacteria and one third caused by a virus. In addition, a large European study reported mortality in 2% of children with severe community-acquired infections admitted to the hospital.[2] In this cohort, causal microorganisms were only identified in 50% of the children of which the most common pathogens were *Neisseria meningitidis*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and group A streptococcus.

Infections can also cause morbidity in children. Almost one third of children admitted to ICU with severe sepsis had disability at discharge.[18] These adverse outcomes can be largely prevented by early recognition of severe infections and in-time treatment with antibiotics.[19,20] Therefore, it is essential for physicians to early identify serious bacterial infections.

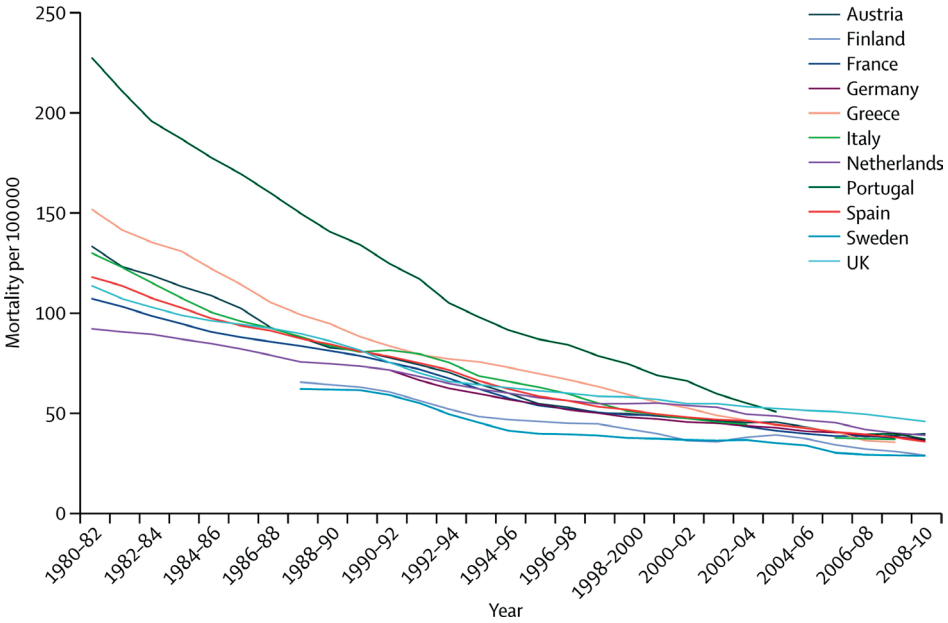

Figure 1 | Trends in mortality in children aged 0-14 years in 11 European Union countries, 1980-2010. [9]
Source: WHO Mortality Database, 2012

Recognition of serious illness in children

Physicians use alarming symptoms and vital signs in their assessment of the febrile child (Figure 3). Several studies and guidelines exist which have evaluated alarming signs and symptoms in predicting the risk of serious illness in children, and in predicting the risk for serious bacterial infection in particular.[5,7,22] Vital signs like temperature, heart rate, respiratory rate and capillary refill time are recommended in the widely used NICE guideline as part of the routine assessment of the febrile child.[22,23] The application of these measurements, however, is limited in European EDs as only 50% of children had full assessment of the four measurements. Heart rate was most commonly measured in 73% of febrile children while blood pressure is not routinely measured.[23]

Unlike in adult care, blood pressure is not part of the routine assessment in febrile children. Previous studies report rates of blood pressure measurements ranging from 8-30%

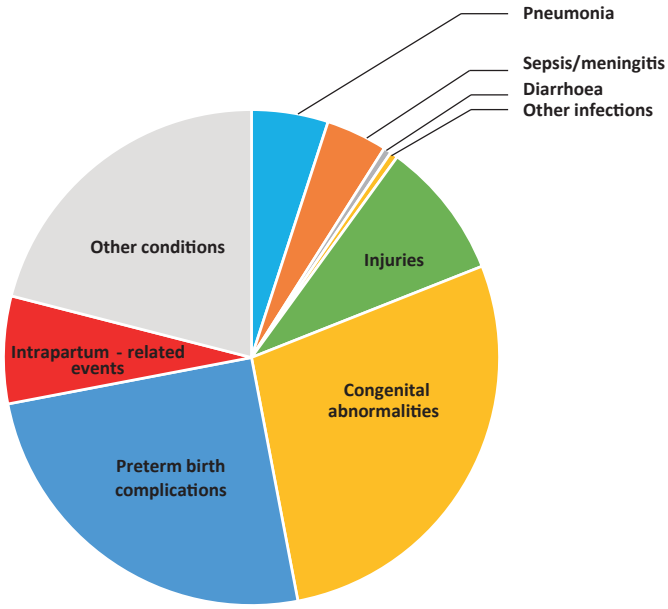

Figure 2 | Cause-specific mortality fractions for <5 years in developed countries [21] - 10% of mortality due to infectious causes

for febrile children at the ED.[24,25] The paediatric assessment of circulation has focused on capillary refill and heart rate as circulatory parameters. Children first increase their heart rate to preserve cardiac output whereas hypotension is considered a late sign of deterioration.[26,27] Furthermore, obtaining blood pressure measurement of good quality is more complex due to the need for age-appropriate blood pressure cuffs and the interference movements of limbs.[28] In addition, the definition of hypotension is unclear since varying clinical cut-offs for hypotension exist. Nonetheless, hypotension is used to diagnose shock and monitor the effect of interventions. In addition, systolic blood pressure is included as variable in Parshuram's bedside early warning score and several scoring systems for sepsis.[29-31] It is unclear whether routine blood pressure measurement at the ED could improve identification of serious illness.

Besides using single predictors as heart rate or blood pressure, identification of serious ill children might improve by combining different vital signs. The Shock Index, defined as the ratio between heart rate and systolic blood pressure, combines properties of both heart rate and blood pressure. In adults, the Shock Index has shown predictive ability for hospitalisation after ED visit and in-hospital mortality.[32] The Shock Index could have additional value at the paediatric ED although research in children has been limited to specific disease groups as septic shock and trauma.

Vital signs
• Temperature • Heart rate • Respiratory rate • Oxygen saturation • Capillary refill time • Systolic blood pressure

Red alarming signs		
•	Colour	Pale, mottled, ashen, blue
•	Activity	- No response to social cues - Ill appearance - Does not wake or if roused does not stay awake - Weak, high-pitched or continuous cry
•	Respiratory	- Grunting - Tachypnea >60/min - Moderate/severe chest indrawing
•	Circulation and hydration	Reduced skin turgor
•	Other	- Age < 3 months & temperature ≥38 C - Non-blanching rash - Bulging fontanelle - Neck stiffness - Focal neurological signs - Status epilepticus - Focal seizures

Figure 3 | Vital sings and red alarming signs for the assessment of the febrile child [22]

Challenges in diagnosing bacterial infection

Vital signs are helpful in identifying children with serious illness, but distinguishing children with bacterial infections from those with self-limiting viral infections is challenging due to similarities of presenting clinical symptoms. In addition, the typical alarming signs for bacterial infection become mostly evident later in the disease course.[20] The large majority of infections in children are respiratory tract infections, which are predominantly caused by viruses and do not need treatment with antibiotics. The incidence of invasive bacterial infections defined as meningitis, sepsis or bacteraemia is very low (range 0.1-0.4%) in febrile children at the ED.[5,24,33] The challenge in early recognition of invasive bacterial infections can resemble finding the needle in a haystack. Physicians prescribe antibiotics in order not to miss one child with invasive bacterial infection, whereas due to the low prevalence of invasive bacterial infections, this leads to overuse of antibiotics, adverse events and eventually to emerging antimicrobial resistance.[3,34-37] Emergency

care of febrile children can be improved by focusing on 1) improved diagnosis by the use of host response biomarkers and 2) improvement of antibiotic prescription.

Host response biomarkers

The response of the host to a pathogen results in activation of inflammatory pathways. C-reactive protein (CRP) and procalcitonin are inflammatory markers which are currently used at the ED as markers for bacterial infections.[38-41] C-reactive protein is much cheaper than procalcitonin [price per test CRP €4; procalcitonin €31][42], and both can be measured at the point-of-care.[43-45] Although these biomarkers are extensively studied, concerns regarding their accuracy exist. First, CRP and procalcitonin are both part of a non-specific inflammation response which limits discrimination between viral and bacterial infection, and other causes of febrile illnesses. Previous studies have shown that elevated levels for CRP or procalcitonin increase the likelihood for bacterial infection.[46] However, high levels also occur in viral infections and in non-infectious causes of diseases.[47] In addition, timing from the disease-onset should be taken into account. CRP reaches its peak after 36-48 hours of illness whilst procalcitonin peaks at 6 hours. Measurement of CRP in the first 24 hours could result in false-negative results.

Since CRP and procalcitonin measure non-specific inflammation, recent studies focus on proteomic and transcriptomic approaches to identify novel biomarkers.[48-51] For example, the PERFORM project (Personalised Risk assessment in Febrile illness to Optimise Real-life Management across the European Union) is a large prospective multicentre cohort which aims to identify and validate new discriminators for bacterial and viral infection in febrile children.[52] Costs and available resources will limit the application of new biomarkers to all febrile children. Therefore, prediction models will be needed to identify risk groups in which biomarkers can improve diagnosis.

Invasive bacterial infections cause a complex host response by activating inflammatory and coagulation pathways. Critically ill children are at risk of adverse outcomes as morbidity and mortality. Host response biomarkers can also have predictive value of adverse outcomes in this population. Secondary infections are an important cause of morbidity and mortality in critically ill patients. Critically ill patients are at risk for prolonged period of immunosuppression, which could lead to an increased risk of acquiring a secondary infection. Monocytic human leukocyte antigen-DR (mHLA-DR) expression is a biomarker which estimates immunosuppression: a prolonged decrease in mHLA-DR expression has been associated with acquisition of secondary infections and mortality in small studies.[53-56] Larger studies on mHLA-DR expression involving infectious critically

ill children with both bacterial and viral infections are lacking. Besides its potential in predicting adverse outcomes, mHLA-DR expression could be able to identify patients who will benefit from immunostimulatory therapies including interferon gamma and granulocyte-macrophage colony– stimulating factor (GM-CSF).[57-61]

Apart from the inflammatory response, the host-response induces pro-coagulant pathways and reduces anti-coagulant pathways which could result in coagulation disorders.[62] Various gene polymorphisms involved in coagulation are related with susceptibility to infections as well as mortality following infection.[63-65] Meningococcal infections are known for purpura, a sign of disseminated intravascular coagulation (DIC), which are associated with morbidity and mortality. In non-meningococcal infections purpura do not commonly occur.[66] Each pathogen might induce a different coagulation host-response. However, the variation of haemostasis proteins across specific pathogen groups and their relation with mortality has not yet been described.

Antibiotic prescription

In Europe, the average antibiotic consumption for systemic use is 19.8 defined daily doses (DDD) per 1000 inhabitants per day of which 91% is consumed in primary care and 9% in hospital care.[67] In the United States, 5% of total antibiotics is prescribed in emergency care.[68] Similar number were reported in the United Kingdom with 7% of antibiotic consumption of hospital out-patients.[69] European data on the amount of antibiotics prescribed in emergency care are not available.

Antimicrobial steward programs aim to optimise antibiotic prescription and strategies targeted at the ED setting have been limited.[70] Paediatric-focused stewardship programmes at the ED are needed.[71] Previous studies have shown that guideline implementation at the ED can improve antibiotic prescription in febrile children.[72,73] In spite of these guideline implementations, studies in the United States and in Europe have shown overprescription of antibiotics at the paediatric ED.[3,74] One large European study showed that more than one third of children with respiratory tract infections received antibiotics and that this varied from 19% to 64% across EDs in Europe.[3] This variation remained after adjustment for patient characteristics, diagnostics and hospital factors. Since respiratory tract infections are mainly caused by viruses, this variation in prescribing suggests overtreatment of antibiotics in children with respiratory tract infection. More insight in antibiotic prescribing patterns in the febrile paediatric ED population including the use of broad-spectrum antibiotics and appropriateness of antibiotic prescriptions could improve compliance to guidelines and antibiotic stewardship programs.

Most hospitals have guidelines in place which inform clinicians regarding type, duration and prescription mode of antibiotics. However, guidance on decisions to start antibiotics are general and lack individual precision. Therefore, clinical decision-making can impact antibiotic prescribing at the individual level by classifying patients at low-risk for bacterial infections. The Feverkidstool is the most extensively evaluated decision rule for paediatric pneumonia and specifies the individual risk for bacterial pneumonia.[4,75,76] The Feverkidstool advices withholding of antibiotic in patients at low/intermediate-risk for bacterial pneumonia. Its impact has been studied in a recent multicenter stepped-wedge cluster randomised trial in EDs in the Netherlands. In children <5 years with suspected lower respiratory tract infections, the Feverkidstool did not result in overall reduction of antibiotic prescribing, but it did achieve less therapy failure amongst high-risk patients. In children at low/intermediate-risk for bacterial pneumonia, the Feverkidstool safely reduced antibiotic prescriptions.[77] The impact of implementation of the FKT in European EDs with different prescription rates and patient populations is unknown.

Aims and outline

This thesis aims to improve diagnostic strategies and antibiotic treatment of febrile children. In particular, this thesis aims to answer the following research questions.

- Can systolic blood pressure improve recognition of serious illness at the Emergency Department?
- Can we develop a clinical prediction model to identify invasive bacterial infections and to identify children who might benefit from new biomarkers to improve diagnosis?
- Can host response biomarkers better predict adverse outcomes of critically ill children with serious infections?
- Can we identify strategies to improve antibiotic prescription in children with fever at European EDs?

In **Part II**, we study the value of routine blood pressure measurement in the ED. In c**hapter 2** we identify evidence-based reference values for low blood pressure and compare these with existing definitions for systolic hypotension in children. In **chapter 3**, we evaluate the predictive value of hypotension in addition to tachycardia and assess the utility of the Shock Index based on the combination of heart rate and systolic blood pressure for serious illness in children. In **chapter 4**, we derive reference values for the Shock Index, and determine the diagnostic value of the Shock Index for serious illness in febrile children.

In **Part III**, we study host response biomarkers. First, in **chapter 5**, we develop and validate a clinical prediction model including CRP to identify invasive bacterial infections and identify patient groups who might benefit from new biomarkers. Next, we evaluate the prognostic value of mHLA-DR expression as an immunologic marker, and the prognostic value of haemostasis proteins. In **chapter 6,** we assess the longitudinal association between mHLA-DR expression and the acquisition of secondary infections in infectious critically ill children. In **chapter 7,** we describe the variation of haemostasis proteins in hospitalised children with infections caused by different pathogens and describe haemostasis protein in relation to disease severity and mortality.

In **Part IV**, we evaluate current practices of antibiotic prescription in febrile children in EDs in Europe. In **chapter 8**, we investigate the variation and appropriateness of antibiotic prescription in febrile children visiting 12 EDs in 8 European countries. In **chapter 9**, we simulate the potential impact of a decision rule based on clinical signs and CRP (the Feverkidstool) on antibiotic prescription in febrile children with suspected lower respiratory tract infections in different European EDs.

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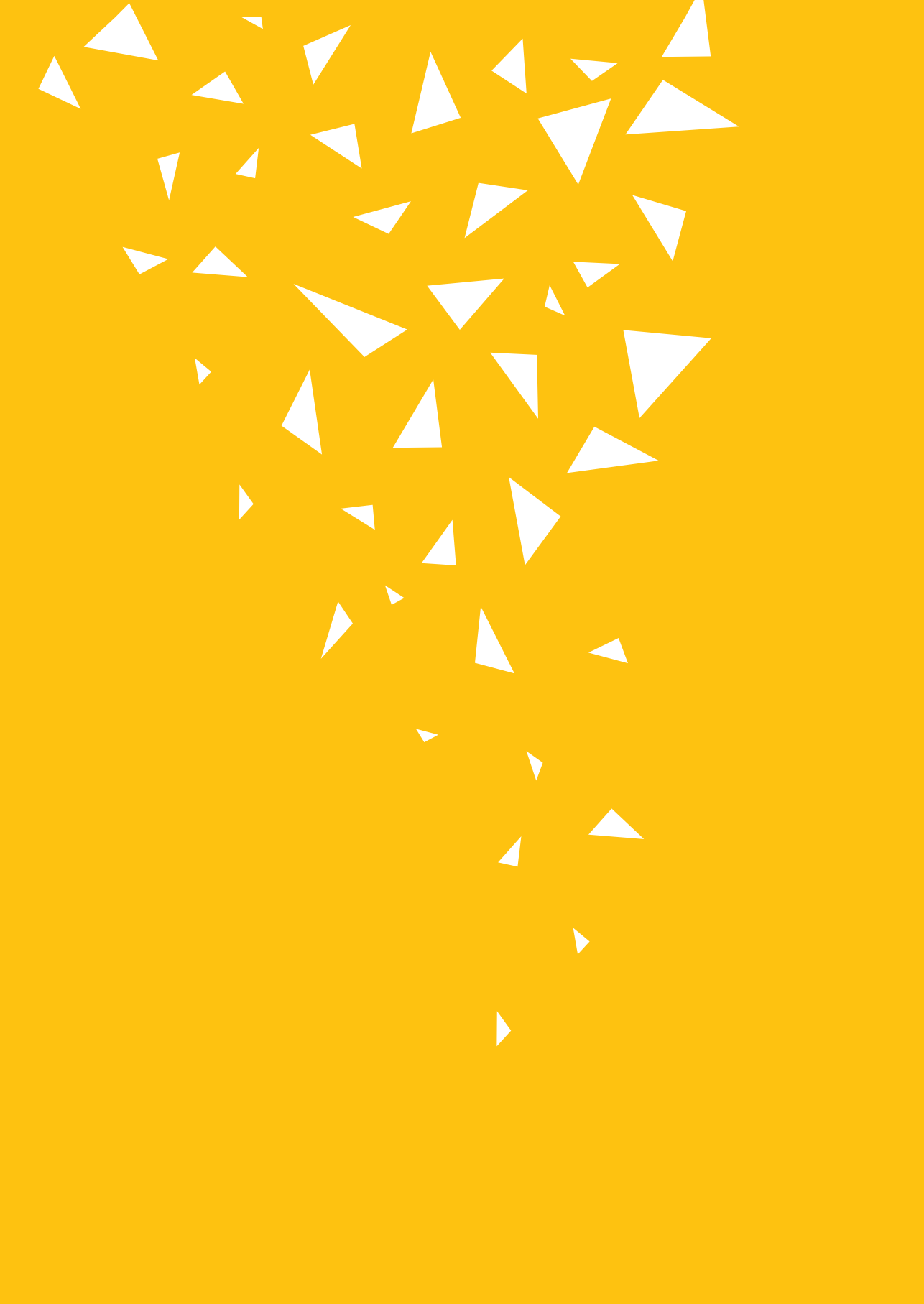
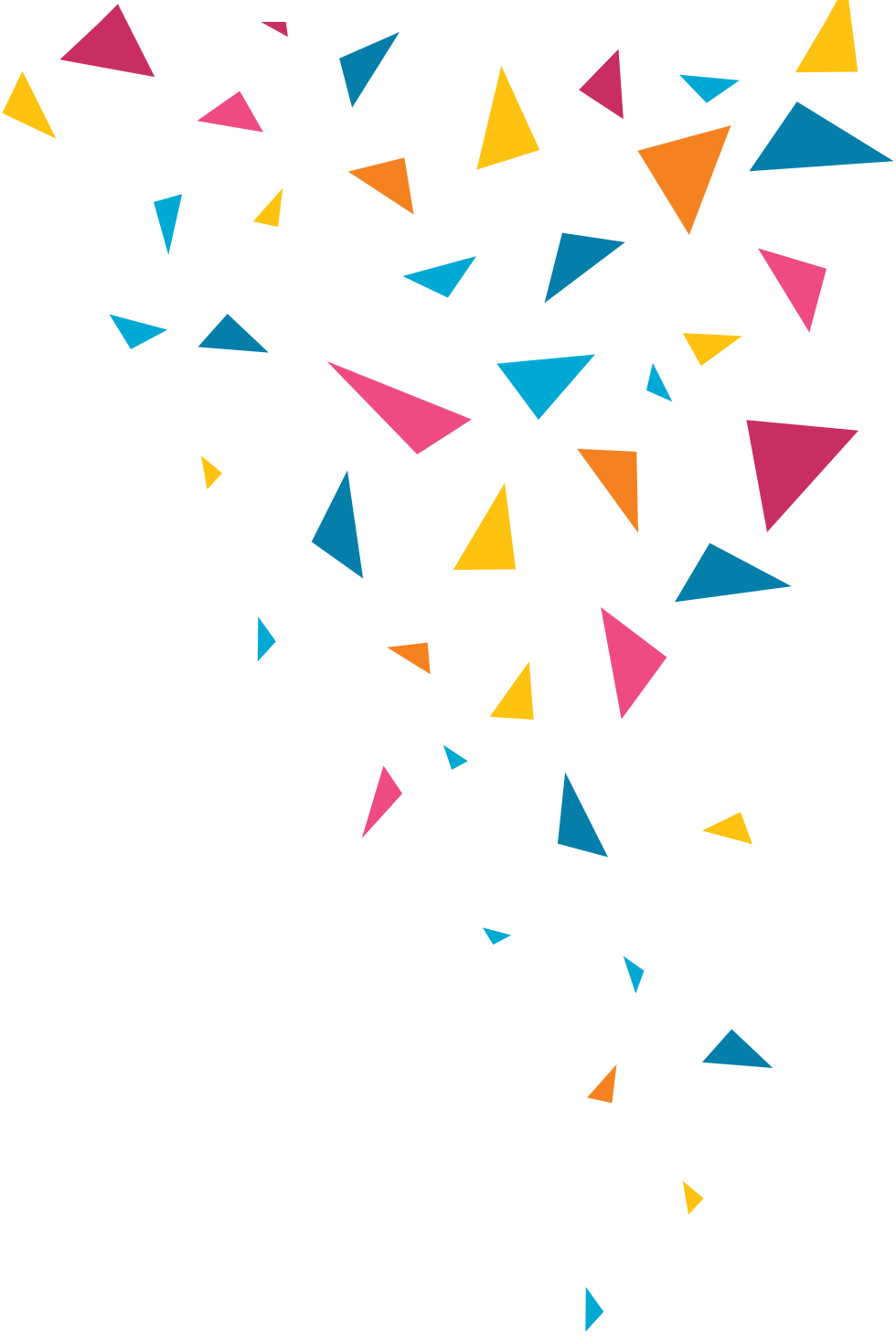


Part II

Vital signs for early recognition of serious illness



2

A comparison of clinical paediatric guidelines for hypotension with population-based lower centiles: a systematic review

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Abstract

Background Different definitions exist for hypotension in children. In this study, we aim to identify evidence-based reference values for low blood pressure and to compare these with existing definitions for systolic hypotension.

Methods We searched online databases until February 2019 (including MEDLINE, EMBASE, Web-of-science) using a comprehensive search strategy to identify studies that defined age-related centiles (first-fifth centile) for non-invasive systolic blood pressure in healthy children < 18 years. Existing cut-offs for hypotension were identified in international guidelines and textbooks. The age-related centiles and clinical cut-offs were compared and visualized using step charts.

Results Fourteen studies with population-based centiles were selected, of which 2 addressed children < 1 year. Values for the fifth centile differed 8 to 17 mmHg for age. We identified 13 clinical cut-offs of which only 5 reported accurate references. Age-related cut-offs for hypotension showed large variability (ranging from 15 to 30 mmHg). The clinical cut-offs varied in agreement with the low centiles. The definition from Paediatric Advanced Life Support agreed well for children < 12 years but was below the fifth centiles for children > 12 years. For children > 12 years, the definition of Parshuram's early warning score agreed well, but the Advanced Paediatric Life Support definition was above the fifth centiles.

Conclusions The different clinical guidelines for low blood pressure show large variability and low to moderate agreement with population-based lower centiles. For children < 12 years, the Paediatric Advanced Life Support definition fits best but it underestimates hypotension in older children. For children > 12 years, the Advanced Paediatric Life Support overestimates hypotension but Parshuram's cut-off for hypotension in the early warning score agrees well. Future studies should focus on developing reference values for hypotension for acutely ill children.

Introduction

Vital signs are important in the recognition of acutely ill children. One parameter associated with serious illness is hypotension.[1-3] Because normal blood pressure values vary with age, accurate age-related reference values are needed to correctly identify hypotension in children and guide interventions.

Blood pressure can be measured by invasive, oscillometric and auscultatory methods. In addition, various outcome measures for blood pressure exist as mean arterial pressure, and diastolic and systolic blood pressure. Paediatric guidelines propose different definitions of hypotension, and in general use cut-off values of systolic blood pressure.[4-6] Although not based on evidence, several guidelines use the fifth percentile of systolic blood pressure in healthy children as cut-off for hypotension.[4,7,8] Moreover, it is unclear how well these guidelines discriminate between normal and low blood pressure. To date, no study has summarized the available evidence on reference values of low systolic blood pressure in children.

This study aims to identify population-based reference values for non-invasive low blood pressure in healthy children and to compare these with cut-offs for hypotension defined by existing paediatric guidelines.

Methods

Search strategy and selection of population-based studies

We systematically searched databases including MEDLINE, EMBASE, and other databases (1950 to 14 February 2019) to identify primary studies that defined lower centiles for non-invasive systolic blood pressure measurement in healthy children [Additional file 1: detailed search strategy]. Studies that were included were published in English, recorded blood pressure and defined age-related centiles for systolic blood pressure (first to fifth centile) on a minimum of 100 children aged < 18 years. Studies were excluded if populations involved children with underlying diseases, or studies reporting on premature neonates, measurements during anaesthesia, exercise or orthostasis. We excluded populations from low- and middle-income countries since factors influencing blood pressure levels such as body composition and nutrition, are different compared to high-income countries.[9] We excluded abstracts, reviews and commentaries, and studies reporting on lower centiles solely derived from mathematical analysis. One researcher (NH) conducted the first selection and two researchers (NH, JZ) independently conducted the second and third selection. Disagreements were discussed and agreed upon consensus or discussed with 3rd researcher (HM) for majority decision.

Data extraction and analysis

For the selected studies, data were extracted by one researcher (NH) and included country, population, setting, sample size, age range, blood pressure measurement method and age-specific centiles (P1-P5). We included the centiles for non-overweight children and for the median height if blood pressure centile values were reported for different height categories. The age-specific fifth centiles were summarized using weighted medians and interquartile ranges for age categories which involved three or more studies. If sample sizes were only given for age ranges > 1 year, we estimated the sample size per age group by dividing the total sample size by the number of years.

Quality assessment

No specific tool exists for quality assessment of observational studies.[10] The Quality Assessment of Diagnostic Accuracy Studies-2 checklist was the most appropriate to use for these observational studies.[11] This checklist covers risk of bias and applicability judgments on four domains: patient selection, index test, reference standard and flow and timing. For each question studies were classified as high, low or unclear. Disagreements were agreed upon consensus.

Cut-off values for hypotension from clinical guidelines

We selected a sample of clinical cut-offs for hypotension by consulting experts, well-known textbooks and resuscitation, emergency care and sepsis guidelines. Clinical cut-offs included recommended target values for hypotension defined by systolic blood pressure. For each clinical cut-off, we determined the presence of a literature reference and whether this reference agreed with the cut-off values. To compare clinical cut-offs with the population-based centiles identified in the literature, we plotted the age-specific fifth centile values in a step chart separate for boys and girls. Data analyses were performed in SPSS version 25.0 and R version 3.4.

Results

Population-based studies

Our systematic search identified 7625 studies. After the study selection process, we included 14 studies in the final selection that defined lower centiles for non-invasive systolic blood pressure measurement in healthy children (Fig. 1). The median samples size was 5362 (IQR 1760-11,940). Seven out of 14 studies used an automatic oscillometric device for blood pressure measurement. Two studies included children aged < 1 year (Table 1). Studies included populations from Europe (n = 8), North America (n = 3), Australia (n =2) and Asia (n = 1). Four studies excluded overweight patients. For

development of the centiles, 11 studies used the average of multiple blood pressure measurements and 3 studies used only the first measurement. Blood pressure centiles were stratified by gender (n = 12), height (n = 4), ethnicity (n = 1) and overweight vs non-overweight (n = 2). Studies most frequently reported the fifth centile (n = 13), in which the third centile (n = 2) and first centile (n = 3) were also reported separately. One study only reported the first and third centiles. The fifth centiles of the population-based studies showed variation ranging across the age groups from 7 and 17 mmHg for boys (figure 2) and 7 to 22 mm Hg for girls [Additional file 2]. Median values and interquartile ranges of the lower 5th centiles are provided in Additional file 3 and 4.

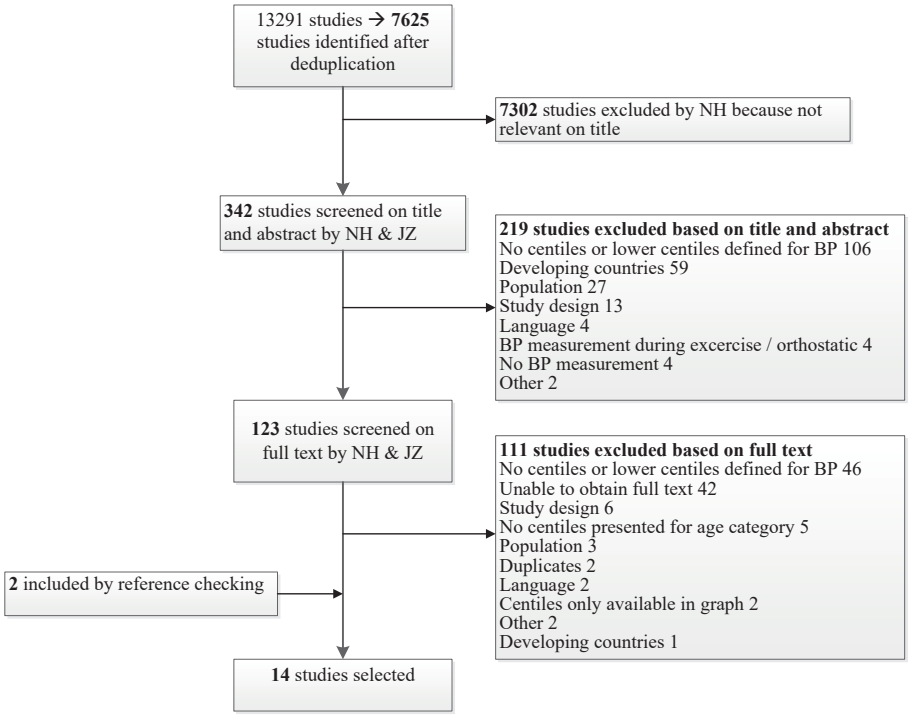

Figure 1 | Study selection process
Legend: BP, blood pressure

Quality of the population studies was generally good. No concerns regarding applicability were found in 12 out of 14 studies. Six studies had high risk of bias in the patient flow and timing domain, due to poor reporting of how missing data were handled (Table 2, Fig. 3).

Table 1 | Characteristics of included studies

Author	Country	Inclusion	Exclusion	Age range (years)	Setting	Sample size	Method of measurement	Defined BP centiles	Determinants of age-specified centiles	Measurement used for analysis	Main outcome
Antal *et al.* (2004) [34]	Hungary	Secondary school	Using antihypertensive medication	15-18	Community setting	6345	Oscill.	P3, P5	Sex	first measurement	Assessment of age- and gender-specific anthropometric parameters and blood pressure values
Barba *et al.* (2014) [35]	8 EU country	Non-overweight children	Overweight	2-10.9	Unspecified	13,547	Oscill.	P1, P3	Sex, height	Mean of first and second measurement	Provide oscillometric blood pressure reference values
Blake *et al.* (2000) [36]	Australia	Cohort from a tertiary perinatal centre. Follow-up at age 1, 3 and 6 years	x	1-6	Unspecified	2876	Oscill.	P5	Sex	Mean of two measurements	To develop age- and gender-specific reference ranges for BP
Grajda et al. (2017) [37]	Poland	Healthy pre-school children	Congenital, chronic or acute disorders and medication affecting growth or BP levels.	3-6	Community setting	4378	Oscill.	P1, P5	Sex, height	Mean of second and third measurement	To develop age- and gender-specific ranges for BP in pre-school children
Hediger *et al.* (1984) [38]	USA	Black adolescents	x	11-17	Unspecified	621	Auscul.	P5	Sex	Mean of two measurements	Percentiles for black adolescents for resting BP and 60-second pulse rate

Table 1 | Characteristics of included studies (continued)

Author	Country	Inclusion	Exclusion	Age range (years)	Setting	Sample size	Method of measurement	Defined BP centiles	Determinants of age-specified centiles	Measurement used for analysis	Main outcome
Kent *et al.* (2007) [25]	Australia	Term infants	Congenital anomalies, birth weight <3rd percentile, sepsis, NICU admission. Maternal hypertension, diabetes, use of illicit substances.	0 -1	Hospital: postnatal clinical, other in a non-clinical room	406	Oscill.	P5	X	Mean of three measurements	Normative BP during first year of life of healthy infants
Karmar *et al.* (2014) [39]	Sweden	Children, junior school	Physical health problems, medication that affects BP	6-16	Community setting	1470	Oscill.	P5	Sex	Mean of second and third measurement	Cross-sectional normative casual BP standards
Krzyzaniak *et al.* (2009) [40]	Poland	School children	x	7-18	Community setting	6447	Auscul.	P5	Sex, height	Mean of two measurements on three different days	To develop age- and gender-specific reference ranges
Lurbe *et al.* (1994) [41]	Spain	Normotensive children	Systemic and renal disease	6-16	Primary care	248	Oscill.	P5	Sex, casual and ambulatory BP	Mean of three measurements and means of daytime measurements	Assess reference values of ambulatory blood pressure
Rosner *et al.*(2008) [42]	USA	11 large pediatric blood pressure studies (based on Pediatric Task Force database)[22]	Overweight	1-17	Unspecified	36,914	Auscul.	P1,P5	Sex, height	first measurement	Norms for childhood BP among normal-weight children

Table 1 | Characteristics of included studies (continued)

Author	Country	Inclusion	Exclusion	Age range (years)	Setting	Sample size	Method of measurement	Defined BP centiles	Determinants of age-specified centiles	Measurement used for analysis	Main outcome
Sarganas (2018) [23]	Germany	Healthy children and adolescents	Chronic conditions or medication influencing growth or BP. Overweight (BMI>90th centile).	3-17	Community setting	14,836	Oscill.	P1, P5	Sex, height	Mean of two measurements	Fifth percentile of BP according to age, sex and height.
Satoh et al. (2016) [24]	Japan	Full-term singleton newborns	Twin newborns, miscellaneous abnormalities, missing Apgar score, condition during BP measurement	0	Hospital	2628	Oscill.	P5	Sex	first measurement	Estimate BP and pulse rate in healthy newborns
Schwandt et al. (2015) [43]	Germany	German parents	Metabolic, cardiovascular, endocrine, malignant disorder, specific medication, non-German ethnicity.	3-18	Community setting	22,051	Auscul.	P3, P5	Sex, overweight and non-overweight	Mean of two measurements	Develop auscultatory BP growth charts
Weiss *et al.* (1973) [44]	USA	Non-institutionalized children	x	6-11	Hospital: one visit	7119	Auscul.	P5	Sex, race	Mean of two measurements	Distribution of BP level 6-11 years

Auscul., auscultatory; BP, blood pressure; EU, European Union; NICU, Neonatal intensive care unit; Oscill, oscillometric; P1, First centile; P3, Third centile; P5, fifth centile; USA, United states of America

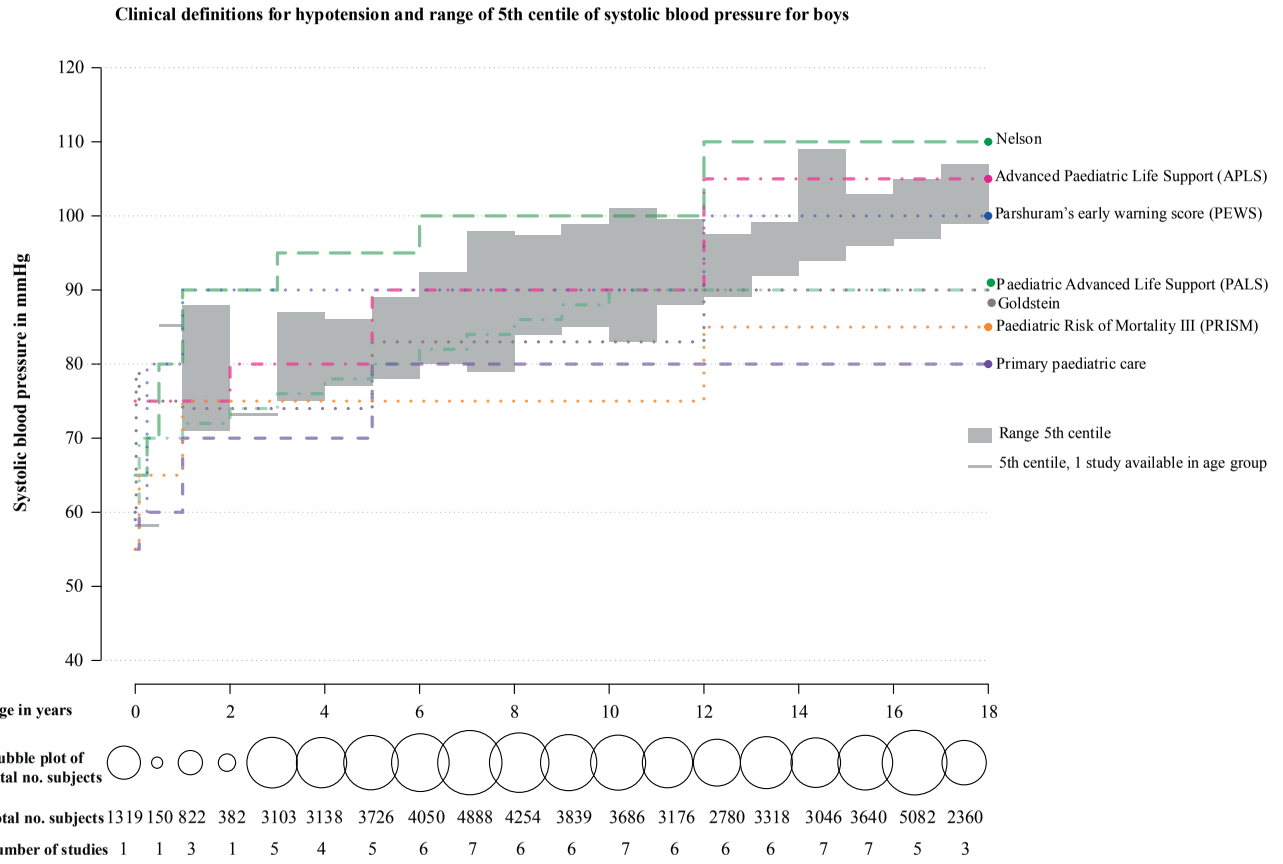

Figure 2 | Clinical definitions for hypotension and range of 5th centile of systolic blood pressure for boys

Cut-off values for hypotension from clinical guidelines

We identified 13 clinical cut-offs for hypotension of which 8 referred to a literature reference [Additional file 5]. Five cut-offs provided an accurate literature reference [7,12-15], of which four out of five referred to the fifth centile of healthy children. In two textbooks the values of the literature reference did not agree with the provided cut-offs.[16,17] One literature reference could not be obtained.[18] Age-specific cut-off values for hypotension showed large differences, ranging from 15 to30 mmHg (Fig. 2, Additional file 5).

Comparison of population-based studies with cut-off values for hypotension from clinical guidelines

The clinical hypotension cut-offs showed poor to moderate agreement with the lower centiles derived from population-based studies (Fig. 2). The frequently used hypotension cut-off from Advanced Paediatric Life Support (APLS)[6] showed moderate agreement for children < 12 years, but was above the highest fifth centile values for children > 12 years. The cut-off from Paediatric Advanced Life Support (PALS) agreed well for children < 12 years but was below the fifth centile values for children > 12 years. The cut-off of Parshuram's early warning score (PEWS) agreed well for children > 12 years.[19] Three other cut-offs were mostly below the fifth centiles (Goldstein, Primary paediatric care and Paediatric Risk of Mortality III (PRISM III))[15,16,20] and one cut-off had higher values (Nelson).[21]

Table 2 | Quality Assessment of the studies

Quality Assessment of the studies							
	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Antal[34]	Low	Low	n/a	Unclear	Low	Low	n/a
Barba[35]	Low	Low	n/a	High	Low	Low	n/a
Blake[36]	Low	Low	n/a	High	Low	Low	n/a
Grajda[37]	Low	Low	n/a	Low	Low	Low	n/a
Hediger[38]	Low	Low	n/a	High	Low	Low	n/a
Kent[25]	Low	Low	n/a	High	Low	Low	n/a
Karmar[39]	Low	Low	n/a	High	Low	Low	n/a
Krzyzaniak[40]	Unclear	Low	n/a	Low	Low	Low	n/a
Lurbe[41]	High	Low	Low	Low	Low	Low	n/a
Rosner[42]	Unclear	High	n/a	Low	Unclear	Low	n/a
Sarganas[23]	Low	Low	n/a	Low	Low	Low	n/a
Satoh[24]	Unclear	Low	n/a	High	Unclear	Low	n/a
Schwandt[43]	Low	Low	n/a	Low	Low	Low	n/a
Weiss[44]	Low	Low	n/a	Low	Low	Low	n/a

n/a: not applicable

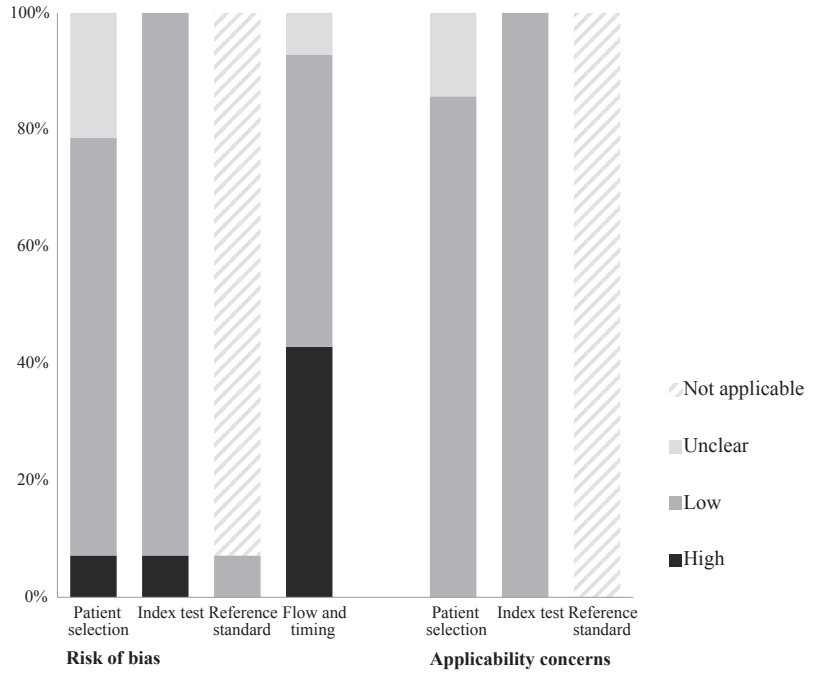

Figure 3 | Quality assessments of the studies

Discussion

This systematic review demonstrates large variation among commonly used paediatric reference values for systolic hypotension. In general, the clinical guidelines are not based on available evidence and showed variable agreement with existing population-based blood pressure centiles. The reviewed literature addressing population-based centiles showed limited studies in children < 1 year of age.

Reference ranges of blood pressure are influenced by multiple factors such as age, gender, height, ethnicity and method of measurement.[22] In the literature, low centiles for blood pressure are often presented for different ages and in some cases for height. To facilitate interpretation, guidelines provide simplified cut-off values for hypotension for various age groups. For early recognition of acutely ill children, these simplified reference values are essential for clinicians.

The evidence for clinically used cut-offs for hypotension is mostly unclear as only five clinical cut-offs for hypotension reported accurate literature references. Our systematic search shows availability of population-based centiles that could provide evidence for lower reference values of blood pressure. Although not evidence based, we propose that

clinical cut-offs for hypotension should not exceed the fifth centile. Clinical cut-offs that are generally below the fifth centile may possibly be too low, while clinical cut-offs that are generally above the fifth centile may be too high. These high clinical cut-offs may classify too many patients incorrectly as hypotensive since by definition five percent of healthy children will fall below this centile. In children < 12 years the values of PALS have good agreement with the low centiles, but for children age > 12 years the PALS could possibly be too low.

Our results are in line with a previous study that compared three clinical cut-offs with the fifth centile, based on a mathematical analysis of a large sample of healthy children.[4] They reported that the fifth centile for systolic blood pressure was generally below three clinical cut-offs for hypotension. Sarganas et al. found that low centiles from a German and US population were higher than the PALS definition in children > 13 years.[23] In contrast to the previous studies, our study conducted an exhaustive systematic search for population-based centiles in all ages and compared them with a large sample of cut-offs for hypotension that are widely used in clinical practice. Our study identified only two studies that provided blood pressure centiles in children < 1 year including one study in new-borns and one at age of 6 months. [24,25] Therefore, more studies providing reference values of blood pressure in children < 1 year are required.

Reference values based on healthy children may not be accurate for acutely ill children, as pain and distress could increase blood pressure values. In addition, cuff size, movement of limbs, crying and uncooperativeness influence the measured values. In the interpretation of the measured values, these factors should be accounted for.

There is no consensus which definition of hypotension should be used for assessment of acutely ill children. Hypotension defined by APLS, PALS and PEWS, showed an association with serious illness, adjusted for tachycardia. These definitions, however, lacked sensitivity for serious illness.[3] In our systematic review, the PALS cut-off showed the best agreement with the values based on healthy children with an average of 4 mm Hg difference from the weighted median of the population-based fifth centiles. In addition, current guidelines do not agree on treatment targets for blood pressure after identification of hypotension in critically ill children. The goal for treatment target of blood pressure is to maintain adequate tissue perfusion. The guideline of International Liaison Committee on Resuscitation recommends targeting systolic blood pressure values higher than the fifth percentile for children who are post-cardiac arrest, [26] whilst the APLS and the surviving sepsis campaign[1] advise to maintain normal blood pressure for age without defining specific measures. The American College of Critical Care medicine recommends to use the 50th centile of the mean arterial pressure (MAP) and to use perfusion pressure

(MAP- central venous pressure) to guide treatment.[12] Some evidence is available suggesting higher MAP levels are needed to improve outcome in traumatic brain injury and central nervous system infections in children.[2,27] Trials in adult critically ill patients with septic shock showed that targeting higher mean arterial pressure levels of 75-85 mm Hg did not influence mortality or other adverse events.[28,29] Future trials will need to evaluate different blood pressure measures and targets in acutely ill children and relate those to interventions and relevant clinical outcomes.

Our review focused on systolic blood pressure and did not include mean arterial blood pressure or diastolic blood pressure. Although the mean arterial pressure is often used in critical care, we focused on systolic hypotension for general illness, since in general, clinical guidelines only report hypotension definitions of systolic blood pressure.

Strengths and limitations

Major strengths of this study are the use of an extensive search strategy, the overview of low reference values of blood pressure in healthy children covering all ages and the comparison with a diverse sample of clinical cut-offs of hypotension that are widely used in practice. Although we used a sensitive search strategy in multiple databases, it is possible we have not included all available data. Since we focused on lower age-related centiles, we excluded studies that reported blood pressure centiles solely for height or body mass index.

This study has some limitations. First, the selected sample of clinical definitions was not exhaustive and various blood pressure cut-offs in early warning scores and mortality scores were not included. We selected Parshuram's early warning score and the PRISM III mortality score as these have been validated and are commonly used in practice. We acknowledge that these cut-offs are part of a score containing other clinical markers. In addition, the PRISM III score has been developed specifically for predicting mortality in critically ill children.

Second, blood pressure is determined by height and we only included blood pressure values for the median height value. However, height is usually not available in the assessment of acutely ill children and none of the clinical guidelines accounted for height. Third, we focused on non-invasive measurement methods including oscillometric and auscultatory measurements. Oscillometric measured values could be different than auscultatory measurements.[30] As different devices were used in the studies and their validity in assessment of low blood pressure is unknown, we combined centiles for oscillometric and auscultatory measurements. Fourth, since non-invasive blood pressure measurements could overestimate hypotension when compared to invasive

arterial measurement, generalization of our study to invasive measurements should be undertaken with caution. [31-33]

Conclusion

Large variation exists among paediatric cut-offs for hypotension. In general, these clinical definitions are not evidence-based and have variable agreement with existing population-based blood pressure lower centiles.

For children < 12 years, the PALS definition agreed well. For children > 12 years, the PEWS agreed well but the PALS cut-off possibly underestimates and the APLS overestimates hypotension. Future studies should focus on developing reference values for hypotension for acutely ill children.

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Additional files

Available as online web appendix on the Critical Care website:

- Additional file 5 Clinical cut-offs for hypotension

Additional file 1 – Systematic search strategy

Table 1 Systematic search strategy

Database	Number of references	After deduplication
Embase.com	5225	5089
Medline (ovid)	4854	1688
Web-of-science	2725	585
Cochrane	141	23
Cinahl (ebsco)	21	12
Lilacs	71	57
Scielo	25	3
Proquest	29	24
Google scholar	200	144
Total	**13291**	**7625**

Embase.com

(('blood pressure'/de OR 'blood pressure measurement'/exp OR 'blood pressure monitoring'/exp OR 'blood pressure variability'/exp) AND ('statistical analysis'/de OR 'statistical distribution'/exp OR statistics/exp)) OR (normotension* OR ((norm* OR healthy OR population OR nomogram* OR curve* OR centile* OR survey* OR distribut* OR statistic* OR trend* OR differen* OR varia* OR 'z score' OR reference* OR standard*) NEAR/9 ('blood pressure' OR 'blood pressures' OR bp))):ab,ti) AND (child/exp OR newborn/exp OR adolescent/exp OR adolescence/exp OR (adolescen* OR infan* OR newborn* OR (new NEXT/1 born*) OR child* OR pediatric* OR paediatric*):ab,ti) AND ('cohort analysis'/exp OR 'population research'/exp OR 'population group'/de OR 'cross-sectional study'/exp OR 'longitudinal study'/exp OR population/de OR (cohort* OR population* OR (cross NEXT/1 section*) OR longitudinal*):ab,ti)

Medline (ovid)

(((exp "blood pressure"/ OR exp "Blood Pressure Determination"/) AND ("Statistics as Topic"/ OR exp "Statistical Distributions"/ OR statistics/)) OR (normotension* OR ((norm* OR healthy OR population OR nomogram* OR curve* OR centile* OR survey* OR distribut* OR statistic* OR trend* OR differen* OR varia* OR "z score" OR reference* OR standard*) ADJ9 ("blood pressure" OR "blood pressures" OR bp))).ab,ti.) AND (exp child/ OR exp infant/ OR adolescent/ OR exp pediatrics/ OR (adolescen* OR infan* OR newborn* OR (new ADJ born*) OR child* OR pediatric* OR paediatric*).ab,ti.) AND ("Cohort Studies"/ OR "Population Groups"/ OR "Cross-Sectional Studies"/ OR "Longitudinal Studies"/ OR population/ OR (cohort* OR population* OR (cross ADJ section*) OR longitudinal*).ab,ti.)

Web-of-science

TS=(((normotension* OR ((norm* OR healthy OR population OR nomogram* OR curve* OR centile* OR survey* OR distribut* OR statistic* OR trend* OR differen* OR varia* OR "z score" OR reference* OR standard*) NEAR/9 ("blood pressure" OR "blood pressures" OR bp)))) AND ((adolescen* OR infan* OR newborn* OR (new NEAR/1 born*) OR child* OR pediatric* OR paediatric*)) AND ((cohort* OR population* OR (cross NEAR/1 section*) OR longitudinal*)))

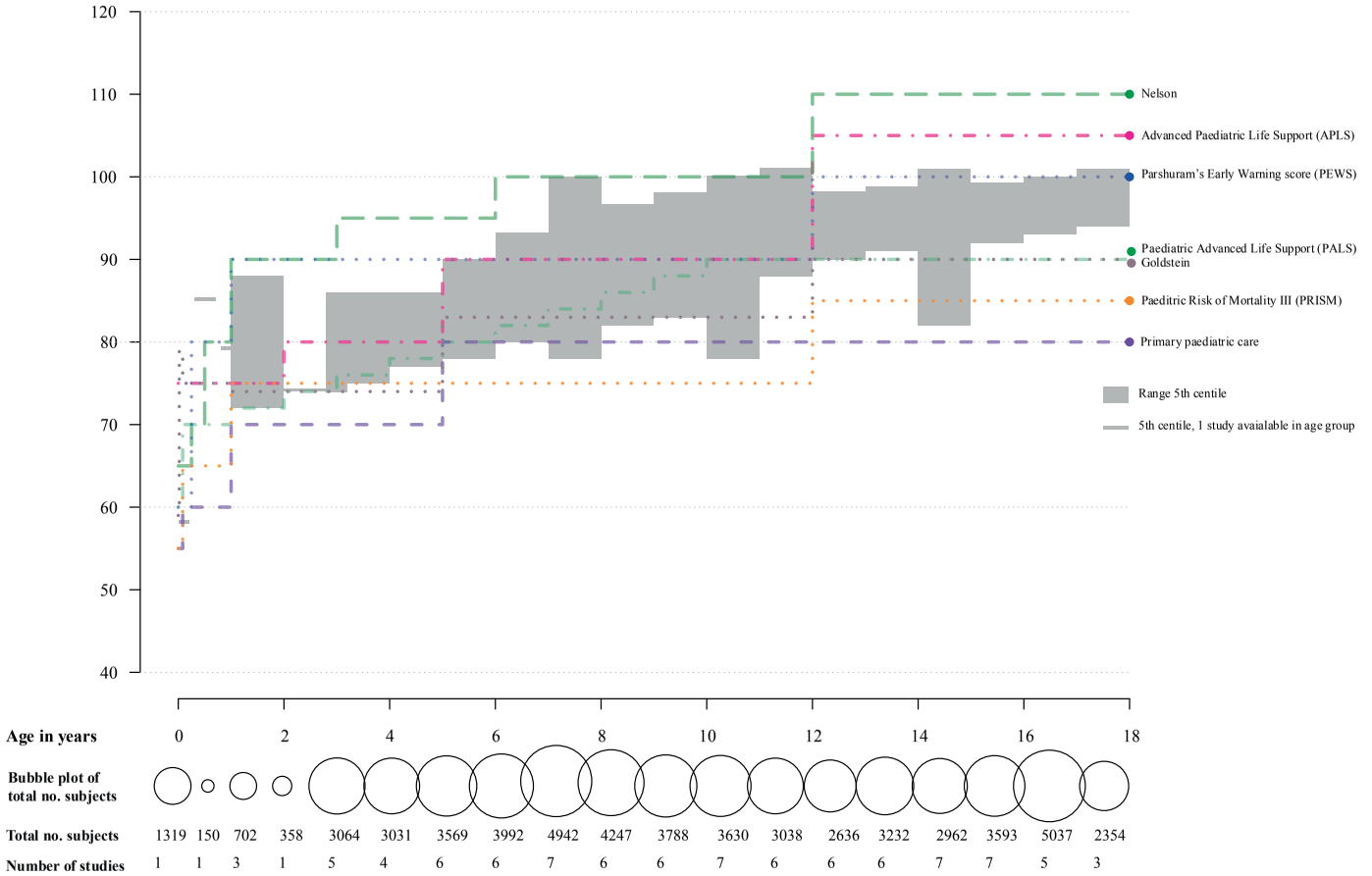
Cochrane

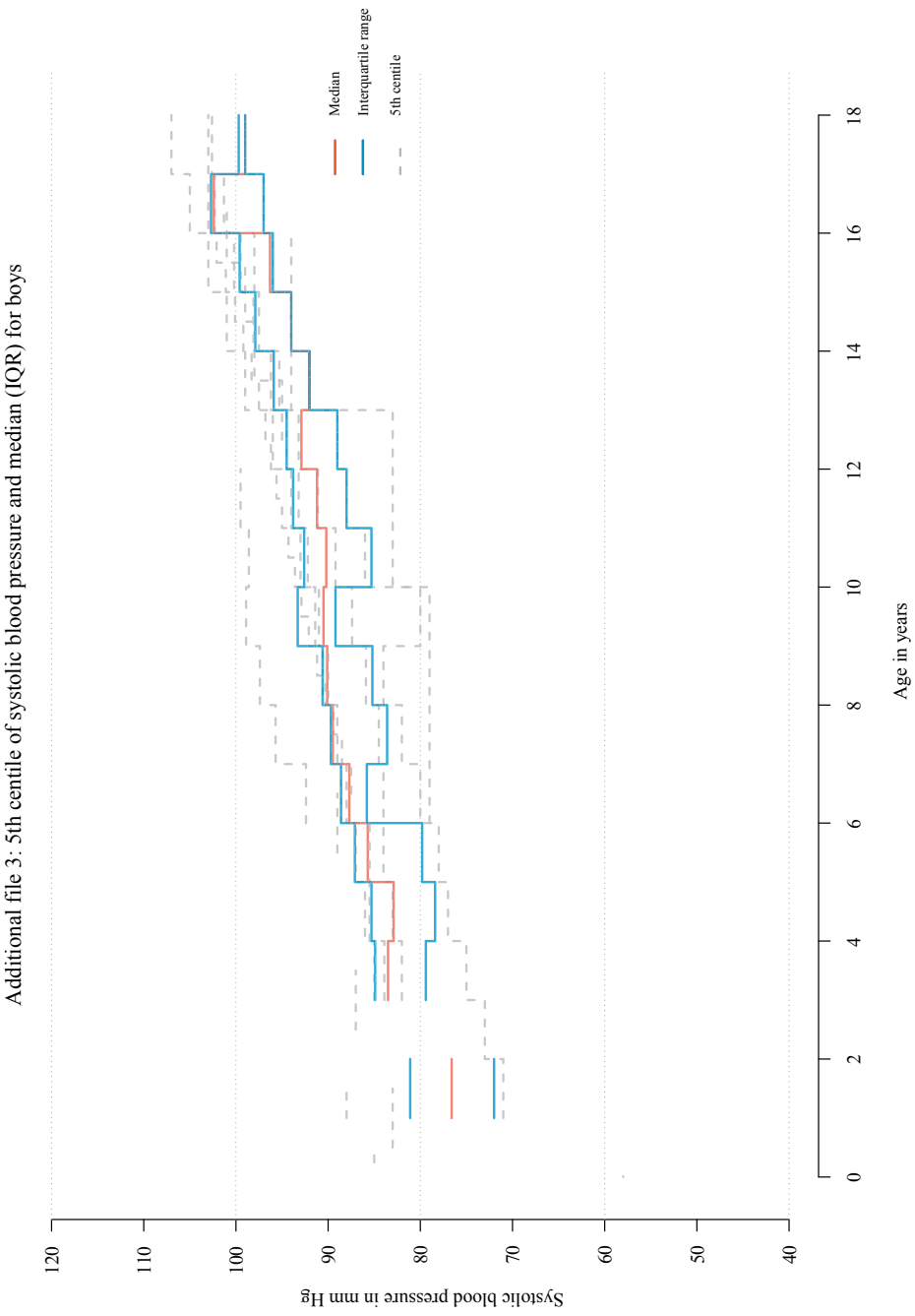
((normotension* OR ((norm* OR healthy OR population OR nomogram* OR curve* OR centile* OR survey* OR distribut* OR statistic* OR trend* OR differen* OR varia* OR 'z score' OR reference* OR standard*) NEAR/9 ('blood pressure' OR 'blood pressures' OR bp))):ab,ti) AND ((adolescen* OR infan* OR newborn* OR (new NEXT/1 born*) OR child* OR pediatric* OR paediatric*):ab,ti) AND ((cohort* OR population* OR (cross NEXT/1 section*) OR longitudinal*):ab,ti)

Cinahl (ebsco)

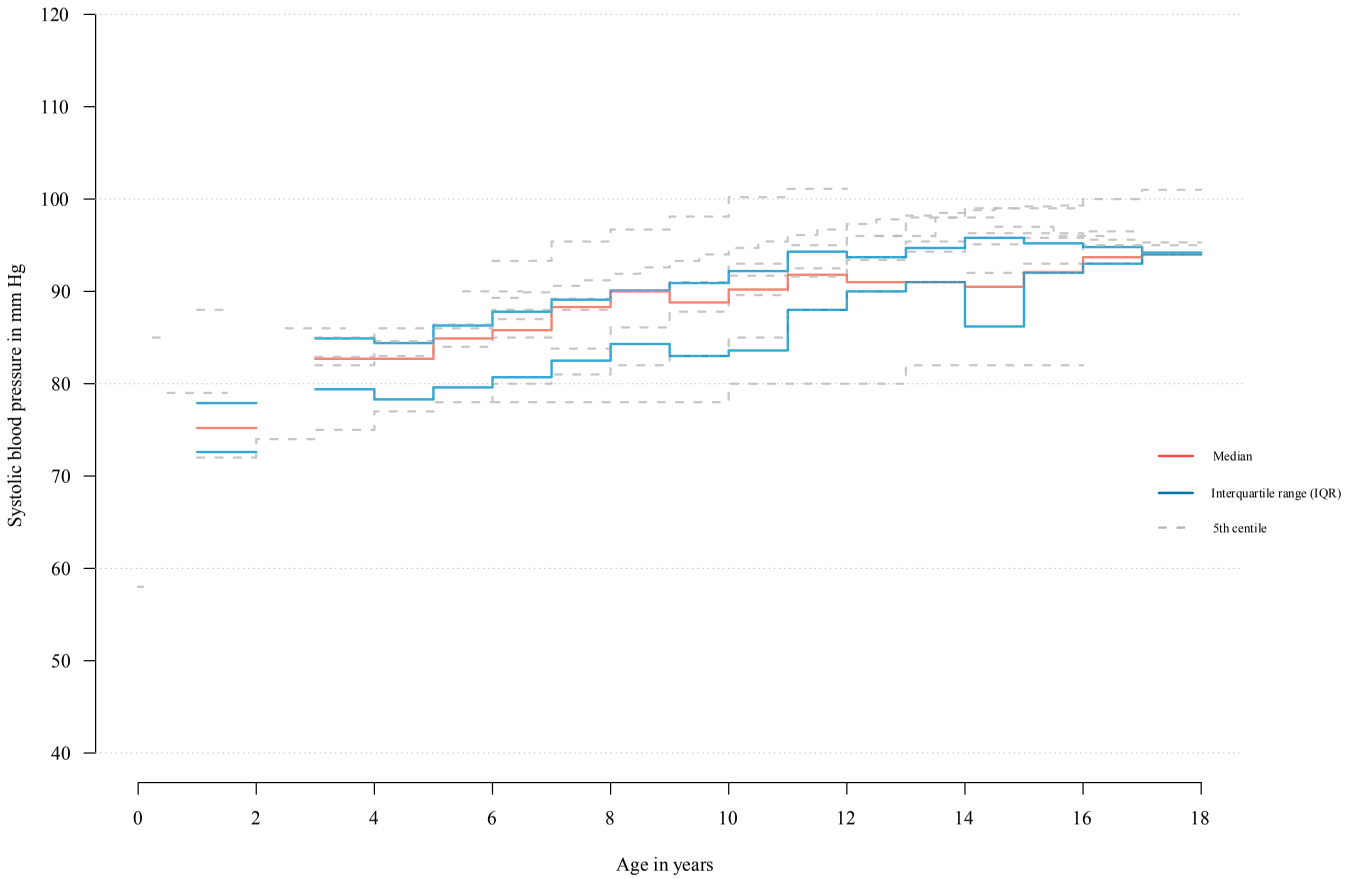
(((MH "blood pressure+" OR MH "Blood Pressure Determination+" OR MH "Blood Pressure Devices+") AND (MH "Statistics")) OR SU (normotension* OR ((norm* OR healthy OR population OR nomogram* OR curve* OR centile* OR survey* OR distribut* OR statistic* OR trend* OR differen* OR varia* OR "z score" OR reference* OR standard*) N3 ("blood pressure" OR "blood pressures" OR bp)))) AND (MH

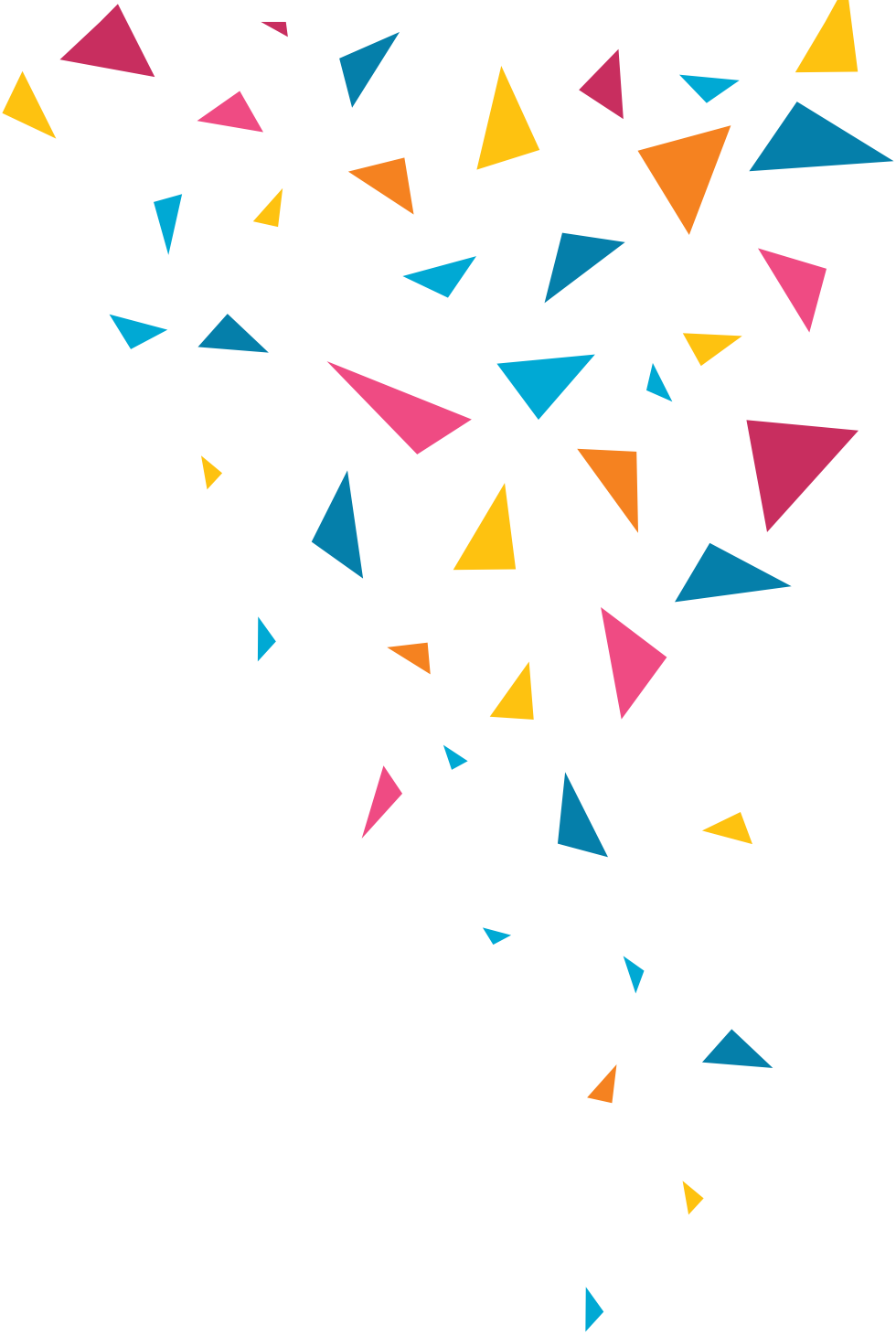
Additional file 2 Clinical definitions for hypotension and range of 5th centile of systolic blood pressure for girls according to age




Additional file 3: 5th centile of systolic blood pressure and median (IQR) for boys
Median
Interquartile range
5th centile
Systolic blood pressure in mm Hg
120
110
100
90
80
70
60
50
40
Age in years
0
2
4
6
8
10
12
14
16
18

Additional file 4: 5th centile of systolic blood pressure and median (IQR) for girls





3

Association between hypotension and serious illness in the Emergency Department: an observational study

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Abstract

Background The value of routine blood pressure measurement in the ED is unclear.

Objective To determine the association between hypotension in addition to tachycardia and the shock index for serious illness.

Design Observational study

Setting University ED (2009-2016)

Participants, methods and main outcomes Routine data collected from consecutive children <16 years. Using logistic regression, we assessed the association between hypotension (adjusted for tachycardia) and shock index (ratio heart rate/blood pressure) for serious illness. The predictive accuracy (sensitivity, specificity) for hypotension and shock index was determined for serious illness, defined as intensive care unit (ICU) and hospital admission.

Results We included 10,698 children with measured blood pressure. According to three age-adjusted clinical cut-offs (Advanced Paediatric Life Support, Pediatric Advanced Life Support and Paediatric Early Warning Score), hypotension was significantly associated with ICU admission when adjusted for tachycardia (range OR 2.6-5.3). Hypotension showed low sensitivity (range: 0.05-0.12) and high specificity (range: 0.95-0.99) for ICU admission. Combining hypotension and tachycardia did not change the predictive value for ICU admission. Similar results were found for hospitalisation. Shock index was associated with serious illness. However, no specific cut-off value was identified in different age groups.

Conclusions Hypotension, adjusted for tachycardia, is associated with serious illness, although its sensitivity is limited. Shock index showed an association with serious illness, but no acceptable cut-off value could be identified. Routine blood pressure measurement in all children to detect hypotension has limited value in the ED. Future studies need to confirm which patients could benefit from blood pressure measurement.

What is already known on this topic

- Hypotension is considered a late feature of serious illness in children and different reference values exist for hypotension.
- In the adult ED population, high shock index is associated with mortality, severity of illness and hospital admission.

What this study adds

- Hypotension has additional value over tachycardia, but due to its low sensitivity clinical relevance is limited.
- High shock index is associated with serious illness in different age groups. Acceptable cut-off values could not be identified.
- Blood pressure measurement for detection of hypotension is suggested to be of limited value in all children attending the ED.

Introduction

Vital signs are essential for recognizing serious illness in children in the emergency department (ED). However, the frequency of BP measurement varies widely (23-87%) and no consensus exists on performing routine blood pressure (BP) measurement to detect hypotension.[1-3] Accurate age-related cut-offs are needed to assess hypotension as incorrect cut-offs may lead to false positive or false negative results. Although pediatric guidelines provide different definitions of low BP, it is unclear which BP cut-off should be used in the ED.[4-6]

Moreover, the predictive value of hypotension for serious illness is unclear in the diverse ED population. In children, hypotension is considered a late sign of deterioration and is used for diagnosis of shock. Children increase heart rate to preserve cardiac output.[7,8] Since abnormal heart rate occurs in an earlier phase, the additional value of routine BP over heart rate in prediction of serious illness could be limited in the ED.

Another measure of haemodynamic status is shock index, the ratio of heart rate to systolic BP, which is associated with mortality and disease severity in adults.[9-11] In small cohorts of children, elevated shock index has been associated with injury severity in trauma and mortality in septic shock.[12-15] However, the shock index in all paediatric ED patients has not yet been evaluated and could be an important predictor in children.

This study aims to study the additional value of BP measurement: 1) to determine the predictive capability of hypotension in addition to tachycardia, and 2) to assess the utility of shock index for serious illness in children. This observational study is based on routine BP measurements in the ED using electronic health records.

Methods

Design

We applied three commonly used clinical definitions for hypotension on data from a prospective study of children visiting the ED to determine the predictive value of hypotension in addition to tachycardia for serious illness. Second, we studied the predictive ability of the shock index. This was a secondary analysis in a study validating the Manchester Triage System (MTS).[16,17]

Setting

The observational study included all children <16 years who presented consecutively at the ED of Erasmus MC-Sophia Children's Hospital (Rotterdam, The Netherlands) between August 2009 and December 2016. This inner-city university hospital receives approximately 7,000 children annually.

Data collection

Data of patient characteristics, vital signs, triage level and disposition were automatically derived from electronic health records that were completed by trained nurses during triage. Heart rate was measured using pulse oximeters and BP using the oscillometric infinity M540 monitor (Draeger medical inc., Telford, USA). BP was measured on medical indication at the discretion of the nurse or attending physician.

Outcomes and definitions

Serious illness was defined as admission to the ICU or hospital following ED visit. Indications for ICU admission include requirement of advanced respiratory support ((non-) invasive ventilation, high flow oxygen); inotropes or continuous intravenous antiepileptics; tracheal cannula; acute or threatening failure of >2 organ systems which expected to last >24 hours or in a child <1 year.[17] We selected three age-adjusted clinical cut-offs to define hypotension to demonstrate the range in clinical practice: Advanced Paediatric Life Support (APLS)[18], Paediatric Advanced Life Support (PALS)/septic shock screening tool[19,20], and the Paediatric Early Warning score (PEWS) [21](table 1). Heart rate was categorized as tachycardia versus no tachycardia according to the same reference as the BP cut-off (appendix 1). Children with bradycardia (5.9%-7.4%) were defined as no tachycardia. Age was categorized as 0-1 year, 1-2 years, 2-5 years, 5-12 years and 12-16 years. Triage urgency was determined by MTS version 3.[22] Ill appearance was assessed by the nurse on a 2-point scale: ill versus non-ill appearance.

Data analysis

Our sample was limited to patients with measured heart rate and BP. Children who died in the ED were excluded (n=34). The value of BP measurement could be limited in this group, since the majority (94%) were triaged as emergencies. Outliers were verified in patient records. First, we assessed the relation between BP and heart rate, using scatterplots. To facilitate analysis across age-groups, we standardised heart rate and BP using Z-scores, which were calculated separately for the different age categories. Second, we assessed the association between hypotension and serious illness using the three clinical cut-offs for hypotension. We used univariable logistic regression to evaluate the association of different BP cut-offs with ICU or with hospital admission, and adjusted for tachycardia in a multivariable model.

Table 1 | Definition of hypotension per different age groups for systolic blood pressure in mm Hg

Age range	APLS[18]	PEWS [34]	PALS [19,20]
<4 weeks	<75	≤60	<60
4 weeks - 6 weeks	<75	≤60	<70
6 weeks - 3 months	<75	≤60	<70
3-6 months	<75	≤80	<70
6-12 months	<75	≤80	<70
1-2 years	<75	≤90	<72
2-3 years	<80	≤90	<74
3-4 years	<80	≤90	<76
4-5 years	<80	≤90	<78
5-6 years	<90	≤90	<80
6-7 years	<90	≤90	<82
7-8 years	<90	≤90	<84
8-9 years	<90	≤90	<86
9-10 years	<90	≤90	<88
10-12 years	<90	≤90	<90
12-13 years	<105	≤100	<90
13-14 years	<105	≤100	<90
14-16 years	<105	≤100	<90

APLS, advanced paediatric life support; PEWS, pediatric early warning score; PALS, pediatric advanced life support

We determined the predictive value of hypotension for ICU admission and hospitalisation by calculating sensitivity, specificity, positive and negative likelihood ratios.[23] To study the predictive value of hypotension in addition to tachycardia, we calculated the predictive value of 1) hypotension; 2) tachycardia; 3) the combination of tachycardia and hypotension; and 4) either hypotension or tachycardia. Positive likelihood ratios >5 and negative likelihood ratios <0.2 were considered relevant.[24]

The normal range of shock index (ratio heart rate to BP) is age dependent.[25] Therefore, we stratified the analysis for shock index by age. To assess the association of shock index, we used univariable logistic regression. To facilitate interpretation, the odds ratios (OR) present the odds for 0.1 unit increase in shock index. Next, the discriminative ability was presented by the area under the curve (AUC) of receiver operating characteristics. We used Youden's index to identify the optimal cut-off value to assess the predictive value. [26] We merged the age groups into <2 years, 2-10 years and >10 years to ensure sufficient numbers for statistical analysis. To explore age-adjusted cut-off values for high shock index, we defined a cut-off by dividing the APLS tachycardia value to the APLS hypotension value for each age group (Appendix 2).

Subgroup analyses were performed in patients with ill appearance, fever (temperature >38.0 C) and patients presenting with surgical problems including major trauma, head injury, limb problems, wounds, torso injuries and assault [27].

Data analyses and visualization were performed in SPSS version 24.0 and R. The Medical Ethical Committee approved the study and waived the requirement for informed consent.

Results

During the study period, 45,495 children (58.6% male) presented to the ED, 891 (2.0%) were triaged as emergencies. A total of 10,698 patients had BP and heart rate measured. In this sample, 3907 (36.5%) children were admitted to the general ward and 631 (5.9%) were admitted to the ICU (table 2). Patients with BP measurement were older, had higher urgency level and were more often admitted compared to children without BP measurement (appendix 3). The prevalence of hypotension ranged from 1.2% to 5.3% depending on the cut-off used (appendix 4). In children with hypotension according to APLS, 13.9% was admitted to the ICU and 33.5% was hospitalized.

Our study found no association between Z-scores of heart rate and BP in any of the age categories (Pearson correlation: 0.04-0.18) (figure 1). In particular, no clear relation was observed between low BP and high z-scores for heart rate.

Hypotension, as a sole predictor, had an association with ICU admission (range OR 2.56-5.27) and hospital admission (range OR 1.4.66–2.66). The association between hypotension and serious illness remained significant after adjustment for tachycardia. In this analysis, the PALS cut-off for hypotension showed the strongest association with ICU admission and hospitalisation (table 3).

The cut-offs for hypotension showed a low sensitivity and a high specificity for serious illness (table 4). For ICU-admission, specificity ranged between 0.95-0.99 and sensitivity between 0.05-0.12. The positive likelihood ratios ranged from 2.38 to 5.06 and the negative likelihood ratios ranged from 0.93 to 0.96. The combination of tachycardia and hypotension did not improve the performance for ICU admission with low sensitivity (0.02-0.08) and high specificity (0.94-0.98). The analysis for hospital admission showed similar results.

Table 2 | Characteristics of visits at the pediatric Emergency Department of Sophia Children's Hospital from 2009 - 2016

	Total	Patients with blood pressure and heart rate measured	Patients with hypotension according to APLS
	n=45,495	**n=10,698**	**N=504**
Male; n %	26338 (57.9)	5872 (54.9)	219 (43.5)
Age in years; median, (IQR)	4.3 (1.4 - 9.8)	7.74 (3.6 – 7.7)	13.0 (6.67 – 14.5)
Age category; n (%)			
0 - 1 year	8734 (19.2)	920 (8.6)	78 (15.5)
1 - 2 years	5736 (12.6)	668 (6.2)	7 (1.4)
2 - 5 years	10154 (22.3)	2091 (19.5)	14 (2.8)
5 - 12 years	13503 (29.7)	4101 (38.3)	80 (15.9)
12 - 16 years	7368 (16.2)	2918 (27.3)	325 (64.5)
MTS urgency; n (%)			
Emergent / Very urgent	6433 (14.2)	2572 (24.0)	155 (30.7)
Urgent	19873 (43.7)	5026 (47.0)	199 (39.5)
Standard/ Non urgent	17711 (38.9)	2922 (27.3)	163 (27.0)
Missing	1478 (3.2)	178 (1.7)	14 (2.8)
Disposition; n (%)			
Admission general ward	8848 (19.4)	3276 (30.6)	169 (33.5)
Intensive care	1132 (2.5)	631 (5.9)	70 (13.9)
Died	34 (0.1)	- [1]	- [1]
Discharge	34913 (76.7)	6719 (62.8)	261 (51.8)
Other	401 (0.9)	61 (0.6)	4 (0.8)
Missing	167 (0.4)	11 (0.1)	0 (0.0)
Shock index; mean (sd)			
0 - 1 year		1.52 (0.48)	
1 - 2 years		1.25 (0.31)	
2 - 5 years		1.11 (0.26)	
5 - 12 years		0.89 (0.24)	
12 - 16 years		0.76 (0.22)	

APLS, advanced paediatric life support; IQR, interquartile range; MTS, Manchester Triage System; sd, standard deviation
1: children who died were excluded

Average values for shock index decreased with age. Stratified by age, shock index was associated with ICU admission (range OR 1.07-1.22) and hospitalisation (range OR 1.06-1.19) (table 3). The discriminative ability for shock index was poor for admission to ICU (range AUC 0.59-0.63) or admission to the hospital (range AUC 0.58-0.62) (appendix 5). The identified cut-offs per age group had low sensitivity (range 0.27-0.42) and moderate specificity (range 0.79-0.91) for ICU admission. None of the identified shock index cut-offs had acceptable positive or negative likelihood ratios (appendix 6).

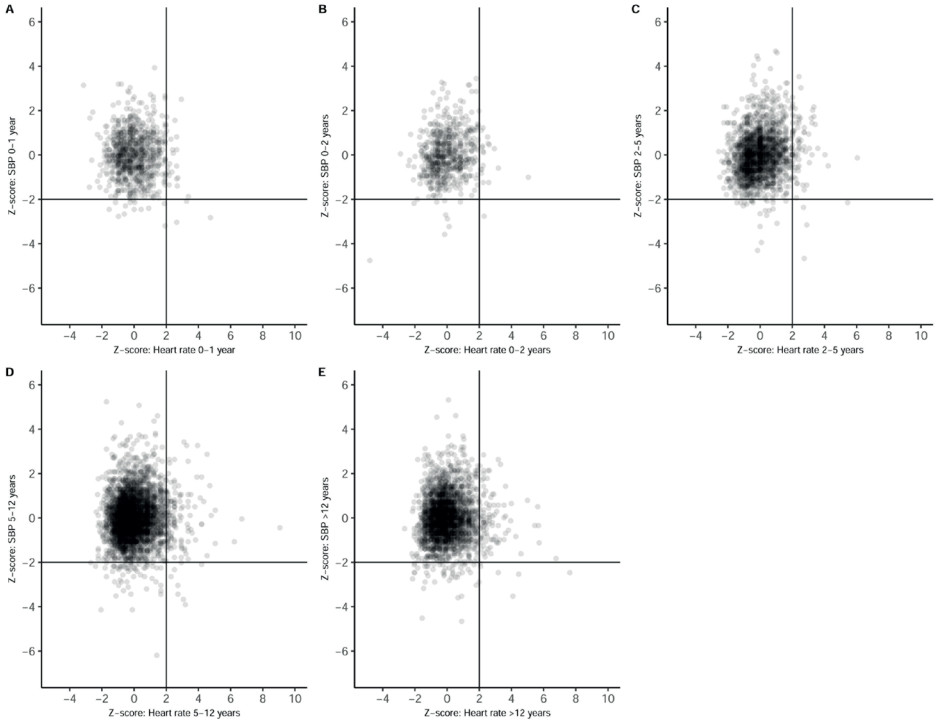

Figure 1 | Scatterplots of z-scores of heart rate and systolic blood pressure (SBP) for different age categories (A; 0-1 year, B; 1-2 years, C; 2-5 years, D; 5-12 years, E; 12-16 years)

The APLS shock index cut-off performed similarly with low sensitivity and high specificity (appendix 7). The positive likelihood ratio was 3.86 (95%CI 3.1- 4.8) and negative likelihood ratio was 0.89 (95%CI 0.87-0.92).

In febrile children, patients with ill appearance and surgical patients, the hypotension and shock index cut-offs showed similar performance. For shock index, the highest AUC was found for febrile patients aged >10 years for ICU admission (0.75 95%CI 0.63-0.87) (appendix 8).

Discussion

In our observational cohort, hypotension has a significant association with serious illness when corrected for tachycardia. However, hypotension showed low sensitivity and high specificity for serious illness in children with routinely measured BP in the ED. The combination of hypotension and tachycardia did not further improve the sensitivity. In

Table 3 | Logistic regression analysis for ICU and hospital admission

	Patients with hypotension/ tachycardia	ICU admission		Hospital admission	
		OR	95% C.I.	OR	95% C.I.
APLS [18]					
hypotension	n=504	2.77	2.12-3.62	1.61	1.34-1.92
tachycardia (APLS)	n=1692	2.46	2.06-2.94	2.62	2.36-2.91
hypotension adjusted for tachycardia		2.68	2.05-3.51	1.56	1.30-1.88
PALS/septic shock screening tool [19,20]					
Hypotension	n=133	5.27	3.51-7.91	2.66	1.87-3.77
tachycardia (septic shock screening tool)	n=1709	1.80	1.49-2.18	1.91	1.72-2.12
hypotension adjusted for tachycardia		4.99	3.32-7.52	2.52	1.77-3.59
PEWS [34]					
hypotension	n=571	2.56	1.98-3.31	1.46	1.24-1.73
tachycardia (PEWS)	n=4113	2.02	1.72-2.37	2.16	1.99-2.34
hypotension adjusted for tachycardia		2.54	1.96-3.29	1.46	1.23-1.73
*Shock index**					
Age 0-1 year		1.09	1.06-1.14	1.14	1.09-1.18
Age 1-2 years		1.07	0.99-1.16	1.07	1.02-1.22
Age 2-5 years		1.08	1.02-1.15	1.06	1.02-1.09
Age 5-12 years		1.13	1.08-1.19	1.14	1.11-1.18
Age >12 years		1.22	1.15-1.29	1.19	1.15-1.24

*Odds ratios present each 0.1 increase in shock index
APLS, advanced paediatric life support; CI, confidence interval; ICU, intensive care unit; PALS, pediatric advanced life support; PEWS, paediatric early warning score

addition, although shock index was associated with serious illness, acceptable cut-off values could not be identified for different age groups.

Accurate reference values for abnormal vital signs are essential to avoid misclassification. Values based on healthy children may not be accurate for children in the ED, as ill children may present with pain and distress which influences heart rate and BP values. Expert-based cut-offs for low BP are currently used. However, these are not based on large studies and show large variation and are therefore not a good alternative. For example, more than 50% of the children with hypotension according to the APLS were discharged home following ED visit. Two recent studies presented BP reference ranges and distributions for critically ill children but validated reference values for the paediatric ED population are lacking.[28,29]

Hypotension is considered a late sign of illness that is preceded by an increase in heart rate. To preserve cardiac output, children compensate by elevating heart rate and sys-

Table 4 | Predictive value for different cut-offs of hypotension and/or tachycardia for ICU admission and hospital admission

	ICU admission				Hospital admission			
	Sensitivity (95% CI)	**Specificity (95% CI)**	**Positive likelihood ratio (95% CI)**	**Negative likelihood ratio (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Positive likelihood ratio (95% CI)**	**Negative likelihood ratio (95% CI)**
Predictive value for hypotension								
APLS	0.11 (0.09-0.14)	0.96 (0.95-0.96)	2.57 (2.03-3.27)	0.93 (0.90-0.96)	0.06 (0.05-0.07)	0.96 (0.96-0.97)	1.57 (1.32-1.86)	0.98 (0.97-0.99)
PALS / sepsis	0.05 (0.04-0.07)	0.99 (0.99-0.99)	5.06 (3.43-7.46)	0.96 (0.94-0.98)	0.02 (0.02-0.03)	0.99 (0.99-0.99)	2.62 (1.86-3.70)	0.99 (0.98-0.99)
PEWS	0.12 (0.09-0.14)	0.95 (0.95-0.95)	2.38 (1.89-2.99)	0.93 (0.90-0.96)	0.07 (0.06-0.07)	0.95 (0.95-0.96)	1.43 (1.22-1.68)	0.98 (0.97-0.99)
Predictive value for tachycardia								
APLS	0.30 (0.27-0.34)	0.85 (0.84-0.86)	2.02 (1.78-2.29)	0.82 (0.78-0.87)	0.24 (0.23-0.26)	0.89 (0.88-0.89)	2.23 (2.04-2.43)	0.85 (0.83-0.87)
PALS / sepsis	0.25 (0.22-0.28)	0.85 (0.84-0.85)	1.60 (1.39-1.85)	0.89 (0.85-0.93)	0.22 (0.20-0.23)	0.87 (0.87-0.88)	1.71 (1.57-1.87)	0.89 (0.88-0.91)
PEWS	0.55 (0.51-0.59)	0.63 (0.62-0.64)	1.46 (1.36-1.58)	0.72 (0.66-0.79)	0.50 (0.49-0.52)	0.68 (0.67-0.69)	1.58 (1.51-1.66)	0.73 (0.71-0.76)
Predictive value for tachycardia AND hypotension								
APLS	0.05 (0.03-0.07)	0.99 (0.99-0.99)	6.52 (4.26-9.96)	0.96 (0.94-0.98)	0.02 (0.02-0.03)	0.99 (0.99-0.99)	6.54 (4.05-10.6)	0.98 (0.98-0.99)
PALS / sepsis	0.02 (0.01-0.04)	0.99 (0.99-0.99)	11.9 (6.16-23.3)	0.98 (0.97-0.99)	0.01 (0.00-0.01)	0.99 (0.99-0.99)	5.21 (2.35-11.6)	0.99 (0.99-0.99)
PEWS	0.08 (0.06-0.09)	0.98 (0.98-0.98)	4.16 (3.06-5.66)	0.94 (0.92-0.96)	0.04 (0.03-0.04)	0.99 (0.98-0.99)	3.06 (2.35-3.99)	0.97 (0.97-0.98)
Predictive value for tachycardia OR hypotension								
APLS	0.37 (0.33-0.40)	0.81 (0.81-0.82)	1.98 (1.77-2.21)	0.78 (0.73-0.83)	0.28 (0.27-0.29)	0.85 (0.85-0.86)	1.96 (1.81-2.11)	0.84 (0.82-0.86)
PALS / sepsis	0.27 (0.24-0.31)	0.84 (0.83-0.84)	1.69 (1.48-1.93)	0.87 (0.83-0.91)	0.23 (0.22-0.24)	0.87 (0.85-0.87)	1.73 (1.59-1.88)	0.89 (0.87-0.91)
PEWS	0.59 (0.55-0.63)	0.59 (0.59-0.60)	1.45 (1.35-1.56)	0.69 (0.63-0.76)	0.53 (0.51-0.54)	0.65 (0.64-0.66)	1.51 (1.44-1.58)	0.73 (0.69-0.75)

APLS, advanced paediatric life support; PALS, pediatric advanced life support; PEWS, paediatric early warning score; CI, confidence interval; ICU, intensive care unit

temic vascular resistance. When this compensatory mechanism is inadequate, BP could drop which may indicate shock.[7] Our study showed that heart rate and BP were not correlated. In particular, high Z-scores of heart rate did not correlate with low Z-scores of BP. Moreover, irrespective of tachycardia, cut-offs for hypotension showed a significant association with serious illness.

We focused on tachycardia as this is an early indicator of critical illness and these children could benefit from measuring BP. Bradycardia, however, indicates irreversible shock. Seriously ill children with bradycardia present with lack of perfusion resulting in cardiopulmonary arrest.[30] Therefore, BP measurement could have limited additional value in children with bradycardia. Furthermore, we did not analyse other predictors of serious illness. In practice, however, heart rate and BP are evaluated with other clinical markers which can be more sensitive predictors for serious illness. Future studies should focus on the combination of BP and other clinical predictors to evaluate the additional value of BP in practice.

Shock index is associated with mortality in children with septic shock.[12,13] Research on shock index in the emergency departments has mainly focused on injured patients.[14,15] No reference values exist for the whole age range in children. Acker et al. proposed age-adjusted cut-offs according to normal vital signs for children >4 year. However, a recent study showed that 2.3% of healthy children had abnormal values according to this definition.[6] Our study found an association between shock index and serious illness in different age groups. For children >12 year a 0.1 unit increase in shock index relates to odds of 1.22 for ICU admission. However, the discriminative ability for shock index was poor. In general, neither of the identified cut-off values had both acceptable sensitivity and specificity.

We focused our analysis on high shock index values to detect severe illness. We acknowledge that low shock index values are also abnormal. Due to the vasopressor response, patients with increased intracranial pressure will have low heart rate and high BP leading to low shock index values.

Although hypotension showed high specificity for serious illness, the sensitivity was very low, regardless of the used definition. The combination of hypotension and tachycardia did not improve the sensitivity or the specificity for predicting serious illness. The PALS[19] had good rule-in value having good specificity and high positive likelihood ratios. However, for early recognition of severely ill children in the ED, it is important to rule out serious illness. Hypotension and tachycardia lack these characteristics, having low sensitivity and poor negative likelihood ratios for serious illness. Considering that

accurate BP measurement is time consuming for nurses[31], these results suggest limited value of routine BP measurement in all children attending the ED.

Strengths of this study are the use of three hypotension cut-offs that are widely used in clinical practice. In addition, our analyses were based on a large cohort of pediatric ED patients with all ages and different presenting problems. We used routine data and therefore our results are representative of clinical practice.

This study has some limitations. First, patients were included when BP and heart rate were measured. This selected group is more severely ill, comprising older children, more high urgent cases and more ICU admissions. This could potentially bias our findings. However, this reflects measurement of BP in the practice of the ED. The frequency of BP investigation and the increase with age and urgency was similar to previous studies.[1,2] In addition, the population of our tertiary university hospital consists of more children with comorbidities and more severely ill children. In settings with low prevalence of serious illness, less yield could be expected. Second, we used hospital admission and ICU admission to define serious illness. These outcomes are widely used in literature and applicable to large datasets.[21,32,33] As reasons for ICU-admission following ED visit include life threatening conditions, the presence of hypotension could have influenced the decision for ICU admission. Hospital admission could occur for various conditions as fractures or bronchiolitis which are unlikely to develop low BP. Furthermore, accurate measurement of BP in children in the ED is challenging. Movement of limbs and uncooperativeness interfere with the measurements. Moreover, the correct cuff size and technique need to be applied. Therefore, the quality of BP measurement should be taken into account.

Finally, our study aimed to evaluate the value of routine BP measurements in children for the recognition of serious illness. We acknowledge that BP measurement may be indicated in the ED for diagnostics, detection of hypertension, follow-up or therapy monitoring.

Conclusion

Our observational study demonstrates that hypotension is associated with serious illness, independent of heart rate. Although the specificity of hypotension is high, the sensitivity for serious illness is very low. The combination of hypotension and tachycardia did not further improve the sensitivity. Shock index is related to serious illness, however we could not identify acceptable cut-off values. These findings suggest limited value of measuring routine BP to detect hypotension in all attending children. Future

studies need to investigate which specific patients could benefit from BP measurement and should focus on developing accurate reference values for hypotension and shock index that are applicable in the ED.

Supplemental information

Available as online web appendix on the website of Archives of Disease in Childhood:

Appendix 8 – Results of subgroup analysis in children with fever, ill appearance, surgical

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Supplemental information

Appendix 1 – Definitions of tachycardia

Table 1 | Definitions of tachycardia per different age groups in heart rate/minute

APLS[1]	
0-3 m	>170
3 m-1.5 y	>160
1.5 y-2 y	>155
2-3 y	>150
3-4 y	>140
4-6 y	>135
6-8 y	>130
8-12 y	>120
12-14 y	>115
>14 y	>110
PEWS [2]	
0 -3 m	≥ 150
3- 12 m	≥ 150
1- 4 y	≥ 120
4-12 y	≥ 110
>12 y	≥ 100
Septic shock identification tool[3]	
0- 3 m	>205
≥ 3m - 2 y	>190
≥ 2 - 10 y	>140
≥ 10 - 13 y	>100
> 13y	>100

m, month; y, year

Appendix 2 – Definition of high shock index APLS

Table 2 | Cut off high shock index according to APLS (hypotension value/tachycardia value)

<4 weeks	2.27
4 weeks - 6 weeks	2.27
6 weeks - 3 months	2.27
3-6 months	2.13
6-12 months	2.13
1-1.5 years	2.13
1.5-2 years	2.07
2-3 years	1.88
3-4 years	1.75
4-5 years	1.69
5-6 years	1.50
6-7 years	1.44
7-8 years	1.44
8-10 years	1.33
10-12 years	1.28
12-13 years	1.10
13-14 years	1.10
14-16 years	1.05

Appendix 3 – Characteristics patients with no blood pressure/heart rate measurement

Table 3 | Characteristics of patients without blood pressure and heart rate measurement

	N=34,763
Male; n %	20,444 (58.8)
Age in years; median, (IQR)	3.40 (1.16-8.45)
Age category; n (%)	
0 - 1 year	7799 (22.4)
1 - 2 years	5067 (14.6)
2 - 5 years	8057 (23.2)
5 - 12 years	9394 (27.0)
12 - 16 years	4446 (12.8)
MTS urgency; n (%)	
Emergent / Very urgent	3828 (11.0)
Urgent	14847 (42.7)
Standard/ Non urgent	14786 (42.5)
Missing	1299 (3.7)
Disposition; n (%)	
Admission general ward	5572 (16.0)
Intensive care	501 (1.4)
Died	- [1]
Discharge	28194 (81.1)
Other	340 (1.0)
Missing	156 (0.4)

IQR, interquartile range; MTS, Manchester Triage System

[1]Children who died were excluded

Appendix 4 – Frequencies of hypotension and tachycardia

Table 4 | Frequencies of hypotension and tachycardia according to 3 different cut-offs (N=10,698)

Hypotension; n (%)	
APLS	504 (4.7)
PALS/septic shock screening tool	133 (1.2)
PEWS by Parshuram	571 (5.3)
Tachycardia; n (%)	
APLS	1692 (15.8)
PALS/septic shock screening tool	1709 (18.0)
PEWS by Parshuram	4113 (38.4)

Appendix 5 – Area under the curve (AUC) for the reveiver operating characteristics with 95% CI for shock index

Table 5 | Area under the curve (AUC) for the receiver operating characteristics with 95% CI for shock index

	ICU admission	Hospital admission
All ages	0.63 (0.60-0.65)	0.63 (0.60-0.65)
0-2 years (n=1588)	0.63 (0.58-0.67)	0.62 (0.59-0.64)
2-10 years (n=5011)	0.56 (0.52-0.60)	0.58 (0.56-0.59)
10-16 years (n=4099)	0.59 (0.54-0.64)	0.58 (0.56-0.59)

CI, confidence interval; ICU, Intensive care unit

Appendix 6 – Predictive value for high cut-offs of shock index

Table 6 | Predictive value for cut-offs of shock index according to different age groups

ICU admission	Shock index Cut-off*	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
0-2 years (n=1588)	1.63	0.42 (0.35-0.49)	0.79 (0.77-0.81)	2.00 (1.65-2.44)	0.74 (0.65-0.83)
2-10 years (n=5011)	1.18	0.36 (0.79-0.81)	0.80 (0.79-0.81)	1.83 (1.53-2.18)	0.79 (0.72-0.88)
10-16 years (n=4099)	1.05	0.27 (0.21-0.34)	0.91 (0.91-0.92)	3.16 (2.45-4.07)	0.79 (0.73-0.87)
Hospital admission					
0-2 years (n=1588)	1.45	0.48 (0.45-0.51)	0.72 (0.69-0.76)	1.74 (1.52-1.99)	0.72 (0.67-0.78)
2-10 years (n=5011)	1.13	0.34 (0.32-0.37)	0.79 (0.77-0.80)	1.63 (1.49-1.79)	0.83 (0.80-0.86)
10-16 years (n=4099)	0.91	0.30 (0.28-0.33)	0.82 (0.81-0.84)	1.73 (1.54-1.94)	0.84 (0.81-0.88)

CI, confidence interval; ICU, intensive care unit
*Cut-off value determined by Youden's index

Appendix 7 Predictive value of high shock index: cut-off defined by APLS abnormal vital signs

Table 7 | Predictive value of high shock index cut-off by APLS*

ICU admission	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Total	0.14 (0.11-0.17)	0.96 (0.96-0.97)	3.86 (3.1-4.8)	0.89 (0.87-0.92)
Ill appearance	0.22 (0.17-0.28)	0.92 (0.90-0.93)	2.71 (1.95-3.75)	0.85 (0.79-0.92)
Fever	0.20 (0.14-0.28)	0.92 (0.91-0.93)	2.49 (1.71-3.65)	0.87 (0.79-0.95)
Surgical	0.04 (0.02-0.08)	0.99 (0.99-0.00)	4.80 (1.74-13.3)	0.97 (0.94-1.0)
Hospital admission				
Total	0.08 (0.07-0.08)	0.98 (0.97-0.98)	3.33 (2.75-4.03)	0.95 (0.94-0.96)
Ill appearance	0.13 (0.11-0.15)	0.96 (0.94-0.97)	3.03 (1.95-4.72)	0.91 (0.88-0.94)
Fever	0.15 (0.13-0.17)	0.95 (0.94-0.96)	3.23 (2.48-4.22)	0.89 (0.87-0.92)
Surgical	0.02 (0.01-0.03)	0.99 (0.99-0.99)	2.79 (1.05-7.41)	0.99 (0.98-1)

CI, confidence interval; ICU, intensive care unit

*APLS cut-off calculated by dividing APLS tachycardia value to APLS hypotension value for each age group

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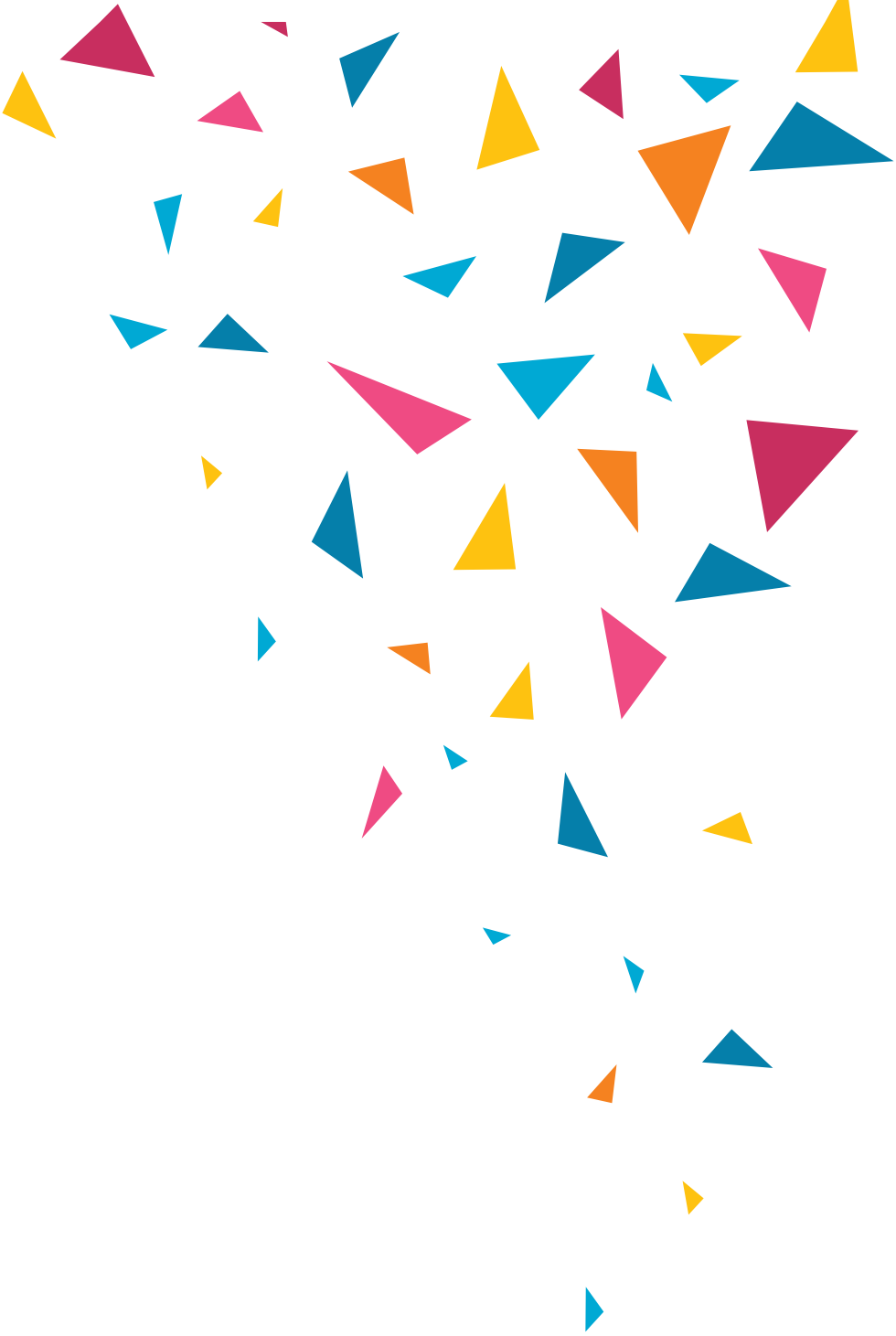

4

Shock Index in the early assessment of febrile children at the Emergency Department: a prospective multicentre study

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Abstract

Objective 1) To derive reference values for the Shock Index (heart rate/systolic blood pressure) based on a large Emergency Department (ED) population of febrile children, and 2) to determine the diagnostic value of the Shock Index for serious illness in febrile children.

Design/Setting Observational study in 11 European EDs (2017-2018)

Patients Febrile children with measured blood pressure

Main outcome measures Serious bacterial infection (SBI), invasive bacterial infection (IBI), immediate life-saving interventions (ILSI) and ICU admission. The association between high Shock Index (>95th centile) and each outcome was determined by logistic regression adjusted for age, sex, referral, comorbidity and temperature. Additionally, we calculated sensitivity, specificity, and negative/positive likelihood ratios (LRs).

Results Of 5,622 children, 461 (8.2%) had SBI, 46 (0.8%) had IBI, 203 (3.6%) were treated with ILSI and 69 (1.2%) were ICU admitted. High Shock Index was associated with SBI (aOR: 1.6 [95%CI:1.3-1.9]), ILSI (aOR 2.5 [95%CI 2.0-2.9]), ICU admission (aOR:2.2 [95%CI:1.4-2.9]) but not with IBI (aOR: 1.5 [95%CI 0.6-2.4]). For the different outcomes, sensitivity for high Shock Index ranged from 0.10 to 0.15, specificity ranged from 0.95 to 0.95, negative LRs ranged from 0.90 to 0.95 and positive LRs ranged from 1.8 to 2.8.

Conclusions High Shock Index is associated with serious illness in febrile children. However, its rule-out value is insufficient which suggests that the Shock Index is not valuable as screening tool for all febrile children at the ED.

What is already known on this topic

- Shock Index (heart rate /systolic blood pressure) is a proposed non-invasive measure for hemodynamic assessment
- In children, high Shock Index is associated with major trauma, and hospitalisation following Emergency Department (ED) visit.
- Shock Index reference values and the value of the Shock Index to identify serious illness for febrile children attending the ED are unknown

What this study adds

- In this cohort of febrile children at the ED, we provide reference values for the Shock Index
- High Shock Index is associated with serious illness in febrile children, but its low sensitivity makes it not valuable as a screening tool
- Our study suggests that the Shock Index is not valuable as a routine screening tool in the early assessment of febrile children at the ED

Background

Early recognition of serious illness is of critical importance in febrile children who attend the Emergency Department (ED). Correct identification enables timely treatment of children with serious bacterial infections (SBI) and children in need of intensive care unit (ICU) admission which improves patient outcomes.[1-4] A recent review has studied the Shock Index, heart rate divided by systolic blood pressure (BP), as haemodynamic marker to predict disease severity in children and adults at the ED.[5] Shock Index in adults has been studied in specific disease groups including trauma and myocardial infarction, and in a large general ED study in which high Shock Index >1.3 at triage has been associated with hospital admission and in-hospital mortality.[6] In paediatrics, evidence of the Shock Index is limited to children with trauma [7-10], children with septic shock [11-13], and a single-centre general ED population.[14] To our knowledge, the Shock Index as a potential non-invasive measure in the early assessment for recognition of serious illness including need for immediate life-saving interventions (ILSI) and SBI, has not yet been evaluated. In addition, the association of the Shock Index with ICU admission in febrile children in a multicentre cohort is still unknown.

Like other vital signs, the normal ranges of the Shock Index are age-dependent. Population-based centiles for Shock Index, have been published for healthy children >8 years. [15] Since fever increases heart rate values, reference values based on healthy children may not be generalizable to acutely ill children with fever attending the ED.[16,17] In order to facilitate interpretation for clinical practice, clinical cut-off values are needed to classify children with high Shock Index.

We aimed 1) to derive reference values for the Shock Index based on this large ED population, and 2) to determine the diagnostic value of the Shock Index for serious illness in febrile children attending European EDs.

Methods

Study design

This is a secondary analysis of the MOFICHE study (Management and Outcome of Febrile children in Europe), embedded in the PERFORM project (Personalized Risk assessment in Febrile illness to Optimize Real-life Management across the European Union).[18] The MOFICHE study is an observational multicenter study assessing the management and outcome of febrile children in Europe using routine data. Details of the study design are described previously.[19]

In short, children from 0-18 years presenting with fever (temperature >=38.0 °C) or with fever <72 hours before ED visit were included. Twelve EDs from eight European countries participated as part of the PERFORM project: Austria, Germany, Greece, Latvia, the Netherlands (n=3), Spain, Slovenia, and the United Kingdom (n=3). The participating hospitals were either university (n=9) or large teaching hospitals (n=3), and all were partners of the PERFORM consortium. Data were collected from January 2017 until April 2018 for at least one year. For the current study, we selected patients with routine BP measurement at the ED. For one ED (London, UK), BP measurements were not available and all visits from this ED were excluded.

Data collected were part of routine care and included sex, mode of referral (self-referral, GP, private paediatrician, emergency medical services or other), comorbidity (chronic condition expected to last ≥1 year)) [20], alarming signs from the National Institute for Health and Care Excellence guideline on fever [21] including consciousness (alert, voice, pain, unresponsive) and ill appearance as assessed by the physician, and vital signs: first measurement of temperature, heart rate, non-invasive systolic BP, capillary refill time. Heart rate was measured by pulse oximeters and systolic blood pressure using oscillometric devices. In addition, we collected diagnostics (C-reactive protein value (CRP) and blood cultures, cerebral spinal fluid cultures and other cultures) collected at the ED or 1st day of hospital admission. Further, we collected treatment with ILSI at the ED, defined as airway and breathing support (non-rebreathing mask, (non-invasive)-ventilation, intubation), emergency procedures (chest needle decompression, pericardiocentesis, or open thoracotomy), hemodynamic support (fluid bolus (>10 ml/kg) or blood administration) or emergency medication (naloxone, dextrose, atropine, adenosine, epinephrine, or vasopressors).[22] In addition, we collected data of prescribed antibiotics and general ward admission > 24 hours, or ICU admission following ED visit.

To classify cause of infection in routine ED practice, we used a consensus-based flowchart [19] combining all clinical data and diagnostic results. We used this flowchart to define the presumed cause of infection for each patient (Appendix 1). The diagnosis 'definite bacterial' infection was assigned when pathogenic bacteria were identified by sterile site culture or PCR. Patients were defined as 'probable bacterial' when a bacterial syndrome was suspected, but no bacteria was identified and CRP level was above 60 mg/L.[23]

Outcome measures

Serious illness was defined using four different outcomes: SBI, invasive bacterial infection (IBI), ILSI and all visits requiring ICU admission. Definition of SBI was decided on in a consensus meeting of experts in paediatrics and paediatric infectious disease specialists (PERFORM partners). SBI was defined as patients with 'definite bacterial' or 'probable

bacterial' with focus of infection from the gastro-intestinal tract, lower respiratory tract, urinary tract, bone and joints, central nervous system or sepsis.[24,25] IBI, a subset of SBI, was defined as positive bacterial culture or PCR detection of a single pathogenic bacterium in blood, cerebrospinal fluid or synovial fluid. All cultures that were treated as contaminant and cultures growing contaminants were considered non-IBI.[26] In addition, cultures growing a single contaminant or candida were defined positive in patients with malignancy, immunodeficiency, immunosuppressive drugs or a central catheter, since antimicrobial treatment is recommended in these patient groups.[27]

Data analysis

We described the study population, and compared patients with and without BP measurement and focused the analysis on patients with BP measurement.

Part 1: Shock Index reference values

For the analysis on reference values, we excluded patients with immediate triage urgency as these patients are vitally compromised, and excluded children with missing heart rate values. First, we visualized heart rate and systolic BP by age using scatterplots. Second, we assessed the relation between heart rate and systolic BP using standardized z-scores calculated separately for different age groups: patients >1 year were grouped in 1-year age groups and patients <1 year were grouped in <3 months, 3-6 months and 6 months-1 years. Next, we calculated the Shock Index by dividing heart rate by systolic BP and calculated 95th centile Shock Index values in the different age groups.

Part 2: Diagnostic value of Shock Index for serious illness

We evaluated the diagnostic value of the Shock Index using the following analyses: (1) the additional value of the Shock Index over systolic BP alone, (2) diagnostic performance of Shock Index above the 95th centile for each of the outcomes, and (3) stratified for age, we explored age-appropriate cut-off values of Shock Index for the different outcomes.

First, we assessed the additional value of the Shock Index to systolic BP by comparing a model with solely systolic BP to a model with both Shock Index and systolic BP (likelihood ratio test). Second, we used univariable logistic regression analysis to assess the association of Shock Index above the 95th centile with each of the outcomes. In multivariable analyses we adjusted for age, sex, referral (referred versus self-referred), comorbidity and temperature. A previous study recommends to adjust for age besides the use of age-adjusted vital signs.[28] Next, we calculated the diagnostic performance of Shock Index above the 95th centile for each of the outcomes using sensitivity, specificity, and negative and positive likelihood ratios (LRs). Negative LR <0.2 or positive LR >5 were defined as relevant.[29] Furthermore, we described the 'number needed to detect a disease' which reflects the number needed to be

examined in order to accurately detect on e person with the disease. [30] Next, the discriminative ability of the Shock Index as continuous predictor for the outcomes was presented by area under the curve of receiver operating characteristics (AUROC) in different age groups. We used the following age groups to ensure sufficient numbers of the different outcomes for analysis: <1 year, 1-5 years, 5-10 years and >10 years. We explored age-appropriate cut-off values of the Shock Index for the different outcomes with a high sensitivity. We determined the optimal cut-off as a sensitivity of at least 90% with maximum specificity.

Missing values

Patients with missing data for the outcomes (cause of infection, focus of infection, ICU admission) were excluded from analysis (n=26). Missing values for referral, comorbidity, temperature, heart rate, capillary refill time and consciousness, were multiple imputed including all available information of the patients using the mice package [31] which resulted in 20 imputation sets (details in Appendix 2). In a sensitivity analysis, using a different approach to deal with missing BP data, we selected all EDs with >20% BP measurements and imputed missing BP values. In this subset, we repeated all analyses from part 2. All data analyses were performed in R version 3.6.

Results

Study population

Of 32,766 eligible patients, we included 5,622 patients with BP measurement and complete outcome (2548 female (45.3%), median age 4.2 years [IQR 1.8-8.4]) (Figure 1). Of those, 1338 (23.8%) patients had comorbidity and 2354 patients (41.9%) were referred to the ED. Regarding the outcomes, 461 patients (8.2%) had SBI, 46 (0.8) IBI, 203 (3.6%) patients were treated with ILSI, and 69 (1.2%) were admitted to the ICU (table 1, details in appendix 3). Of the 203 patients with ILSI, 30 (17.8%) were admitted to the ICU. Patients with BP measurement had more often one of the outcomes of serious illness than patients without BP measurement (details in appendix 4).

Part 1 – Shock Index reference values

In our cohort of febrile children, systolic BP values increased with age, whereas heart rate and Shock Index values decreased with age (figure 2a-2d, Appendix 5). The 95th centile for Shock Index was 2.61 for children <3 months and decreased to 1.21 for children aged 17-18 years. Overall, Shock Index values were higher in children with tachycardia or hypotension than in children without tachycardia or hypotension ($p<0.001$). Children with tachycardia or hypotension more often had Shock Index values above the 95th centile (293/1765, 16.6%) than children without tachycardia or hypotension (14/3744, 0.4%).

Table 1 | Clinical characteristics of the study population and for the different outcomes

		Study population, n=5622	Missing	*SBI, n=461*	*IBI, n=46*	*ILSI, n=203*	*ICU admission, n=69*
		n (%)	**n**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
General characteristics	Age in years, median (IQR)	4.2 (1.8-8.5)		5.3 (1.8-12.0)	4.8 (1.3-9.1)	4.1 (1.5-9.2)	2.8 (1.1-5.8)
	Female	2548 (45.3)		228 (49.5)	21 (45.7)	89 (43.8)	36 (52.2)
	Comorbidity	1338 (23.8)	91	167 (36.2)	29 (63.0)	92 (45.3)	28 (40.6)
	Complex comorbidity	530 (9.4)		85 (18.4)	21 (45.7)	53 (26.1)	20 (29.0)
	Referred	2354 (41.9)	110	293 (63.6)	35 (76.1)	152 (74.9)	55 (79.7)
	Triage urgency		264				
	Low: standard, non-urgent	1746 (31.1)		184 (39.9)	6 (13.0)	23 (11.3)	5 (7.3)
	High: immediate, very urgent, intermediate	3612 (64.2)		224 (48.6)	37 (80.4)	159 (78.3)	58 (84.1)
Clinical symptoms	Fever duration in days, median (IQR)	1.5 (0.5-3)	704	1.5 (0.5-3)	0.5 (0.5-3)	0.5 (0.5-1.5)	0.5 (0.5-1.5)
	Ill appearance	868 (15.4)	620	173 (37.5)	22 (47.8)	106 (52.2)	40 (58.0)
	Decreased consciousness	82 (1.5)	90	10 (2.2)	5 (10.9)	42 (20.7)	23 (33.3)
Vital signs	Temperature in °C, median (IQR)	37.6 (36.8-38.4)	480	37.9 (37.1-38.7)	38.4 (37.7-39.2)	38.2 (37.3-39)	38.1 (37.1-38.7)
	Prolonged capillary refill (>3 sec)	105 (1.9)	866	24 (5.2)	3 (6.5)	39 (19.2)	18 (26.1)
	Tachycardia (APLS)	1667 (29.7)	55	199 (43.2)	27 (58.7)	113 (55.7)	38 (55.1)
	Hypotension (APLS)	209 (3.7)		38 (8.2)	3 (6.5)	22 (10.8)	10 (14.5)
	Shock Index, median (IQR)	1.2 (1.0-1.4)	55	1.2 (1.0-1.5)	1.3 (1.9-1.6)	1.3 (1.1-1.6)	1.4 (1.2-1.7)
	Shock Index, > 95th centile for age	310 (5.5)	55	44 (9.5)	6 (13.0)	29 (14.3)	8 (11.6)
Diagnostics and treatment	C-reactive protein in mg/L, median (IQR)	20 (5-61)	3378	91 (38-154)	58 (17-147)	20 (5-75)	19 (4-83)
	Blood cultures performed	967 (17.2)		243 (52.7)	46 (100)	118 (58.1)	44 (63.8)
	Cerebrospinal fluid performed	140 (2.5)		34 (7.4)	8 (17.4)	28 (13.8)	20 (29.0)
	Admission to the ward >24 hours	1159 (20.6)	137	281 (61.0)	34 (73.9)	109 (53.7)	
	Admission to the ICU	69 (1.2)		19 (4.1)	7 (15.2)	43 (21.2)	69 (100)
	Antibiotic treatment following ED visit	1983 (35.3)	55	407 (88.3)	44 (95.7)	151 (74.4)	50 (72.5)

APLS, advanced paediatric life support; ED, emergency department; IBI, invasive bacterial infection; ICU, intensive care unit; ILSI, immediate life-saving intervention; IQR, interquartile range; SBI, serious bacterial infection

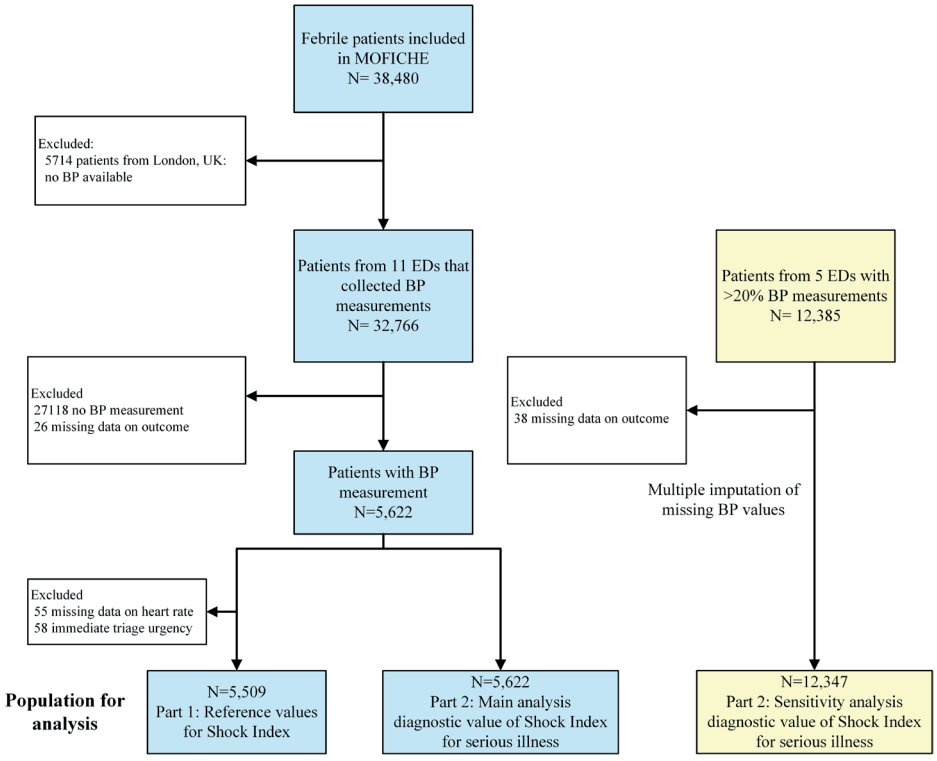

Figure 1 | Flow chart of study population

Part 2 – diagnostic value of Shock Index for serious illness

Overall, 5.5% (310/5622) patients had Shock Index values >95th centile. In patients with SBI, IBI, ILSI or ICU admission, high Shock Index >95th centile occurred in 9.5% (44/461), 13.0% (6/46), 14.3% (29/203) and 11.6% (8/69), respectively (table 1).

Addition of Shock Index to the model with only systolic BP led to a significant improved model for each of the outcomes ($p<0.05$). As a sole predictor, the 95th centile cut-off of Shock Index was associated with SBI (OR 1.9 [95%CI 1.6-2.3]), IBI (OR 2.6 [95%CI 1.7-3.4]), ILSI (OR 3.1 [95%CI 2.7-3.5]) and ICU admission (OR 2.6 [95%CI 1.9-3.3]). For SBI, ILSI and ICU admission, this association remained after adjustment for age, sex, referral, comorbidity and temperature (SBI: aOR 1.6 [95% CI1.3-1.9]; ILSI: aOR 2.5 [95%CI 2.0-2.9]; ICU admission: aOR (2.2 [95%CI 1.4-2.9]), but the association was not significant for IBI (aOR 1.5 [95%CI 0.6-2.4]). The 95th centile cut-off of Shock Index had high specificity (all outcomes 0.95 [95% CI 0.94-0.95]) and positive LRs ranging from 1.8 to 2.8, but had low sensitivity (range 0.10 to 0.15) and poor negative LRs (range 0.90 to 0.95) for the different outcomes (table 2). The number needed to detect a disease for the 95th centile cut-off of Shock Index ranged from10 to 20 for the different outcomes (table 2). Stratified by age,

the AUROC of the Shock Index as continuous predictor ranged 0.55-0.66 for SBI, ranged 0.56-0.74 for IBI, ranged 0.57-0.71 for ILSI, and ranged 0.52-0.73 for ICU admission (table 3). Consequently, when attempting to define age-specific cut-off values these had high sensitivity (>90%) but low specificity (0-54%) for the different outcomes (Appendix 6).

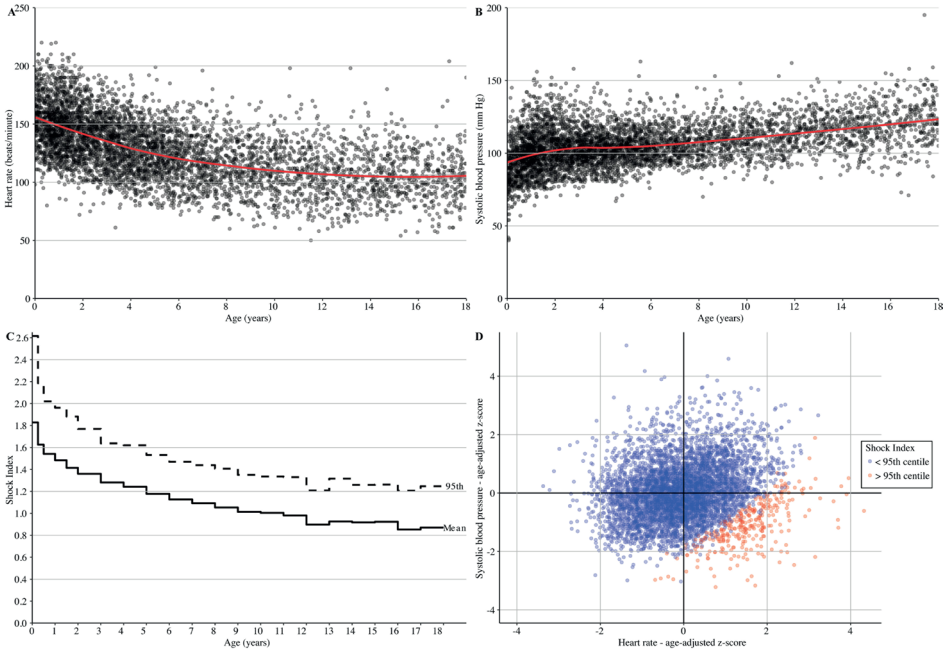

Figure 2 | Scatterplots of heart rate for age (A), Systolic blood pressure for age (B), Step chart of reference values of Shock Index (mean and 95th centile) (C), Scatter plot of age-adjusted z-scores of systolic BP for age-adjusted z-scores of heart rate (D).

Table 2 | Diagnostic value of high Shock Index >95th centile for serious illness, n=5622

	OR (95% CI)	aOR* (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)	Number needed to detect a disease (N)
SBI, n=461	1.9 (1.6-2.3)	1.6 (1.3-1.9)	0.10 (0.07-0.13)	0.95 (0.94-0.95)	1.8 (1.4-2.5)	0.95 (0.93-0.98)	20
IBI, n=46	2.6 (1.7-3.4)	1.5 (0.6-2.4)	0.13 (0.05-0.26)	0.95 (0.94-0.95)	2.4 (1.1-5.1)	0.92 (0.82-1.03)	12.5
ILSI, n=203	3.1 (2.7-3.5)	2.5 (2.0-2.9)	0.15 (0.10-0.20)	0.95 (0.94-0.95)	2.8 (2.0-4.0)	0.90 (0.85-0.95)	10
ICU admission, n=69	2.6 (1.9-3.3)	2.2 (1.4-2.9)	0.13 (0.06-0.23)	0.95 (0.94-0.95)	2.4 (1.3-4.5)	0.92 (0.84-1.01)	12.5

*Adjusted for age, sex, referral, comorbidity and temperature

CI, confidence interval; IBI, invasive bacterial infection; ICU, intensive care unit; ILSI, immediate life-saving intervention; LR, likelihood ratio SBI, serious bacterial infection

Table 3 | Discriminative value of Shock Index (continuous) for serious illness, stratified for age n=5622

	SBI	IBI	ILSI	ICU admission
	AUC (95% CI)	AUC (95% CI)	AUC (95% CI)	AUC (95% CI)
Shock Index (continuous) stratified for age				
<1 year, n=801	0.66 (0.60-0.72)	0.71 (0.56-0.85)	0.70 (0.60-0.80)	0.73 (0.59-0.87)
1-5 year, n=2395	0.54 (0.49-0.59)	0.56 (0.42-0.70)	0.57 (0.51-0.64)	0.58 (0.47-0.68)
5-10 year, n=1330	0.56 (0.50-0.62)	0.68 (0.50-0.86)	0.61 (0.52-0.69)	0.52 (0.36-0.69)
>10 year, n= 1096	0.55 (0.50-0.60)	0.74 (0.63-0.85)	0.71 (0.64-0.79)	0.72 (0.45-0.98)

AUC, area under the curve; CI, confidence interval; IBI, invasive bacterial infection; ICU, intensive care unit; ILSI, immediate life-saving intervention; SBI, serious bacterial infection

The sensitivity analysis including all visits from the 5 EDs with >20% BP measurements (n=12,347) provided similar results for the diagnostic value of Shock Index >95th centile (Appendix 7).

Discussion

In this large European multicentre study, we provided reference values for Shock Index in febrile children attending the ED. In addition, we evaluated the diagnostic value of Shock Index for serious illness defined as SBI, IBI, ILSI and ICU admission. High Shock Index showed an association with serious illness, but its rule-out value was poor.

Tachycardia and delayed capillary refill are early haemodynamic markers of shock, whilst hypotension is considered a late sign. The Shock Index combines the properties of heart rate and systolic BP and could potentially improve identification of acutely ill children at the ED. Previous studies in paediatrics have been studying the role of Shock Index in trauma, septic shock and hospital and ICU admission.[5,7,8,10-14,32,33] In our previous single-centre study, we found an association of high Shock Index for hospital and ICU admission in children with different presentations at the ED.[14] Although this previous study included both febrile and non-febrile children, our study confirms an association of high Shock Index with SBI, ILSI and ICU admission in febrile children.

In adults, Shock Index values of >0.9 are related to hospital admission and mortality [5,6]. In children, reference values and accurate cut-off values for Shock Index are yet unclear. Rappaport et al. have provided reference values of the Shock Index for healthy subjects aged >8 years based on auscultatory BP measurements. [15] Gupta et al. reported Shock Index values in a small study of septic children for the outcome mortality.[13] In this study, we provide reference values of the Shock Index for febrile children attending EDs. These

values could be used as a reference value for clinical practice or further studies, although generalisability of these values to all febrile children or other populations may be limited.

In our sample of patients with measured BP, Shock Index values above the 95th centile cut-off value were associated with SBI, ILSI and ICU admission adjusted for age, sex, referral, comorbidity, and temperature. In this multivariate analysis, Shock Index 95th centile was not significantly associated with IBI although the trend was similar. High Shock Index had high specificity and moderate positive LRs, but had poor rule-out value with low sensitivity and poor negative LRs. Its poor rule-out value, makes the Shock Index not a valuable screening tool at the ED. Although we identified age-specific cut-off values with high sensitivity, none had adequate specificity and therefore leading to high number of false positives. Although this was not the focus of our study, the Shock Index may have additional value in specific high-risk patients or as repeated measurement for monitoring disease course or treatment effect.

Physiologically-based scores have been developed for the early recognition of disease severity in children including scores as quick Sequential Organ Failure Assessment (qSOFA), quick Paediatric Logistic Organ Dysfunction-2 (qPELOD-2) and Liverpool quick Sequential Organ Failure Assessment (LqSOFA).[34-37] In previous ED studies these scores showed high specificity but low sensitivity for serious illness.[36,37] LqSOFA is based on heart rate and capillary refill time as haemodynamic parameters whereas qSOFA and q-PELOD-2 both require BP measurement. Since heart rate and capillary refill time are easy to assess in children, LqSOFA could be more easily implemented than scores that need BP measurement. The low sensitivity of these scores, however, makes them of limited clinical value for routine use at the ED.

Systolic BP measurement is also required for the Shock Index. The National Institute for Health and Care excellence does not advise routine BP measurement in febrile children attending the ED [21], but recommends BP measurement in children with abnormal heart rate or prolonged capillary refill. In our cohort, BP measurement was performed in 1799/7804 (23%) of children with abnormal heart rate or capillary refill. This poor adherence to recommendations agrees with findings of moderate adherence to other vital signs measurements in febrile children in different European EDs.[38]

Strengths of this study include the participation of different EDs in Europe, the detailed data collection and the evaluation of the Shock Index for different definitions of serious illness: SBI, IBI, ILSI and ICU admittance, and adjustment for age, sex, referral, comorbidity, and temperature. Our study has limitations. First, the selection of patients with BP measurement could have led to selection bias. Due to the limited number of BP

measurements in our cohort, multiple imputation of systolic BP in all patients was not possible. In a sensitivity analysis, we imputed systolic BP in all visits of febrile children at the 5 EDs with >20% BP measurement and found similar results. This suggests that the selection of patients with BP measurement did not influence our results. The low proportion of BP measurement in our study reflects clinical practice where guidelines do not advise routine BP measurement in febrile children.[21,38] Patients with BP measurement, however, likely reflect the group in which the Shock Index would potentially be used in clinical practice.

Second, we focused our analysis on high Shock Index since in febrile children we expect the combination of tachycardia and hypotension to be valuable. However, we recognize that hypotension without compensatory high heart rate is a relevant sign of shock which could result in normal Shock Index values. Lastly, the presence of hypotension or tachycardia may have influenced decisions to initiate treatment with ILSI or PICU admission. We acknowledge that Shock Index might not be a complete independent variable for these outcomes.

Conclusions

In this large observational study of 11 European EDs, we provide reference values for Shock Index for febrile children at the ED. High Shock Index was associated with serious illness like SBI, IBI, ILSI and ICU admission. For serious illness, the rule-out value of high Shock Index was not sufficient. Our results suggest that the Shock Index is not valuable as a routine screening tool in the early assessment of febrile children at the ED.

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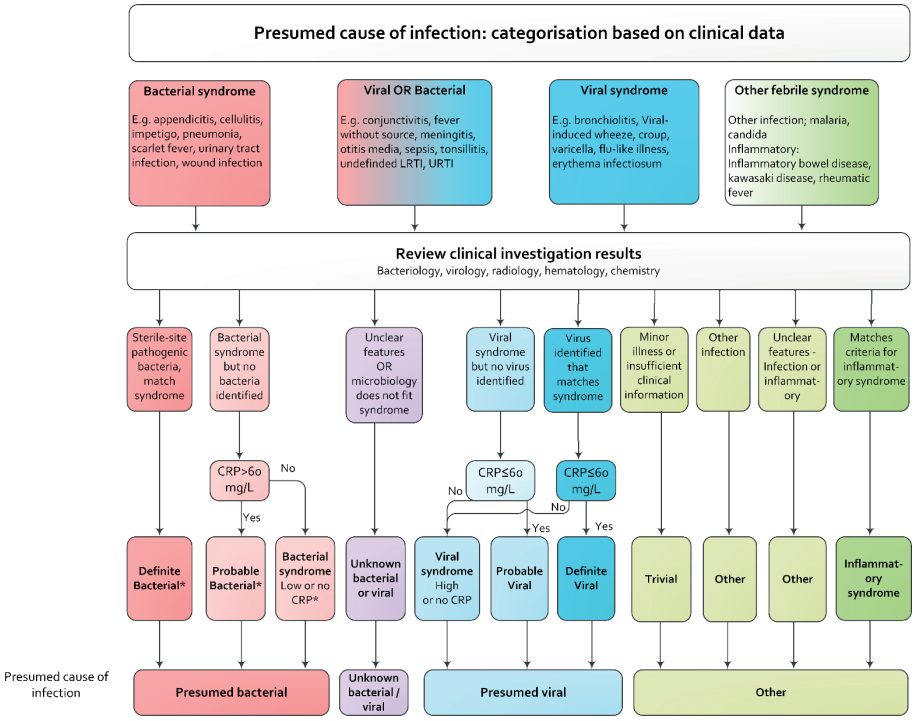
Appendix 1: Flowchart to classify presumed cause of infection



CRP, c-reactive protein; LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection.
*Patients could have identified viral co-infection. (1)

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Appendix 2. Additional methods: multiple imputation

Missing data

For the main analysis, we excluded patients without systolic blood pressure (BP) measurement. We used multiple imputation by chained equations using the MICE package in R to impute referral, comorbidity, temperature, heart rate, capillary refill time and consciousness. We included hospital, all outcome measures and other auxiliary variables influencing case-mix and disease severity in the imputation model. Multiple imputation was performed on all patients (n=32,766). For the statistical analysis where we used the multiple imputation data, results were pooled for a final result. For the main analysis, patients with missing systolic BP measurement were excluded leading to 5648 eligible visits.

For the sensitivity analysis, we used a different approach to deal with missing BP data. We selected the five EDs with >20% BP measurements (n=12,385), and imputed missing BP values in this subset. In this subset we repeated all analysis from part 2.
Proportion of missingness of variables are provided in Table 1 and Appendix 5.

Variables in the multiple imputation model:

General characteristics	Markers of disease severity	Vital signs	Diagnostics	Treatment	Outcomes
Hospital	Triage urgency	Heart rate	CRP-level	Immediate life-saving interventions	Disposition
Age	Fever duration	Respiratory rate	Chest X-ray categories	Oxygen treatment	Final diagnosis
Sex	Ill appearance	Temperature	Urinalysis categories	Inhalation medication	Focus of infection
Referral type (self / GP / emergency services / other)	Work of breathing	Capillary refill time	Blood culture performed	Antibiotic prescription type	Serious bacterial infection
Previous medical care (yes, primary care / yes, this ED / yes other secondary care)	Consciousness	Oxygen saturation	Cerebrospinal fluid performed	Antibiotic prescription mode	Invasive bacterial infection
Season	Meningeal signs	Non-invasive systolic blood pressure			
Comorbidity	Focal neurology				
Complex comorbidity	Non-blanching rash				
	Dehydration				
	Seizures				

Appendix 3. Further details of serious bacterial infections (n=461), invasive bacterial infections (n=46) and immediate-lifesaving interventions (n=203)

Infection focus of serious bacterial infections (n=461)	N (%)
Urinary tract	153 (33.2%)
Lower respiratory tract infection	139 (30.2%)
Gastro intestinal or surgical abdomen	93 (20.2%)
Sepsis	37 (8.0%)
Musculoskeletal	15 (3.3%)
Meningitis / CNS infection	10 (2.2%)
Other	14 (3.0%)

Invasive bacterial infections (n=46)	N (%)
Bacteraemia*	40 (87%)
Bacterial meningitis*	6 (13%)
Bone and joint	2 (4.3%)

*Two patients had both bacteraemia and bacterial meningitis

Immediate life-saving interventions (n=203)*	N (%)
Airway/breathing interventions	100 (49.3%)
Haemodynamic interventions	112 (55.2%)
Emergency medications	52 (26.6%)

*Multiple categories per patients possible

Appendix 4. Patient characteristics of patients with blood pressure measurement and patients without blood pressure measurement

	Blood pressure measured (n=5622)		No blood pressure measured (n=26841)	
	n (%)	Missing	n (%)	Missing
General characteristics				
Age in years, median (IQR)	4.2 (1.8-8.5)		2.6 (1.3-5.2)	
Female	2548 (45.3)		12172 (45.3)	
Comorbidity	1338 (23.8)	91	3831 (14.3)	182
Complex comorbidity	530 (9.4)	91	931 (3.5)	182
Referred	2354 (41.9)	110	11028 (41.1)	1044
Triage urgency		264		879
Low: standard, non-urgent	3612 (64.2)		18670 (69.6)	
High: immediate, very urgent, intermediate	1746 (31.1)		7292 (27.2)	
Clinical symptoms				
Fever duration in days, median (IQR)	1.5 (0.5-3)	704	1.5 (0.5-3)	1676
Ill appearance	868 (15.4)	621	4855 (18.1)	1040
Decreased consciousness	82 (1.5)	90	87 (0.3)	210
Vital signs				
Temperature in °C, median (IQR)	37.6 (36.8-38.4)	480	37.7 (37.0-38.4)	2432
Hypoxia <95%	2920(5.2)	211	935 (3.5)	5204
Prolonged capillary refill (>3 sec)	105 (1.9)	866	254 (0.9)	3004
Tachycardia (APLS)	1667 (29.7)	55	5537 (20.6)	3372
Diagnostics and treatment				
CRP in mg/L, median (IQR)	20 (5-61)	3378	17 (5-47)	13021
Blood cultures performed	967 (17.2)		1798 (6.7)	
Cerebrospinal fluid performed	140 (2.5)		198 (0.7)	
Antibiotic treatment following ED visit	1983 (35.2)	55	8305 (30.9)	398
Admission to the ward >24 hours	1159 (20.6)	137	5415 (20.2)	328
Serious illness				
Serious bacterial infection	461 (8.2)		1683 (6.3)	
Invasive bacterial infection	46 (0.8)		82 (0.3)	
Admission to the ICU	69 (1.2)		76 (0.3)	
Immediate life-saving interventions	203 (3.6)		212 (0.8)	

APLS, advanced paediatric life support; CRP, C-reactive protein; ICU, intensive care unit; IQR, interquartile range; NA, not applicable

Appendix 5. Shock Index reference values according to age, n=5509

Age group	N	Shock Index Mean (SD)	Shock Index 95th centile
<3m	181	1.83 (0.48)	2.62
3-6m	163	1.63 (0.34)	2.19
6m-1y	430	1.54 (0.29)	2.02
1-2y	753	1.45 (0.29)	1.96
2-3y	574	1.36 (0.25)	1.88
3-4y	549	1.28 (0.22)	1.77
4-5y	462	1.24 (0.23)	1.64
5-6y	406	1.18 (0.21)	1.62
6-7y	276	1.13 (0.21)	1.53
7-8y	234	1.09 (0.21)	1.47
8-9y	196	1.05 (0.22)	1.44
9-10y	185	1.01 (0.20)	1.41
10-11y	166	1.00 (0.20)	1.35
11-12y	157	0.98 (0.21)	1.34
12-13y	139	0.90 (0.19)	1.33
13-14y	127	0.93 (0.24)	1.21
14-15y	159	0.92 (0.21)	1.32
15-16y	122	0.92 (0.21)	1.26
16-17y	99	0.85 (0.21)	1.26
17-18y	131	0.87 (0.23)	1.21

SD, standard deviation; m, months; y, year

Appendix 6. Shock Index cut-off values for the different outcomes, stratified for age groups

Serious bacterial infection	Shock Index cut-off value*	Sensitivity	Specificity	Negative LR	Positive LR
Age <1 year	1.37	0.91	0.24	0.37	1.20
Age 1-5 year	1.12	0.90	0.18	0.54	1.10
Age 5-10 year	0.81	0.91	0.08	1.21	0.98
Age >10 year	0.67	0.90	0.11	0.88	1.02

Invasive bacterial infection	Shock Index cut-off value*	Sensitivity	Specificity	Negative LR	Positive LR
Age <1 year	1.43	1.00	0.31	0.00	1.45
Age 1-5 year	1.19	0.92	0.29	0.29	1.28
Age 5-10 year	0.79	0.92	0.07	1.26	0.98
Age >10 year	0.93	0.91	0.54	0.17	1.98

Immediate life-saving intervention	Shock Index cut-off value*	Sensitivity	Specificity	Negative LR	Positive LR
Age <1 year	1.40	0.91	0.27	0.34	1.24
Age 1-5 year	1.06	0.91	0.12	0.78	1.03
Age 5-10 year	0.96	0.92	0.25	0.33	1.22
Age >10 year	0.79	0.92	0.29	0.29	1.29

ICU admission	Shock Index cut-off value*	Sensitivity	Specificity	Negative LR	Positive LR
Age <1 year	1.32	0.94	0.18	0.33	1.14
Age 1-5 year	1.11	0.90	0.17	0.56	1.09
Age 5-10 year	0.68	0.93	0.02	4.25	0.94
Age >10 year	0.53	1.00	0.01	0.00	1.01

* minimal sensitivity >=90% and maximal specificity

Appendix 7. Sensitivity analysis for febrile children in 5 EDs with >20% SBP measurement (n=12347)

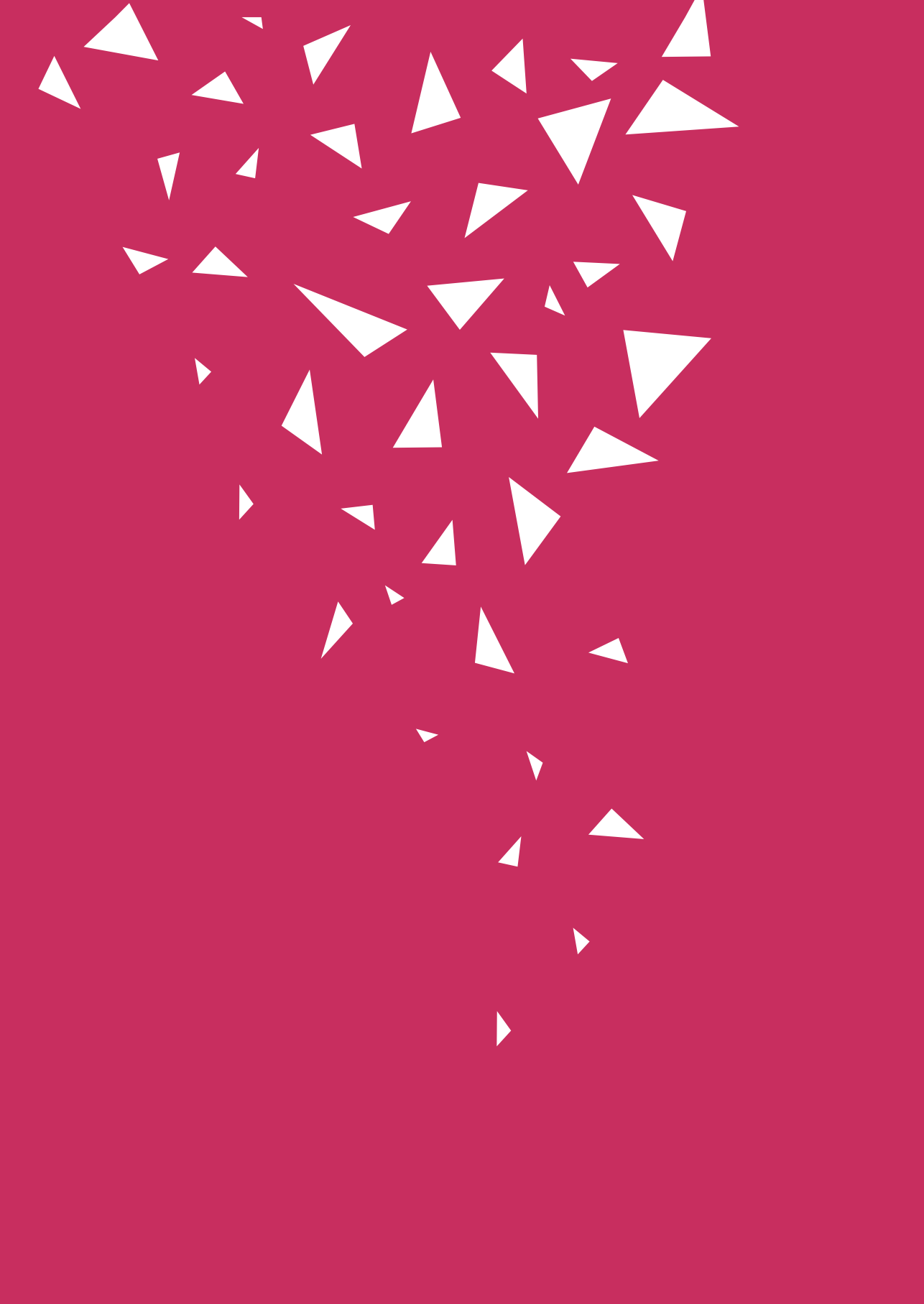
Univariate and multivariate analysis of Shock Index >95th centile values for serious illness (n=12347)		
	Shock Index >95th centile value	
	OR (95% CI)	Adj. OR (95% CI)*
SBI n=643	1.7 (1.2-2.4)	1.4 (1.0-2.0)
IBI n=81	2.0 (0.8-4.8)	1.7 (0.7-4.1)
ILSI n=336	2.6 (1.8-3.8)	2.4 (1.6-3.6)
ICU admission n=90	2.9 (1.5-5.5)	3.0 (1.5-5.8)

*Adjusted for age, sex, referral (y/n), comorbidity (y/n), temperature
Adj, adjusted; CI, confidence interval; ICU, intensive care unit; OR, odds ratio

Diagnostic performance of high Shock Index >95th centile for serious illness (n=12347)				
	Sensitivity (95% CI)	Specificity (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
SBI	0.08 (0.06-0.10)	0.97 (0.96-0.97)	2.4 (1.8-3.2)	0.95 (0.93-0.97)
IBI	0.10 (0.04-0.19)	0.97 (0.96-0.97)	2.9 (1.5-5.7)	0.93 (0.87-1.00)
ILSI	0.13 (0.09-0.17)	0.97 (0.96-0.97)	3.9 (2.9-5.3)	0.90 (0.87-0.94)
ICU admission	0.14 (0.08-0.23)	0.97 (0.96-0.97)	4.3 (2.6-7.20)	0.89 (0.81-0.96)

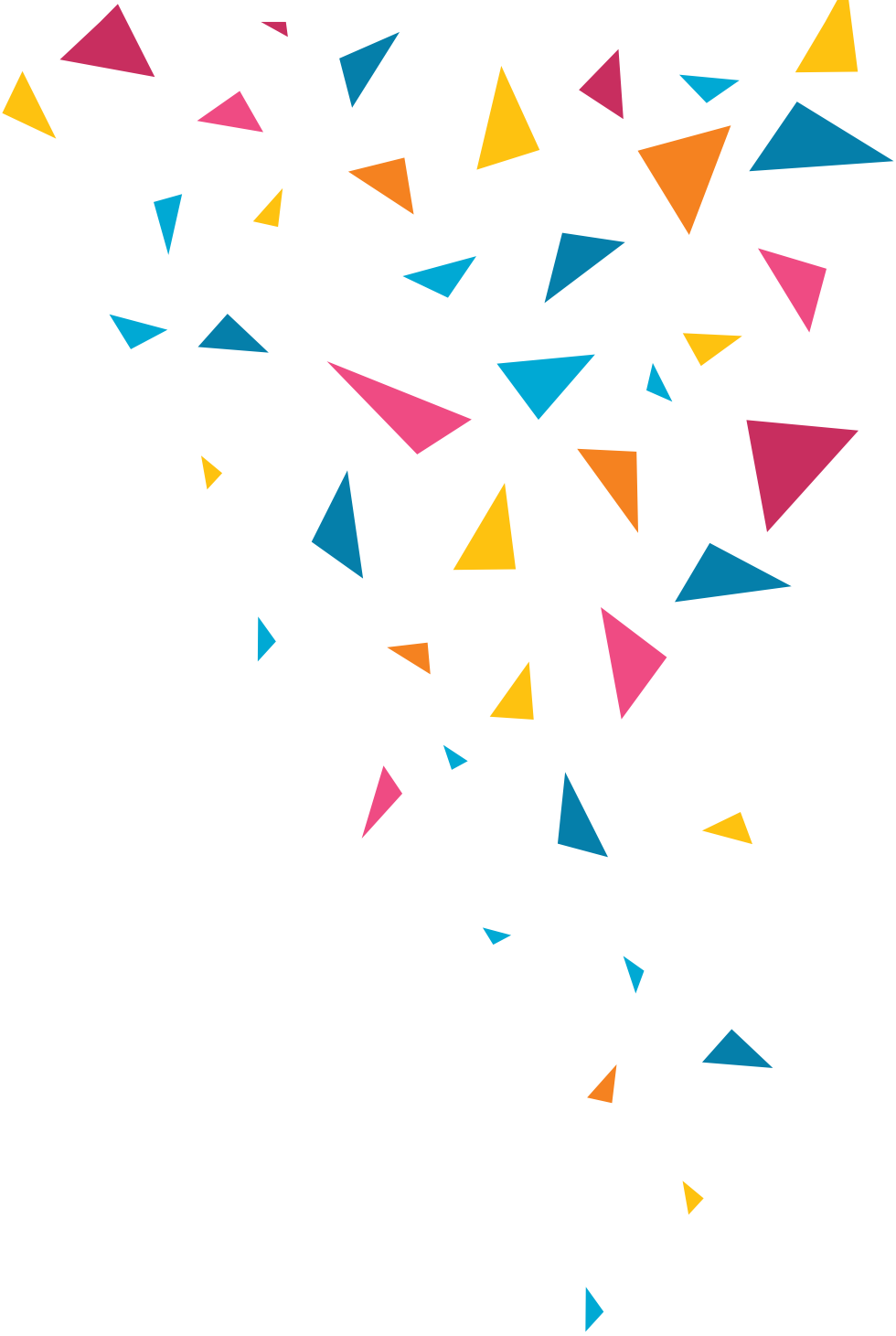
Discriminative value of Shock Index (continuous) for serious illness, stratified for age n=12347				
	SBI AUC (95% CI)	IBI AUC (95% CI)	ILSI AUC (95% CI)	ICU admission AUC (95% CI)
Shock Index (continuous) stratified for age				
<1 year, n=2337	0.63 (0.57-0.68)	0.71 (0.58-0.84)	0.69 (0.61-0.77)	0.71 (0.59-0.83)
1-5 year, n=6064	0.55 (0.51-0.60)	0.56 (0.42-0.69)	0.59 (0.54-0.65)	0.57 (0.46-0.67)
5-10 year, n=2484	0.53 (0.46-0.59)	0.65 (0.50-0.81)	0.56 (0.48-0.64)	0.53 (0.36-0.69)
>10 year, n= 1462	0.59 (0.53-0.65)	0.63 (0.46-0.80)	0.66 (0.59-0.74)	0.73 (0.48-0.98)

AUC, area under the curve; CI, confidence interval; IBI, invasive bacterial infection; ICU, intensive care unit; ILSI, immediate life-saving intervention; SBI, serious bacterial infection



Part III

Biomarkers in febrile children



5

Development and validation of a prediction model for invasive bacterial infections in febrile children at European Emergency Departments: MOFICHE a prospective observational study

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On behalf of the PERFORM consortium (Personalised Risk assessment in febrile children to optimise Real-life Management across the European Union).

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doi: 10.1136/archdischild-2020-319794.

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Abstract

Objectives: To develop and cross-validate a multivariable clinical prediction model to identify invasive bacterial infections (IBI) and to identify patient groups who might benefit from new biomarkers.

Design: Prospective observational study.

Setting: 12 emergency departments (EDs) in 8 European countries.

Patients: Febrile children aged 0-18 years .

Main outcome measures: IBI, defined as bacteraemia, meningitis and bone/joint infection. We derived and cross-validated a model for IBI using variables from the Feverkidstool (clinical symptoms, C reactive protein), neurological signs, non-blanching rash and comorbidity. We assessed discrimination (area under the receiver operating curve) and diagnostic performance at different risk thresholds for IBI: sensitivity, specificity, negative and positive likelihood ratios (LRs).

Results: Of 16 268 patients, 135 (0.8%) had an IBI. The discriminative ability of the model was 0.83 (95% CI 0.80 to 0.87) and 0.77 (95% CI 0.73 to 0.81) in pooled cross-validations. The model performed well for the rule-out threshold of 0.1% (sensitivity 0.97 (95% CI 0.93 to 0.99), negative LR 0.1 (95% CI 0.0 to 0.2) and for the rule-in threshold of 2.0% (specificity 0.94 (95% CI 0.94 to 0.95), positive LR 8.3 (95% CI 6.8 to 10.0)). The intermediate thresholds of 0.1-2.0% performed poorly (ranges: sensitivity 0.56-0.93, negative LR 0.15-0.51, specificity 0.51-0.87, positive LR 1.9-4.4) and comprised 9784 patients (60%).

Conclusions: The rule-out threshold of this model has potential to reduce antibiotic treatment whilst the rule-in threshold could be used to target treatment in febrile children at the ED. In more than half of patients at intermediate risk, sensitive biomarkers could improve identification of IBI and potentially reduce unnecessary antibiotic prescriptions.

What is already known on this topic

- In children, distinction between invasive bacterial and self-limiting infections on only clinical symptoms is unreliable leading to overuse of antibiotics on the one hand, but to missed invasive bacterial infections in others.
- Several clinical prediction models including biomarkers have been developed to help decision making by risk prediction of patients at high risk or low risk for bacterial infections, but none predicts the outcome invasive bacterial infections in older children or includes children with chronic conditions.

What this study adds

- We derived and externally validated a clinical prediction model based on clinical predictors from the Feverkidstool (clinical symptoms, C reactive protein) and non-blanching rash, neurological symptoms and comorbidity, to early recognise invasive bacterial infections with data from a large observational European-wide study of febrile children 0-18 years.
- The rule-out threshold of this model could reduce antibiotic prescription and invasive diagnostics, while the rule-in threshold could be useful to target early treatment for invasive bacterial infections.
- In more than half of the patients at intermediate risk, sensitive new biomarkers could reduce diagnostic uncertainty and improve identification of invasive bacterial infections.

Introduction

Children presenting at the Emergency Department (ED) still die from treatable invasive bacterial infections (IBI) due to delayed or missed diagnosis.[1-3] For not missing one child with IBI, antibiotics are prescribed in children with self-limiting viral infections.[4] The distinction between bacterial and viral infections based solely on clinical signs and symptoms is unreliable. Although C reactive protein (CRP) and procalcitonin are currently used as markers for bacterial infections, they measure non-specific inflammation and immunologic responses. Recent studies focus on proteomic and transcriptomic approaches for finding new discriminators of bacterial and viral infections.[5-8] Due to costs and limited resources, it is not feasible to apply new biomarkers to all febrile children. Therefore, prediction models are needed to identify risk groups where biomarkers can improve diagnosis.

Clinical prediction models that include clinical signs and CRP or procalcitonin have been developed to assist decision making in treatment of febrile children,[9-15] and have focused on young infants to differentiate between patients at high or low-risk for IBI (bacteraemia, meningitis, bone/joint infections). No clinical prediction models for IBI exists for older children who are also at risk for IBI.[16 17] The Feverkidstool, developed for children <16 years, predicts risks for pneumonia and other serious bacterial infections which besides IBIs also includes bacterial infections of the urinary tract, gastro-intestinal tract and soft tissue.

Although the Feverkidstool is extensively validated, the original population only included 21 IBI cases and important predictors for IBI such as non-blanching rash or neurologic symptoms were not included. Several models yet exist for prediction of bacterial pneumonia and the impact of the original Feverkidstool on antibiotic use in respiratory tract infections is proven.[18] Therefore, another model for bacterial pneumonia is not required. Furthermore, prediction of urinary tract infections may be less relevant as sensitive laboratory tests (urinalysis) are readily available for accurate diagnosis at ED visit. In addition, the Feverkidstool is developed in previous healthy children and is therefore not applicable for children with chronic conditions with higher-risk of IBI. Hence, a new tool is required for early risk assessment of IBI in febrile children including all age ranges (0-18 years) and chronic conditions.

We aim 1) to derive and cross-validate a clinical prediction model including CRP to identify IBIs in febrile children presenting to different European EDs, and 2) to identify patient groups which might benefit from new biomarkers.

Methods

Study design

This study is embedded in MOFICHE (Management and Outcome of Febrile children in Europe), an observational multicenter study, which is part of PERFORM (PErsonalized Risk assessment in Febrile illness to Optimize Real-life Management across the European Union, www.perform2020.org).

Children from 0-18 years with temperature >=38.0°C or fever <72 hours before ED visit were included. Twelve EDs participated in this study: Austria, Germany, Greece, Latvia, the Netherlands (n=3), Spain, Slovenia, and the United Kingdom (n=3).[19] Data were collected for at least one year from January 2017 to April 2018. Details of the study design have been described previously.[20]

For this study, we selected patients with CRP measurement and excluded patients with working diagnosis of urinary tract infections after first assessment at the ED.[21] To identify IBI at the earliest opportunity, we included only the first ED visit for patients with IBI who repeatedly visited the ED within the same disease episode. Data were analysed according a statistical analysis plan (online supplemental appendix 1).

Collected data included age, sex, comorbidity (chronic condition expected to last ≥1 year)[22], warning signs for identifying risk of serious illness (National Institute for Health and Care Excellence (NICE))[23] (consciousness, ill appearance, work of breathing, meningeal signs, focal neurology, non-blanching rash, dehydration) and vital signs (heart rate, respiratory rate, oxygen saturation, temperature, capillary refill time). We collected CRP level (point-of-care or laboratory assay) and microbiologic cultures (blood, cerebrospinal fluid and other) ordered at the ED or at the first day of hospital admission on indication of the physician. Further, we collected data of prescribed antibiotics and admission following ED visit.

Outcome

IBI included bacterial meningitis, bacteraemia and bacterial bone/joint infections, defined as culture or PCR detection of a single pathogenic bacterium in blood, cerebrospinal or synovial fluid. All cultures that were treated as contaminant and cultures growing contaminants were considered non-IBI (online supplemental appendix 2).[24] Cultures growing a single contaminant or candida were defined positive in patients with malignancy, immunodeficiency, immunosuppressive drugs or a central catheter, since antimicrobial treatment is needed in these patients.

Model development

Descriptive and univariate logistic regression analyses were performed for children with and without IBI.

Sample size was estimated based on Riley et al.[25] Assuming 16 predictors, a prevalence of 0.8% and an expected R^2 of 0.0135 (15% of maximum achievable R^2), a sample size of 10587 with 85 cases would be sufficient. For model development [26 27], we considered predefined variables with predictive value for IBI: (1) variables in the Feverkidstool[9] (age, sex, temperature, fever duration, tachypnea and tachycardia defined by Advanced Pediatric Life Support[28], oxygen saturation <94%, capillary refill ≥ 3 s, work of breathing, ill appearance and CRP value), (2) NICE warnings signs (consciousness, meningeal signs, focal neurology, status epilepticus, non-blanching rash)[23] and (3) complex chronic condition (≥2 body systems, malignancy or immunocompromised).[22] Consciousness, meningeal signs and focal neurology were combined into a composite variable abnormal neurology. Linearity of continuous variables was assessed using restricted cubic splines. As in the Feverkidstool, age was modelled linear piecewise for children <1 year and children >1 year and a logarithmic transformation for CRP was used. Outliers were truncated at the 0.01 percentile for temperature (35.7 °C) and the 0.99 percentile for CRP (215 mg/L) and fever duration (8 days).

Variable selection was not influenced by the results of the univariate logistic regression analysis, but was performed using least absolute shrinkage and selection operator (LASSO) which reduces the degree of overfitting by shrinking large regression coefficients (detailed methods in online supplemental appendix 3).[29 30] The final model was developed on data from all the 12 EDs. For the cross-validation, we created 5 ED groups; 1 group combined the data from the 8 EDs with <10 IBI cases, and 4 groups were based on data from EDs with >10 IBI cases per ED: Slovenia, the Netherlands (n=2) and the UK (online supplemental appendix 4). Next, in cross-validation the model was repeatedly derived on four ED groups and validated on the fifth ED group, leading to five different cross-validations.[31] The five cross-validations were pooled using a random-effects model. This cross-validation determines model performance most accurately but also provides information on the heterogeneity of performance across different settings. This cross-validation is therefore superior to a single external validation.[13 31] We assessed the discriminative ability by the area under the receiver operating curve (AUC), and calibration, the agreement between predicted risks and observed cases. We explored the impact of difference in case-mix heterogeneity on the discriminative ability of the model in the internal-external cross-validation. We used decision curve analysis to evaluate the net benefit of the prediction model.[32] At different cut-offs for the individual probability of IBI according to the model, we assessed sensitivity, specificity, negative and positive likelihood ratios (LRs) . Missing values for the covariates were multiple imputed using the MICE package, resulting in 20 imputation sets (details in

Online supplemental appendix 3). Sensitivity analysis was performed in the population where missing CRP values were imputed. All analyses were performed in R v3.6.

Results

Of 38 480 patients, 17 213 patients had CRP measurements. Patients with CRP measurements were more often ill-appearing and admitted than patients without CRP measurements (online supplemental appendix 5). We excluded 939 urinary tract infections and 6 repeated visits in the same disease period of IBI patients, resulting in 16,268 patients. Of those, most common infections were the upper respiratory tract (45%), lower respiratory tract (18%), gastro-intestinal tract (14%) and undifferentiated fever (9%). IBI was diagnosed in 135 patients (0.8%), and comprised 119 bacteraemias, 15 bacterial meningitis and 9 bone/joint infections (8 patients had concurrent infections). Main pathogens included *Streptococcus pneumoniae* (21%), *Staphylococcus aureus* (19%), *Escherichia coli* (10%), *Neisseria meningitidis* (7%) and coagulase-negative staphylococcus (7%) (figure 1, online supplemental appendix 6). Complex chronic conditions were present in 37% of

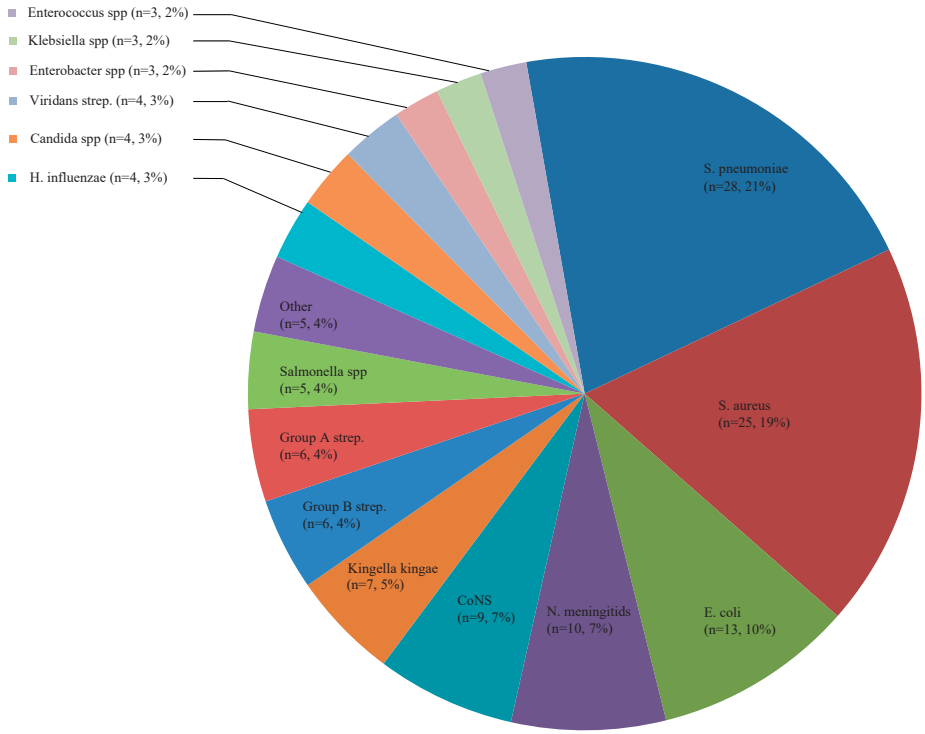

Figure 1 | Identified pathogens for invasive bacterial infections (n=135)
Legend: spp, species; CoNS, coagulase-negative staphylococci

patients with IBI versus 6% of patients without IBI. IBI incidence varied from 0.1 to 5.6% of patients per ED (online supplemental appendix 4).

Table 1 | Characteristics of patients with invasive bacterial infections and patients without invasive bacterial infections

	Invasive bacterial infection (n=135)		No invasive bacterial infection (n=16133)	
	n (%)	Missing	n (%)	Missing
Age in years, median (IQR)	3.2 (0.8-6.0)		2.8 (1.4-6.0)	
Female	76 (56.2)		8932 (55.4)	
Underlying chronic condition		2		89
Any	68 (50.4)		3005 (18.6)	
Complex	50 (37.0)		1008 (6.2)	
Referred	96 (71.1)	3	8633 (53.5)	936
Triage urgency		5		477
Low: standard, non-urgent	41 (30.4)		9242 (57.3)	
High: immediate, very urgent, intermediate	89 (65.9)		6414 (39.8)	
Feverkidstool				
Temperature in °C, median (IQR)	38.0 (37.4-38.7)	3	37.8 (37.0-38.5)	764
Fever duration in days, median (IQR)	0.5 (0.5-3)	5	1.5 (0.5-3)	817
Tachypnea (APLS)	38 (28.1)	37	3345 (20.7)	3919
Tachycardia (APLS)	81 (60.0)	5	5578 (34.6)	821
Hypoxia <95%	4 (2.9)	13	749 (4.6)	2373
Prolonged capillary refill (>3 s)	8 (5.9)	29	305 (1.9)	2311
Increased work of breathing	11 (8.1)	40	887 (5.5)	2136
Ill appearance	60 (44.4)	13	4398 (27.3)	610
CRP in mg/L, median (IQR)	61 (21-144)		16 (5-45)	
NICE Warning signs				
Decreased level of consciousness	6 (4.4)		137 (0.8)	141
Meningeal signs	8 (5.9)	24	116 (0.7)	845
Focal neurology	2 (1.5)	29	95 (0.6)	1249
Status epilepticus	0 (0.0)	8	49 (0.3)	887
Rash: petechiae/non blanching	10 (7.4)	25	640 (3.9)	1183
Blood cultures performed	134 (99.3)		3002 (18.6)	
CSF performed	25 (18.5)		381 (2.4)	
Admission to the ward >24 hours	111 (82.2)	1	5879 (36.4)	159
Admission to the ICU	10 (7.4)		125 (0.8)	17
Antibiotic treatment following ED visit	126 (93.3)		5804 (35.9)	197
LSI: airway, breathing or hemodynamic support	16 (11.9)		343 (2.1)	

APLS, advanced paediatric life support; CRP, C-reactive protein; CSF, cerebrospinal fluid; ED, emergency department; ICU, intensive care unit; IQR, interquartile range; LSI, lifesaving intervention

Patients with IBI were similar in age and sex compared to patients without IBI. CRP-level was higher in the IBI group (median 62 mg/L, IQR:21-144) than in the non-IBI group (median 16 mg/L, IQR:5-45) (p<0.01) (Table 1). The majority of IBIs were treated with antibiotics (n=126, 93.3%) at first ED visit and all were treated with antibiotics in the disease course. The associations of the sole predictors with IBI are provided in online supplemental appendix 7.

The final model is presented in table 2. This model discriminated well (AUC: 0.84 (95% CI 0.81 to 0.88)). In the cross-validation, the model discriminated moderate to well (range AUC: 0.76-0.81) yielding a pooled AUC of 0.78 (95% CI 0.74 to 0.82) (figure 2). Calibration was poor to moderate for the different cross-validations (range slope: 0.45 to 0.81, range intercept -1.2 to 1.0) (online supplemental appendix 8). Apparent calibration was improved by adding an ED-specific variable for high (>2%) vs low (<2%) incidence of IBI (online supplemental appendix 9).

Table 2 | Model specification of multivariate logistic model for IBI

		Coefficients	OR
	(Intercept)	-9.16	0.00
Feverkidstool	Male	-0.19	0.83
	Age < 1 year*	-2.53	0.08
	Age ≥ 1 year*	0.00	1.00
	Temperature	-0.05	0.95
	Fever duration in days	-0.15	0.86
	Tachypnea	-0.44	0.65
	Tachycardia	0.69	2.00
	Hypoxia	-0.87	0.42
	Increased work of breathing	-0.31	0.73
	Ill appearance	0.87	2.38
	Ln CRP	0.76	2.14
NICE warning signs	Abnormal neurology	1.54	4.66
	Non-blanching rash	1.38	3.96
Comorbidity	Complex chronic condition	2.41	11.1

*Age <1 year and age ≥ 1 year were calculated linear-piecewise:
The risk of children aged < 1 year was calculated: $\beta_{(age <1 year)}\times$ age in years.
The risk of children age ≥ 1 year was calculated: $\beta_{(age <1 year)} \times 1 +$ (age in years-1) $\times \beta$ ($_{age \geq 1 in years}$).
CRP, C-reactive protein; ln, natural log

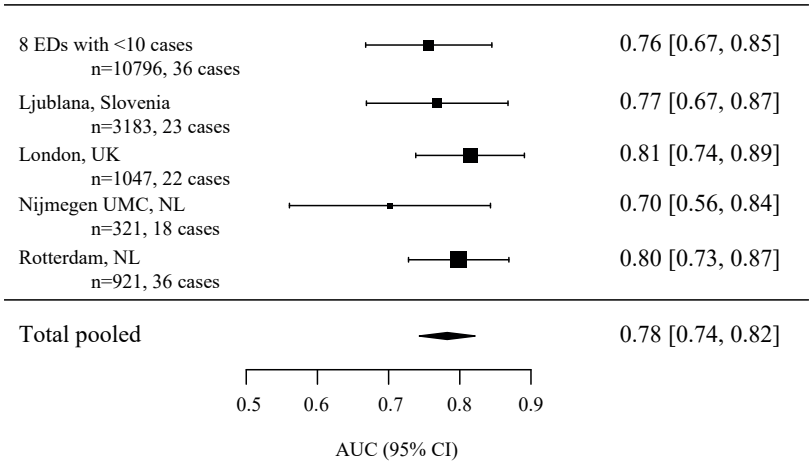

Figure 2 | Discriminative value of the prediction model for invasive bacterial infection for five internal-external cross-validations. The model was repeatedly derived on four ED groups, and validated on the fifth ED group which was left out from the derivation. The five cross-validations were pooled using a random-effects model. More details are provided in Figure A in Online supplemental appendix 3.
Legend: ED, emergency department; CI, confidence interval; UK, united kingdom; NL, the Netherlands; UMC, University Medical Centre

The diagnostic performance was good for the rule-out threshold of 0.1% with sensitivity of 0.97 (95% CI 0.93 to 0.99) and negative LR of 0.09 (95% CI 0.03 to 0.23) (table 3, online supplemental appendix 10). For the rule-in threshold of 2.0%, the model had specificity 0.94 (95% CI 0.94 to 0.95) and positive LR of 8.4 (95% CI 6.9 to 10.0). The intermediate thresholds of 0.1-2.0% performed poorly (ranges: sensitivity 0.59-0.93, negative LR 0.14-0.57, specificity 0.52-0.88, positive LRs 1.9-4.8) and comprised 9784 (60.1%) patients. The rule-in threshold misclassified four patients with IBI from three different EDs, including

Table 3 | Diagnostic performance of the prediction model for different risk-thresholds for invasive bacterial infection

Risk Thresholds	N below threshold (%)	N above threshold (%)	Sensitivity (95% CI)	Negative LR (95% CI)	Specificity (95% CI)	Positive LR (95% CI)
0.1%	5,495 (33.8)	10,773 (66.2)	0.97 (0.93-0.99)	0.09 (0.03-0.23)	0.34 (0.33-0.35)	1.5 (1.4-1.5)
0.2%	8,461 (52.0)	7,807 (48.0)	0.93 (0.87-0.96)	0.14 (0.08-0.26)	0.52 (0.52-0.53)	1.9 (1.9-2.1)
0.25%	9,416 (57.9)	6,852 (42.1)	0.90 (0.84-0.95)	0.17 (0.10-0.28)	0.58 (0.58-0.59)	2.2 (2.0-2.3)
0.5%	12,200 (75.0)	4,068 (25.0)	0.76 (0.67-0.83)	0.32 (0.24-0.44)	0.75 (0.75-0.76)	3.1 (2.8-3.4)
1.0%	14,224 (87.4)	2,044 (12.6)	0.59 (0.50-0.67)	0.47 (0.39-0.58)	0.88 (0.87-0.88)	4.8 (4.1-5.6)
2.0%	15,279 (93.9)	989 (6.1)	0.48 (0.39-0.57)	0.55 (0.47-0.65)	0.94 (0.94-0.95)	8.4 (6.9-10)
5%	15,831 (97.3)	437 (2.7)	0.36 (0.37-0.45)	0.65 (0.57-0.74)	0.98 (0.97-0.98)	15 (12-19)

CI, confidence interval; LR, likelihood ratio

two patients with arthritis, and two patients with a sinusitis and pneumonia resulting in bacteraemia. Three of these patients had CRP levels <10 mg/L and symptoms <1 day.

In sensitivity analysis involving the population with imputed CRP levels (n=37093, IBI n=135), model development yielded similar coefficients (online supplemental appendix 11).

Discussion

Based on the Feverkidstool and important predictors for early recognition of IBI, we derived and cross-validated a clinical prediction tool, in febrile children at different European EDs. The prediction model discriminated well between patients with and without IBI. The risk-threshold of 0.1% has good rule-out value for IBI and thus decreases the risk of missing an IBI. The higher risk thresholds of >2.0% have good rule-in value and these thresholds can be used to identify patients at high-risk of IBI to target treatment. The large number of patients with intermediate risk of 0.1-2.0% for IBI is expected to benefit most from sensitive biomarkers.

Strengths of this study include the participation of 12 European EDs based in 8 countries with a broad population of febrile children of all ages and chronic conditions. Furthermore, we performed five cross-validations which provided us insight in heterogeneity between EDs, and improves the generalisability of our results. Second, we included a large number of IBI cases, whilst previous studies did not have sufficient cases to define a prediction model exclusively for IBI.[9-11] Furthermore, our model involves accessible predictors as clinical symptoms and CRP level, which will facilitate implementation in practice. We provide clinical case examples of the model (online supplemental appendix 12) and, to help physicians to use this model in practice, a web-based digital calculator will be developed.

Our study has some limitations. First, we focused our study on patients who had CRP measurement on indication. This involved more severe illness than patients without CRP measurement. However, the CRP group reflect patients with diagnostic uncertainty and is more likely to benefit from a clinical prediction model. All patients with IBI had CRP measurement, leading to inclusion of all eligible IBIs in the main analysis. In our sensitivity analysis, predictors were similar in the model developed on imputed CRP-levels. Therefore, model performance was not influenced by selection of patients with CRP measurement. Second, diagnostic tests were ordered according to usual care. If patients with an IBI did not have cultures taken >24 hours after hospital admission, this was not

included in the data and these patients could have been misclassified as non-IBI. Since diagnostic workup is in general performed at the ED or <24 hours after presentation, this misclassification is minimized. Third, due to the low incidence of IBI, model performance was evaluated in cross-validation with a lower number of cases than is optimal for validation (100 cases).[33 34] Although discrimination of the model was good in the cross-validations, calibration was poor to moderate. The low incidence of IBI and other case-mix differences not taken into account by our model may have influenced model performance in the cross-validation. Our range of IBI incidence (range EDs 0.1-5.6%) was comparable with IBI incidence in other studies including febrile population of all age-ranges (range 0.4-4.5%).[9 11 35] Fourth, due to limited measurements of systolic blood pressure (14.7%) and procalcitonin in our cohort (1.6%), we were not able to include these as predictor. Lastly, data on individual immunisation status were not available and were not included in the model. In the clinical assessment of febrile patients, immunisation status should be taken into account.

Patients with and without IBI were discriminated well in the cross-validations. Calibration was poor to moderate indicating discrepancy between model predictions and the observed risk of IBI. Addition of the ED covariate of low/high incident IBI improved calibration, indicating that model performance is influenced by the likelihood of IBI in the ED. Therefore, ED incidence should be included in the model.

Clinical prediction models involving older children are the Feverkidstool and Irwin's model, and predict pneumonia and other serious bacterial infections separately, whereas our model focuses on IBI. Discrimination of our model in cross-validation (pooled AUC: 0.77 (95% CI 0.73 to 0.81) was better compared to one external validation and similar to another external validation of the Feverkidstool for other serious bacterial infection.[9 11] Unlike our study, these models were not based on an European-wide ED population. We recommend to use the Feverkidstool to guide antibiotic prescription in suspected lower respiratory tract infections [18] and to use our model in febrile children to predict IBI. These two models, the original Feverkidstool and our model will be integrated in one electronic decision tool. For both implementation of the Feverkidstool and our model, measurement of (point-of-care) CRP is necessary. We do not recommend CRP measurement in all febrile children, but since CRP-level is an important discriminator in bacterial and viral illness, measurement should be easily accessible to aid in the decision-making process at the ED.

Missing and undertreatment of IBI in children can lead to morbidity and mortality. Current practice is to start antibiotic treatment in patients at risk for bacterial infection awaiting culture results which take >48 hours. Since the low incidence of IBI, this leads

to overuse of antibiotics and resources. The balance of not missing IBIs and overtreating self-limiting infections is delicate. Therefore, clinical prediction models can help in decision making at the ED. Our study showed that the low-risk threshold can be helpful to rule-out IBI and to reduce invasive diagnostics and antibiotic use.

Starting early treatment is key to prevent adverse outcomes due to IBI. The high-risk threshold of >2.0% can be used for targeted treatment with intravenous antibiotics. Although our model was able to identify 38% of the study population as low or high risk, diagnostic uncertainty exist for the intermediate group (60%). In our study, this intermediate group with diagnostic uncertainty was estimated as 25% of the population of febrile children presenting to the ED, including patients without CRP measurement. Additional diagnostics including procalcitonin, repeated CRP measurement[36] or novel sensitive biomarkers may be helpful in the decision-making for this intermediate-risk group. The potential benefit of additional diagnostics using these risk-thresholds will need to be evaluated in future studies.

Conclusion

Based on the Feverkidstool and important clinical predictors, we derived and cross-validated a clinical prediction model for early detection of IBI in febrile children in an European wide cohort. Where the rule-in threshold of this model could target early treatment to reduce adverse outcomes from IBI, the rule-out threshold has the potential to reduce unnecessary use of invasive diagnostics and antibiotics. However, more than half of the population was at intermediate risk. In this group, sensitive, new biomarkers could improve identification of IBI and could potentially reduce unnecessary antibiotic use.

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Appendix

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Appendix 1 | Statistical analysis plan

Appendix 5 | Patient characteristics of patients with CRP measurement and patients without CRP measurement

Appendix 6 | Details of patients with complex chronic conditions

Appendix 11 | Sensitivity analysis: model development on population with imputed CRP-level (n=37093)

Appendix 12 | Clinical case examples

Appendix 2: Definition of contaminants

Definition of contaminants
Micrococcus
Coagulase-negative staphylococci
Propionibacterium species
Alpha-haemolytic streptococci (except pneumococcus)
Corynebacterium species (diphteroids)
Bacillus species
Pseudomonas (except P. aeruginosa)
Other environmental non-fermenting gram-negative rods

Appendix 3: Additional methods on data analysis

Multiple imputation

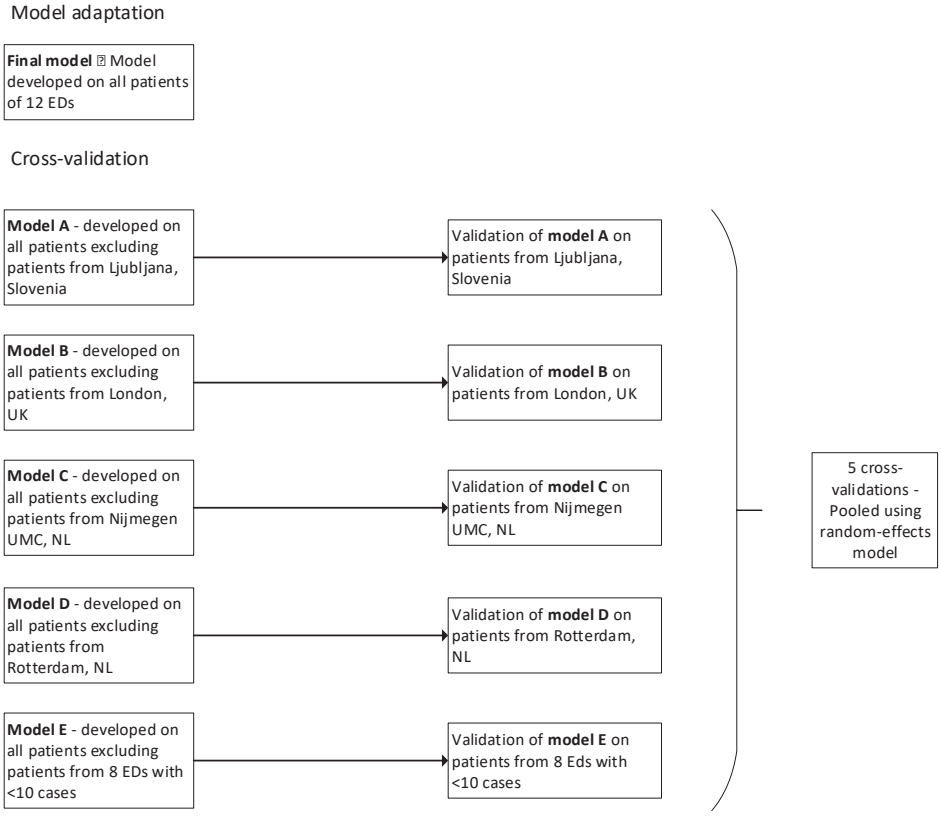
Missing data were multiple imputed using the MICE package in R v3.4. The imputation model included the outcome variable IBI, all considered predictors, ED and other auxiliary variables related to casemix and disease severity (specific details of the multiple imputation model are proved in the Statistical Analysis Plan). The imputation process resulted in 20 imputation sets. For all the statistical analysis, apart from the model development in LASSO (least absolute shrinkage and selection operator), results were pooled for a final result.(1) The LASSO was applied to a stacked dataset containing all imputed data.(2) To adjust for the inflated sample size we assigned each record a weight of 1/20 (20 is number of imputed datasets).

Model development and internal-external cross-validation

For model development (3, 4), we considered predefined variables with predictive value for IBI: 1) variables in the Feverkidstool(5) (age, sex, temperature, fever duration, tachypnea and tachycardia defined by Advanced Pediatric Life Support(6), oxygen saturation <94%, capillary refill >=3 seconds, work of breathing, ill appearance and CRP value), 2) NICE warnings signs which were not included in the Feverkidstool (consciousness, meningeal signs, focal neurology, status epilepticus, non-blanching rash)(7) and 3) complex chronic condition (condition in ≥2 body systems, malignancy or immunocompromised). (8) Level of consciousness, meningeal signs and focal neurology were combined into a composite variable abnormal neurology. Linearity of continuous variables was assessed using restricted cubic splines. As in the Feverkidstool, age was modelled linear piecewise for children <1 year and children >1 year and a logarithmic transformation for CRP was used. Outliers were truncated at the 0.01 percentile for temperature (35.7 °Celsius) and the 0.99 percentile for CRP (215 mg/L) and fever duration (8 days).

Variable selection was not influenced by the results of the univariate logistic regression analysis, but was performed using least absolute shrinkage and selection operator (LASSO).(9, 10) This approach aims to reduce the degree of overfitting by shrinking large regression coefficients and performs variable selection.(10) The lambda to derive the final model was estimated using 10 times 10-fold cross-validation. We used internal-external cross-validation in EDs with >10 IBI cases (four EDs) and EDs with <10 IBI cases (eight EDs) were combined in one group leading to five ED groups (appendix 5). In internal-external cross-validation The model was repeatedly derived on all ED groups except one, and validated on the remaining ED group (see figure A below).(11) Unlike splitting data in a derivation and validation set, this method uses all available data for the model development and uses cross-validation to validate the model five times. This cross-validation determines model performance most accurately but also provides information on the heterogeneity of performance across different settings. This internal-external cross-validation is therefore superior to a single external validation.(11, 12) We assessed the discriminative ability by the area under the receiver operating curve (AUC), and calibration, the agreement between predicted risks and observed cases., was evaluated by calibration plots. We explored the impact of difference in case-mix heterogeneity on the discriminative ability of the model in the internal-external cross-validation. Sensitivity, specificity, negative and positive likelihood ratios (LR) were evaluated at different cut-offs for the individual probability of IBI according to the model. We explored cut-offs for ruling-out (negative LR <0.2) or ruling-in IBI (positive LR >5).(13) Missing values for the covariates were multiple imputed (MICE). Sensitivity analysis was performed in the population where missing CRP values were imputed. All analyses were performed in R v3.6.

Figure A


Model adaptation
Final model Model developed on all patients of 12 EDs
Cross-validation
Model A - developed on all patients excluding patients from Ljubljana, Slovenia
Validation of **model A** on patients from Ljubljana, Slovenia
Model B - developed on all patients excluding patients from London, UK
Validation of **model B** on patients from London, UK
Model C - developed on all patients excluding patients from Nijmegen UMC, NL
Validation of **model C** on patients from Nijmegen UMC, NL
Model D - developed on all patients excluding patients from Rotterdam, NL
Validation of **model D** on patients from Rotterdam, NL
Model E - developed on all patients excluding patients from 8 EDs with <10 cases
Validation of **model E** on patients from 8 Eds with <10 cases
5 cross-validations - Pooled using random-effects model

Appendix 4: EDs - classification of EDs with low (<2%) and high incidence (>2%) for IBI based on proportion of invasive bacterial infection, and proportion of chronic complex comorbidity per ED

ED	N total included patients	N study population	IBIs N (% of study population per ED)	Chronic complex comorbidity N (% of study population per ED)
Graz, Austria	2241	1987	1 (0.1%)	73 (3.7%)
Athens, Greece	4548	1450	1 (0.1%)	19 (1.3%)
Riga, Latvia	9000	5495	9 (0.2%)	60 (1.1%)
Munich, Germany	1173	456	1 (0.2%)	19 (4.2%)
Nijmegen, CWZ, the Netherlands	423	184	1 (0.5%)	12 (6.5%)
Ljubljana, Slovenia	3667	3183	23 (0.7%)	61 (1.9%)
Liverpool, UK	1623	468	8 (1.7%)	76 (16.2%)
Newcastle, UK	3854	475	9 (1.9%)	41 (8.6%)
London, UK	5714	1047	22 (2.1%)	184 (17.6%)
Santiago de Compostela, Spain	3877	281	6 (2.1%)	9 (3.2%)
Rotterdam, the Netherlands	1683	921	36 (3.9%)	369 (40.1%)
Nijmegen, UMC, the Netherlands	677	321	18 (5.6%)	135 (42.1%)
Total	**38480**	**16268**	**135**	**1058**
EDs with low incidence for IBI (<2%)		13698	53 (0.4%)	367 (2.7%)
EDs with high incidence for IBI (>2%)		2570	82 (3.2%)	364 (14.2%)

ED, emergency department; IBI, invasive bacterial infection; UK, United Kingdom; UMC, university medical centre; CWZ, Canisius Wilhelmina Hospital

Appendix 7: Univariate logistic regression analysis for invasive bacterial infection.

Univariate logistic regression analysis for invasive bacterial infection. N=16268, IBI cases N=135	
Variables	**OR (95%CI)***
Feverkidstool	
Male	1.04 (0.74-1.46)
Age <1 year±	0.25 (0.14-0.43)*
Age >1 year±	1.01 (0.97-1.05)
Temperature in °C	1.34 (1.13-1.59)*
Fever duration in days	0.89 (0.80-0.99)*
Tachypnea (APLS)	1.50 (1.03-2.18)*
Tachycardia (APLS)	2.84 (2.01-4.01)*
o2 saturation <94%	0.65 (0.24-1.75)
Prolonged capillary refill time (>3 sec)	2.62 (1.24-5.56)*
Presence of work of breathing	1.62 (0.90-2.93)
Ill appearance	2.51 (1.76-3.58)*
Ln CRP	1.89 (1.63-2.19)*
NICE alarming signs	
Status epilepticus	No cases
Reduced level of consciousness	4.70 (2.04-10.83)*
Focal neurology	2.30 (0.54-9.71)
Meningeal signs	9.20 (4.54-18.62)*
Abnormal neurology: decreased level of consciousness, presence of meningeal signs or focal neurology	4.81 (2.61-8.91)
Non-blanching rash	2.31 (1.21-4.41)*
Chronic condition	
Complex chronic condition	8.83 (6.19-12.59)*

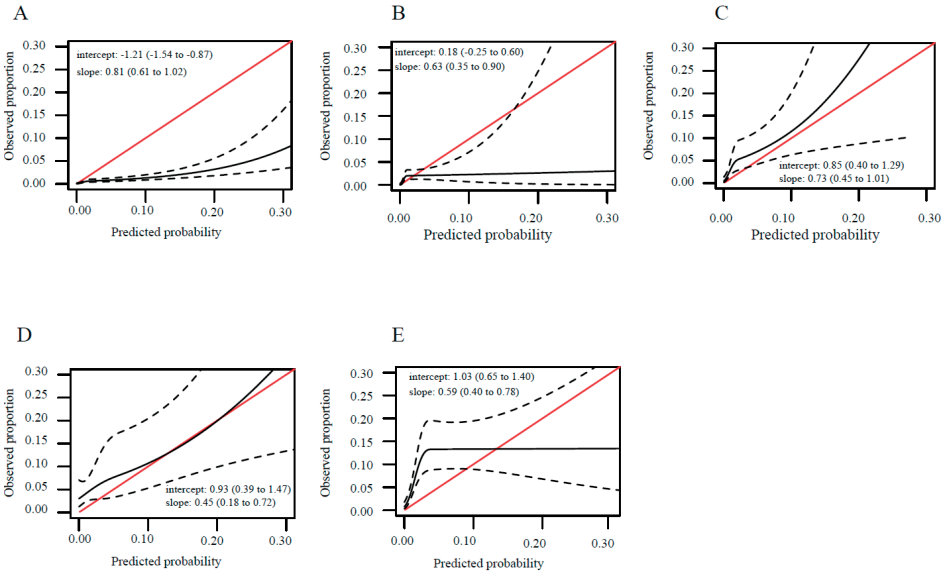
*Significant, $p<0.05$

±The risk of children aged < 1 year was calculated: β(age <1 year)×age in years.

The risk of children aged >1 years was calculated with: β(age <1 year)×1+β(age ≥1 year)×(age in years−1).

APLS, Advanced Paediatric Life Support; CRP, C-reactive protein; ln, natural log

Appendix 8: Calibration plot: observed proportion vs predicted probability of the clinical prediction model for 5 internal-external cross-validations.



The solid red line with a slope of 1 and intercept of 0 represents ideal prediction accuracy. The dotted lines indicate the 95% confidence interval.

A, Model developed on leave-out EDs with <10 cases, validated on EDs with <10 cases

B, Model developed on leave-out Ljubljana (Slovenia), validated on Ljubljana (Slovenia)

C, Model developed on leave-out London (UK), validated on London (UK)

D, Model developed on leave-out Nijmegen (the Netherlands), validated on Nijmegen, UMC (the Netherlands)

E, Model developed on leave-out Rotterdam (the Netherlands), validated on Rotterdam (the Netherlands)

Legend: ED, emergency department; UK, united kingdom; UMC, University Medical Centre

Appendix 9: Model 2 – model specification and performance

In model 2 the variable ED with low/high IBI incidence is added to the model.

Model 2 – model specification

	Model specification of multivariate logistic model for IBI, model 2 with the addition of variable low/high IBI incidence ED		
		Coefficients	**OR**
	(Intercept)	-6.13	0.00
Feverkidstool	Male	-0.16	0.85
	Age < 1 year*	-2.22	0.11
	Age ≥ 1 year*	0.00	1.00
	Temperature	-0.16	0.85
	Fever duration in days	-0.15	0.86
	Tachypnea	-0.47	0.62
	Tachycardia	0.66	1.94
	Hypoxia	-0.81	0.44
	Prolonged capillary refill	-0.31	0.74
	Increased work of breathing	-0.47	0.62
	Ill appearance	1.18	3.26
	Ln CRP	0.75	2.11
NICE warning signs	Abnormal neurology	1.10	3.01
	Non-blanching rash	1.06	2.89
Chronic condition	Complex chronic condition	1.56	4.78
IBI incidence	ED with high IBI incidence (>2%)	1.98	7.26

*Age <1 year and age ≥ 1 year were calculated linear-piecewise:
The risk of children aged < 1 year was calculated: β(age <1 year)× age in years.
The risk of children age ≥ 1 year was calculated: β(age <1 year) × 1 + (age in years-1) × β (age ≥ 1 in years).
CRP, C-reactive protein; IBI, invasive bacterial infection; ln, natural log

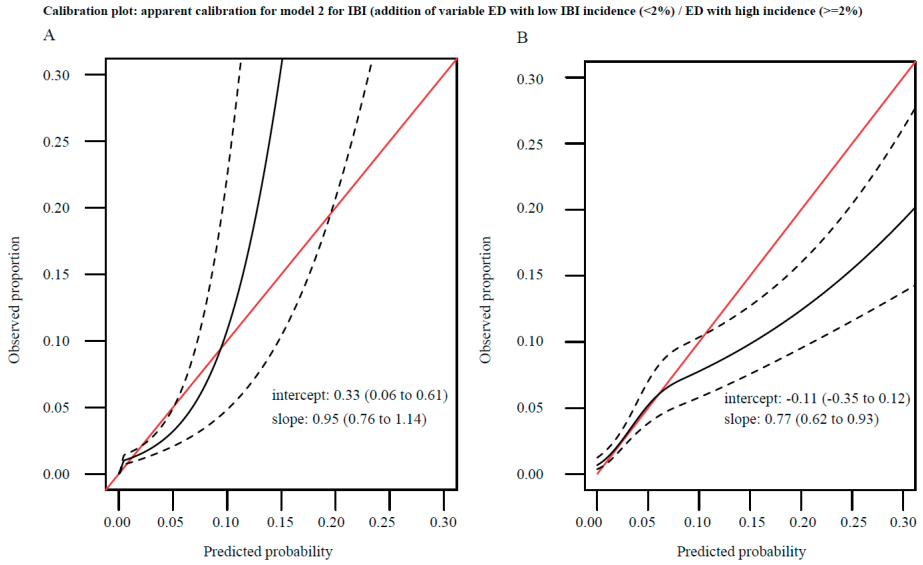
Model 2 – performance

Discrimination:

Development model 2: C-statistic 0.88 (95%CI 0.85-0.90)

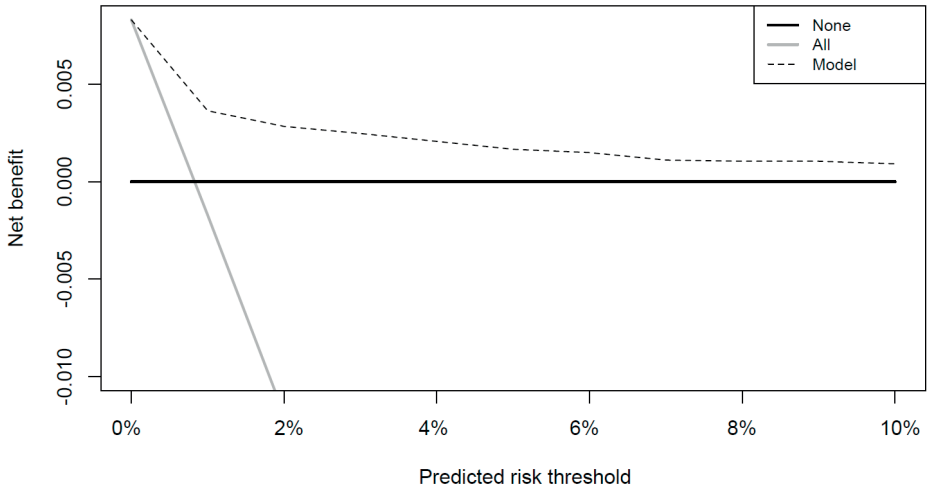
Calibration:

Apparent calibration for model 2 for IBI (addition of variable ED with low IBI incidence (<2%) / ED with high IBI incidence (>=2%)). Risk predictions are calculated on the developed model using all data (n=16268). These risk predictions are calibrated in the two groups: EDs with low IBI incidence (A) and EDs with high IBI incidence (B). ED, emergency department; IBI, invasive bacterial infection



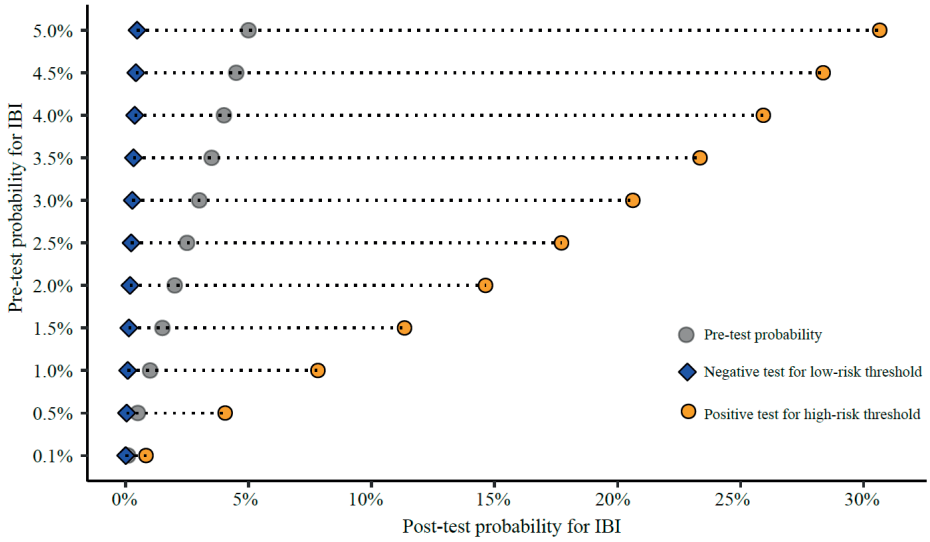
Appendix 10: Performance of the prediction model (model 1)

Decision curve analysis



Post-test probability for varying pre-test probabilities for invasive bacterial infection (IBI)

Negative test for the low-risk threshold (0.1%) and positive test for the high-risk threshold (2.0%)



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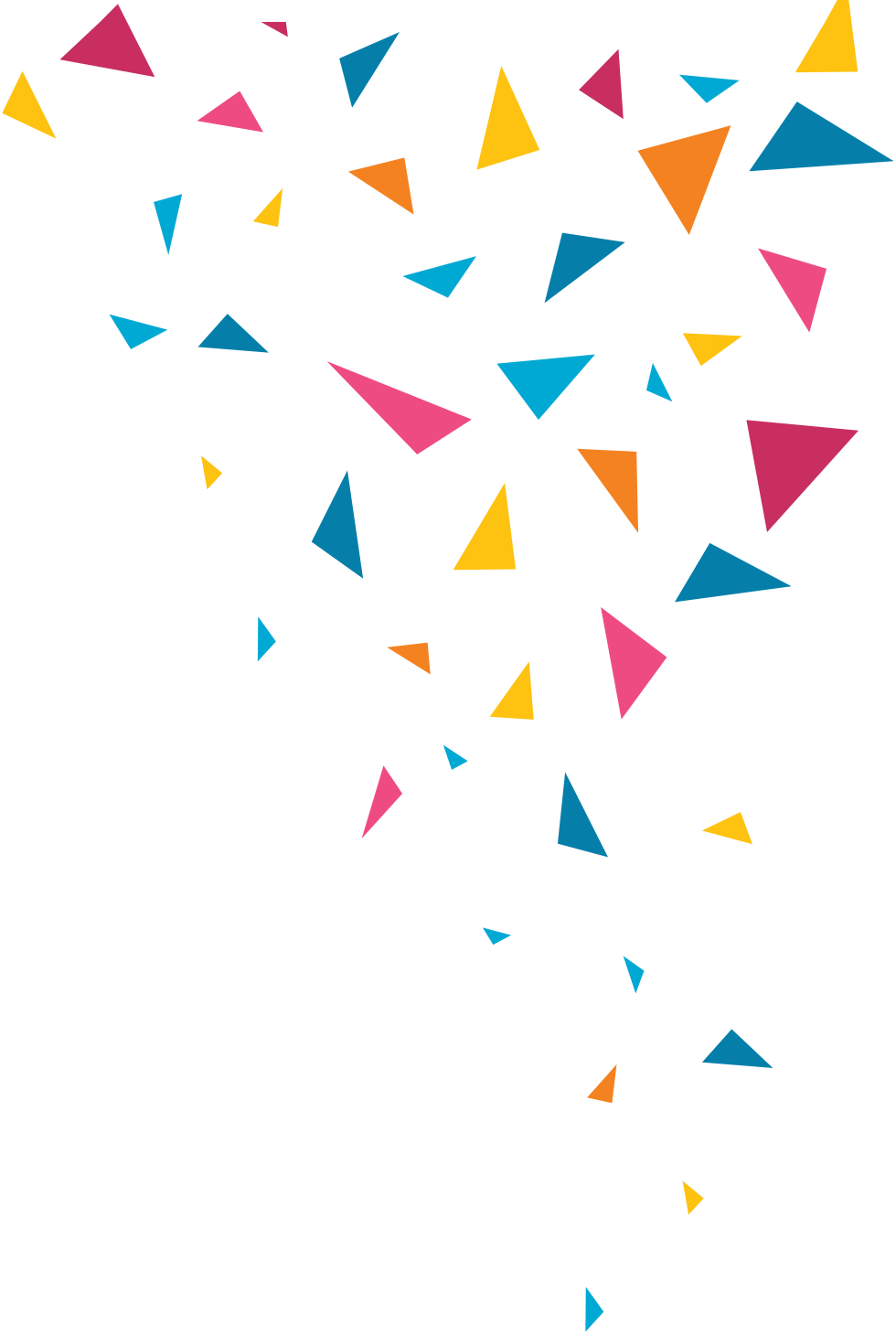

6

Association of monocyte HLA-DR expression over time with secondary infection in critically ill children: a prospective observational study

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Submitted.

Abstract

An impaired immune response could play a role in the acquisition of secondary infections in critically ill children. Human leukocyte antigen-DR expression on monocytes (mHLA-DR) has been proposed as marker to detect immunosuppression, but its potential to predict secondary infections in critically ill children is unclear. We aimed to assess the association between mHLA-DR expression at several timepoints and the change of mHLA-DR expression over time with the acquisition of secondary infections in critically ill children.

In this prospective observational study, children <18 years with fever and/or suspected infection (community-acquired or hospital-acquired) were included at a paediatric intensive care unit in the Netherlands. mHLA-DR expression was determined by flow cytometry on day 1, day 2-3 and day 4-7. The association between delta-mHLA-DR expression (difference between last and first measurement) and secondary infection was assessed by multivariable regression analysis, adjusted for age and Paediatric Logistic Organ Dysfunction-2 score. We included 104 patients at the PICU (median age 1.2 years [IQR 0.3-4.2]), of whom 28 patients (27%) developed a secondary infection. Compared to 93 healthy controls, mHLA-DR expression of critically ill children was significantly lower at all timepoints. mHLA-DR expression did not differ at any of the time points between patients with and without secondary infection. In addition, delta-mHLA-DR expression was not associated with secondary infection (aOR 1.00 [95% CI 0.96-1.04]).

Conclusions Our results confirm that infectious critically ill children have significantly lower mHLA-DR expression than controls. mHLA-DR expression was not associated with the acquisition of secondary infections.

Introduction

Critically ill patients are at risk for a prolonged period of immunosuppression, potentially leading to an increased risk of secondary infections.[1-4] Secondary infections, acquisition of an infection during hospital admission, occur in 11 to 19% of paediatric intensive care unit (PICU) admissions [5-7], causing prolonged hospital stay, morbidity, mortality and costs.[8]

Immunosuppression has been established in critically ill patients following trauma, surgery or stroke [9-14], but most studies have focused on adult patients with sepsis and septic shock.[3,15-22]

Monocytic human leukocyte antigen-DR (mHLA-DR) expression has been shown a reliable biomarker in critically ill patients to estimate immunosuppression. In adults, a prolonged decrease in mHLA-DR expression has been associated with acquisition of secondary infection and mortality in small studies [13,19,20,23,24] although one study did not found an association.[25] The largest study to date included >400 adult patients and found lower mHLA-DR values in non-survivors. However, the authors concluded that mHLA-DR expression was not suitable as predictive parameter due to limited discriminative ability.[26]

In children, small studies have confirmed an association of reduced mHLA-DR with secondary infection or mortality in post-operative critically ill children[27], in children with critical illness for multiple reasons [28] and in septic children.[16,18] In 30 paediatric septic patients, Manzoli *et al.* found an association of decreased mHLA-DR expression with mortality but not with secondary infections.[18] On our PICU, critically ill children are admitted with a wide range of infectious diseases including suspected community-acquired infections and hospital-acquired infections. More insight in mHLA-DR expression and the relation with mortality and secondary infections in these children can aid to identify patients who could benefit from immunostimulatory therapies.

Therefore, we performed a large study including children with a wide range of infectious diseases to assess the relation of mHLA-DR expression with adverse outcomes. The association of mHLA-DR expression at several timepoints and the change of mHLA-DR expression over time was studied and related to mortality and the acquisition of secondary infections.

Methods

Study design and population

This is a pre-planned prospective observational study embedded in the PERFORM project (Personalised Risk assessment in Febrile illness to optimise Real-Life Management across the European Union, www. perform2020.org).[29] The overarching aim of PERFORM is to improve diagnosis and management of febrile children by development of a new diagnostic tests to discriminate bacterial from viral infections. For this particular study, we included critically ill children (aged 0-17 years) admitted to the level 3 PICU of Erasmus MC-Sophia Children's hospital (Rotterdam, the Netherlands, mixed surgical/medical) between March 2017 and April 2019. Inclusion criteria included fever (body temperature of ≥ 38° C) and/or a suspected infection in patients who had an arterial or central venous line *in situ*. This comprised both children who were admitted through the Emergency Department (community-acquired infection) and children who were already admitted and developed an infection during hospital admission >48 hours after admission (hospital-acquired infection). Exclusion criteria were presence of chronic conditions that affect immune status (immunodeficiency, malignancy, chronic immunosuppressive medication including corticosteroids) and severe anaemia (haemoglobin < 6.5 g/dL). In addition, we included afebrile healthy controls who underwent elective surgery for a minor condition. Exclusion criteria included fever in the three week prior to surgery or presence of more than one chronic condition. To cover all ages, we aimed to include 20 healthy children in clinically relevant age groups: 0-1 years, 1-2 years, 2-5 years, 5-12 years, and 12-16 years. Informed consent was obtained from children >12 years and parents or legal guardians.

Clinical data

Prospective clinical data were collected from electronic medical records. We collected baseline data on the first day of PICU admission or, in a case of suspected hospital-acquired infection, at the onset of the infectious disease episode on the PICU. Collected data included demographics, chronic conditions, Paediatric risk of Mortality (PRISM) score [30], Paediatric Logistic Organ Dysfunction-2 (PELOD-2) probability of mortality [31] and signs of septic shock (sepsis and cardiovascular organ dysfunction as defined by *Goldstein et al.)*.[32] The cause of the initial infection was classified in presumed bacterial, presumed viral, unknown bacterial/viral or other, according to a published flowchart including clinical signs and symptoms and microbiological cultures/PCR.[33] In addition, for a period of 28 days following inclusion, we collected days of mechanical ventilation, days of inotropic support, survival status and number of PICU-free days at day 28.

Outcome measures

The outcome measures included 28-day mortality and acquisition of a secondary infection defined by the surveillance definition of hospital-acquired infections by Centres for Disease Control and Prevention according to previous studies.[7,28,34] All suspected secondary infections during 28 days of follow-up after inclusion were reviewed by trained clinical researchers (NH, PK, JW) and discussed with one paediatric infectious disease specialist (CV). All researchers were blinded regarding mHLA-DR expression at the time of reviewing.

Blood sampling and measurement of monocytic HLA-DR expression

Blood samples were taken either on PICU admission or at the onset of the infectious episode during PICU stay. Follow-up samples were taken at day 2-3, day 4-7 and once a week with a maximum of 5 blood draws in total. The number of time points in which follow-up sampling could be performed was restricted by logistic reasons, limitations of blood sample volumes and lack of parents' consent. For healthy controls, a single sample was drawn during insertion of the peripheral catheter before start of surgery. Samples were analysed for mHLA-DR expression on a flowcytometer (FACSCanto-II, BectonDickinson) using the Anti-HLA-DR/Anti-Monocyte Quantibriteassay (BD Biosciences), as described previously.[35,36] This assay approach uses an HLA-DR antibody conjugated to phycoerythrin (PE) in a 1:1 ratio as well as a mixture of beads to which a defined amount of PE molecules have been conjugated. In the end, this allows to calculate the number of HLA-DR-PE antibodies bound per cell (AB/c). Laboratory technicians conducting the assay were blinded for the clinical data.

Data analysis

We compared results of mHLA-DR expression of patients with suspected infections vs healthy controls, and patients with and without secondary infections. Differences between the groups were tested for significance with Mann-Whitney U test, Student's t-test or Kruskal-Wallis test when appropriate. To assess the change of mHLA-DR expression over time during PICU admission, we calculated delta-mHLA-DR: the difference of mHLA-DR expression between the latest timepoint and the first timepoint.

In logistic regression analysis, we adjusted for age and baseline PELOD-2 score. We assessed the association of delta-mHLA-DR for acquisition of secondary infections, and for the composite outcome acquisition of secondary infections and/or 28-day mortality. Based on delta-mHLA-DR, patients were classified as improved (delta >20% from baseline), declined (delta < -20% from baseline), or stable (<20% difference from baseline). The delta 20% from baseline was chosen to ensure sufficient numbers for statistical analysis. We tested the interaction between delta-mHLA-DR and delta-mHLA-DR group

(improved, declined, stable) for secondary infection using the likelihood ratio test. In addition, we tested the interaction between delta-mHLA-DR and cause of the initial infection (presumed bacterial, presumed viral, unknown bacterial/viral or other). Lastly, we explored the association of delta-mHLA-DR in the subgroup with low mHLA-DR, defined as values below the 25th centile at baseline in our population. All data analyses were performed in R version 3.6 and a P-value <0.05 was considered significant.

Results

Study population

We included 104 patients with suspected infection and 93 healthy controls with available mHLA-DR measurements. Compared to controls, infectious patients were younger (median 1.2 years [IQR 0.3-4.2] vs 3.6 years [IQR 1.1-9.9]) but were similar in sex ((male: 60% (62/104) vs 66% (61/93)) (additional file 1). Of the 104 infectious patients, the initial infection was a suspected community-acquired infection in 36% (n=37) and a suspected hospital-acquired infection in 64% (n=76). Seven patients died (7%) and 28 patients (27%) acquired a secondary infection after inclusion which occurred after a median of 9 days (IQR 5-16 days) (**Fig. 1**, additional file 2). Compared to patients without a secondary infection, patients with a secondary infection were similar in age, sex, initial infection and baseline PELOD-2 and PRISM score. Patients with a secondary infection, however,

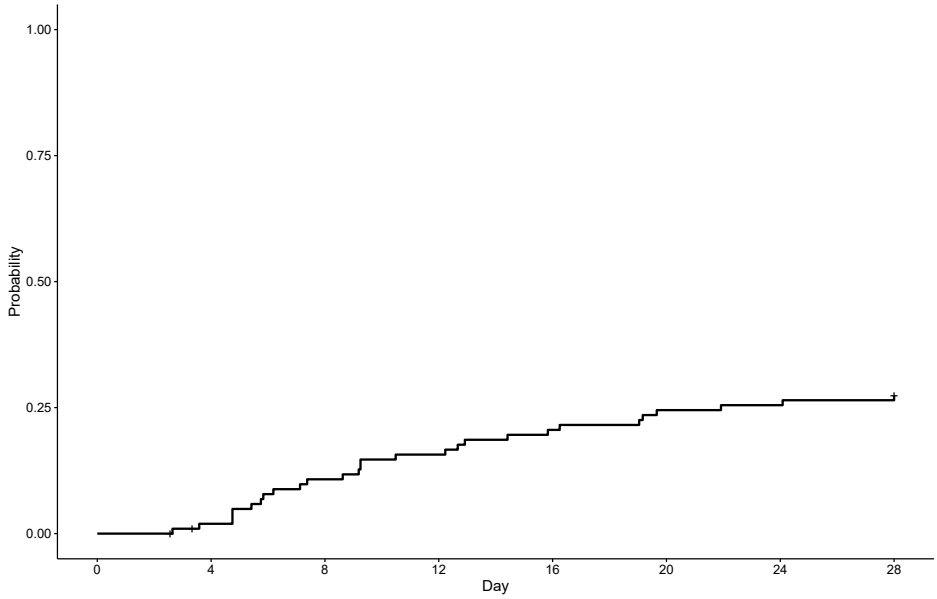

Fig. 1 | Cumulative incidence of secondary infection (n=104)

had more ventilation and inotropes days and were admitted longer to the PICU (Table 1) than patients without a secondary infection. Comparing children with suspected community-acquired and hospital-acquired infections, no differences on baseline PRISM or PELOD-2 scores or occurrence of secondary infections were observed.

Table 1 | Descriptive characteristics of critically ill children with suspected infections (n=104)

	Secondary infection	No secondary infection	p-value
	n=28	**n=76**	
Age in years, median [IQR]	1.3 (0.5-3.1)	1.0 (0.2-4.2)	0.47
Male	18 (64.3)	44 (57.9)	0.72
Any chronic underlying condition*	21 (75.0)	54 (71.1)	0.88
Pulmonary	5 (17.9)	11 (14.5)	
Prematurity	2 (7.1)	7 (9.2)	
Gastro-intestinal		3 (3.9)	
Neurological	2 (7.1)	7 (9.2)	
Cardio	15 (53.6)	36 (47.4)	
Endocrinologic	1 (3.6)		
Genetic	6 (21.4)	9 (11.8)	
PRISM score at day 1, median [IQR]	15 (12-22)	14 (9-19)	0.26
PELOD-2 probability of mortality at day 1, median [IQR]	2.2 (1.3-6.2)	1.4 (0.5-3.5)	0.11
PICU admission duration in days during study period, median [IQR]	19 (9-36)	8 (2-17)	0.003
Days of invasive ventilation during study period	7 (2-18)	2 (0-5)	0.003
Days of inotropes during study period	2 (0-9)	1 (0-2)	0.02
Signs of septic shock at baseline	9 (32.1)	15 (19.7)	0.26
Phenotype initial infection			0.08
Presumed bacterial	13 (46.4)	19 (25.0)	
Presumed viral	3 (10.7)	16 (21.1)	
Unknown bacterial/viral	3 (10.7)	6 (7.9)	
Other	9 (32.1)	35 (46.1)	
Mortality	4 (14.3)	2 (2.6)	0.07

*multiple categories per patient
IQR, interquartile range; PELOD-2, Paediatric Logistic Organ Dysfunction-2; PRISM, Paediatric risk of Mortality;

Monocytic HLA-DR expression

In controls, mHLA-DR expression was 29500 AB/cell (IQR 23800-37600) and was similar across age groups (0-1 years 28000 [IQR 26100-39300]; 1-2 years 34300 [25700-42600]; 2-5 years 33900 [27600-43800]; 5-12 years 28000 [24900-35800]; 12-18 years 23600 [19700-28300]) (**Fig. 2a**). In controls, boys had higher mHLA-DR expression than girls (32900 AB/cell [IQR 25700-43300] vs 25400 [IQR 21400-32200], p<0.01) (**Fig. 2b**), but mHLA-DR expression in critically ill children was similar in girls and boys at all timepoints.

In critically ill children, mHLA-DR expression was significantly lower than in controls at all timepoints (**Fig. 2c**). No differences were observed for baseline mHLA-DR expression in community-acquired infections or hospital-acquired infections.

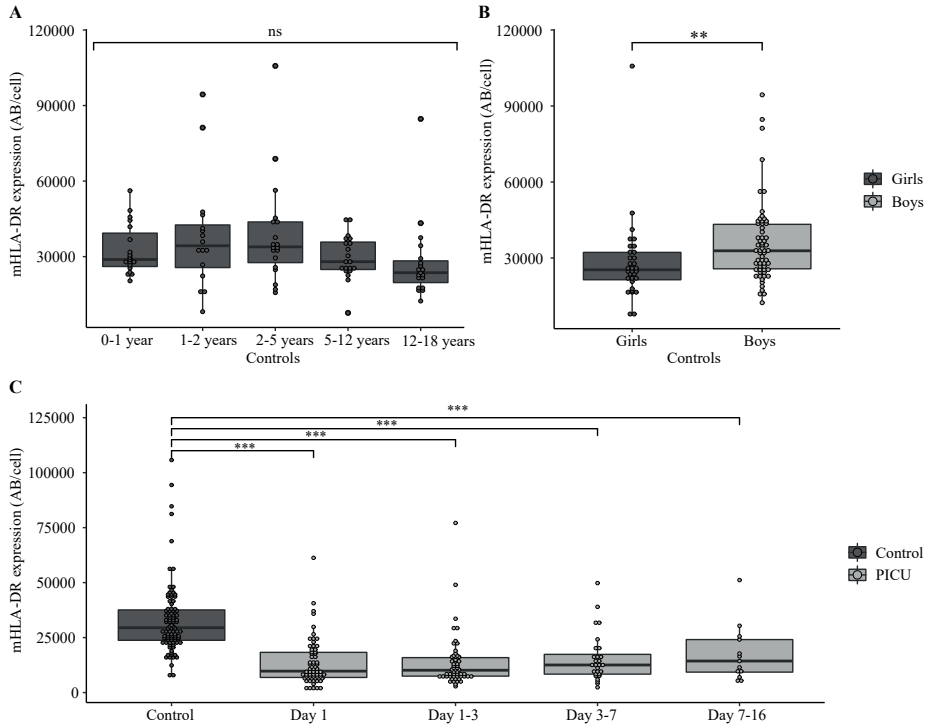

Fig. 2 | Monocyte HLA-DR expression
A: mHLA-DR expression stratified for age group in controls
B: mHLA-DR expression stratified for sex in controls
C: mHLA-DR expression for controls and children with suspected infections at different timepoints
Ns, not significant; *** p<0.001, **p<0.01

Association of monocytic HLA-DR expression with secondary infection

mHLA-DR expression did not differ for patients with and without secondary infections at any of the timepoints (**Fig. 3a**). In patients with two or more measurements (n=47), delta-mHLA-DR varied widely (range -45232 to 40944 AB/cell). Delta-mHLA-DR expression did not differ for patients with and without secondary infection (712000 AB/cell [IQR -496 to 4371] vs (497000 AB/cell [IQR -5161 to 4896]) (**Fig. 3b**). Adjusted for age and PELOD-2 score, delta-mHLA-DR was not associated with acquisition of secondary infection (aOR 1.00 [95% CI 0.96-1.04]), or the composite outcome secondary infection/mortality (aOR 0.99 [0.96-1.04]). Occurrence of secondary infection did not differ for patients classified by delta-mHLA-DR as improved (8/19, 42%), declined (4/14, 29%) or

stable (7/14, 50%), although numbers were small (**Fig. 3c**) (p>0.05 for interaction). In addition, the association of delta-mHLA-DR did not vary for presumed cause of infection (p>0.05 for interaction). In the subgroup of patients with low mHLA-DR at baseline (<7200 AB/cell, n=26), delta-mHLA-DR was not associated with secondary infection (OR 1.0 [95% CI 0.7-1.6]).

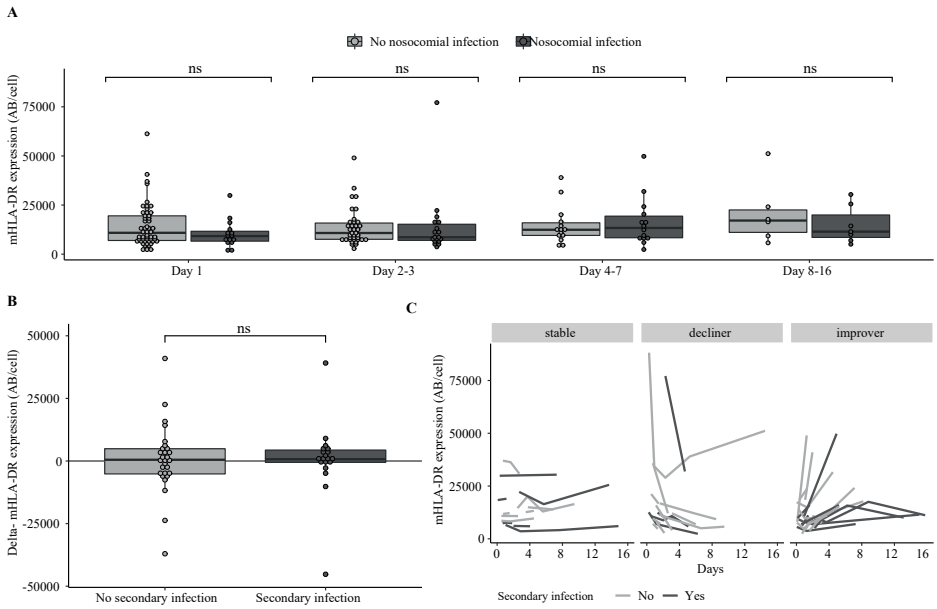

Fig. 3 | Secondary infection
A: mHLA-DR expression stratified for secondary infections at different timepoints
B: delta-mHLA-DR expression in patients with and without secondary infection
C: mHLA-DR expression in secondary infection stratified for delta-mHLA-DR groups (stable, decliner, improver)
Ns, not significant

Discussion

Main findings

In our study of critically ill children with suspected infections, mHLA-DR expression was significantly lower compared to healthy controls. No association was found between mHLA-DR expression and acquisition of secondary infections at any of the timepoints. In addition, change in mHLA-DR expression over time was not associated with the occurrence of secondary infections or the composite outcome secondary infection/mortality.

Our study also provides reference values of mHLA-DR expression in healthy children in different age groups and, similar to previous studies, we found no difference of

mHLA-DR expression for age. Compared to one previous study in children using the same measurement method, we found similar mHLA-DR values in our large sample of controls.[18] Surprisingly, healthy boys had higher mHLA-DR expression compared to girls. A previous study including healthy children did not report any differences in sex for mHLA-DR expression.[28] This sex-difference in mHLA-DR was not observed in critically ill children, although their mHLA-DR levels were also influenced by disease severity. In two studies investigating immune markers in adults, pre-surgical mHLA-DR levels did not differ between men and women.[37,38] As mHLA-DR expression did not differ in the various studied age groups, we did not perform an age-matched analysis.

Previous studies in children have investigated the relation of mHLA-DR expression with secondary infections in general ICU admissions or in septic shock: Boeddha *et al*, performed in our hospital, and Remy *et al.* reported a significant association, whereas Manzoli *et al* did not.[18,28,39] Our study, using a larger cohort of children, did not show an association of mHLA-DR expression with secondary infection. Populations included in previous studies differed: they were based on small number of patients (range 30-37 patients) and included either solely septic patients or a general ICU population, whereas our study included children with suspected infections. We included both (suspected) community-acquired and hospital-acquired infections, to cover the wide range of suspected infections at the ICU. Although these two groups might have different immunological profiles or different risks for secondary infections, we did not observe differences in baseline mHLA-DR expression or occurrence of secondary infections. In our study, the proportion of patients who suffered from a secondary infection (28%) was higher than reported by other studies including general PICU populations (11-19%).[5-7]

Although it is widely established that mHLA-DR expression is a measure for immunosuppression, cut-off values for mHLA-DR defining immunosuppression in both adults and children are yet unclear. In different adult trials which investigated immunostimulatory therapies in septic patients, cut-offs of<8000 mAB/cell and 10,000 AB/cell were used for patient selection.[40,41] Tamulyte *et al.* found that different cut-off values for mHLA-DR expression (2000 / 5000 / 8000 molecules/cell) could not predict mortality, but the cut-offs of 2000 and 5000 could discriminate patients with a longer ICU stay and ventilation days. In their study, mHLA-DR measurement was performed using novel point-of-care flow cytometry. It is yet unclear whether these cut-off values can be extrapolated to children. Manzoli *et al.* suggested that a delta of <1000 AB/cell was associated with mortality in septic children.[18] We hypothesized that different cut-offs may be needed for prediction of secondary infections. Therefore, we performed a subgroup analysis in the lowest mHLA-DR quartile but found no association of change in mHLA-DR expression with the occurrence of secondary infection.

Although we found that mHLA-DR expression is lower in our cohort of critically ill children compared to healthy controls, its clinical value is yet unclear. Our results show that in critically ill children with suspected infections mHLA-DR expression has no additional value to identify patients who are at risk for secondary infection. The immunosuppressed state of critically ill children as measured by mHLA-DR was not related to acquisition of secondary infections. Although this was not the focus of our study, mHLA-DR expression may have value in predicting patients at higher risk for a more complicated disease course, as found in adults.[10] Previous studies have used immunostimulating therapies as granulocyte-macrophage colony-stimulation factor (GM-CSF) to restore mHLA-DR levels and improved patient outcomes.[40-44] In addition, interferon-gamma improved the immune response in a child suffering from refractory candidemia with extremely low mHLA-DR.[45] Currently, these immunostimulating therapies are not routinely used in clinical practice due to the lack of predictive ability on the individual level.[26,46] Hence, future trials in children should focus on accurate identification of immunosuppression using point-of-care devices in the assessment of mHLA-DR and prediction could be improved by using multiple biomarkers involved in other pathways such as programmed death (PD)-1.[47]

The main strengths of this study include its prospective design and inclusion of large number of critically ill children with a wide variety of infections. Second, we collected detailed clinical data with complete follow-up on all patients. In addition, we used a standardized method for mHLA-DR measurement to facilitate comparison with other studies.[35,36] The major limitation of this study is that we were not able to collect follow-up samples in all patients: patients were either discharged from the PICU, had their central line removed, no consent was given for multiple blood sampling, blood volume restrictions were reached or mHLA-DR measurement was not possible due to logistic reasons. It is possible that follow-up samples were collected in critically ill children with more severe disease, which could limit the generalizability of our results to the general PICU population. Furthermore, secondary infections may occur more frequent in patients who have more ventilation days, more ICU days or invasive medical devices such as urinary catheters and central venous catheters. Although we adjusted for PELOD-2 in our analysis, we did not have enough power to include all these confounders in our analysis. Lastly, some patients in our cohort had surgery during the follow-up period which could have influenced mHLA-DR levels.[27]

Conclusion

In this single-centre study of mHLA-DR expression in children admitted to PICU with suspected infections, we confirm that critically ill children have lower mHLA-DR expression than controls. Decreased mHLA-DR and change in mHLA-DR was not associated with the acquisition of secondary infections. Therefore, in this population mHLA-DR expression is not valuable for identifying children at risk for secondary infections.

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Additional file 1: characteristics of critically ill children with suspected infections and controls

	patients (n=104)	**Control (n=93)**	P-value
Age in years, median [IQR]	1.1 [0.2-4.1]	3.6 [1.1-9.9]	<0.01
0-1 year	51 (49.0)	19 (20.4)	
1-2 years	13 (12.5)	16 (17.2)	
2-5 years	16 (15.4)	19 (20.4)	
5-12 years	10 (9.6)	20 (21.5)	
12-18 years	14 (13.5)	19 (20.4)	
Male	62 (59.6)	61 (65.6)	0.6
Type of surgery			
Urology		49 (52.7)	
Plastic surgery		18 (19.4)	
Orthopedic surgery		18 (19.4)	
Other		8 (8.6)	

mHLA-DR levels in controls and per age group

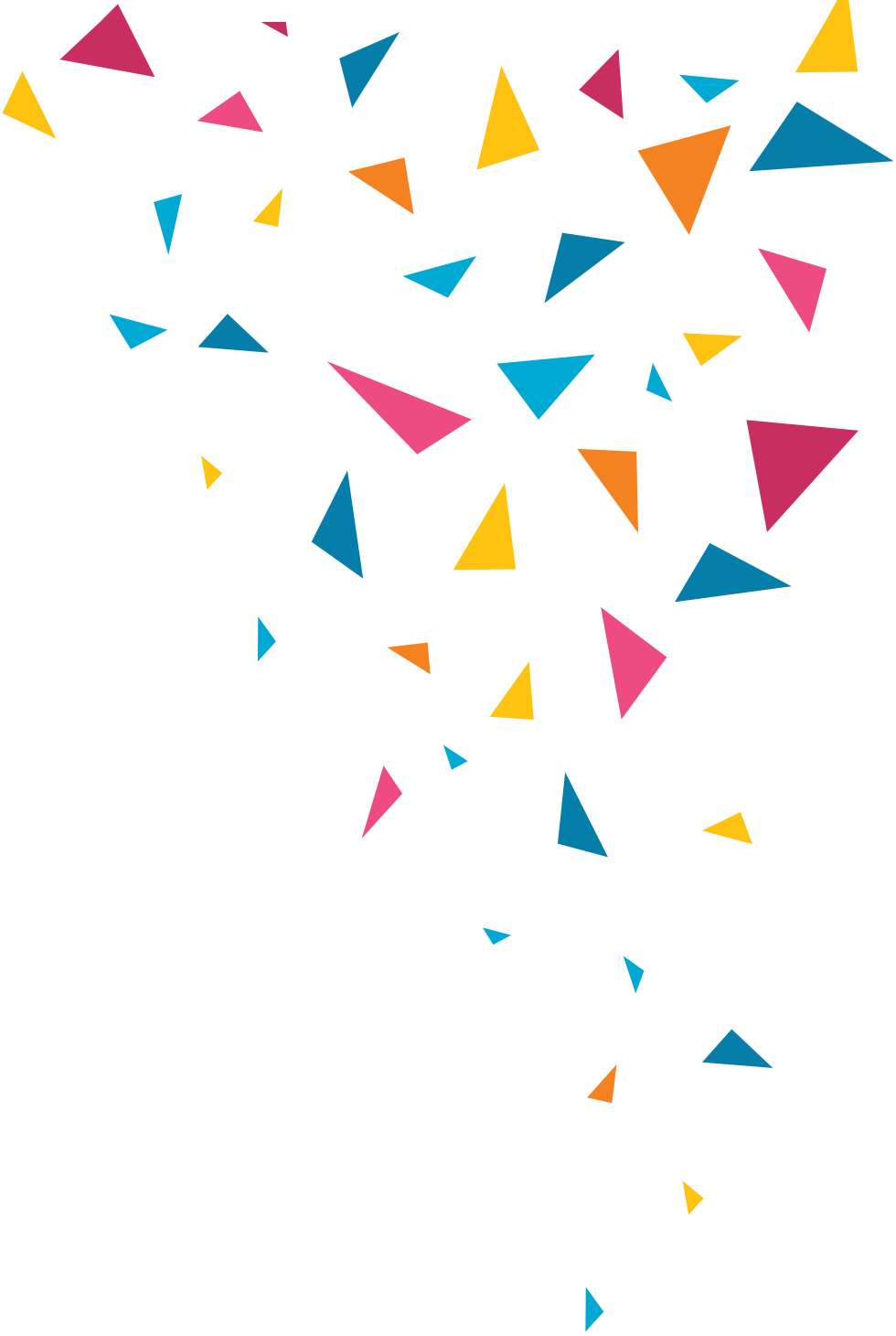
	mHLA-DR AB/cell (/1000)
	Median (IQR)
All controls, n=93	29.5 (23.8-37.6)
0-1 year, n=19	28.9 (26.1-39.3)
1-2 years, n=16	34.3 (25.7-42.6)
2-5 years, n=19	33.9 (27.6-43.8)
5-12 years, n=20	28.0 (24.9-35.8)
12-18 years, n=19	23.6 (19.7-28.3)

mHLA-DR levels in PICU patients

	mHLA-DR AB/cell (/1000)
	Median (IQR)
Day 1 (n=66)	9.7 (6.9-18.3)
Day 1-3 (n=55)	10.2 (7.5-15.9)
Day 4-7 (n=28)	12.5 (8.4-17.4)
Day 8-16 (n=13)	14.4 (9.3-24.1)

Additional file 2: details of secondary infections

	Secondary infections n=28 **n (%)**
Blood stream infection/clinical sepsis	7 (25)
Gastrointestinal system	4 (14.3)
Other lower respiratory tract	1 (3.6)
Pneumonia	4 (14.3)
Surgical site	5 (17.9)
Skin and soft tissue	1 (3.6)
Urinary tract	5 (17.9)
Eye, ear nose, throat or mouth infection	1 (3.6)



7

Haemostasis proteins in invasive meningococcal and non-meningococcal infections: a prospective multicentre study

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**Equal contribution*

Submitted.

Abstract

Background: Coagulation disorders and disseminated intravascular coagulation (DIC) are a well-known feature of meningococcal infection and associated with morbidity and mortality. Little is known however about haemostasis proteins in other childhood bacterial infections. We aimed to describe the variation of haemostasis proteins in children with infections due to *N. meningitidis*, *S. pneumoniae*, *S.aureus* and Group A streptococcus (GAS), and to study haemostasis proteins in relation to mortality and disease severity.

Methods: In this pre-planned sub-analysis of the EUCLIDS study (European Union Childhood Life-threatening Infectious Disease study, 5 countries), we selected patients with invasive bacterial infections due to meningococci (n=83), pneumococci (n=64), *S. aureus* (n=50) and GAS (n=44) with available serum samples collected <48 hours after hospital admission. Fibronectin, plasminogen activator inhibitor type 1 (PAI-1), thrombomodulin and ADAMTS-13 were measured in serum. Additionally, von Willebrand Factor (vWF), Protein C, Protein S and factor IX were measured in citrate plasma available from a subset of patients. Outcome measures included in-hospital mortality and disease severity (need for ventilation/inotropes, Paediatric Index of Mortality score).

Results: Of 241 children with invasive bacterial infections, 21 (8.7%) died and 177 (73.5%) were admitted to paediatric intensive care unit. Mortality rate was similar for the different pathogens. Levels of fibronectin and thrombomodulin differed for the different pathogens ($p<0.05$). Fibronectin levels were lower in GAS infections than in *S. pneumoniae* and *S. aureus* infections, but did not differ from meningococcal infections. Thrombomodulin, levels in meningococcal infections were higher than in *S. aureus* and pneumococcal infections, but similar for GAS infections. Overall, thrombomodulin and ADAMTS-13 were higher in non-survivors whereas fibronectin was lower in non-survivors ($p<0.05$). Discriminative ability was 0.81 (95%CI 0.70-0.92) for thrombomodulin, 0.78 (95%CI 0.69-0.88) for ADAMTS-13 and 0.67 (95%CI 0.55-0.79) for fibronectin. The association of each haemostasis protein did not vary across pathogens for any of the outcome measures (interaction $p>0.05$).

Conclusion: Haemostatic disturbances in invasive paediatric bacterial infections are not limited to meningococcal sepsis, but occur with a comparable severity across infections with different pathogens. Thrombomodulin, ADAMTS-13 and fibronectin were associated with mortality. Our results emphasize the importance of haemostatic disturbances in both meningococcal and non-meningococcal paediatric bacterial infections.

Introduction

Sepsis is an important cause for mortality and morbidity in children and is estimated to contribute to 20% of childhood deaths.(1-5) The inflammatory response to infection induces pro-coagulant and platelet activating pathways, whilst it reduces the functioning of anti-coagulant pathways and fibrinolytic activity.(6) These mechanisms result in coagulation abnormalities ranging from subtle derangements only detectable by highly sensitive assays to widespread deposition of fibrin throughout the microcirculation, manifesting as disseminated intravascular coagulation (DIC). Purpura, a sign of DIC, are considered typical for meningococcal sepsis and are associated with septic shock, multi-organ dysfunction and death. Non-meningococcal infections, in which purpura do not commonly occur, can also cause mild to severe coagulopathies which influence disease severity.(7)

Next to meningococcal infections, infections caused by *Streptococcus pneumoniae, Group A streptococcus* (GAS) and *Staphylococcus aureus* comprise a large group of invasive community-acquired bacterial infections in children.(1, 8, 9) Each pathogen might induce a different coagulation host-response, as bacteria are known to induce platelet activation or promote platelet adhesion, trigger activation of coagulation independently, or interact with the fibrinolytic system.(10-12) For example, *Staphylococcus aureus* secretes coagulases that directly activate thrombin (13) while meningococcal and streptococcal infections have been shown to be associated with activation of the contact pathway.(14)

Although extensive research has been performed in describing haemostasis proteins in meningococcal sepsis or sepsis in general (15-28), the variation of haemostasis protein levels in infections across specific pathogen groups has not yet been described. Therefore, we aimed 1) to study the variation of haemostasis protein levels in children with invasive bacterial infections due to either *Neisseria meningitidis, Streptococcus pneumoniae, Staphylococcus aureus* or *GAS*, and 2) to describe haemostasis protein levels and their relation with mortality and disease severity. Since severe DIC is typical for meningococcal infections, we hypothesize that disturbances in haemostasis protein levels are more pronounced in meningococcal infections than in infections due to non-meningococcal infections.

Methods

Study design and population

This is a pre-planned study embedded in the EUCLIDS project (European Union Childhood Life-threatening Infectious Disease study) a European prospective multicentre cohort study that aimed to evaluate determinants of susceptibility and severity of severe paediatric bacterial infection. The EUCLIDS study design and methods have been described previously.(1, 8) (29) In short, patients <18 years admitted with community-acquired (suspected) sepsis, or severe focal infection were prospectively included in 2012-2016. Informed consent was obtained from parents or carers for inclusion in the study. This sub-analysis focuses on children with sterile culture-proven infections caused by *Neisseria meningitidis, Streptococcus pneumoniae, Staphylococcus aureus* or GAS included in Austria, the Netherlands, Spain, Swiss and the United Kingdom. Patients were selected if they had serum samples taken within 48 hours after study centre admission. In addition, we selected afebrile children as healthy controls (4:1). Collected clinical data included general characteristics, laboratory and microbiological results, disease severity score (Paediatric Index of Mortality-2 (PIM2)) (30), DIC score (31), treatments during admission, and mortality.

Samples and laboratory assay for haemostasis proteins

Venous blood was drawn for collection of serum and citrate-plasma. All samples were kept at -80 degrees Celsius until analysis. We selected 8 proteins for measurement: fibronectin, factor IX, protein C, protein S, thrombomodulin, ADAMTS-13 (a disintegrin and metalloprotease with thrombospondin type-1 motives), von Willebrand Factor (vWF), and plasminogen activator inhibitor type 1 (PAI-1). This selection was based on previously reported associations with disease severity in sepsis (details on proteins are provided in Figure 1 and Appendix 1).(15-27) In serum, we measured fibronectin, thrombomodulin and ADAMTS-13 and PAI-1. In a subset of patients with available citrate-plasma drawn at the same timepoint (n=146), we measured factor IX, protein C, protein S, and vWF. All the proteins were measured by a Luminex assay. Assays for fibronectin, PAI-1, ADAMTS-13 were obtained from R&D systems, Abingdon, UK. Factor IX, protein C, protein S and vWF were measured with the human coagulation 4-plex ProcartaPlex panel 3 (ThermoFisher Scientific, Vienna, Austria). Thrombomodulin levels were measured with a laboratory developed Luminex assay based on capture antibody, detection antibody and recombinant human thrombomodulin (Thrombomodulin DuoSet ELISA assay; R&D Systems, Abingdon, UK), with a lower limit of detection 10 pg/ml.

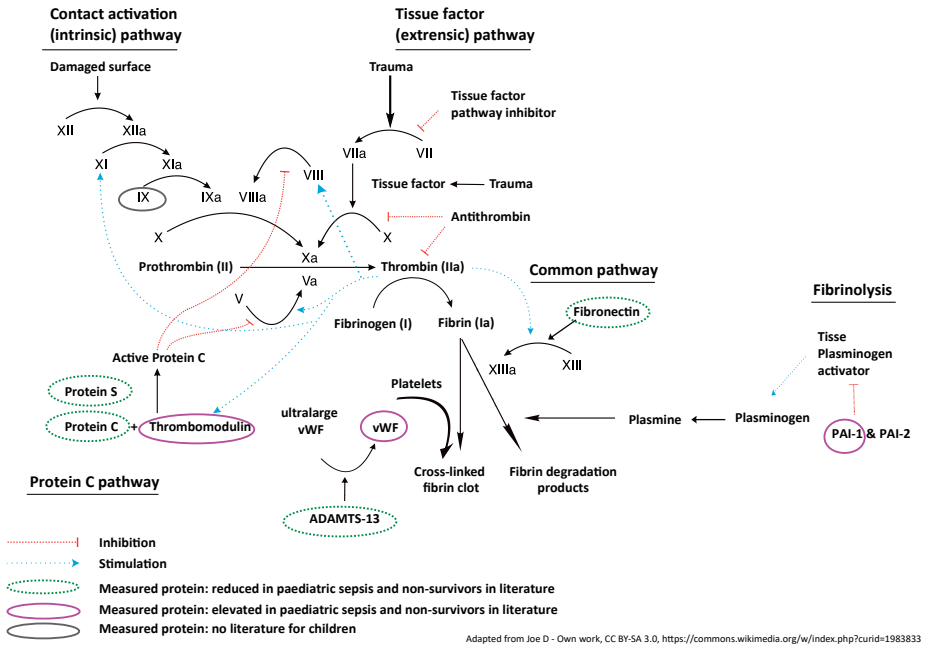

Figure 1 | Schematic simplified presentation of the coagulation pathways

Clinical definitions

The primary outcome measure was in-hospital mortality. Secondary outcome measures comprised markers for disease severity: need for mechanical ventilator support or inotrope/vasoactive drug requirement (32) and Paediatric Index of Mortality-2 (PIM2) score (30). PIM2 scores were only calculated for patients admitted to PICU. DIC scores were calculated for patients with at least one parameter (platelet count, d-dimer, prothrombin time or fibrinogen) measured in routine care +/- 12 hours of the drawn research sample and DIC was defined as a score >=5.(31)

Data analysis

First, we compared clinical characteristics and haemostasis protein levels between the patients with invasive bacterial infections and controls. Second, to study the variation between different pathogens, we compared clinical characteristics and differences in haemostasis protein levels between groups of patients with different causative pathogens. To study whether the association of each protein level depended on pathogen type, we used logistic regression to test the interaction of the pathogen group with the log-transformed value for each protein for the primary outcome measure (mortality) and secondary outcome (need for invasive ventilation/inotropes). We tested the significance of the interaction using the likelihood ratio test. Third, in the total cohort of invasive

bacterial infections, we assessed haemostasis protein levels in relation to mortality and disease severity (need for invasive ventilation/inotropes, PIM2 score). Differences were assessed using Mann-Whitney U test and Kruskal Wallis test for continuous variables, and chi-square test for categorical variables. For continuous outcomes, we assessed correlations with Spearman's rank test. Fourth, for the proteins that differed for survivors and non-survivors, we assessed their discriminative ability for mortality by area under the receiver operating curves (AUROC) and identified cut-off values using the Youden's index. We assessed the predictive performance of these cut-off values for mortality by sensitivity, specificity, and positive/negative likelihood ratios.

Significance level was defined at $p<0.05$. To account for multiple testing of haemostasis proteins, we applied the false discovery rate method (33, 34). All analyses were performed in R version 3.6.

Results

Study population

Out of the 4739 patients included in the EUCLIDS study, 2062 patients had a sterile site proven infection of which 1443 were infections caused by any of the bacteria of interest for the current study. Of those, we included 241 patients with available serum sample collected <48 hours after hospital admission (Appendix 2). This comprised 83 (34.4%) *N. meningitidis* infections, 64 (26.6%) *S. pneumoniae* infections, 50 (20.7%) *S. aureus* infections, and 44 (18.3%) GAS infections. In addition, we collected 64 healthy controls (female (n=30, 47%); median age 5.4 years [IQR 3.1-12.3]). Compared to healthy controls, patients with invasive bacterial infections were younger (median age 3.3 years [IQR 1.3-9.2], $p<0.05$) but had similar sex distribution (110 female (47%)) (Appendix 3). Of 241 patients with invasive bacterial infections, 21 (8.7%) died and 177 (73.5%) were admitted to the PICU (Table 1).

Across the different pathogens, distribution of sex was similar, but patients with *S. aureus* (median 9.9 year [IQR 4.3-13.0] were older than patients with infections due to GAS (median 3.7 years [IQR 1.8-7.6], meningococcus (median 1.8 years [IQR 0.7-5.3]) and pneumococcus (median 2.5 years [IQR 1.3-5.3]). Overall mortality was comparable ($p=0.89$) between the different infections (GAS 7% (n=3), meningococcal 8% (n=7), pneumococcal 11% (n=7) and *S. aureus* 8% (n=4)). For patients admitted to PICU, PIM2 scores were similar across the pathogen groups. DIC scores differed between the different infections with higher abnormal DIC scores in the meningococcal patients (meningococcal 2 [IQR 2-4], pneumococcal 2 [IQR 0-2], *S. aureus* 1.5 [IQR 0-2]), GAS: median 2 [IQR 0-2]). Incidence of DIC (score >5), however, was similar across pathogens ($p=0.38$).

Table 1 | Descriptive characteristics for all patients and stratified for *Neisseria meningitidis, Streptococcus pneumoniae, Staphylococcus aureus* and Group A streptococcus

	Invasive bacterial infections n=241	**Missing**	*Neisseria meningitidis* n=83	*Streptococcus pneumoniae* n=64	*Staphylococcus aureus* n=50	*Group A streptococcus* n=44	P-value
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
General characteristics							
Female	112 (46.9)		33 (39.8)	25 (39.1)	25 (50)	27 (61.4)	0.07
Age in years, median [IQR]	3.3 (1.3-9.2)		1.8 (0.7 - 5.3)	2.5 (1.3 - 5.3)	9.9 (4.3 - 13.0)	3.7 (1.8 - 7.6)	<0.01
Immunizations up to date	179 (74.3)	52 (21.6)	63 (75.9)	50 (78.1)	32 (64)	34 (77.3)	0.56
Any chronic underlying condition	102 (42.3)		27 (32.5)	30 (46.9)	27 (54)	18 (40.9)	0.08
Duration of symptoms at presentation in days, median [IQR]	2.7 (1.5-5.6)	36 (14.9)	1.5 (1.1-2.6)	3.7 (2.1 - 6.8)	3.6 (2.4 - 4.9)	4.6 (2.3 - 7.7)	<0.01
Severity of illness							
Sepsis	159 (66.0)		65 (78.3)	36 (56.2)	25 (50.0)	33 (75)	<0.01
Admitted to PICU	177 (73.4)		73 (88)	40 (62.5)	24 (48)	40 (90.9)	<0.01
Need for invasive ventilation or inotropes	123 (51.0)		54 (65.1)	20 (31.2)	17 (34)	32 (72.7)	<0.01
Need for invasive ventilation	106 (44.0)	37 (15.4)	45 (54.2)	18 (28.1)	13 (26)	30 (68.2)	<0.01
Days on invasive ventilation, median [IQR]	4 (3 - 7)		5 (3 - 6)	3 (2 - 9)	4 (3 - 12)	4 (2 - 8)	0.7
Need for inotropes	105 (43.6)	38 (15.8)	51 (61.4)	12 (18.8)	15 (30)	27 (61.4)	<0.01
Days on inotropes, median [IQR]	3 (2 - 5)		3 (2-4)	2 (4-5)	3 (3-6)	4 (2 - 5)	0.51
PIM2 score (%), median [IQR]	3.5 (0.8 -11.5)		3.5 (0.8 -12.6)	2.7 (0.8 - 8.9)	2.6 (0.8 - 4.1)	6.2 (1.1 -12.3)	0.32
PICU free days at day 28, median [IQR]	25 (21-28)	2/177	24 (21 - 26)	26 (21 - 28)	28 (20 - 28)	23 (17-26)	<0.01
Death	21 (8.7)		7 (8.4)	7 (10.9)	4 (8)	3 (6.8)	0.89
Routine chemistry and haematological tests*							
Platelets (10^9/L), median [IQR]	174 (103 - 270)	54 (22.4)	140 (86 - 196)	249 (172 - 385)	196 (137 -254)	174 (66 -293)	<0.01
D-dimer (ng/mL), median [IQR]	5733 (2650 - 11711)	219 (90.9)	7970 (2384 -22819)	3189 (1595 - 4783)	5090 (3380 -8930)	4497 (4195 - 31929)	0.5

Table 1 | Descriptive characteristics for all patients and stratified for *Neisseria meningitidis, Streptococcus pneumoniae, Staphylococcus aureus* and Group A streptococcus (continued)

	Invasive bacterial infections n=241	**Missing**	*Neisseria meningitidis* n=83	*Streptococcus pneumoniae* n=64	*Staphylococcus aureus* n=50	*Group A streptococcus* n=44	P-value
PT (s), median [IQR]	19 (14 - 27)	128 (53.1)	22 (18 - 31)	15 (13 -24)	16 (13 -26)	15 (12 - 20)	<0.01
Fibrinogen (g/L), median [IQR]	3.9 (2.5 - 5.5)	146 (60.6)	3.6 (2.6 -4.7)	4.9 (3.5 -6.6)	4.9 (3.2 - 6.1)	2.9 (1.8 - 4.7)	0.09
DIC score >=5	18 (7.5)	53 (22.0)	10 (12.0)	2 (3.1)	3 (6)	3 (6.8)	0.38
DIC score, median [IQR]	2 (0-2)	53 (22.0)	2 (2-4)	2 (0-2)	1.5 (0-2)	2 (0-2)	<0.01

*+/- 12 hours from blood sample

DIC, disseminated intravascular coagulation; IQR, interquartile range; PICU, paediatric intensive care unit; PIM, paediatric index of mortality; PT, prothrombin time

Variation of haemostasis protein levels across pathogens

In patients, levels of PAI-1, thrombomodulin, ADAMTS-13 and vWF were higher than in controls, whereas levels of fibronectin and protein C were lower in patients than in controls. Levels of factor IX and protein S did not differ for patients and controls (Appendix 4). Between the four pathogen groups, levels of fibronectin and thrombomodulin differed across the pathogens ($p<0.05$, Figure 2, Appendix 5). Levels of the other proteins did not vary for the different pathogens. Thrombomodulin levels in *N. meningitidis* infections were higher than in *S. aureus* and *S. pneumoniae* infections. Fibronectin levels were lower in GAS infections compared to *S. aureus* and *S. pneumoniae* infections.

Haemostasis protein levels and relation with mortality and disease severity

In all patients, thrombomodulin and ADAMTS-13 were higher in non-survivors compared to survivors whereas levels of fibronectin were lower in non-survivors than in survivors. PAI-1, factor IX, protein C, protein S and vWF were not related to survival status (Figure 3). The association of each protein for mortality or need for invasive ventilation/inotropes did not vary between the different pathogens (interaction $p > 0.05$).

The discriminative ability (AUROC) between survivors and non-survivors was 0.67 (95%CI 0.55-0.79) for fibronectin, 0.81 (95%CI 0.70-0.92) for thrombomodulin and 0.78 (95%CI 0.69-0.88) for ADAMTS-13. For fibronectin, thrombomodulin and ADAMTS-13 the optimal cut-offs were 23 ug/mL, 2733 pg/mL and 20 ng/mL, respectively. The cut-off for thrombomodulin yielded the best predictive performance (specificity 0.89 [95% CI 0.84-0.92]; sensitivity 0.67 [95% CI 0.43-0.85]; positive LR 5.8 [95%CI 3.6-9.4]; negative LR 0.38 [95%CI 0.21-0.69]) (Table 2).

Table 2 | Predictive performance of fibronectin, thrombomodulin and ADAMTS-13 for mortality, n=241

	Sensitivity (95% CI)	Specificity (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Fibronectin, <=23 ug/mL	0.76 (0.53-0.92)	0.71 (0.64-0.77)	2.6 (1.9-3.6)	0.34 (0.16-0.73)
Thrombomodulin >=2733 pg/mL	0.67 (0.43-0.85)	0.89 (0.84-0.92)	5.8 (3.6-9.4)	0.38 (0.21-0.69)
ADAMTS-13, >=20 ng/mL	0.95 (0.76-1.00)	0.53 (0.47-0.60)	2.0 (1.7-2.4)	0.09 (0.01-0.61)

CI, confidence interval; LR, likelihood ratio

Compared to patients without invasive ventilation/inotropes, patients in need for invasive ventilation/inotropes had higher levels of thrombomodulin and lower levels for fibronectin, factor IX, protein C and protein S. Levels of fibronectin, thrombomodulin, factor IX, protein C and protein S were correlated with PIM2 score although correlations were not strong (Appendix 6).

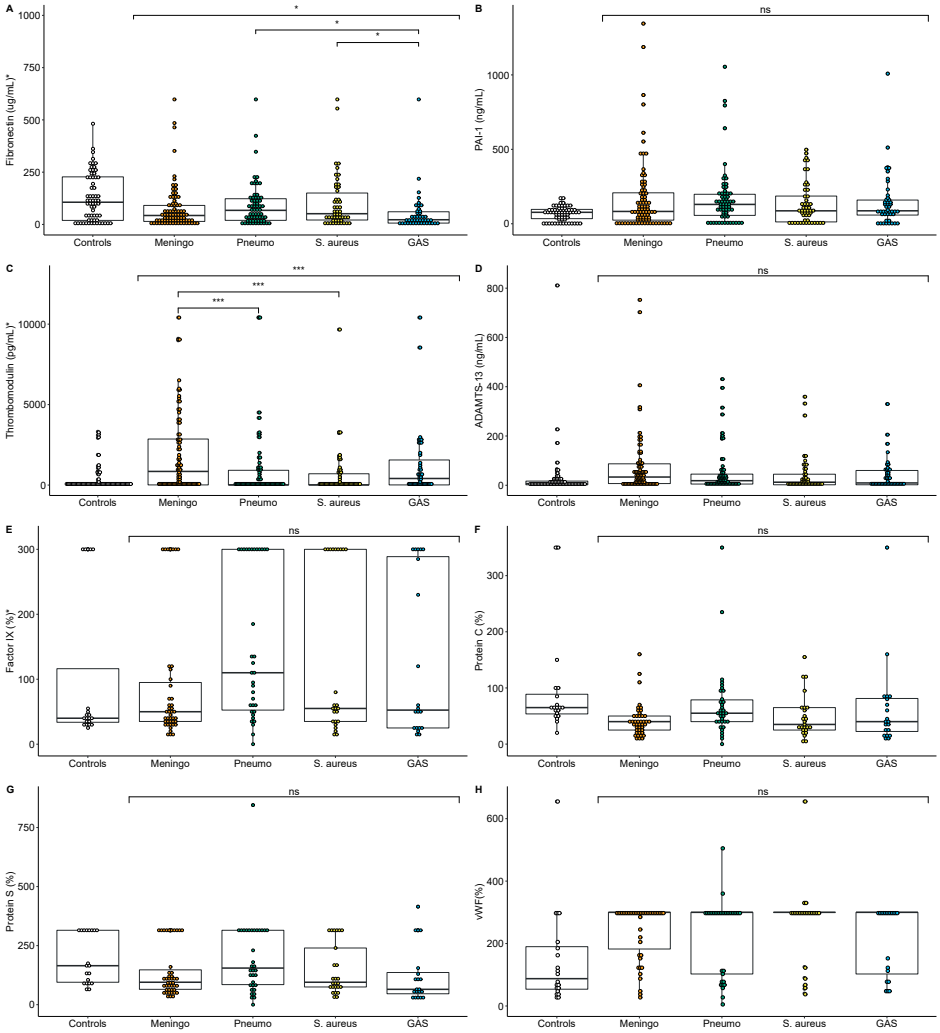

Figure 2 | Haemostasis protein levels in controls and different pathogens
A, Fibronectin; B, PAI-1; C, Thrombomodulin; D, ADAMTS-13; E, Factor IX; F, Protein C; G, Protein S; H, vWF
*Extreme values for Fibronectin, thrombomodulin and Factor IX were truncated to the 99th centile.
ns, not significant, *p-value≤0.05, **p-value≤0.01, ***p-value≤0.001
p-values are adjusted for multiple testing.

Discussion

In this large European cohort, we studied the variation of haemostasis protein levels across invasive paediatric community-acquired infections caused by *N. meningitidis, S. pneumoniae, S. aureus* and GAS, and assessed the association of haemostasis protein levels with mortality and disease severity. Mortality was similar across all pathogen

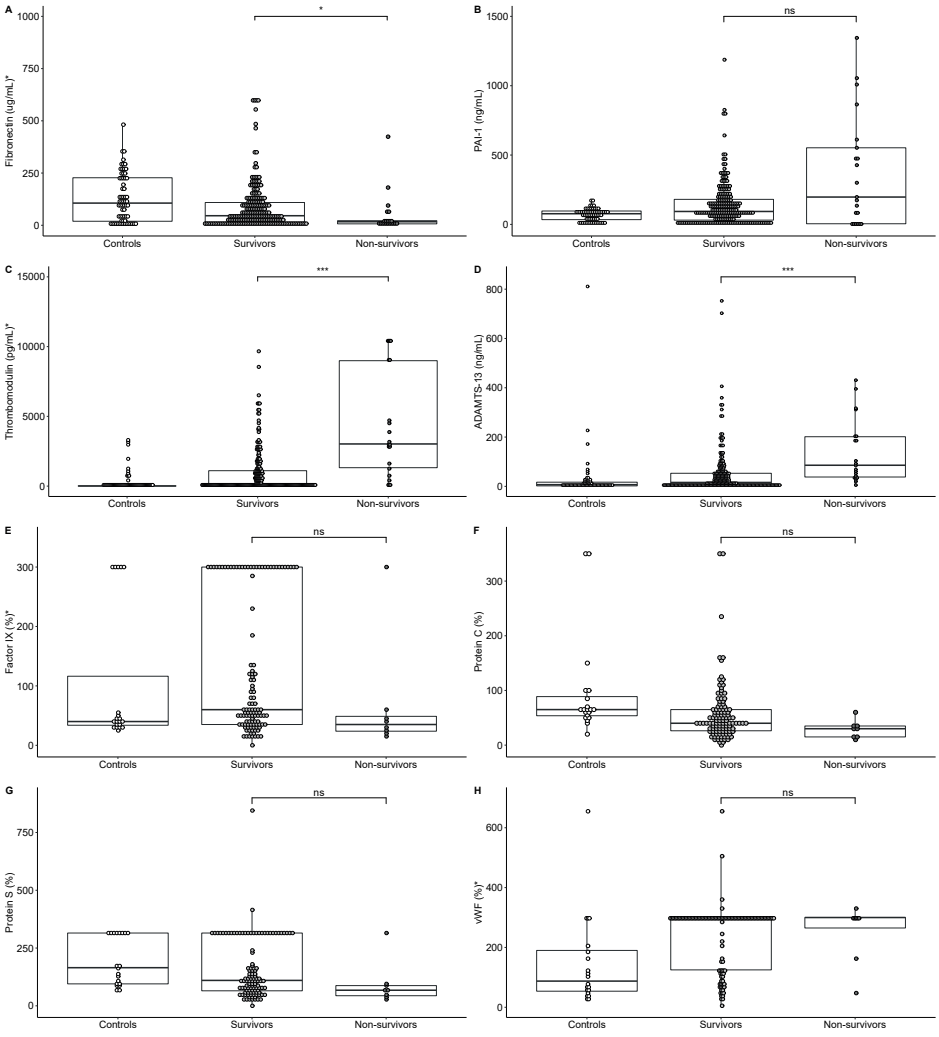

Figure 3 | Haemostasis protein levels in controls, survivors and non-survivors
A, Fibronectin; B, PAI-1; C, Thrombomodulin; D, ADAMTS-13; E, Factor IX; F, Protein C; G, Protein S; H, vWF
*Extreme values for Fibronectin, thrombomodulin and Factor IX were truncated to the 99th centile.
ns, not significant, *p-value≤0.05, **p-value≤0.01, ***p-value≤0.001
p-values are adjusted for multiple testing.

groups. Haemostatic derangements were not limited to meningococcal infections, but occurred with a comparable frequency and severity in non-meningococcal infections. Higher levels of thrombomodulin and ADAMTS-13, and lower levels of fibronectin were associated with mortality in children admitted to hospital with invasive infections.

Haemostatic disturbances and purpura fulminans are typical for meningococcal infections. Although the highest DIC scores were more frequent in patients with meningococcal disease, we found similar frequency and severity of haemostatic disturbances across the different pathogens. Apart from thrombomodulin, the levels of haemostasis proteins did not differ specifically for meningococcal infections. Haemostatic derangements are therefore not only typical for meningococcal infections but can also occur in non-meningococcal infections. While less visible on physical examination than purpura fulminans, haemostatic derangements may result in microthrombi which could negatively influence microcirculatory perfusion and reduce oxygen delivery thereby influencing disease severity.(35) In our study data on purpura or petechiae, skin necrosis and amputations were unfortunately not available.

Thrombomodulin is an endothelial cell surface glycoprotein activating the protein C pathway and is shed from the surface after endothelial injury.(36-38) Serum thrombomodulin is a sensitive marker for endothelial injury, and is associated with mortality and severity of disease.(17, 20, 39) Our study confirms previous findings and shows higher thrombomodulin levels in non-survivors compared to survivors, and good discriminative ability of thrombomodulin levels for non-survivors and survivors. Additionally, serum thrombomodulin is correlated to PIM2 scores and is higher in patients needing invasive ventilation or inotropes, thereby underscoring a clear association of high thrombomodulin with severity of disease.

This study is the first to compare thrombomodulin levels across different pathogen groups. A study in adults with sepsis reported higher thrombomodulin levels in patients with DIC compared to those without, but the disease-causing pathogens were not reported.(39) In our cohort, thrombomodulin levels in meningococcal infections were higher compared to *S. pneumonia* and *S. aureus* infections, but the presence of DIC did not differ between pathogen groups. Possibly, higher thrombomodulin levels in meningococcal infections could reflect more endothelial injury, without leading to more DIC. Importantly, our study shows the potential of serum thrombomodulin as prognostic marker for disease severity and mortality.

Fibronectin is a glycoprotein which is part of the host response to infection and triggers the coagulation process. It interacts with phagocytes, the reticulo-endothelial system and the complement system to prevent invasion of bacterial pathogens.(40-44) Previous studies showed that plasma fibronectin levels are lower in meningococcal sepsis, and one study found especially lower levels in non-survivors.(16) Another study reported similar fibronectin levels in survivors and non-survivors which might be explained by low number of cases (15, 45). Our study of mixed bacterial infections confirms the de-

creased serum levels of fibronectin in non-survivors compared to survivors. Fibronectin levels were especially low in GAS infections. The origin and role of low fibronectin levels is yet unclear. Low fibronectin may be genetically determined, lowered by decreased production, depleted by binding to bacteria or lost from the intravascular department due to capillary leak. In addition, low fibronectin in itself could lead to reduced protection to invasion of bacteria.(43, 44) Future studies need to further unravel the role of fibronectin in the pathogen-host interaction.

In our study, ADAMTS-13 was higher in bacterial infections compared to healthy children, and higher in non-survivors than in survivors. This is in contrast to previous studies which found lower plasma ADAMTS-13 in paediatric meningococcal sepsis than in healthy adults, and also lower plasma ADAMTS-13 in non-survivors compared to survivors.(22, 23) Comparability of these studies is not straightforward due to differences in measurement (assay, serum/plasma), controls (children vs adults) and patients (septic shock vs hospital admissions). Future studies will need to address the role of ADAMTS-13 in severe paediatric infections.

Our study showed no significant association of PAI-1 levels with mortality. Although we observed a trend for higher PAI-1 levels in non-survivors, previous studies in paediatric meningococcal infections found higher levels of PAI-1 in in non-survivors (25, 46). A possible explanation is that our study focused on children with different pathogens that needed hospital admission whereas previous studies have focused on meningococcal infections only, with more severe disease reflected by higher mortality rate (up to 29%). (25, 46) Another possible explanation for an absent association with mortality is that we measured PAI-1 in serum, whereas previous studies measured PAI-1 in citrate plasma. Indeed, additional measurements of PAI-1in citrate plasma samples (from the same moment as the serum samples) revealed poor correlation (n=30, Spearman r = 0.47, data not shown) between PAI-1 level in serum and citrate plasma samples.

The inflammatory response as result of infection can lead to coagulation abnormalities, ranging from subtle to more severe derangements. As the overall incidence of DIC was low in our population, our results confirm that disturbances in haemostatic proteins may not necessarily lead to DIC. Disturbances in haemostatic proteins do influence disease severity as our results show that increased thrombomodulin and ADAMTS-13 levels as well as decreased fibronectin levels were associated with mortality.

Strengths of our study include the large prospective European cohort of paediatric bacterial infections caused by different pathogens. Second, we used detailed clinical data to study haemostasis proteins in relation to mortality and disease severity. Third,

haemostasis proteins were chosen according to reported associations with disease severity in literature.

Our study has limitations. First, we selected patients with samples drawn within 48 hours after study centre admission. As our population consists of almost 75% of PICU admissions, our results may not be generalizable to all bacterial infections admitted to the general ward. The higher proportion of PICU admissions, however, may reflect the population at risk for development of haemostatic abnormalities. In addition, the different timings of the measurement and influence of treatment effects during admission were not taken into account. However, as ≥ 70% of the samples included were obtained within the first 24 hours of hospital admission, we consider the influence of sample timing and treatment influences to be limited. Furthermore, plasma required for measurement of factor IX, protein C, protein S and vWF was only available from a subset of the included patients with similar distribution of pathogens. However, this subset consisted of sicker patients with higher rate of PICU admissions and more requirement of ventilation/inotropes (appendix 7).

Conclusion

In this large European cohort of severe invasive paediatric bacterial infections, haemostasis disturbances were not limited to meningococcal infections, but occurred with a comparable frequency and severity in non-meningococcal sepsis. Mortality was similar across all pathogens. In all infections, high thrombomodulin levels, high ADAMTS-13 levels and low fibronectin levels were associated with mortality. Thrombomodulin levels discriminated well for mortality. Our results emphasize the importance of haemostatic disturbances in severe paediatric bacterial infections.

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Appendix

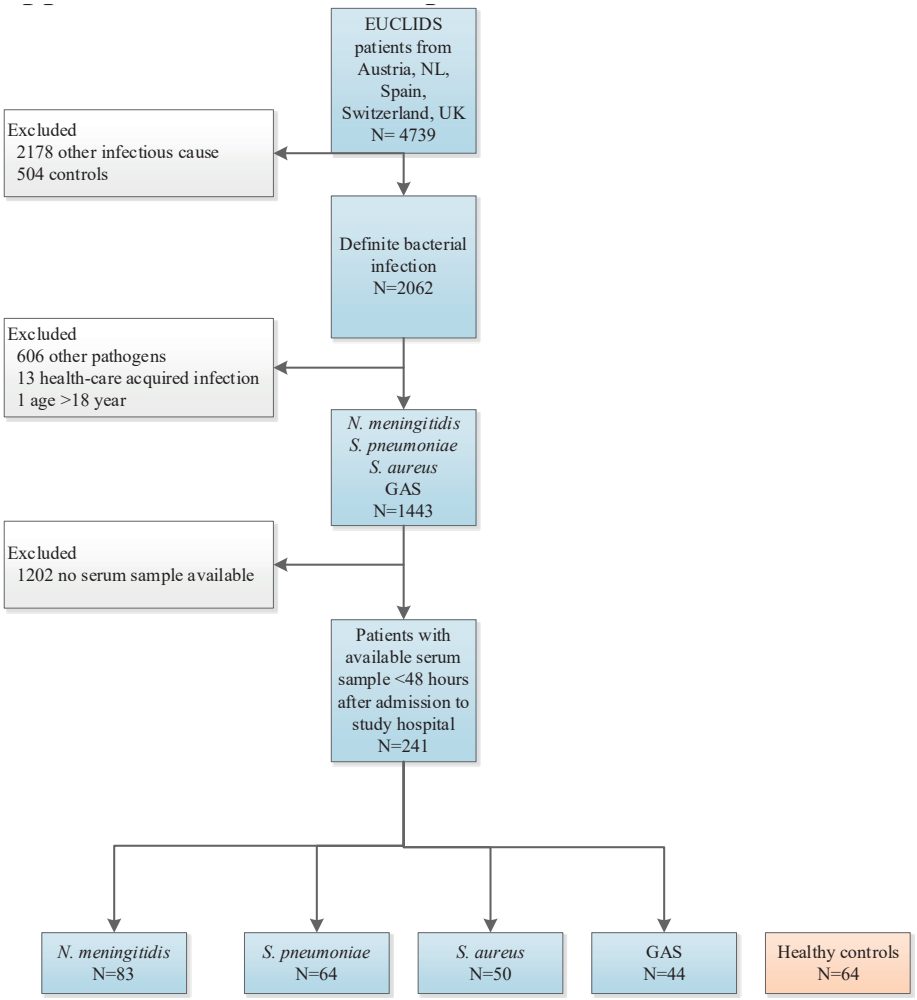
Appendix 1 - Overview of coagulation proteins and their relation with paediatric sepsis and childhood mortality in literature

	Paediatric sepsis*	Non-survivors^	References
Common pathway			
Fibronectin	↓	↓	(15, 16)
Contact activation (intrinsic) pathway			
Factor IX	No data±	No data	(47)
Protein C pathway			
Protein C	↓	↓	(17-19)
Protein S	↓	↓	(17, 18)
Thrombomodulin	↑	↑	(17, 20, 21)
Platelet adhesion			
ADAMTS-13	↓	↓	(22, 23)
vWF	↑	↑	(23)
Fibrinolysis			
PAI-1	↑	↑	(24-26)

*Compared to controls; ^Compared to survivors; ± Only literature in adults

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Appendix 2 – Flowchart of patient selection



Appendix 3 – Descriptive characteristics healthy controls vs invasive bacterial infections

	Invasive bacterial infections n=241	Healthy controls n=64	P-value
Female	110 (45.6)	30 (46.9)	0.97
Age in years, median [IQR]	3.3 (1.3-9.2)	5.4 (3.1-12.3)	<0.01
Ethnicity*			0.22
European	185 (76.8)	47 (73.4)	
Asian	13 (5.4)	4 (6.3)	
African	12 (4.9)	8 (12.5)	
Other	20 (8.3)	4 (1.7)	

*Available data: Invasive bacterial infections n=230, healthy controls n=63
IQR, interquartile range

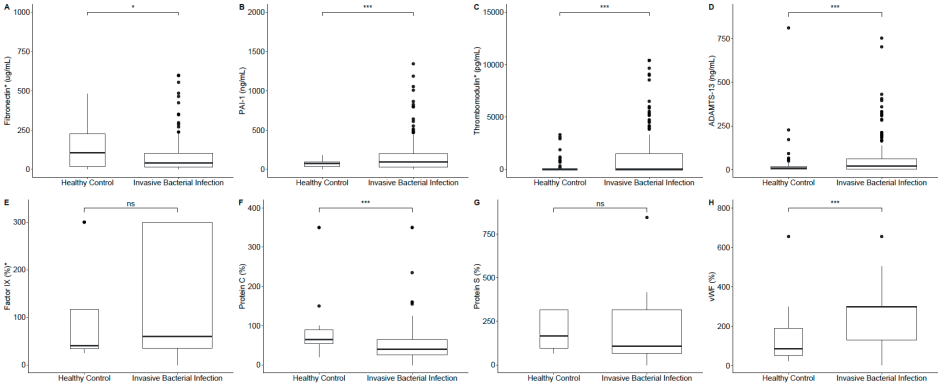
Appendix 4 – Levels of haemostasis proteins

Table Appendix 4A – Invasive bacterial infections vs healthy controls

	Invasive bacterial infections n=241	Healthy controls n=64	P-value §
Serum	median (IQR)	median (IQR)	
Fibronectin (ug/ml)	41 (15 - 103)	106 (19-228)	0.02
PAI-1 (ng/ml)	96 (30 - 197)	77 (34-97)	0.00
Thrombomodulin (pg/ml)	10 (10 - 1490)*	10 (10-10)	0.00
ADAMTS13 (ng/ml)	21 (2-61)^	7 (2-17)	0.00
Citrate plasma	**n=122**	**n=20**	
Factor IX (%)	60 (35 - 300)	40 (34-116)	0.36
Protein C (%)	40 (25 -65)	65 (54-89)	0.00
Protein S (%)	108 (65-315)	165 (95-315)	0.08
vWF (%)	300 (131 - 300)	88 (54-190)	0.00

*n=239, ^n=240.
§ Adjusted for multiple testing.
ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; IQR, interquartile range; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Figure Appendix 4A – Haemostasis protein levels in controls and patients with invasive bacterial infections



A, Fibronectin; B, PAI-1; C, Thrombomodulin; D, ADAMTS-13; E, Factor IX; F, Protein C; G, Protein S; H, vWF
*Extreme values for Fibronectin, thrombomodulin and Factor IX were truncated to the 99th centile.
ns; not significant. **p-value≤0.05, **p-value≤0.01, ***p-value≤0.001
p-values are adjusted for multiple testing.

Table Appendix 4B – Survivors vs non-survivors

	Survivors n=220	**Non-survivors n=21**	P-value*
Serum	median (IQR)	median (IQR)	
Fibronectin (ug/ml)	46 (15-109)	17 (7-22)	0.03
PAI-1 (ng/ml)	93 (31-180)	197 (5-552)	0.06
Thrombomodulin (pg/ml)	10 (10-1105)	3022 (1320-8990)	0.00
ADAMTS13 (ng/ml)	16 (2-53)	86 (38-201)	0.00
Citrate plasma	**n=114**	**n=8**	
Factor IX (%)	60 (35-300)	35 (24-49)	0.10
Protein C (%)	40 (26-65)	30 (15-35)	0.08
Protein S (%)	110 (65-315)	68 (44-88)	0.12
vWF (%)	300 (125-300)	300 (265-300)	0.52

*Adjusted for multiple testing
ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; IQR, interquartile range; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Table Appendix 4C – No invasive ventilation/inotropes vs need for invasive ventilation or inotropes

	No invasive ventilation/ inotropes n=118	Need for invasive ventilation or inotropes n=123	P-value*
Serum	median (IQR)	median (IQR)	
Fibronectin (ug/ml)	59 (26-131)	28 (9-74)	0.00
PAI-1 (ng/ml)	110 (22-190)	86 (35-198)	0.65
Thrombomodulin (pg/ml)	10 (10-729)	640 (10-2641)	0.00
ADAMTS13 (ng/ml)	16 (2-60)	27 (4-64)	0.44
Citrate plasma	**n=49**	**n=73**	
Factor IX (%)	110 (50-300)	50 (30-115)	0.01
Protein C (%)	55 (35-80)	35 (25-55)	0.01
Protein S (%)	160 (75-315)	90 (60-135)	0.03
vWF (%)	300 (85-300)	300 (245-300)	0.06

*Adjusted for multiple testing

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; IQR, interquartile range; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Appendix 5 – Coagulation proteins in invasive bacterial infections: stratified by pathogen group

	Group A streptococcus n=44	*Neisseria meningitidis* n=83	*Streptococcus pneumoniae* n=64	*Staphylococcus aureus* n=50	P-value*
Serum	median (IQR)	median (IQR)	median (IQR)	median (IQR)	
Fibronectin (ug/ml)	22 (6 - 61)	43 (14 - 91)	67 (20 - 123)	51 (21 - 150)	0.03
PAI-1 (ng/ml)	87 (58 - 159)	83 (24 - 208)	130 (56 -198)	87 (12 -186)	0.52
Thrombomodulin (pg/ml)	409 (10 -1559)	847 (10 - 2861)	10 (10 - 918)	10 (10 - 703)	0.00
ADAMTS13 (ng/ml)	9 (2 - 60)	33 (7 - 87)	19 (5 - 45)	12 (2 - 45)	0.13
Citrate plasma	**n=20**	**n=43**	**n=34**	**n=25**	
Factor XI (%)	53 (25 - 289)	50 (35 -95)	110 (53 -300)	55 (35 - 300)	0.11
Protein C (%)	40 (23 -81)	40 (25 -50)	55 (40 -79)	35 (25 -65)	0.11
Protein S (%)	65 (46 -136)	95 (65 -148)	155 (85 -315)	95 (75 -240)	0.14
vWF (%)	300 (103 - 300)	300 (183 - 300)	300 (103 -300)	300 (300 - 300)	0.38

*P-values adjusted for multiple testing

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; IQR, interquartile range; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Appendix 6 - Correlation of coagulation proteins with PIM2 score in all invasive bacterial infections (n=241)

	PIM2 score	
	Spearman's rho	P-value*
Fibronectin	-0.25	0.00
PAI-1	-0.01	0.94
Thrombomodulin	0.2	0.02
ADAMTS-13	0.13	0.15
Factor IX	-0.24	0.04
Protein C	-0.25	0.03
Protein S	-0.29	0.01
vWF	0.14	0.25

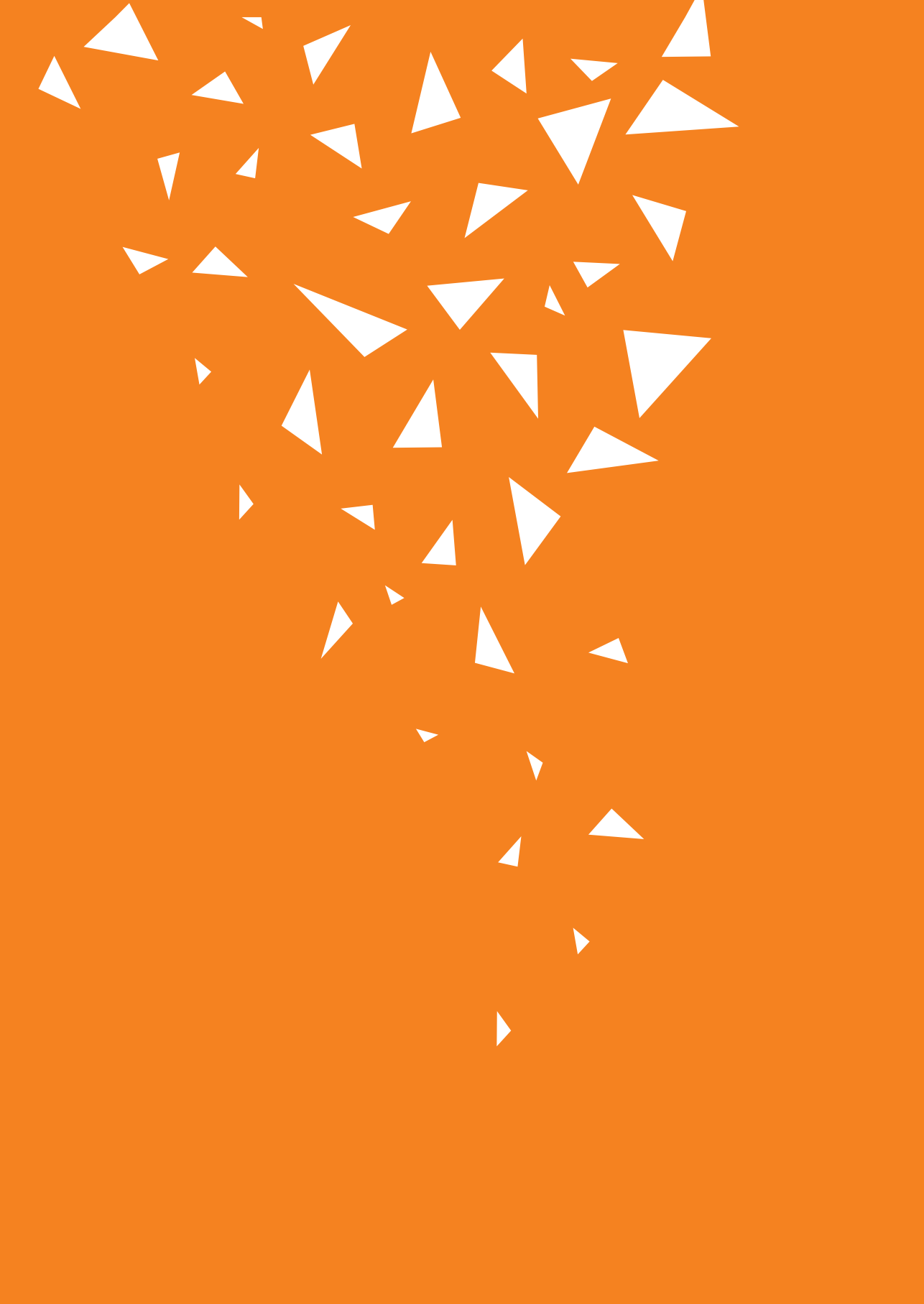
*P-values adjusted for multiple testing

ADAMTS-13, a disintegrin and metalloprotease with thrombospondin type-1 motives; IQR, interquartile range; PAI-1, plasminogen activator inhibitor type 1; vWF, von Willebrand Factor

Appendix 7 – Characteristics of patients with available citrate plasma (n=122) and patients without available citrate plasma (n=110)

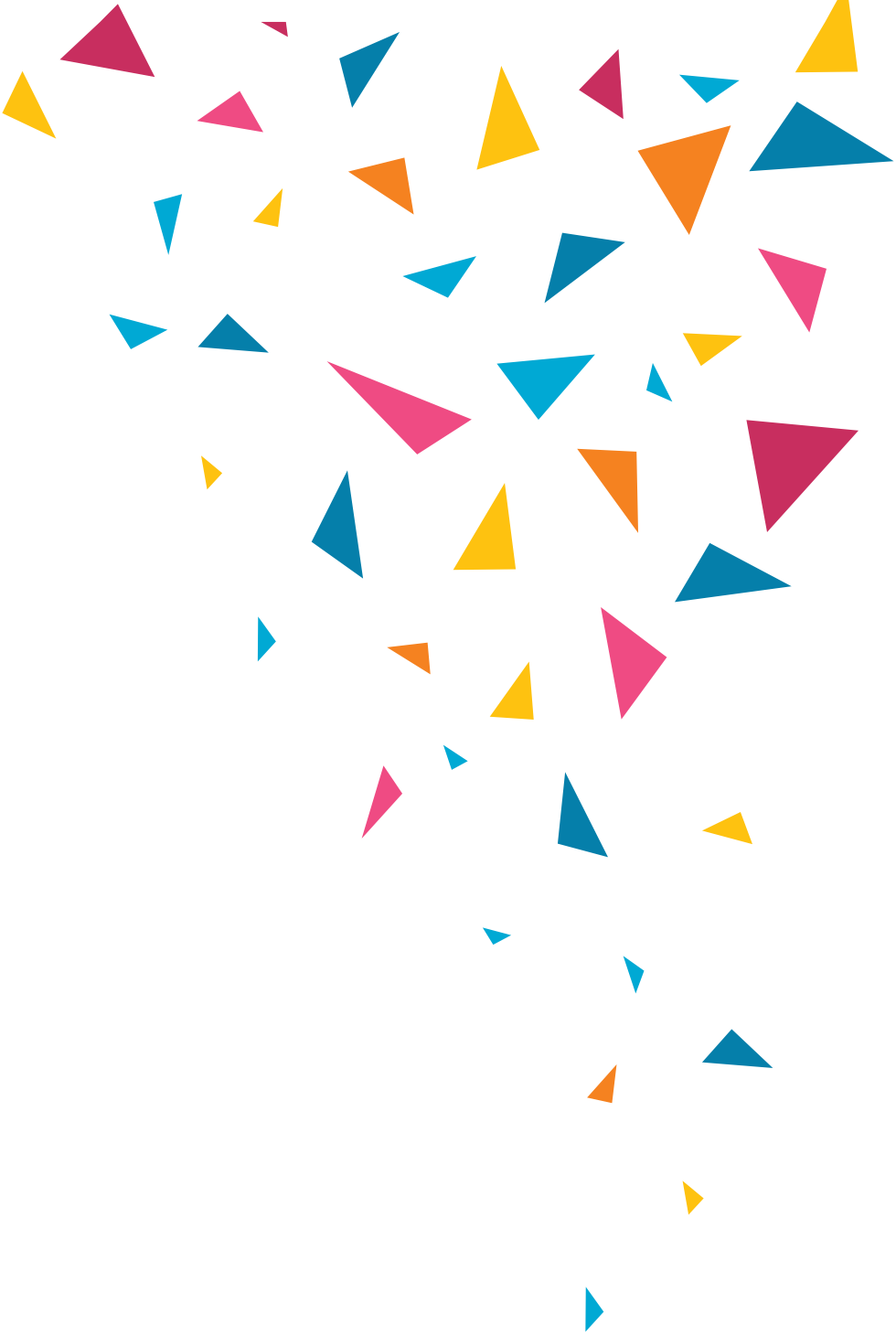
	All invasive bacterial infections	Citrate plasma available	Citrate plasma not available	P-value
	n=241	n=122	n=110	
Severity of illness				
Admitted to PICU	177 (73.4)	103 (84.8)	74 (62.2)	<0.001
Need for invasive ventilation or inotropes		73 (59.8)	50 (42.0)	<0.01
PIM2 score (%), median [IQR]		3.5 (1.0-10.9)	3.5 (0.8-11.7)	p=0.67
Death	21 (8.7)	8 (6.6)	13 (10.9)	0.33
Pathogen				
Neisseria meningitidis	83 (34.4)	43 (35.2)	40 (33.6)	p=0.88
Streptococcus pneumoniae	64 (26.6)	34 (27.9)	30 (25.2)	
Staphylococcus aureus	50 (20.7)	25 (20.5)	25 (21.0)	
Group A streptococcus	44 (18.3)	20 (16.4)	24 (20.2)	

IQR, interquartile range; PICU, paediatric intensive care unit; PIM, paediatric index of mortality



Part IV

Antibiotic prescription in emergency care for febrile illness



8

Variation in antibiotic prescription rates in febrile children presenting to emergency departments across Europe (MOFICHE): A multicentre observational study

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Abstract

Background: The prescription rate of antibiotics is high for febrile children visiting the emergency department (ED), contributing to antimicrobial resistance. Large studies at European EDs covering diversity in antibiotic and broad-spectrum prescriptions in all febrile children are lacking. A better understanding of variability in antibiotic prescriptions in EDs and its relation with viral or bacterial disease is essential for the development and implementation of interventions to optimise antibiotic use. As part of the PERFORM (Personalised Risk assessment in Febrile illness to Optimise Real-life Management across the European Union) project, the MOFICHE (Management and Outcome of Fever in Children in Europe) study aims to investigate variation and appropriateness of antibiotic prescription in febrile children visiting EDs in Europe.

Methods and findings: Between January 2017 and April 2018, data were prospectively collected on febrile children aged 0–18 years presenting to 12 EDs in 8 European countries (Austria, Germany, Greece, Latvia, the Netherlands [*n* = 3], Spain, Slovenia, United Kingdom [*n* = 3]). These EDs were based in university hospitals (*n* = 9) or large teaching hospitals (*n* = 3). Main outcomes were (1) antibiotic prescription rate; (2) the proportion of antibiotics that were broad-spectrum antibiotics; (3) the proportion of antibiotics of appropriate indication (presumed bacterial), inappropriate indication (presumed viral), or inconclusive indication (unknown bacterial/viral or other); (4) the proportion of oral antibiotics of inappropriate duration; and (5) the proportion of antibiotics that were guideline-concordant in uncomplicated urinary and upper and lower respiratory tract infections (RTIs). We determined variation of antibiotic prescription and broad-spectrum prescription by calculating standardised prescription rates using multilevel logistic regression and adjusted for general characteristics (e.g., age, sex, comorbidity, referral), disease severity (e.g., triage level, fever duration, presence of alarming signs), use and result of diagnostics, and focus and cause of infection. In this analysis of 35,650 children (median age 2.8 years, 55% male), overall antibiotic prescription rate was 31.9% (range across EDs: 22.4%–41.6%), and among those prescriptions, the broad-spectrum antibiotic prescription rate was 52.1% (range across EDs: 33.0%–90.3%). After standardisation, differences in antibiotic prescriptions ranged from 0.8 to 1.4, and the ratio between broad-spectrum and narrow-spectrum prescriptions ranged from 0.7 to 1.8 across EDs. Standardised antibiotic prescription rates varied for presumed bacterial infections (0.9 to 1.1), presumed viral infections (0.1 to 3.3), and infections of unknown cause (0.1 to 1.8). In all febrile children, antibiotic prescriptions were appropriate in 65.0% of prescriptions, inappropriate in 12.5% (range across EDs: 0.6%–29.3%), and inconclusive in 22.5% (range across EDs: 0.4%–60.8%). Prescriptions were of inappropriate duration in 20% of oral prescriptions (range across EDs: 4.4%–59.0%). Oral prescriptions were not concor-

dant with the local guideline in 22.3% (range across EDs: 11.8%–47.3%) of prescriptions in uncomplicated RTIs and in 45.1% (range across EDs: 11.1%–100%) of prescriptions in uncomplicated urinary tract infections. A limitation of our study is that the included EDs are not representative of all febrile children attending EDs in that country.

Conclusions: In this study, we observed wide variation between European EDs in prescriptions of antibiotics and broad-spectrum antibiotics in febrile children. Overall, one-third of prescriptions were inappropriate or inconclusive, with marked variation between EDs. Until better diagnostics are available to accurately differentiate between bacterial and viral aetiologies, implementation of antimicrobial stewardship guidelines across Europe is necessary to limit antimicrobial resistance.

Why was this study done?

- Respiratory infections, which are mainly caused by viruses, account for the majority of antibiotic use in children. In children with respiratory infections, antibiotic prescription rates vary across emergency departments (EDs) in Europe.
- In order to optimise antibiotic prescriptions, it is important to better understand variability and appropriateness in antibiotic prescriptions.

What did the researchers do and find?

- In this prospective observational study, we included routine information of 35,650 children (median age 2.8 years) with fever attending 12 different EDs in Europe and calculated the proportion of antibiotic prescriptions and broad-spectrum antibiotic prescriptions. We adjusted for differences in population including age, comorbidity, disease severity, and focus and cause of infection.
- Across EDs, antibiotic prescription rates ranged between 22.4% and 41.6%, and of these prescriptions, broad-spectrum antibiotic rates ranged between 33.0% and 90.3%. Standardised antibiotic prescription rates ranged between 0.77 and 1.35, and standardised rates of broad-spectrum antibiotics ranged between 0.65 and 1.75.
- Prescriptions that were inappropriately indicated ranged from 0.6% to 29.3%, and inconclusive prescriptions ranged from 0.5% to 61.7%. The proportion of oral prescriptions with inappropriate duration ranged from 4.4% to 59.0%.

What do these findings mean?

- In this study we found variation of prescription of antibiotics and broad-spectrum antibiotics between EDs in children with fever, even when correcting for age, comorbidity, disease severity, diagnostics, and focus and cause of infection.
- Variation was especially large in prescriptions for viral infections and infections of unknown cause.
- In this cohort of febrile children, one-third of prescriptions were of inappropriate or inconclusive indication, with variation between EDs. In addition, guideline concordance for respiratory and urinary infections varied widely across EDs.
- Generalisation of these results to all EDs in Europe should be undertaken with caution.
- Implementation of guidelines is needed to improve appropriate prescription of antibiotics, whilst new biomarkers will further improve antibiotic prescription.

Introduction

Fever is one of the most common reasons for children to visit the emergency department (ED), and most visits are accounted for by self-limiting infections [1,2]. The proportion of children with a serious bacterial infection that needs treatment with antibiotics ranges from 7% to 13%, while antibiotic prescription rates in febrile children at EDs are between 19% and 64% [3-5]. Inappropriate antibiotic use, including the unnecessary use of broad-spectrum antibiotics, remains high in children, promoting the emergence of antimicrobial resistance [6-9]. Inappropriate antibiotic prescriptions were described in around 30% of outpatient prescriptions. However, these outpatient settings mainly involve primary care, and limited studies are available on specific emergency care [6,10].

Large variability exists between countries in antibiotic prescriptions in inpatient and outpatient settings, according to several large studies [6,8,11-14]. In general, these large studies did not adjust for differences in populations. In children, previous studies have demonstrated substantial variation of antibiotic use in general outpatient settings in the United States and Europe, indicating possible overuse of antibiotics [5,6,10,15].

A literature review on antibiotic prescription rates and their determinants in febrile children in emergency care found large heterogeneity of studied populations, which limited the ability to draw conclusions [16]. One recent European study, focusing solely on EDs, showed significant differences in antibiotic prescription rates in otherwise healthy children with respiratory tract infections (RTIs) [5]. Large studies at EDs across Europe are lacking that cover antibiotic and broad-spectrum prescriptions in all febrile children, including patients with comorbidity, patients with detailed clinical information, and patients in different diagnostic groups. Additionally, previous studies have addressed appropriate prescribing based on diagnosis coded with the International Classification of Diseases [6,10,17]. This classification, however, may not accurately take into account bacterial versus viral aetiology. Antibiotic prescription rates for viral and bacterial disease using a structured classification have not yet been investigated at EDs.

A better understanding of variability in antibiotic prescriptions in EDs and its relation with bacterial or viral disease, taking into account differences in case mix, is essential for the development and implementation of interventions to optimise antibiotic use. In addition, knowledge regarding variation of prescribing in infections where antibiotic prescription is inappropriate, such as prescriptions in viral disease, prescriptions of inappropriate duration, or prescriptions that are not concordant with guidelines, could target and improve implementation of antimicrobial stewardship guidelines at the ED level.

In this study, we aim to investigate the variation and appropriateness of rates and types of antibiotic prescription in febrile children attending 12 different EDs in Europe.

This is a main analysis of the MOFICHE (Management and Outcome of Fever in Children in Europe) study, which is embedded in the PERFORM (Personalised Risk assessment in Febrile illness to Optimise Real-life Management across the European Union) project (https://www.perform2020.org) [18]. MOFICHE is an observational multicentre study that studies the management and outcome of febrile children in Europe using routine data. The overall aim of PERFORM is to improve management of febrile children and to improve diagnosis through development of new diagnostic tests to discriminate viral and bacterial infections in children.

Methods

Study design

MOFICHE is a prospective observational study using data that are collected as part of routine care. This study is reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline (S1 Text), and data were analysed using an a priori statistical analysis plan (S2 Text). The study was approved by the ethics committees in the participating hospitals, and the need for informed consent was waived (S3 Text).

Study population and setting

Children aged 0–18 years presenting with fever (temperature ≥ 38.0 °C) or a history of fever (fever within 72 hours before ED visit) were included. Twelve EDs from 8 European countries participated in this study: Austria, Germany, Greece, Latvia, the Netherlands (n = 3), Spain, Slovenia, and the United Kingdom (n = 3). The EDs were included because they all participated in the PERFORM project. Characteristics of these EDs are described in S4 Text and in a previous publication [19]. In short, the participating hospitals were either university hospitals (n = 9) or large teaching hospitals (n = 3), and 11 EDs had paediatric intensive care facilities. Nine EDs were paediatric focused, and 3 EDs served both children and adults. Care for febrile children was supervised by general paediatricians (7 EDs), by paediatric emergency physicians (2 EDs), or by a general paediatrician or a (paediatric) emergency physician (3 EDs). All data were available in electronic healthcare records in 5 EDs, 1 ED used paper records, and 6 EDs used a combination of paper and electronic healthcare records.

Data were collected from January 2017 until April 2018, and for at least 1 year at each site to include all seasons. The period of data collection per month ranged from 1 week per month to the whole month in the participating hospitals (S4 Text).

Sample size

We expected to include 40,000 children with at most 5% missing data. Pilot data showed an overall antibiotic prescription rate of 30%. Applying 10 events per variable, this study is large enough to analyse over 1,000 determinants for the outcome antibiotic prescription [20]. We performed a post hoc sample size estimation for a desired width of the 95% confidence interval (CI) of standardised antibiotic prescription rate per ED. The expected width of the CI of the standardised prescription rate was below 0.5 for the smallest ED.

Data collection

Data were collected as part of routine ED care. The local research team entered data from patient records in an electronic case record form (eCRF) [21]. Collected data included age, sex, season, referral, comorbidity (chronic condition expected to last at least 1 year) [22], triage urgency, fever duration, fever measured at ED, presence of "red traffic light" symptoms for identifying risk of serious illness (alarming signs) (from the National Institute for Health and Care Excellence [NICE] guideline on fever [23]: decreased consciousness, ill appearance, work of breathing, meningeal signs, focal neurology, non-blanching rash, dehydration, status epilepticus), previous antibiotic use, vital signs (heart rate, respiratory rate, oxygen saturation, temperature, capillary refill time), laboratory results (white blood cell count, C-reactive protein [CRP], urinalysis), imaging (chest X-ray and other imaging), microbiological investigations (cultures and respiratory viral tests), and disposition (intensive care unit admission, general ward admission or discharge). We collected data on antibiotics prescribed in the ED or started on the first day of hospital admission (type, route of administration, and duration). The focus of infection was categorised as upper respiratory tract (otitis media, tonsillitis/pharyngitis, other), lower respiratory tract, gastrointestinal tract, urinary tract, skin, musculoskeletal, sepsis, central nervous system, flu-like illness, childhood exanthem, inflammatory syndrome, undifferentiated fever, or other.

To date, no reference standard exists to classify the cause of infection in routine ED practice [24]. The PERFORM consortium adapted the consensus-based flowchart from Herberg and colleagues [25,26], combining all available clinical data, investigation results such as CRP, cultures, and imaging. This flowchart was used to define the presumed cause of infection for each patient visit: definite bacterial, probable bacterial, bacterial syndrome, unknown bacterial/viral, viral syndrome, probable viral, definite viral, trivial, inflammatory syndrome and other (Fig 1). The diagnosis definite bacterial infection was assigned

only when a sterile site culture identified pathogenic bacteria. The diagnosis 'probable bacterial infection' was assigned when a bacterial syndrome was suspected but no bacteria were identified and CRP was above 60 mg/l. Patients with clinical bacterial symptoms and CRP ≤ 60 mg/l or no CRP were classified as 'bacterial syndrome'. Children with suspected viral infections were classified as 'viral syndrome' (no CRP or CRP > 60 mg/l) or 'definite viral' (CRP ≤ 60 mg/l) when a virus was identified that matched the clinical symptoms. Children with a viral syndrome and CRP ≤ 60 mg/l, but no identified virus, were classified as 'probable viral'. Children who did not fit these definitions were classified as unknown bacterial/viral. Children with mixed infections (bacterial and viral co-infection) were classified as bacterial. Children with trivial infections, inflammatory syndrome or other infections were classified as 'other'.

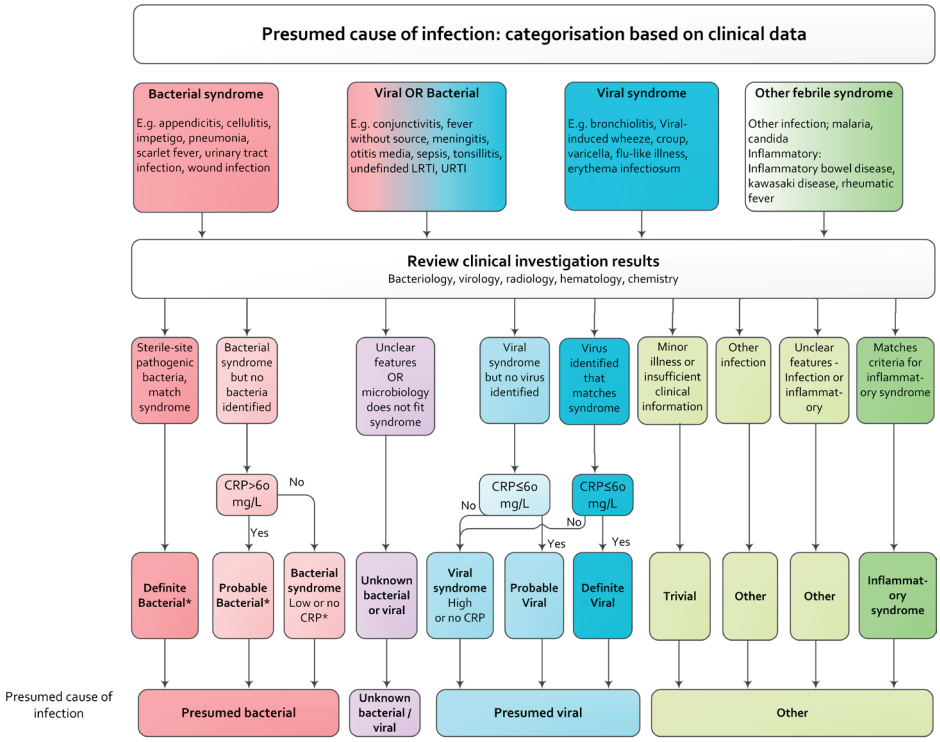

Fig 1 | Categorisation of presumed cause of infection.
CRP, C-reactive protein; LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection.
*Patients could have identified viral co-infection.

We aimed to improve data quality and standardised data collection by using a training module for the local clinical and research teams to optimise clinical assessment and data collection for febrile children. This training module included clarification of the individual alarming signs and classification examples of common diagnoses. Furthermore, entry

guidelines for the eCRF were available, monthly teleconferences and biannual meetings were organised, and quarterly reports of data quality for each ED were discussed. These consortium teleconferences also included discussion of difficult cases.

Antibiotic classification

Antibiotics were categorised using the Anatomical Therapeutic Chemical classification including beta-lactamase sensitive penicillins (J01CE); beta-lactamase resistant penicillins (J01CF); penicillins with extended spectrum (J01CA); combinations of penicillins including beta-lactamase inhibitors (J01CR); macrolides (J01FA); first-generation, second-generation, and third-generation cephalosporins (J01DB, J01DC, J01DD); trimethoprim and sulphonamides (J01EA01, J01EE01); aminoglycosides (J01GB); quinolones (J01MA); glycopeptides (J01XA); and other antibiotics.

In addition, we compared the prescription of narrow-spectrum and broad-spectrum antibiotics. We explored the definitions reported in previous studies on antibiotic classification and used an expert opinion panel including paediatric infectious disease specialists and general paediatricians (PERFORM partners), to establish the final classification into broad-spectrum and narrow-spectrum for all systemic antibiotics [5,6,11,15,27,28]. Narrow-spectrum antibiotics comprised penicillins (e.g., amoxicillin) and first-generation cephalosporins. Broad-spectrum antibiotics included penicillins with beta-lactamase inhibitor combinations (e.g., amoxicillin/clavulanic acid), macrolides, aminoglycosides, glycopeptides, and second-generation and third-generation cephalosporins. Prescriptions of both broad-spectrum and narrow-spectrum antibiotics in the same patient were considered broad-spectrum. Topical antibiotics were not included. Details of this classification are presented in S5 Text.

Outcomes

We assessed various aspects of antibiotic prescription: (1) antibiotic prescription rate; (2) the proportion of antibiotics that were broad-spectrum versus narrow-spectrum; (3) the proportion of antibiotics of 'likely appropriate' indication (presumed bacterial), 'likely inappropriate' indication (presumed viral), or 'inconclusive' indication (unknown bacterial/viral); (4) the proportion of oral antibiotics of inappropriate duration; and (5) the proportion of oral antibiotics that matched the antibiotic type in the local guideline ('guideline-concordant') in uncomplicated urinary and upper and lower RTIs. Antibiotic prescriptions were classified as likely appropriate in presumed bacterial infections (definite bacterial, probable bacterial, bacterial syndrome), likely inappropriate in presumed viral infections (definite viral, probable viral, viral syndrome), and inconclusive in unknown bacterial/viral infections or other infections. Inappropriate duration was defined as >10 days for treatment of tonsillitis with beta-lactamase sensitive penicillins

(J01CE) and >7 days for all other prescriptions according to recommendations by international guidelines [29-31]. In addition, guideline-concordant prescription in patients with uncomplicated RTIs and uncomplicated urinary tract infections was defined according to the local guideline (S6 Text). Uncomplicated infections were defined as infections in previously healthy children who did not receive therapeutic antibiotic treatment before the ED visit.

Data analysis

Missing values were assumed to be missing at random, and therefore we used multiple imputation by chained equations with the MICE package in R for the regression analysis. We excluded patients with missing data on antibiotic prescription, presumed cause of infection, and focus of infection [32]. Only the first visit was included for patients who visited the ED again within 5 days.

First, we performed a descriptive analysis of the frequency of antibiotic prescription and broad-spectrum and narrow-spectrum prescription, including ranges across EDs. For all outcomes, we calculated the overall proportion and proportion per ED. Second, we used multilevel logistic regression with a random intercept for each ED to study variation between EDs in antibiotic prescription, broad-spectrum prescription versus narrow-spectrum prescription, and intravenous/intramuscular versus oral prescriptions [33]. In an adjusted model we corrected for patient-level factors and for hospital-level factors influencing antibiotic prescribing. Patient-level factors were selected a priori according to the literature [3,4,23,34,35] and included general characteristics (age, sex, season, comorbidity, referral [referred versus self-referred]), markers for disease severity such as triage urgency (high urgency [immediate, very urgent, urgent] versus low urgency [standard, non-urgent]), fever duration in days, fever measured at ED visit (≥38 °C), and presence of NICE guideline "red traffic light" alarming signs (0, 1, ≥2). We investigated diagnostics, including CRP (not performed or <20, 20–60, or >60 mg/l) [25,36], chest X-ray (not performed, normal, abnormal), and urinalysis (not performed, normal, abnormal [positive for leukocyte esterase and/or nitrite]). Furthermore, we included focus of infection (upper respiratory tract, lower respiratory tract, gastrointestinal, urinary tract, undifferentiated fever, skin/musculoskeletal, sepsis/central nervous system, flu-like illness/childhood exanthem, inflammatory/other) and diagnostic groups according to cause as classified by the flowchart in Fig 1: presumed bacterial (definite bacterial, probable bacterial, bacterial syndrome), unknown bacterial/viral, presumed viral (definite viral, probable viral, viral syndrome), and other.

For the hospital-level factors, we explored variables that varied between hospitals and were related to antibiotic prescribing [19,37-40]: total number of ED visits, supervision, avail-

ability of point-of-care tests (streptococcal antigen test and CRP), and primary care during out-of-office hours. We included hospital-level factors if they improved the model using univariate analysis. Linearity of continuous variables was tested using restricted cubic splines. Specifications of the adjusted model are presented in Table 1 and in S7 Text.

Table 1 | Variables in the adjusted model.

Category	Variables
Patient-level factors	
General characteristics	Age*, sex, season, comorbidity, referral
Disease severity	Triage urgency, fever duration, fever measured at ED, presence of NICE alarming signs, previous antibiotic use°
Diagnostics	C-reactive protein, chest X-ray, urinalysis
Infection	Focus of infection, cause of infection
Hospital-level factors[±]	Total number of ED visits, supervision, availability of point-of-care tests (streptococcal antigen test and C-reactive protein), primary care during out-of-office hours[±]

*Age was modelled using restricted cubic splines (3 knots).
°Previous antibiotic use was added in the models with outcome broad-spectrum versus narrow-spectrum prescription.
[±]None of the hospital-level factors were significant, and therefore they were not included in the final model.
ED, emergency department; NICE, National Institute for Health and Care Excellence.

Variation in antibiotic prescription rates between EDs was determined by 2 measures: standardised prescription rates and median odds ratios (MORs). We calculated standardised antibiotic prescription rates using indirect standardisation, where the expected number of antibiotic prescriptions was standardised to the average ED. Standardised antibiotic prescription ratios are the ratio between observed antibiotic prescriptions in an ED and the expected antibiotic prescriptions in an ED. The expected number of antibiotic prescriptions was estimated through the adjusted model, by summing the predicted probabilities from the adjusted model of antibiotic prescription for each of the patients. Standardised rates > 1 indicate higher prescription rates than expected, and standardized rates < 1 indicate lower prescription rates than expected. We visualised standardised rates in a heat map.

The MOR is a measure of variation between high- and low-prescribing clusters of EDs. The MOR reflects the difference in probability of receiving antibiotics comparing similar patients attending an ED with high antibiotic prescribing and an ED with low antibiotic prescribing. If the MOR is equal to 1.00, there is no variation between clusters, and if the MOR is high, this indicates important between-cluster variation [41,42].

Stratified analyses were performed in patients with and without comorbidities. Also, since antimicrobial resistance patterns vary greatly between European countries, standardised rates of broad-spectrum versus narrow-spectrum antibiotic prescription were compared with antimicrobial resistance data of invasive isolates on a national level and at the hospital level [11,43] (S8 Text). Correlations were calculated using the 2-tailed Spearman's rank coefficient (ρ). A *p*-value below 0.05 was considered significant.

Appropriateness of antibiotic prescriptions

We calculated standardised rates for antibiotic prescription and broad-spectrum prescription in groups of presumed viral infections, presumed bacterial infections, and unknown bacterial/viral infections. Next, we assessed the proportion of all antibiotic prescriptions that were likely appropriate, likely inappropriate, and inconclusive. For all oral prescriptions, we calculated the proportion of prescriptions that were both inappropriate in indication (likely inappropriate) and of inappropriate duration, and the proportion of prescriptions that were either inappropriate in indication or inappropriate in duration. In uncomplicated RTIs and urinary tract infections, we calculated the proportion of all oral prescriptions that were inappropriate for all the 3 measures (indication, duration, and guideline concordance), and the proportion of prescriptions that were inappropriate in any of the 3 measures. R version 3.4 was used for the analysis and visualisation of the data.

Results

Study population

Of the total population of 38,480 patients, we excluded 738 patients based on missing data of antibiotics or diagnosis, and the repeated visit of 2,092 patients to the same ED. Compared to patients with complete outcome data, patients with missing data were similar in age, sex, comorbidity, and admission rate (S9 Text). In addition, there were no differences in completeness of outcomes and diagnosis between discharged and admitted patients.

For the analysis, we included 35,650 febrile children (median age 2.8 years [IQR 1.3–5.6], 54.6% male). The different EDs varied substantially in patients who were referred (range: 4.9%–99.2%), were ill appearing (range: 0.8%–47.4%), or had any comorbidity (range: 5.1%–65.3%) (Table 2). The most common infections were upper respiratory tract (*n* = 18,783, 52.7%), lower respiratory tract (*n* = 5,167, 14.5%), gastrointestinal tract (*n* = 3,694, 10.4%), and undifferentiated fever (*n* = 2,784, 7.8%). The incidence of sepsis and central nervous system infections was low (*n* = 270, 0.8%). The majority of the children had a

presumed viral infection (*n* = 20,383, 57.2%); presumed bacterial infections occurred in 22.1% of the patients (definite bacterial, 4.1%; probable bacterial/bacterial syndrome, 18.1%), and unknown bacterial/viral infections in 14.6% (*n* = 5,200) (Table 3).

Table 2 | Patient characteristics of the study population (*n* = 35,650).

Characteristic	*n* (%) or median (IQR)	Range across EDs (%)	Missing, *n* (%)
Age in years	2.77 (1.32–5.59)		
Male	19,476 (54.6)	51.5–59.1	1 (0.0)
Comorbidity	5,889 (16.5)	5.1–65.3	326 (0.9)
Season			1,111 (3.1)
Winter	12,665 (35.5)	26.8–53.2	
Spring	9,054 (25.4)	18.2–31.2	
Summer	5,767 (16.2)	9.5–23.5	
Autumn	8,164 (22.9)	6.9–31.4	
Triage urgency			1,059 (2.9)
High: immediate, very urgent, urgent	12,251 (34.4)	8.3–88.5	
Low: standard, non-urgent	22,340 (62.7)	10.1–91.6	
Referred	15,104 (42.4)	4.9–99.2	1,110 (3.1)
Fever duration in days	1.5 (0–3)		2,449 (6.9)
NICE "red traffic light" alarming signs			
Ill appearance	5,567 (15.6)	0.8–47.4	1,525 (4.3)
Work of breathing	2,987 (8.4)	3.2–25.7	4,482 (12.6)
Dehydration	1,763 (4.9)	0.4–15.2	6,323 (17.7)
Rash: petechiae/non-blanching	1,039 (2.9)	1.4–5.8	3,963 (11.1)
Decreased consciousness	188 (0.5)	0.1–3.8	334 (0.9)
Meningeal signs	132 (0.4)	0.1–1.7	1,807 (5.1)
Focal neurology	121 (0.3)	0.0–2.6	2,224 (6.2)
Status epilepticus	60 (0.2)	0.0–1.9	1,099 (3.1)
C-reactive protein (CRP)			
No CRP performed	19,578 (54.9)	7.9–93.2	
<20 mg/l	8,729 (24.5)	3.2–58.4	
20–60 mg/l	4,191 (11.8)	1.9–24.9	
>60 mg/l	3,152 (8.8)	1.6–30.2	
Chest X-ray			
No	30,662 (86.0)	78.6–93.8	
Normal	1,931 (5.4)	0.9–10.0	
Abnormal	3,057 (8.6)	2.9–12.8	
Urinalysis			
No	26,691 (74.9)	60.8–91.4	
Normal	7,210 (20.2)	7.1–29.8	
Abnormal	1,749 (4.9)	1.5–9.5	

ED, emergency department; IQR, interquartile range; NICE, National Institute for Health and Care Excellence.

Table 3 | Patient characteristics of the study population: Outcomes, *n* = 35,650.

Outcome	*n* (%) or median (IQR)	Range across EDs (%)
Therapeutic antibiotics use in last 7 days*	3,592 (10.1)	6.6–15.6
Antibiotic treatment duration, days	7 (5–10)	
Antibiotics prescribed at ED visit or first day of hospital admission*	11,371 (31.9)	22.4–41.6
Narrow-spectrum	5,401 (15.2)	3.1–23.2
Broad-spectrum	5,887 (16.5)	9.5–34.7
Antibiotic administration*		
Oral	7,636 (21.4)	10.4–34.2
Intravenous/intramuscular	3,564 (9.9)	1.7–21.3
Admission*	9,000 (25.2)	4.5–54.2
ICU admission*	147 (0.4)	0.1–4.3
Focus of infection		
Upper respiratory tract	18,783 (52.7)	25.7–70.0
Lower respiratory tract	5,167 (14.5)	8.5–26.4
Gastrointestinal/surgical abdomen	3,694 (10.4)	6.0–19.2
Undifferentiated fever	2,784 (7.8)	1.8–18.8
Flu-like illness/exanthem	1,753 (4.9)	2.0–11.9
Urinary tract	1,231 (3.5)	1.2–5.8
Soft tissue/musculoskeletal	876 (2.5)	0.5–6.8
Sepsis/central nervous system	270 (0.8)	0.0–3.9
Inflammatory	136 (0.4)	0.0–1.3
Other	957 (2.7)	1.2–8.4
Cause of infection		
Presumed viral	20,383 (57.2)	37.3–71.4
Definite bacterial	1,451 (4.1)	1.6–10.9
Probable bacterial/bacterial syndrome	6,438 (18.1)	4.7–31.8
Unknown bacterial/viral	5,200 (14.6)	1.6–37.9
Other	2,178 (6.1)	1.1–30.9

*Missing: therapeutic antibiotic use in last 7 days, 681/35,650 (1.9%); antibiotic duration, 1,980/11,371 (17.4%); broad-spectrum versus narrow-spectrum antibiotics, 83/11,371 (0.7%); antibiotic administration, 171/11,371 (1.5%); admission and ICU admission, 25/35,650 (0.1%).
ED, emergency department; ICU, intensive care unit; IQR, interquartile range.

Overall antibiotic prescriptions

The overall antibiotic prescription rate was 31.9% (*n* = 11,371), of which 67.2% (7,636/11,731) were oral administrations and 31.3% (3,564/11,371) were administered intravenously or intramuscularly (153 children received a single dose at the ED). One-third of patients were treated with antibiotics for over 7 days (3,534/9,391, 37.6%) (S1 Fig). The types of antibiotics most often prescribed were penicillins with extended spectrum (3,220/11,371, 28.3%), combinations of penicillins with beta-lactamase inhibitors (2,309/11,371, 20.3%),

and beta-lactamase sensitive penicillins (2,001/11,371, 17.6%). Half of the prescribed antibiotics were broad-spectrum agents (5,887/11,371, 51.7%). The most prescribed broad-spectrum antibiotics were combinations of penicillins with beta-lactamase inhibitors (2,309/11,371, 20.3%), second-generation cephalosporins (1,154/11,371, 10.1%), and third-generation cephalosporins (1,097/11,371, 9.6%) (Table 4; Fig 2).

Table 4 | Frequencies of antibiotic classes and ranges across EDs (*n* = 11,371).

Antibiotic class	*n* (%)	Range across EDs (%)
Beta-lactamase sensitive penicillins (e.g., benzylpenicillin)	2,001 (17.6)	0.1–32.5
Beta-lactamase resistant penicillins (e.g., flucloxacillin)	167 (1.5)	0.0–8.1
Penicillins with extended spectrum (e.g., amoxicillin)	3,220 (28.3)	2.6–61.6
Combinations of penicillins with beta-lactamase inhibitors (e.g., amoxicillin with clavulanate)	2,309 (20.3)	1.4–59.0
Macrolides (e.g., azithromycin)	638 (5.6)	2.9–11.0
First-generation cephalosporins	167 (1.4)	0.0–9.8
Second-generation cephalosporins	1,154 (10.1)	0.0–25.6
Third-generation cephalosporins	1,097 (9.6)	1.1–25.1
Trimethoprim and sulphonamides	128 (1.1)	0.0–5.1
Aminoglycosides	205 (1.8)	0.0–15.6
Quinolones	51 (0.4)	0.0–2.8
Glycopeptides	31 (0.3)	0.0–2.7
Other	120 (1.1)	0.0–4.6
Missing	83 (0.7)	0.0–3.6

ED, emergency department.

Variation of overall antibiotic prescription and broad-spectrum prescription

The proportion of febrile children receiving an antibiotic prescription ranged from 22.4% to 41.6% across EDs, and the proportion of those prescriptions that were for broad-spectrum agents ranged from 33.0% to 90.3%. Of the broad-spectrum agents, penicillins with beta-lactamase inhibitors had the largest variation (range 1.4%–59.0%), but other broad-spectrum agents varied as well (range 17.3%–37.0%). Fig 3 presents the standardised prescription rates from the adjusted model. None of the hospital-level factors was related with antibiotic prescription (*p*-value range: 0.14–0.77). After correction for general patient characteristics (age, sex, season, comorbidity, referral), disease severity (triage urgency, fever duration, fever measured at ED, alarming signs), diagnostics, focus of infection, and cause of infection, variability of antibiotic prescriptions remained between EDs in the adjusted model (range of standardised prescription rates: 0.77–1.35; MOR 2.41). Variation was also observed for intravenous versus oral

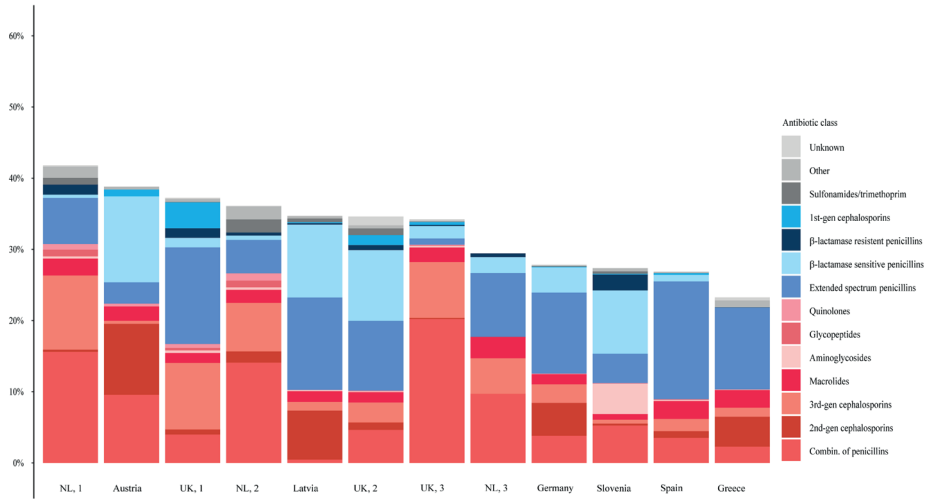

Fig 2 | Antibiotic classes of prescribed antibiotics across EDs, *n* = 35,650. Red shades indicate broad-spectrum classes, blue shades indicate narrow-spectrum classes and grey shades indicate unclassified classes and prescriptions of unknown class.
EDs are sorted by antibiotic prescription rate.
ED, emergency department; NL, the Netherlands; UK, United Kingdom.

EDs	Antibiotics vs no antibiotics n=35650	Intravenous vs oral n=11200	Broad vs narrow* n=11288
UK, 3	1.35 (1.34-1.36)	1.31 (1.29-1.34)	1.75 (1.73-1.77)
UK, 2	1.34 (1.32-1.35)	1.23 (1.20-1.26)	0.67 (0.66-0.69)
UK, 1	1.19 (1.17-1.21)	1.31 (1.27-1.34)	0.78 (0.76-0.80)
Greece	1.17 (1.16-1.19)	0.88 (0.85-0.91)	1.08 (1.06-1.10)
NL, 1	1.12 (1.09-1.15)	0.98 (0.94-1.01)	1.08 (1.05-1.11)
NL, 2	1.05 (1.03-1.07)	0.86 (0.83-0.88)	1.10 (1.08-1.12)
Spain	0.99 (0.97-1.00)	1.01 (0.95-1.07)	0.98 (0.95-1.00)
Germany	0.97 (0.94-0.99)	0.71 (0.67-0.75)	0.99 (0.96-1.02)
NL, 3	0.96 (0.92-1.00)	0.91 (0.86-0.97)	0.94 (0.89-0.99)
Austria	0.94 (0.93-0.96)	0.29 (0.27-0.31)	1.17 (1.14-1.19)
Latvia	0.84 (0.84-0.85)	1.20 (1.19-1.22)	0.65 (0.64-0.66)
Slovenia	0.77 (0.76-0.78)	1.15 (1.13-1.17)	0.68 (0.67-0.70)

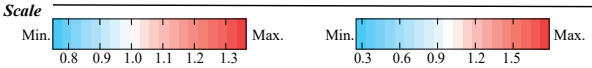

Fig 3 | Heat map of standardised prescription rates by ED (95% CI).
All adjusted for age, sex, season, comorbidity, referral, triage urgency, fever measured at ED, fever duration, alarming signs, CRP, chest X-ray, urinalysis, focus of infection, and cause of infection. EDs are ordered according to standardised antibiotic prescribing rate, from low to high on the left vertical axis. The coloured boxes represent rank of standardised rate for each ED: Red indicates rates > 1, blue indicates rates < 1, and rates equal to 1 are white.
*Also adjusted for previous antibiotic use.
ED, emergency department; NL, the Netherlands; UK, United Kingdom.

administration (range of standardised rates: 0.29–1.31; MOR 2.60) and prescription of broad-spectrum antibiotics versus narrow-spectrum antibiotics (range of standardised rates: 0.65–1.75; MOR 3.20). Stratified for comorbidity, standardised antibiotic prescription rates and broad-spectrum rates were comparable in children with and without comorbidity. Higher standardised rates for broad-spectrum antibiotics were not related to higher antimicrobial resistance percentages on a national level or on a hospital level (S8 Text). Results of variation of antibiotic and broad-spectrum prescriptions for RTIs are provided in S10 Text.

Variation of antibiotic and broad-spectrum prescriptions in viral infections, bacterial infections, and unknown bacterial/viral infections

The antibiotic prescription rate was 6.9% (1,418/20,383) for presumed viral infections (range across EDs: 0.4%–18.9%), 88.8% (1,289/1,451) for definite bacterial infections (range across EDs: 83.5%–96.2%), 94.7% (6,097/6,438) for probable bacterial / bacterial syndrome infections (range across EDs: 81.2%–99.3%), and 45.2% (2,348/5,200) for unknown bacterial/viral infections (range across EDs: 1.7%–79.3%) (S2 Fig).

Adjusted for general characteristics, disease severity, diagnostics, and focus of infection, we observed variation for antibiotic prescriptions in presumed viral infections (range of standardised rates: 0.05–3.29; MOR 4.91) and unknown bacterial/viral infections (range of standardised rates: 0.05–1.78; MOR 4.78) (Fig 4). Antibiotic prescriptions varied less for patients with presumed bacterial infections (range of standardised rates: 0.91–1.06; MOR 2.32). The proportion of broad-spectrum prescriptions was 74.1% (1,037/1,399) for presumed viral infections (range across EDs: 38.9%–91.4%), 68.5% (880/1,284) for definite bacterial infections (range across EDs: 39.2%–96.0%), 43.2% for probable bacterial/bacterial syndrome infections (2,628/6,081, range across EDs: 28.5%–86.3%), and 51.6% (1,191/2,306) for unknown bacterial/viral infections (range across EDs: 20.0%–95.7%) (S2 Fig). After adjustment, differences for broad-spectrum versus narrow-spectrum antibiotics remained for presumed viral infections (range of standardised rates: 0.57–1.54; MOR 2.59), presumed bacterial infections (range of standardised rates: 0.66–1.86; MOR 3.09), and unknown bacterial/viral infections (range of standardised rates: 0.44–1.64; MOR 3.70) (S3 Fig).

Variation in prescriptions of appropriate indication and appropriate duration

Of all antibiotic prescriptions, 65.0% (7,386/11,371) were determined to be likely appropriate (range across EDs: 23.7%–98.9%), 12.5% (1,418/11,371) were likely inappropriate (range across EDs: 0.6%–29.3%), and 22.6% (2,567/11,371) were inconclusive (range across EDs: 0.5%–61.7%).

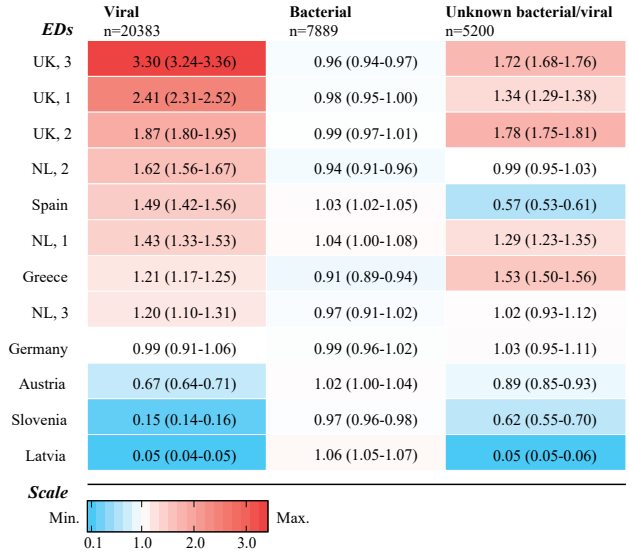

Fig 4 | Heat map of standardised antibiotic prescription rates by ED for presumed viral, presumed bacterial, and unknown bacterial/viral infections (95% CI).
All adjusted for age, sex, season, comorbidity, referral, triage urgency, fever duration, alarming signs, CRP, chest X-ray, urinalysis, and focus of infection. EDs are ordered according to standardised antibiotic prescribing rate, from low to high on the left vertical axis. The coloured boxes represent rank of standardised rate for each ED: Red indicates rates > 1, blue indicates rates < 1, and rates equal to 1 are white.
ED, emergency department; NL, the Netherlands; UK, United Kingdom.

Oral antibiotic prescriptions with inappropriate duration were found in 20.0% (1,525/7,636) of prescriptions, and this ranged from 4.4% to 59.0% across EDs (Fig 5). Of all oral antibiotic prescriptions, 2.1% (134/7,636) were of both inappropriate indication and inappropriate duration (range across EDs: 0.0%–8.4%), whereas 30.0% (2,294/7,636) were either of inappropriate indication or of inappropriate duration (range across EDs: 11.3%–69.9%).

Variation of appropriate prescriptions in uncomplicated RTIs and urinary tract infections

In uncomplicated RTIs, oral prescriptions were not guideline-concordant in 22.3% (973/4,373) of prescriptions (range across EDs: 11.8%–47.3%) (Fig 5). In this group, the proportion of prescriptions that were inappropriate in all 3 measures (indication, duration, and guideline concordance) was 0.7% (31/4,373), whilst 42.3% (1,850/4,373) were inappropriate in any of the 3 measures (range across EDs: 15.7%–80.9%). In uncomplicated urinary tract infections, oral prescriptions were not concordant with the local guideline in 45.1% of prescriptions (152/337) (range across EDs: 11.1%–100%), and 65.9% (222/337) were inappropriate in any of the 3 measures (range across EDs: 11.1%–100%).

Fig 5 | Heat map of inappropriateness of antibiotic prescriptions across EDs.
EDs are ordered according to proportion of inappropriately indicated prescriptions, from low to high on the left vertical axis. The coloured boxes represent rank of proportion for each ED: Red indicates the highest proportion, and white indicates the lowest proportion.
ED, emergency department; NL, the Netherlands; UK, United Kingdom.

Discussion

In this large prospective multicentre study, we found diversity in antibiotic prescriptions, and in particular broad-spectrum antibiotic prescriptions, for febrile children attending different EDs in Europe. After adjustment for general characteristics, disease severity, diagnostics, and focus of infection, we observed minor variation in antibiotic prescriptions for bacterial infections, and larger variability in antibiotic prescriptions for viral infections and unknown bacterial/viral infections. Moreover, one-third of all antibiotic prescriptions were of inappropriate or inconclusive indication, and 20% of oral prescriptions were of inappropriate duration, with large variation across EDs. Between EDs, the

proportion of oral prescriptions that were not concordant with the local guideline varied from 12% to 47% in RTIs and from 11% to 100% in urinary tract infections.

Our study supports previous studies that reported variable antibiotic prescribing for all febrile children, but found less variation than a previous study in children with RTIs across 28 European EDs (range of standardised rates: 0.5–2.0) [5]. In contrast to this study, our study corrected for aetiology of infection—bacterial, viral, or unknown—based on a standardised flowchart. Studies in the US on diversity in outpatient antibiotic prescribing found regional differences in both antibiotic and broad-spectrum prescribing [6,10,17]. These studies, however, were not focused on ED visits alone since all ambulatory visits were included.

The Access, Watch, and Reserve (AWaRE) classification has recently been used to classify global antibiotic prescriptions in 2 studies assessing oral formulations and use of inpatient antibiotics in children [14,44,45]. These studies confirmed variable patterns of antibiotic prescribing between countries, but did not adjust for differences in population and did not report data of emergency care visits. Further, the AWaRE classification led to a substantial proportion of unclassified antibiotics in our study population (12.2%; range across EDs: 1.9%–26.9%) and absence of the reserve category.

Previous studies in the US have evaluated appropriateness of antibiotic prescribing in children defined by ICD codes. Poole et al. [46] found that prescriptions were in general not indicated in 32% of emergency care visits in children. Additionally, overall prescription of first-line antibiotics (amoxicillin, amoxicillin-clavulanate) ranged from 50% to 78% for RTIs in children [46-48]. We found a similar rate of guideline-concordant prescriptions in RTIs (78%), whilst guideline concordance was defined differently for most EDs: amoxicillin and narrow-spectrum penicillins according to the local guideline. One ED (UK, 3) used amoxicillin-clavulanate as first-line for RTIs. Our study is the first to our knowledge to evaluate appropriateness of antibiotic prescribing in febrile children visiting different EDs in Europe, using a structured flowchart categorising viral, bacterial, and unclassified infections, and taking local guidelines into account.

Strengths of this European multicentre study include the large sample size, detailed patient information, recruitment in a diverse range of ED settings in 8 EU countries, and recruitment over a full year to reflect seasonal variation. Furthermore, a rigorous, standardised structured assessment of all cases was carried out to establish the presumed cause of infection, using a consensus-based flowchart taking into account clinical syndrome, CRP, and culture results. Previous studies have addressed appropriate prescribing for diagnoses based on ICD codes. This classification, however, may not

accurately take into account bacterial or viral aetiology [6,10,17,46]. Our large sample size enabled adjustment for hospital- and patient-level factors influencing antibiotic use in the EDs [19].

This study has some limitations. First, the included EDs are not representative of all febrile children attending the ED in that country. The EDs participating in this study are university hospitals or large teaching centres with intensive care unit facilities involved in paediatric infectious disease research collaborations. Fever and sepsis guidelines were available in all EDs [19]. Therefore, these EDs represent a high standard of care, and generalisation of our findings to smaller hospitals or to a regional or national level should be undertaken with caution. However, we corrected for the most important confounders including comorbidities, multiple markers of disease severity, and focus and presumed cause of infection. Second, although the experience of the physician (resident or consultant) and clinician specialty are related with antibiotic prescription [49,50], we could not adjust for physician background at the patient level. However, we evaluated the contribution of supervision to antibiotic prescription at the hospital level. In our study, supervision was not related to antibiotic prescription. Our efforts to improve data quality by training clinical and research staff might have influenced common clinical practice. Since this training focused on awareness of alarming signs in the clinical assessment of the febrile child, it is unlikely that it influenced antibiotic prescription. Furthermore, we only included the first visit of patients who repeatedly visited the ED, since data collection did not include secondary visits in all EDs.

Differences in antibiotic prescribing could be influenced by differences in immunisation coverage. In our study, countries with lower coverage for pneumococcal vaccinations (<90%) (Germany, Slovenia) did not have higher antibiotic prescriptions at the ED [19,51].

We found large variation in broad-spectrum prescriptions across the different EDs. Increased antimicrobial resistance rates could possibly explain higher broad-spectrum prescribing. We compared broad-spectrum rates with national data for antimicrobial resistance and hospital methicillin resistance rates. Interestingly, EDs based in countries with higher antimicrobial resistance on a population level (e.g., Greece, Spain) prescribed less broad-spectrum agents than expected in the ED. These hospitals with higher burden of national antimicrobial resistance may perceive more problems with antimicrobial resistance and might feel a greater pressure to reduce antibiotic prescriptions in the ED. It should be noted that antibiotic prescribing in the ED will not be representative of antibiotic prescription patterns of primary care in the community.

The diversity in antibiotic prescribing across different EDs appears not to be associated with antimicrobial resistance or immunisation coverage. Although the ideal antibiotic prescription rate is unknown, the diversity in antibiotic prescribing suggests overprescribing. Prescription rates were above the average incidence of serious bacterial infections. We found variation in antibiotic prescription rates, even when adjusting for general characteristics, disease severity, diagnostics, and focus and cause of infection.

This suggests room for improvement in reduction of antibiotic prescriptions and especially broad-spectrum prescriptions at the ED. EDs with higher antibiotic prescription rates did not necessarily prescribe more broad-spectrum antibiotics. The ED with the highest standardised broad-spectrum rate (UK, 3) did not have a high proportion of inappropriate prescriptions for RTIs. In only this ED, amoxicillin-clavulanate (broad-spectrum) was the first-choice agent for uncomplicated RTIs, which could explain the higher broad-spectrum rate in this ED. Studies demonstrated that use of narrow-spectrum antibiotics compared to broad-spectrum antibiotics leads to similar clinical outcomes and to fewer adverse events [28,52]. Unnecessary use of broad-spectrum antibiotics potentially increases resistance rates even further.

In addition, diversity of antibiotic prescription increased with diagnostic uncertainty. After adjustment for general characteristics, disease severity, diagnostics, and focus of infection, we observed minor variation in antibiotic prescriptions for bacterial infections, and larger variability in antibiotic prescriptions for viral infections and unknown bacterial/viral infections. In general, EDs with higher antibiotic prescription rates in viral infections also had higher antibiotic prescription rates in unknown bacterial/viral infections. This indicates that overprescribing in viral infections is linked to higher prescriptions in unknown bacterial/viral infections. Diagnostic uncertainty in patients with an unclear cause of infection could be reduced by improved targeted antibiotic prescription from new diagnostic signatures of bacterial and viral infection.

We evaluated appropriateness in indication, duration, and guideline concordance. Ideally, EDs should target 100% appropriateness in these 3 aspects of antibiotic prescribing. In our study, we did not observe a clear association between inappropriately indicated prescriptions and prescriptions of inappropriate duration. This indicates that guideline implementations should focus on these different aspects of appropriate antibiotic prescribing to ensure prescriptions of appropriate indication, duration, and antibiotic selection. Furthermore, quality improvement initiatives should be emphasised in EDs with higher proportions of inappropriate prescriptions. In addition, future antimicrobial stewardship interventions across Europe should focus on reducing broad-spectrum treatment and antibiotic use in viral infections.

To conclude, we found substantial variation in antibiotic prescriptions and especially broad-spectrum antibiotic prescriptions in European EDs after adjustment for patient characteristics, disease severity, diagnostics, and focus and cause of infection. The proportion of antibiotic prescriptions in bacterial infections was comparable between EDs, but diversity was especially large in antibiotic prescriptions for viral infections and unknown viral/bacterial infections. This variation indicates overprescription of antibiotics in these groups of patients. Furthermore, indications of prescriptions were inappropriate or inconclusive in one-third of prescriptions, and this proportion varied between EDs. In respiratory and urinary infections, guideline concordance of prescriptions varied widely across EDs. Until better diagnostics are available to accurately differentiate between bacterial and viral aetiologies, we strongly urge the implementation of antimicrobial stewardship guidelines to reduce antibiotic prescription in febrile children across Europe.

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Supporting information

Available as online web appendix from the website of PLOS Medicine

S1 Text. STROBE checklist.

S2 Text. Statistical analysis plan.

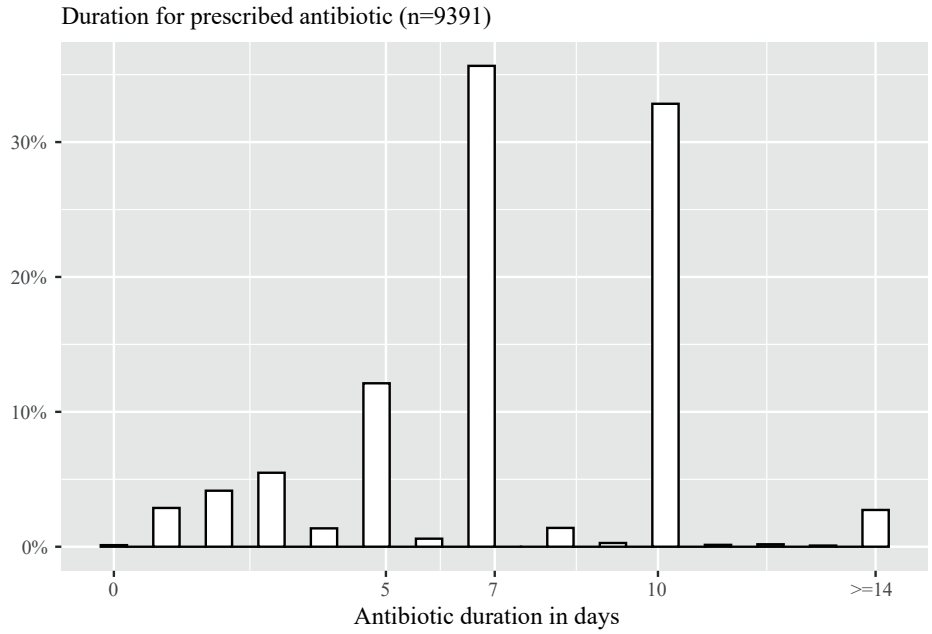
S3 Text. Ethics committees of participating hospitals.

S4 Text. Hospital characteristics.

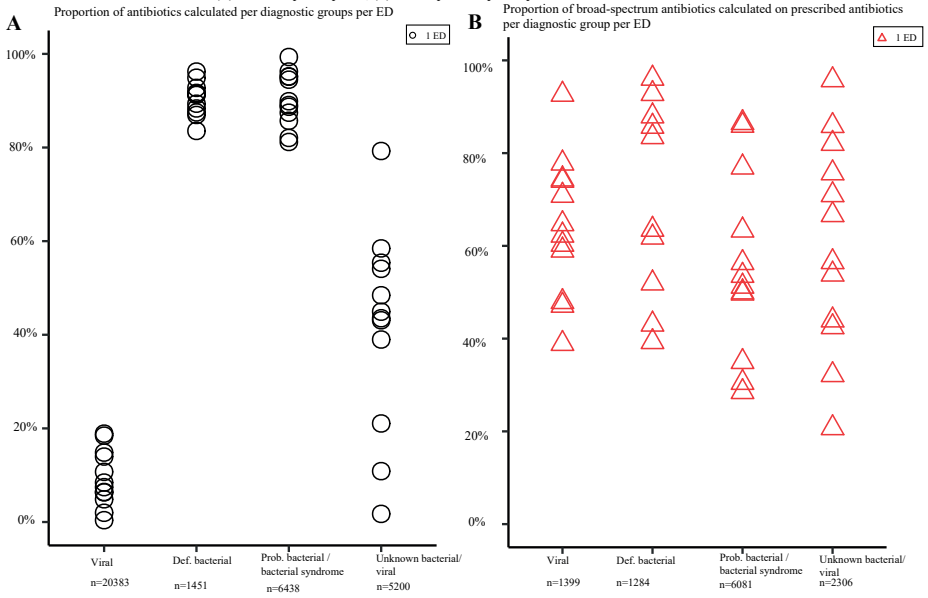
S7 Text. Details of the adjusted model.

S9 Text. Descriptive characteristics of cases with complete outcomes and cases with missing outcomes.

S1 Fig:


Duration for prescribed antibiotic (n=9391)
30%
20%
10%
0%
0
5
7
10
>=14
Antibiotic duration in days

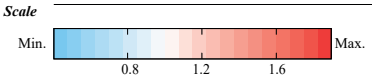
S2 Fig: Range of antibiotic prescriptions and broad−spectrum prescriptions per emergency department (ED) for viral, bacterial, and unknown bacterial/viral infections. (A) antibiotic prescriptions; (B) broad-spectrum prescriptions.



S3 Fig - Heat map of standardized broad-spectrum vs narrow-spectrum rates for viral, bacterial or unknown bacterial/viral infections (95% CI)

All adjusted for age, gender, season, comorbidity, fever duration, warning signs, CRP, focus of infection and previous antibiotic use.
*Also adjusted for triage urgency and focus of infection

EDs	**Viral*** n=1399	**Bacterial*** n=7365	**Unknown bacterial/viral** n=2306
UK, 3	1.54 (1.51-1.57)	1.86 (1.83-1.89)	1.64 (1.61-1.68)
Greece	1.15 (1.11-1.20)	1.08 (1.03-1.12)	0.86 (0.84-0.89)
NL, 2	1.07 (1.02-1.11)	1.10 (1.07-1.13)	1.09 (1.04-1.14)
NL, 1	0.97 (0.89-1.05)	1.04 (0.99-1.08)	1.13 (1.07-1.19)
Spain	0.96 (0.90-1.03)	0.98 (0.95-1.01)	0.94 (0.84-1.04)
UK, 1	0.96 (0.91-1.01)	0.81 (0.78-0.85)	0.56 (0.53-0.60)
NL, 3	0.95 (0.85-1.04)	0.94 (0.88-1.01)	0.84 (0.74-0.94)
Austria	0.94 (0.88-1.00)	1.21 (1.18-1.24)	1.05 (1.00-1.10)
Germany	0.93 (0.84-1.02)	0.95 (0.90-0.99)	0.98 (0.89-1.07)
UK, 2	0.92 (0.88-0.97)	0.77 (0.75-0.80)	0.44 (0.42-0.46)
Slovenia	0.76 (0.69-0.83)	0.69 (0.67-0.70)	0.93 (0.79-1.08)
Latvia	0.57 (0.48-0.67)	0.66 (0.65-0.67)	0.81 (0.67-0.94)



Supplemental file 5 - Broad-spectrum and narrow-spectrum definition

Definition of broad-spectrum and narrow-spectrum antibiotics (MOFICHE)[1-6]

Narrow		Broad	
Beta-lactamase sensitive penicillins	benzylpenicillin (pen G), pheneticillin, benzathine, phenoxymethylpenicillin, benzathine benzylpenicillin, phenoxymethylpenicillin (pen V)	***Combinations of penicillins, including beta-lactamase inhibitors***	ampicillin and beta-lactamase inhibitor, amoxicillin and beta-lactamase inhibitor, piperacillin and beta-lactamase inhibitor
Beta-lactamase resistant penicillins	cloxacillin, flucloxacillin, oxacillin	***2nd- generation cephalosporins***	cefaclor, cefprozil, cefuroxime
Penicillins with extended spectrum	amoxicillin, ampicillin, piperacillin	***3rd –generation cephalosporins***	cefotaxime, ceftazidime, cefixime, ceftriaxone, cefdinir, cefpodoxime
1st -generation cephalosporins	cefazolin, cefadroxil, cefalexin,	***Macrolides***	erythromycin, azithromycin, clarithromycin, josamycin, midecamycin
Sulfonamides/ trimethoprim	trimethoprim	***Sulfonamides/ trimethoprim***	sulfamethoxazole-trimethoprim
Nitrofurans	furazolidone, nitrofurantoin	***Tetracyclines***	doxycycline, tetracycline
Other	colistin (polymxin), tazobactam	***Lincosamides***	clindamycin
		Carbapenems	meropenem
		Quinolones	ciprofloxacin, levofloxacin, ofloxacin
		Aminoglycosides	gentamicin, tobramycin, amikacin, neomycin
		Glycopeptides	vancomycin, teicoplanin
		Imidazole derivates	metronidazole
		Other	rifampicin

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Supplemental file 6 – Local guidelines of antibiotic treatment

1st choice antibiotic for uncomplicated infections:

otitis media, lower respiratory tract infections, other respiratory infections:

All EDs (apart from UK, 3): 1st choice amoxicillin

UK, 3: 1st choice amoxicillin-clavulanate

1st choice antibiotic for uncomplicated infections:

tonsillitis/pharyngitis:

All EDs (apart from UK, 3): penicillin. In these EDs, amoxicillin was also defined as appropriate treatment for tonsillitis/pharyngitis.

UK, 3: <8 year: amoxicillin-clavulanate. >8 year: penicillin

Local recommendations for oral treatment of urinary tract infections

Infection	Recommended antibiotic type	ED
Urinary tract	Amoxicillin/clavulanic acid	Germany, Munich
		NL, Rotterdam
		NL, Nijmegen, Canisius
		NL, Nijmegen, Radboud
	Cefuroxime	Greece, Athens
		Latvia, Riga
		Spain, Santiago
	Cephalexine	UK, Liverpool
		UK, London
		UK, Newcastle
	Cephalexine OR Amoxicillin/clavulanic acid	Austria, Graz
	Suflonamides/trimethoprim OR nitrofurantoin	Slovenia, Ljubljana

Supplemental file 8 - National and hospital antimicrobial resistance data

Methods:

Since antimicrobial resistance patterns vary greatly between European countries, standardized rates of broad-spectrum vs narrow-spectrum antibiotics were compared with antimicrobial resistance data of invasive isolates for specific pathogens on a national level and the local hospital [1, 2]: staphylococcus aureus (methicillin resistance) for all patients, Streptococcus pneumoniae (resistance for penicillins and macrolides) for patients with lower respiratory tract infections and Escherichia coli (combined resistance for fluoroquinolones, 3rd-generation cephalosporins and aminoglycosides) for patients with urinary tract infections. Since antimicrobial resistance patterns could vary between hospitals in the same country, we also compared high broad-spectrum prescriptions with data of the local methicillin resistance for invasive isolates of S. aureus. For hospital resistance data, we focused on methicillin resistance for S. aureus as these data were uniformly collected. Correlations were calculated using the two-tailed Spearman's Rank coefficient (ρ). A p-value below 0.05 was determined as significant.

Results:

Higher standardized rates for broad-spectrum antibiotics were not related to higher antimicrobial resistance percentages on a national level (resistance S. aureus (ρ -0.07 95% CI (-0.62 to 0.53), p=0.83), S. pneumoniae (ρ -0.31 (95% CI -0.77 to 0.35), p=0.35) and E. coli (ρ 0.07 95%CI (-0.52 to 0.62), p=0.82) or on a hospital level (methicillin resistance rates for S. aureus ρ 0.12 (95% CI -0.48 to 0.65), p=0.70).

National antimicrobial resistance data extracted from ECDC 2017[1]

N: number of isolates tested

S. aureus - resistance to methicillin

Country	N	Resistance %	95% CI
Austria	3158	5.9	(5-7)
Germany	12021	9.1	(9-10)
Greece	822	38.4	(35-42)
Latvia	210	5.7	(3-10)
Netherlands	2694	1.5	(1-2)
Slovenia	576	9	(7-12)
Spain	1804	25.3	(23-27)
United Kingdom	8883	6.9	(6-7)

S. Pneumoniae - resistance to penicillins and macrolides

Country	N	Resistance %	95% CI
Austria	457	3.3	(2-5)
Germany	1803	2.4	(2-3)
Greece*	-	-	-
Latvia	28	3.6	(0-18)
Netherlands	1297	1.1	(1-2)
Slovenia	216	6.5	(4-11)
Spain	676	12.4	(10-15)
United Kingdom	3885	2	(2-2)

*Data from Greece not available

E.coli - combined resistance to fluoroquinolones, 3rd generation cephalosporins and aminoglycosides

Country	N	Resistance %	95% CI
Austria	5071	3.3	(3-4)
Germany	20610	3.7	(3-4)
Greece	1463	9.8	(8-11)
Latvia	197	11.2	(7-16)
Netherlands	6681	1.9	(2-2)
Slovenia	1381	6.3	(5-8)
Spain	5551	5.5	(5-6)
United Kingdom	26808	4.1	(4-4)

Hospital antimicrobial resistance rates for S. aureus - resistance to methicillin for invasive isolates

Country	N	N Resistant isolates	Resistance %	Population
Austria	237	22	9%	Children & adults
Germany	13	0	0%	<18 y
Greece	23	5	22%	<18 y
Latvia	5	1	20%	<18 y
Slovenia	16	0	0%	<18 y
Spain	11	0	0%	<18 y
NL, 1 and NL, 3	55	1	2%	Children & adults
NL, 2	37	2	5%	<18 y
UK, 1	50	10	20%	<18 y
UK, 2	61	3	5%	<16 y
UK, 3	68	5	7%	<16 y

NL, the Netherlands; UK, United Kingdom, y, year

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2. World Health Organization. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. : World Health Organization; 2017 [cited 2019 25-03-2019]. Available from: https://www.who.int/medicines/areas/rational_use/PPLreport_2017_09_19.pdf?ua=1.

Supplemental file 11 - Variation of antibiotic and broad-spectrum prescription in lower respiratory, otitis media, tonsillitis/pharyngitis and other upper respiratory tract infections

Results:

Antibiotic and broad-spectrum prescriptions varied for respiratory tract infections. In particular, antibiotic prescriptions varied for otitis media (80.7%, range EDs 39.8-92.5%) and tonsillitis/pharyngitis (51.3%, range EDs 14.8-82.3%) (Fig A). After adjustment of general characteristics, disease severity, CRP and cause of infection, variability remained in antibiotic prescription rates for patients with lower respiratory tract infection (range standardized rates 0.71-1.32, MOR 4.14), otitis media (range standardized rate: 0.73-1.78, MOR 4.48), tonsillitis/pharyngitis (range standardized rate: 0.63-1.85, MOR 3.54) and other upper respiratory tract infections (range standardized rate 0.39-2.77, MOR 3.14) (Fig B). These differences increased for broad-spectrum vs narrow-spectrum antibiotics in the majority of the different groups (range standardized rates: lower respiratory tract: 0.50-1.71, otitis media: 0.42-3.82, tonsillitis/pharyngitis 0.34-3.43, other upper respiratory 0.39-1.51) (Fig C).

Figure A: Range of antibiotic prescriptions (A) and broad−spectrum prescriptions (B) per emergency department (ED) for lower and upper respiratory tract infections

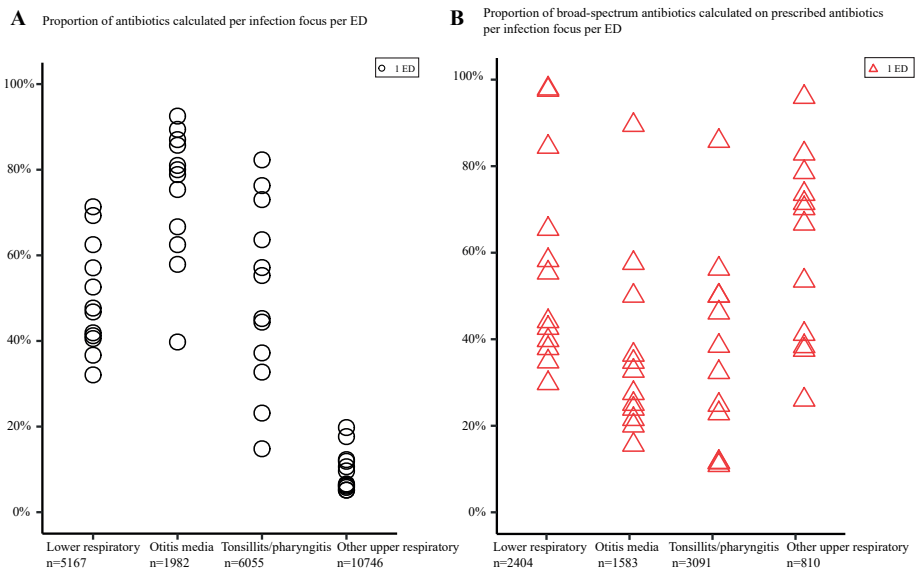


Figure B - Heat map of standardized antibiotic prescription rates stratified for lower respiratory, otitis media, tonsillitis/pharyngitis and other upper respiratory tract infections (95% CI)

All adjusted for age, sex, season, comorbidity, fever duration, warning sings, CRP, cause of infection.
*Also adjusted for chest X-ray and triage urgency

EDs	**Lower respiratory*** n=5167	**Otitis media** n=1982	**Tonsillitis/pharyngitis** n=6055	**Other upper respiratory** n=10746
UK, 2	1.32 (1.28-1.36)	1.45 (1.40-1.50)	1.85 (1.81-1.89)	1.50 (1.41-1.58)
UK, 3	1.31 (1.28-1.34)	1.78 (1.72-1.84)	1.12 (1.10-1.14)	2.77 (2.68-2.86)
UK, 1	1.24 (1.19-1.30)	1.10 (0.96-1.24)	1.33 (1.27-1.39)	1.55 (1.44-1.67)
NL, 2	1.20 (1.15-1.24)	0.73 (0.67-0.78)	1.21 (1.12-1.30)	1.34 (1.27-1.41)
NL, 1	1.14 (1.07-1.21)	1.01 (0.88-1.13)	1.05 (0.89-1.21)	1.55 (1.45-1.66)
Germany	1.07 (1.01-1.12)	0.81 (0.76-0.87)	0.93 (0.89-0.98)	1.00 (0.93-1.07)
Spain	1.03 (0.99-1.06)	0.99 (0.97-1.02)	0.84 (0.82-0.87)	1.26 (1.18-1.33)
Greece	1.02 (1.00-1.05)	1.71 (1.67-1.76)	1.08 (1.05-1.11)	1.30 (1.25-1.35)
NL, 3	0.99 (0.91-1.06)	0.95 (0.83-1.08)	0.69 (0.54-0.85)	0.92 (0.77-1.08)
Latvia	0.81 (0.79-0.82)	0.91 (0.89-0.93)	0.83 (0.82-0.84)	0.55 (0.53-0.57)
Slovenia	0.79 (0.77-0.81)	0.94 (0.90-0.99)	0.63 (0.60-0.65)	0.39 (0.36-0.42)
Austria	0.71 (0.67-0.74)	1.23 (1.17-1.29)	1.08 (1.05-1.10)	0.54 (0.50-0.58)

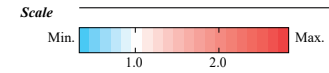
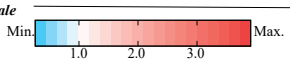


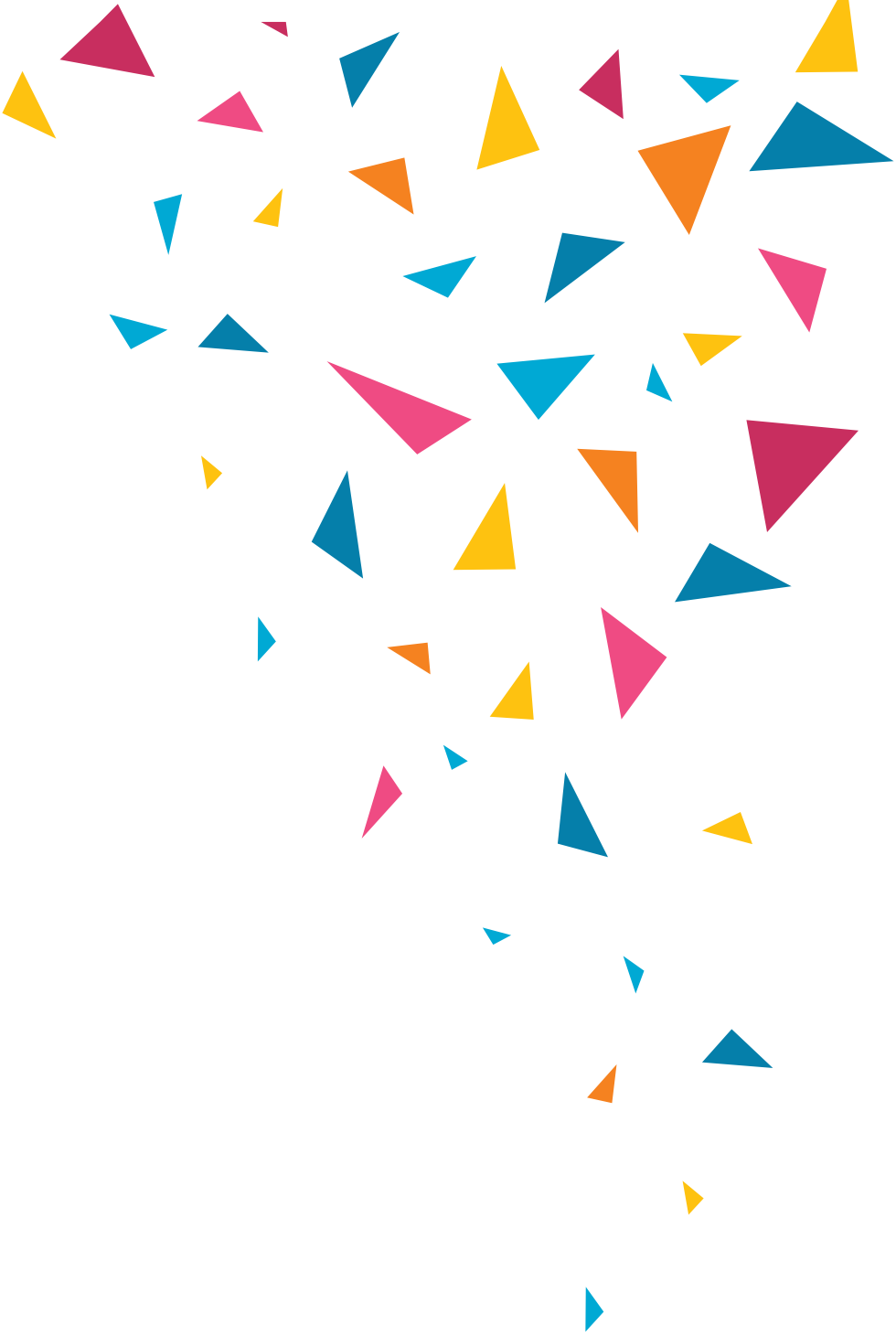
Figure C - Heat map of standardized broad-spectrum vs narrow-spectrum prescription rates stratified for lower respiratory, otitis media, tonsillitis/pharyngitis and other upper respiratory tract infections (95% CI)

All adjusted for age, sex, season, comorbidity, fever duration, warning sings, CRP, cause of infection and previous antibiotic use

*Also adjusted for chest X-ray

EDs	**Lower respiratory*** n=2404	**Otitis media** n=1583	**Tonsillitis/pharyngitis** n=3091	**Other upper respiratory** n=810
UK, 3	1.71 (1.67-1.75)	3.82 (3.68-3.96)	3.43 (3.36-3.50)	1.51 (1.46-1.56)
Austria	1.52 (1.45-1.59)	1.07 (0.97-1.16)	1.22 (1.18-1.26)	1.09 (0.99-1.18)
NL, 2	1.06 (1.02-1.11)	1.41 (1.26-1.55)	1.35 (1.21-1.48)	1.18 (1.11-1.25)
Greece	0.94 (0.88-1.00)	0.83 (0.78-0.87)	1.40 (1.33-1.47)	1.13 (1.08-1.18)
Germany	0.94 (0.88-1.00)	0.91 (0.79-1.03)	1.16 (1.05-1.27)	0.66 (0.58-0.73)
NL, 1	0.90 (0.84-0.97)	0.99 (0.82-1.17)	1.05 (0.82-1.27)	1.17 (1.07-1.26)
UK, 1	0.73 (0.68-0.77)	0.61 (0.45-0.76)	0.69 (0.63-0.76)	0.82 (0.74-0.89)
Spain	0.71 (0.67-0.75)	1.06 (1.01-1.10)	0.57 (0.52-0.62)	0.72 (0.66-0.79)
UK, 2	0.70 (0.67-0.73)	0.60 (0.55-0.65)	0.38 (0.36-0.41)	0.69 (0.62-0.75)
NL, 3	0.63 (0.56-0.71)	1.11 (0.87-1.35)	1.06 (0.73-1.39)	1.03 (0.82-1.23)
Latvia	0.54 (0.52-0.55)	0.42 (0.40-0.44)	0.35 (0.33-0.36)	0.40 (0.36-0.43)
Slovenia	0.50 (0.48-0.52)	0.48 (0.44-0.53)	0.59 (0.54-0.65)	0.96 (0.88-1.05)





9

Impact of a clinical decision rule on antibiotic prescription for children with suspected lower respiratory tract infections presenting to European emergency departments: a simulation study based on routine data

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Abstract

Background: Discriminating viral from bacterial lower respiratory tract infections (LRTIs) in children is challenging thus commonly resulting in antibiotic overuse. The Feverkidstool, a validated clinical decision rule including clinical symptoms and C-reactive protein, safely reduced antibiotic use in children at low/intermediate risk for bacterial LRTIs in a multicentre trial at emergency departments (EDs) in the Netherlands.

Objectives: Using routine data from an observational study, we simulated the impact of the Feverkidstool on antibiotic prescriptions compared with observed antibiotic prescriptions in children with suspected LRTIs at 12 EDs in 8 European countries.

Methods: We selected febrile children aged 1 month to 5 years with respiratory symptoms and excluded upper respiratory tract infections. Using the Feverkidstool, we calculated individual risks for bacterial LRTI retrospectively. We simulated antibiotic prescription rates under different scenarios: (1) applying effect estimates on antibiotic prescription from the trial; and (2) varying both usage (50%–100%) and compliance (70%–100%) with the Feverkidstool's advice to withhold antibiotics in children at low/intermediate risk for bacterial LRTI (≤10%).

Results: Of 4938 children, 4209 (85.2%) were at low/intermediate risk for bacterial LRTI. Applying effect estimates from the trial, the Feverkidstool reduced antibiotic prescription from 33.5% to 24.1% [pooled risk difference: 9.4% (95% CI: 5.7%–13.1%)]. Simulating 50%–100% usage with 90% compliance resulted in risk differences ranging from 8.3% to 15.8%. Our simulations suggest that antibiotic prescriptions would be reduced in EDs with high baseline antibiotic prescription rates or predominantly (>85%) low/intermediate-risk children.

Conclusions: Implementation of the Feverkidstool could reduce antibiotic prescriptions in children with suspected LRTIs in European EDs.

Introduction

Discriminating viral from bacterial aetiology in lower respiratory tract infections (LRTIs) is challenging, due to similarities in clinical symptoms and the absence of a gold standard.[1] Despite the implementation of national guidelines,[2] antibiotic prescription rates for LRTIs are high and vary widely (27%–84%) at European emergency departments (EDs), suggesting overtreatment.[2,3] Unnecessary antibiotic prescriptions can lead to adverse effects, additional costs and antimicrobial resistance.[4–6] Therefore, unnecessary antibiotic prescriptions should be reduced in children at low risk for bacterial LRTIs.

Clinical decision rules can be useful in reducing antibiotic prescribing.[7,8] Nijman *et al.*[9] developed the Feverkidstool, which predicts serious bacterial infections and specifies the individual probability of children having bacterial pneumonia, based on clinical parameters and C-reactive protein (CRP) level. To reduce antibiotic treatment, the Feverkidstool advises to withhold antibiotic prescription for patients at low/intermediate risk for having bacterial LRTI. The Feverkidstool has been extensively validated[8–11] and its effect on antibiotic prescriptions was evaluated in a stepped-wedge cluster-randomized multicentre study in EDs in the Netherlands.[12] In this intervention trial, antibiotic prescription in usual care was compared with antibiotic prescription using the advice of the Feverkidstool: withholding antibiotics for patients at low/intermediate risk for bacterial pneumonia (≤10%) or antibiotic prescription at the discretion of the physician for patients at high risk (>10%). This did not result in overall reduction of antibiotic prescribing in all patients, but it did achieve a reduction of antibiotic prescription in low/intermediate-risk patients as well as less therapy failure amongst high-risk patients. Moreover, in low/intermediate-risk patients the withholding of antibiotics did not influence therapy failure and thus was shown to be safe. The proportion of low/intermediate-risk patients was lower in the intervention trial than was estimated in the power calculations. The authors discussed that the potential effect of the Feverkidstool is related to the proportion of low/intermediate-risk patients and that its effect might be larger in settings with more low/intermediate-risk patients or higher baseline prescription rates.

Besides the differences in patient population, the potential impact of the Feverkidstool on antibiotic prescription is influenced by differences in uptake, including usage and compliance rates. In both clinical trials and observational studies at EDs, clinical decision rules were calculated in 50%–93% (usage rate),[12–17] whilst the treatment advice was followed in 80%–96% (compliance rate).[2,10,12–14,16,18] In addition, it is not evident that the effects from the intervention trial can be extrapolated to other European countries due to differences in the proportion of low/intermediate-risk patients and baseline prescription rates in LRTIs at European EDs.[8]

A clinical study to assess the prospective impact of the Feverkidstool in European EDs would be expensive and time-consuming and would expose children to additional investigations, whereas a simulation study is an efficient method to evaluate its effect under different scenarios for the uptake of the decision rule and, on top of that, its effect in different patient populations.[19,20] Using routine data, this study aims to simulate the potential impact of the Feverkidstool on antibiotic prescription rates in children with suspected LRTIs at European EDs compared with observed antibiotic prescriptions.

Patients and methods

Study design and population

This study is a secondary analysis of data collected as part of the Management and Outcome of Fever in Children in Europe (MOFICHE) study, which is embedded in the Personalized Risk assessment in Febrile illness to Optimize Real-life Management across the European Union (PERFORM) project (www.perform2020.org). MOFICHE is an observational study performed in 12 EDs in university or large teaching hospitals in 8 different European countries: Austria, Germany, Greece, Latvia, the Netherlands (n=3), Spain, Slovenia and the UK (n=3). Study design and details regarding these EDs have previously been described.[21,22] The STROBE reporting guideline was followed.

In short, MOFICHE included routine data of children aged <18 years with a temperature of ≥38.0°C measured at the ED or a history of fever in the 72 h before the ED visit. For this study, we focused the main analysis on children >1 month to 5 years of age with suspected LRTI. Following inclusion and exclusion criteria of the intervention trial, we selected children with respiratory symptoms, defined as coughing and/or increased work of breathing. We excluded children with a single clinical focus of upper respiratory tract infection, children with therapeutic antibiotic treatment up to 7 days prior to the ED visit and children with relevant comorbidity, i.e. a condition in ≥2 organ systems, or immunodeficiency, malignancy, cardiac condition, psychomotor delay or prematurity (born before gestational age of 32 weeks and <1 year of age at the time of presentation).[9,12] For subanalysis, we also included children aged 5–12 years and 12–18 years with suspected LRTIs according to aforementioned inclusion and exclusion criteria.

Collected data included age, sex, comorbidity,[23] type of referral (self-referral, GP, private paediatrician, emergency medical services or other) and triage urgency. In addition, we collected the presence of ill appearance, vital signs (heart rate, respiratory rate, oxygen saturation, temperature, capillary refill time) and diagnostic data including laboratory results (CRP level), imaging and microbiological results. We collected the presumed

focus of infection by the physician after assessment at the ED, and hospital admission or ICU admission following the ED visit. We recorded antibiotic prescription (type, route of administration) at the ED or in the first 24 h of hospital admission.[21]

Outcome

The primary outcome was the difference between observed antibiotic prescription rates and antibiotic prescription rate after simulating the implementation of the Feverkidstool in different scenarios.

Missing data

Vital signs marked as normal were given a normal value based on age-adjusted Advanced Paediatric Life Support (APLS) ranges.[24] CRP values marked as normal were given a value in the range 0–8 mg/L.[25] Missing values of the predictor variables of the Feverkidstool, including missing CRP level, were multiple imputed (MICE package). The imputation model included covariates of the Feverkidstool and auxiliary variables associated with urgency, disease severity, diagnostics, working diagnosis and antibiotic treatment. Patients with missing values for antibiotic prescription were excluded from analysis.

Simulation

We retrospectively calculated individual risk scores of having a bacterial LRTI based on the original Feverkidstool algorithm. The Feverkidstool included the following variables: age <1 year; age ≥1 year; sex; fever duration; temperature; tachypnoea and tachycardia defined by APLS;[24] oxygen saturation <94%; capillary refill time ≥3 s; increased work of breathing; ill appearance; and CRP level (details in Table S1, available as Supplementary data at *JAC* Online).[9] A risk threshold of 10%, based on earlier research,[8,9,12] was used to classify patients at low/intermediate risk (≤10%) or high risk (>10%) for bacterial LRTI. Characteristics of the low/intermediate-risk versus high-risk groups were compared using chi-squared tests, independent *t*-tests and Mann–Whitney *U*-tests. Results with a *P* value <0.05 were deemed significant.

The effect of the Feverkidstool on antibiotic prescriptions was simulated using five strategies: (1) applying the effect estimates on antibiotic prescription from the intervention trial; (2) sensitivity analysis showing the effect of different combinations of usage and rates of compliance with the Feverkidstool's advice; (3) subgroups of each separate ED; (4) the transferability of the Feverkidstool's effect to older age groups (5–12 years, 12–18 years) and; (5) sensitivity analysis on complete cases for CRP data. The differences between observed prescription rates with simulated prescription rates were quantified by risk differences (RDs) and risk ratios (RRs).[26] All simulations were calculated separately for each of the 12 EDs and were pooled using a random-effects model (metafor package).

For the first simulated strategy, we simulated antibiotic prescription rates under the assumption that implementation of the Feverkidstool would have equal effect on antibiotic prescription as in the intervention trial.[12] In the trial, the pre-intervention prescription rate was 17% in the low/intermediate-risk group and 47% in the high-risk group. The adjusted ORs for antibiotic prescription after implementing the Feverkidstool were 0.31 (95% CI: 0.12–0.81) for the low/intermediate-risk group and 2.28 (95% CI: 0.84–6.17) for the high-risk group. To estimate the overall prescription rate after simulating the implementation of the Feverkidstool, we sampled ORs (n=1000) based on the results from the intervention trial (estimated effect and standard error) and applied these to the routine data to obtain the simulated prescription rate and associated uncertainty after implementing the Feverkidstool. Separate ORs were sampled for the low/intermediate-risk and high-risk groups.

For the sensitivity analysis, we simulated the effect of the Feverkidstool on antibiotic prescription for varying usage rates (50%–100%) combined with varying compliance rates (70%–100%). These rates were chosen according to published impact studies of clinical decision rules in the ED: usage rates (50%–93%)[12–17] and compliance rates (80%–96%) where the average compliance rates was ±90%.[2,10,12–14,16,18] Usage and compliance rates were modelled using a uniform random distribution on patient level, meaning that every patient had the same probability of usage or compliance. The usage rate was modelled as the percentage of patients for whom the Feverkidstool risk score was calculated. For these children, the compliance rate was modelled as the percentage of patients for whom physicians followed the advice of the Feverkidstool. Compliance resulted in withholding of antibiotics for low/intermediate-risk patients, whilst non-compliance resulted in antibiotic prescriptions to low/intermediate-risk patients despite the advice to withhold them. In high-risk patients, we assumed that antibiotic treatment was as observed in the data. For this analysis of varying compliance rates, we assumed that the simulated antibiotic rates could not exceed observed prescription rates.

Third, we simulated the effect estimates of the intervention trial in each ED separately to provide insight on the Feverkidstool's effect in populations with different antibiotic prescription rates and different distribution of low/intermediate-risk patients. Fourth, we evaluated the transferability of the Feverkidstool's effect to older age groups with suspected LRTIs including 5–12 years and 12–18 years. Last, since we imputed CRP level for the main analyses, a sensitivity analysis was performed on complete cases: all analyses were repeated in children with CRP data available. Statistical analyses were performed in R version 3.6.

Ethics

The study was approved by all the participating hospitals. No informed consent was needed for this study. Ethics Committee details for each country are as follows: Austria (Ethikkommission Medizinische Universitat Graz, ID: 28-518 ex 15/16); Germany (Ethikkommission Bei Der LMU München, ID: 699-16); Greece (Ethics committee, ID: 9683/18.07.2016); Latvia (Centrala medicinas etikas komiteja, ID: 14.07.201 6. No. Il 16-07 -14); Slovenia (Republic of Slovenia National Medical Ethics Committee, ID: ID: 0120-483/2016-3); Spain (Comité Autonómico de Ética de la Investigación de Galicia, ID: 2016/331); The Netherlands (Commissie Mensgebonden onderzoek, ID: NL58103.091.16); and the UK (Ethics Committee, ID: 16/LO/1684, IRAS application no. 209035, Confidentiality advisory group reference: 16/CAG/0136). In the UK, an 'opt-out' procedure was used for this study.

Results

Study population

Of 38480 febrile children, 13984 patients aged 1 month to 5 years with respiratory symptoms were eligible for the main analysis. We excluded 7896 (56.5%) patients with solely upper respiratory infections, 429 (3.1%) with relevant comorbidity, 675 (4.8%) patients due to antibiotic treatment in the week prior to the ED visit and 46 (0.3%) with missing information on antibiotic prescription. This resulted in 4938 included patients [female: n=2122, 42.9%, median age 1.8 years (IQR: 0.9–2.9)] (Table S2). Supplemental oxygen was provided to 459 (9.3%) patients. Following their ED visit, 2038 patients (41.3%) were admitted to a general ward and 29 (0.6%) to an ICU. CRP level was measured for 2409 patients [48.8%, median CRP level: 19 mg/L (IQR: 5–52)]. Characteristics of patients with and without CRP measurement are provided in Table S3.

Simulation of the Feverkidstool resulted in a median risk score of 2.9% (IQR: 1.5%–6.3%) for bacterial LRTI. Characteristics of the low/intermediate-risk group (n=4209, 85.2%) and the high-risk group (n=729, 14.8%) for bacterial LRTI are presented in Table 1. Compared with high-risk patients, low/intermediate-risk patients were more often self-referred and more frequently triaged as low urgency (P<0.01). High-risk patients had a higher need for oxygen therapy and higher admission rates to the ward or the ICU (P<0.01) than low/intermediate-risk patients.

Table 1 | Descriptive characteristics of the study population stratified by risk groups based on the Feverkidstool risk score for bacterial LRTI

	Low/intermediate-risk group (≤10%) *N*=4209	Missing values *n* (%)	High-risk group (>10%) *N*=729	Missing values *n* (%)
Female, *n* (%)	1785 (42.4)		337 (46.2)	
Age, years, median (IQR)	1.7 (0.9–2.9)		1.9 (1.3–2.8)	
Simple comorbidity, *n* (%)	487 (11.6)	61 (1.5)	124 (17.0)	9 (1.2)
Way of referral, *n* (%)		82 (1.9)		13 (1.8)
Self-referral	2270 (53.9)		240 (32.9)	
GP or private paediatrician	897 (21.3)		307 (42.1)	
Emergency medical service	579 (13.8)		105 (14.4)	
Other healthcare professionals	381 (9.1)		64 (8.8)	
High triage urgency[a], *n* (%)	1584 (37.6)	122 (2.9)	387 (53.1)	46 (6.3)
Clinical symptoms				
Ill appearance, *n* (%)	680 (16.2)	218 (5.2)	292 (40.1)	53 (7.3)
Coughing, *n* (%)	4012 (95.3)	100 (2.4)	673 (92.3)	31 (4.3)
Fever duration, days, median (IQR)	1.5 (0.5–3)	341 (8.1)	3 (1.5–5)	54 (7.4)
Temperature, °C, median (IQR)	37.6 (36.9–38.3)	250 (5.9)	38.3 (37.5–39.0)	53 (7.3)
Increased work of breathing, *n* (%)	1214 (28.8)	327 (7.8)	459 (63.0)	67 (9.2)
Tachypnoea, *n* (%)	1342 (31.9)	785 (18.7)	416 (57.1)	176 (24.1)
Tachycardia, *n* (%)	1455 (34.6)	288 (6.8)	453 (62.1)	39 (5.4)
Capillary refill time ≥3 s, *n* (%)	69 (1.6)	480 (11.4)	18 (2.5)	134 (18.4)
Hypoxia, *n* (%)	86 (2.0)	485 (11.5)	328 (45.0)	55 (7.5)
Management				
Chest X-ray performed, *n* (%)	1293 (30.7)	1 (0.0)	425 (58.3)	2 (0.3)
CRP, mg/L, median (IQR)	13 (4–35)	2939 (54.0)	64 (29–129)	296 (32.6)
Oxygen therapy, *n* (%)	252 (5.9)	14 (0.33)	207 (28.4)	8 (1.1)
Airway/breathing lifesaving interventions[b], *n* (%)	68 (1.6)		45 (6.2)	
Haemodynamic interventions[c], *n* (%)	27 (0.6)		10 (1.4)	
Admission to ward, *n* (%)	1519 (36.1)	5 (0.1)	519 (71.2)	1 (0.1)
Admission to ICU, *n* (%)	16 (0.4)		13 (1.8)	

[a]High triage urgency included patients with urgency levels of 'immediate', 'very urgent' and 'urgent'.
[b]Airway/breathing lifesaving interventions are defined as the need for a non-rebreathing mask, non-invasive ventilation, intubation or ventilation.
[c]Haemodynamic lifesaving interventions are defined as the need for IV or intra-ossal fluid resuscitation, intra-ossal access or blood administration

Simulation of effect estimates from the intervention trial on antibiotic prescription

The overall observed antibiotic prescription rate was 33.5% (1656/4938), similar to the weighted prescription rate per ED (33.5%). In low/intermediate-risk patients, the observed antibiotic prescription rate was 29.6% (1247/4209) and in high-risk patients it was 56.1% (409/729). Applying the effects estimates from the intervention trial [adjusted ORs for antibiotic prescription: low/intermediate-risk group: 0.31 (95% CI: 0.12–0.81); high-risk group: 2.28 (95% CI: 0.84–6.17)] reduced overall antibiotic prescriptions from 33.5% to 24.1% [pooled RD: 9.4% (95% CI: 5.7%–13.1%); pooled RR 0.72 (95% CI: 0.63–0.81)].

Varying usage and compliance rates

Simulating the Feverkidstool with 100% usage and compliance reduced overall antibiotic prescriptions from 33.5% to 9.9% [pooled RD: 23.6% (95% CI: 19.2%–28.0%), pooled RR 0.28 (95% CI: 0.22–0.36)]. Both usage rates and compliance rates influenced the effect on antibiotic prescription rate. Simulating usage rates from 50%–90%, combined with 100% compliance with the Feverkidstool, resulted in a reduction of antibiotic prescription [50% usage: pooled RD 11.8% (95% CI: 9.6%–14.0%); 90% usage: pooled RD 21.1% (95% CI: 17.0%–25.1%)] (Figure 1, Table S4). Assuming 100% usage, a minimum compliance rate of 78% was needed to achieve a significant reduction [pooled RD 4.9% (95% CI: 0.2%–9.7%)]. Combining usage rates of 50%–100% with 90% compliance resulted in overall antibiotic reductions ranging from 8.3% to 15.8%.

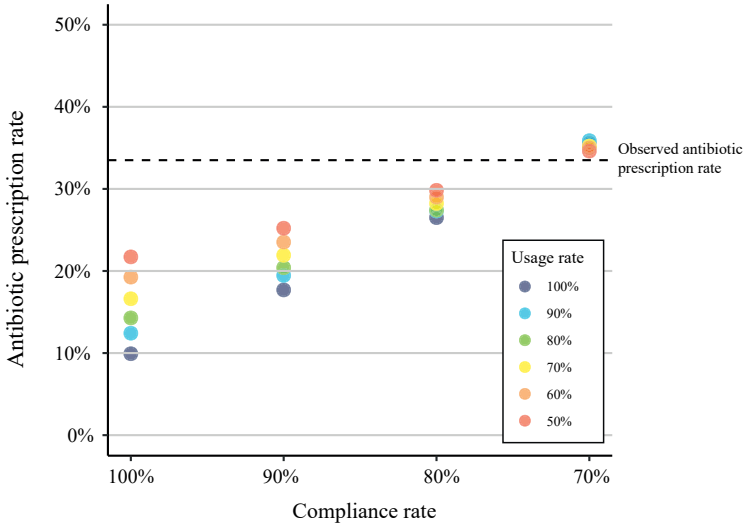
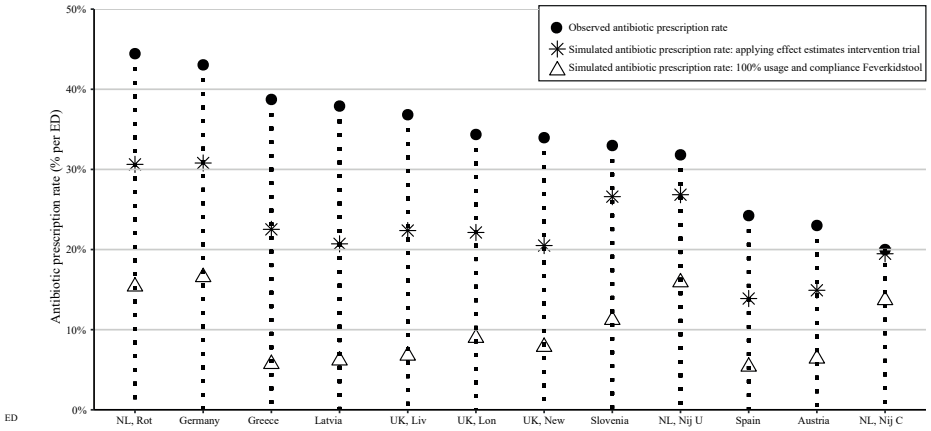


Figure 1 | Antibiotic prescription rate simulated by implementing the Feverkidstool with varying usage and compliance rates. Presented data are based on pooled data from 12 EDs. Detailed information on simulation of the varying usage and compliance rates is presented in Table S4.

Subgroup analysis of each ED

Between EDs, observed overall antibiotic prescription rates varied between 20.0% and 44.4%. Simulation of the effect estimates of the intervention trial resulted in a reduction in all 12 EDs and was significant in 9 EDs [range of RD: 8.1% (95% CI: 0.8%–12.8%)–17.2% (95% CI: 4.1%–25.8%)] (Figure 2). EDs with significant reductions had either large proportions of low/intermediate-risk patients (>85% in 7 EDs) or high observed antibiotic prescription rates (>35% in 5 EDs) (Table S5).



ED	NL, Rot	Germany	Greece	Latvia	UK, Liv	UK, Lon	UK, New	Slovenia	NL, Nij U	Spain	Austria	NL, Nij C
N	117	151	315	926	296	780	586	758	44	557	313	95
% Low/intermediate risk	79.5%	77.5%	88.9%	92.4%	86.8%	85.1%	88.4%	69.5%	72.7%	92.3%	88.5%	77.9%
RR (95% CI) intervention trial	0.69 (0.5-0.96)	0.72 (0.54-0.97)	0.58 (0.33-0.93)	0.55 (0.32-0.89)	0.61 (0.37-0.95)	0.64 (0.43-0.95)	0.60 (0.39-0.92)	0.81 (0.52-1.13)*	0.84 (0.63-1.09)*	0.57 (0.37-0.9)	0.65 (0.44-0.96)	0.97 (0.75-1.17)*
RR (95% CI) 100% usage/compliance	0.35 (0.24-0.5)	0.38 (0.28-0.52)	0.15 (0.1-0.23)	0.16 (0.13-0.21)	0.18 (0.12-0.27)	0.26 (0.21-0.32)	0.23 (0.18-0.3)	0.34 (0.29-0.4)	0.50 (0.3-0.84)	0.22 (0.16-0.30)	0.28 (0.19-0.4)	0.68 (0.50-0.93)

Figure 2 | Antibiotic prescription rates simulated by applying effect estimates from the intervention trial and for 100% usage and compliance for each ED. Liv, Liverpool; Lon, London; New, Newcastle; Nij C, Nijmegen, Canisius; Nij U; Nijmegen, RadboudUMC; NL, Netherlands; Rot, Rotterdam. EDs are sorted according to observed antibiotic prescription rates. Details of the analysis are presented in Table S5.
*RR not significant.

Transferability to older children

In the age range 1 month to 18 years, 6300 children were eligible with suspected LRTIs. Of those, the majority were aged 1 month to 5 years (78.4%; 4938/6300). Children aged 5–12 years accounted for 16.7% (1056/6300) and children aged above 12 years for 4.9% (306/6300) of this population. In children aged 5–12 years and 12–18 years, the observed antibiotic prescription rates were 35.7% (377/1056) and 43.8% (134/306), respectively. In both these age groups, antibiotic prescriptions were reduced by applying effect estimates from the intervention trial [5–12 years: RD 13.4% (95% CI: 2.0%–20.8%); 12–18 years: RD 17.9% (95% CI: 4.0%–27.6%)] (Figure 3, Table S6 and Figure S1).

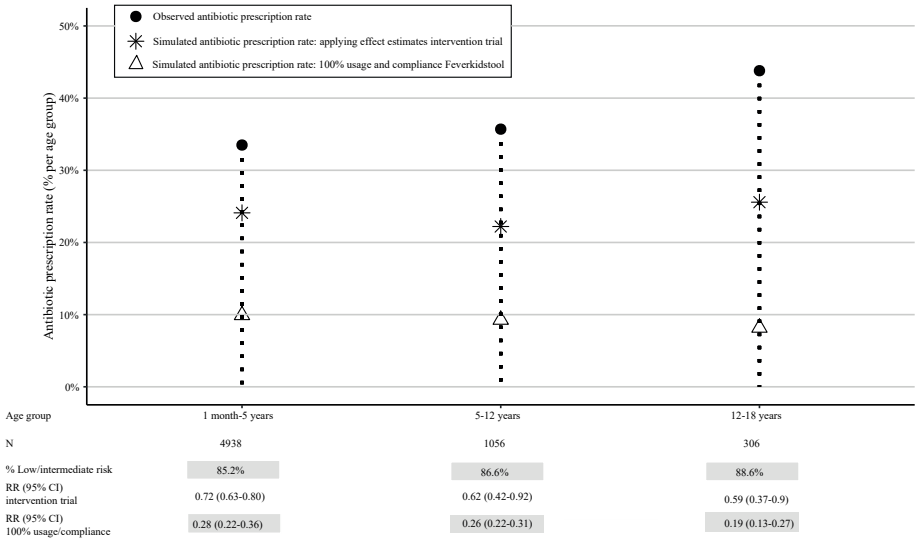


Figure 3 | Antibiotic prescription rates simulated by applying effect estimates from the intervention trial and for 100% usage and compliance with the Feverkidstool for the age group 1 month to 5 years and its transferability to children aged 5–12 years and 12–18 years. Details of this analysis are presented in Table S6.

Complete cases for CRP

The sensitivity analysis involving the population of only children that had CRP performed (n=2409), showed similar results to those found in all analyses [pooled RD intervention trial: 13.5% (95% CI: 10.0%–17.1%); pooled RD 100% usage/compliance: 34.6% (95% CI: 26.8%–42.4%)] (Table S7).

Discussion

Based on the data of routine care of febrile children in EDs in Europe, we simulated the potential effect of implementing the Feverkidstool on antibiotic prescription rates in children with suspected LRTIs compared with observed prescriptions. Simulating the effect estimates of the intervention trial reduced antibiotic prescriptions in routine care from 33.5% to 24.1%,[12] whereas 100% usage of and compliance with the Feverkidstool resulted in a reduction of antibiotic prescriptions to 9.9%. With usage rates varying from 50% to 100% and a compliance rate of 90%, antibiotic prescription reductions ranged from 8.3% to 15.8%. Subgroup analysis showed that the largest reduction of antibiotic prescription was observed in EDs with high antibiotic prescription rates or high prevalence of low/intermediate-risk patients.

Our study has some limitations. First, our study is based on simulating assumptions and, accordingly, results are estimates of the potential impact on antibiotics prescribing. Ideally, to reach maximum level of evidence, a multicentre intervention trial should be performed to assess the broad impact of a clinical decision rule.[27,28] However, it is expensive and time-consuming to conduct an intervention study in multiple European countries. Therefore, simulation using routine data can be used to estimate potential effects after safety of the intervention has previously been established in a previous clinical trial.

Second, we were not able to evaluate the safety of the implementation of the Feverkidstool in our simulation study as follow-up after ED visit was not available. The Feverkidstool proved to be safe in the intervention trial where safety was evaluated by secondary hospitalizations or antibiotic prescriptions, prolonged illness at Day 7, or complications. In low/intermediate-risk patients, implementation of the Feverkidstool did not change safety outcomes in the trial, whilst in high-risk patients fewer secondary antibiotic prescriptions and prolonged duration of fever were observed. Safety is not likely to be different in EDs with lower or higher incidence of bacterial infections, since the clinical decision rule itself takes risk factors for bacterial pneumonia into account. Therefore, we assume that the Feverkidstool could be safely applied. Furthermore, Reilly *et al.*[28] suggest that the safety of a decision rule can be improved by a certain degree of non-compliance. In practice, physicians could overrule the recommendations of a decision rule due to clinical judgement. In our study, we simulated non-compliance by assuming that non-compliance would result in antibiotic prescriptions in low/intermediate-risk patients. This might overestimate antibiotic prescriptions for these patients.

Third, we simulated that all patients had equal probability on usage and compliance rates. We did not take into account that non-compliance might be related to higher predicted individual risks. Fourth, our inclusion criteria for fever (≥38.0°C) differed from that in the intervention trial (≥38.5°C). As temperature is a predictor in the Feverkidstool, this could have reflected a higher proportion of low/intermediate-risk patients in our cohort. It is unlikely that this has influenced calibration of the model as the population in which the Feverkidstool was developed was selected on a temperature of ≥38.0°C. Last, the Feverkidstool requires CRP levels to calculate individual risk scores and CRP measurement for febrile children varied widely (8%–90%) at European EDs.[21] To simulate the potential impact of the Feverkidstool, we imputed CRP values for patients without CRP measurement. We repeated analysis in complete cases of CRP level and found similar results, indicating that imputing CRP level did not influence our results.

The main strength of our study is that we simulated the impact of the Feverkidstool in a large European-wide cohort. Although EDs differed in case mix and baseline antibiotic prescriptions, we observed a reduction of antibiotics at every ED and significant reduction in nine EDs. This increases the generalizability of the potential effect of the Feverkidstool in young febrile children with respiratory symptoms. We believe our effect estimates to be representative for other EDs in Europe with comparable prescription rates and proportion of low/intermediate-risk patients. In the intervention trial, baseline antibiotic prescriptions were relatively low in the low/intermediate-risk group (17%) whereas in our study observed prescription rates were higher (overall 29.6%, range in EDs: 20.0%–44.4%). Our study showed that the potential antibiotic reduction is higher in EDs with higher baseline prescription rates. This agrees with a previous French study with a high baseline prescription rate (32%) where antibiotic prescriptions were significantly reduced by implementing antibiotic guidelines in paediatric respiratory tract infections.[2]

Simulation is an efficient method to collate evidence on impact of clinical decision rules, especially in situations when trials are not feasible. In addition, simulation introduces the possibility of changing assumptions in the models. We estimated the potential clinical impact on antibiotic prescription by applying the effect estimates on antibiotic prescription that were observed in the intervention trial, by varying usage and rates of compliance with the Feverkidstool, and in different age groups. Furthermore, cost-effectiveness analyses could be added to simulation studies[29] and simulation provides the ability to determine target values of usage and compliance rates before implementing the decision model. Next, simulation could also be used to estimate the potential effect on antibiotic prescription in other settings including primary care settings or low/middle-income countries with different baseline risks on bacterial infections.

As expected, our study showed that high usage and compliance were important to reach maximum effect of the Feverkidstool on antibiotic reduction.[28,30] Assuming a usage rate of 60% and a compliance rate of 90%, both frequently described in literature,[13–17] the Feverkidstool led to a prescription reduction of 10.0% (95% CI: 7.5%–12.4%). In practice, a high level of acceptance of CRP measurement and incorporating the clinical decision rule in the electronic hospital system will contribute to higher usage rates.[22]

The treatment decisions according to the Feverkidstool are targeted towards the low/intermediate-risk patients (withholding of antibiotics) whereas in high-risk patients, antibiotics were prescribed at the discretion of the physician. Since individual patient risks are only known after calculation of the Feverkidstool, all eligible patients were included in the intervention trial. As discussed by the authors,[12] the sample size was

reached, but the proportion of low/intermediate-risk patients was lower than that expected in the power calculations. Subsequently, implementation of the Feverkidstool did not reduce overall antibiotic prescriptions, but did result in antibiotic reductions in the subgroup of children at low/intermediate risk. Instead of performing a new trial and exposing children to new risks, simulation is a good alternative to extrapolate trial data to populations with different risk profiles. In our simulation study, the proportion of low/intermediate-risk patients was higher (85%), based on the observed range across EDs of 70%–92%, than in the intervention study (58%). Consequently, our simulations in populations with predominance of low/intermediate risk resulted in reductions of overall antibiotic prescriptions. Our results indicate that reductions in antibiotic prescriptions can be achieved by ensuring a broad use of this tool. In addition, EDs with either high antibiotic prescription rates or many low-risk patients are likely to benefit the most from the implementation of the Feverkidstool.[3] Even in EDs with lower prescription rates, ensuring high usage of and compliance with the Feverkidstool has a substantial effect on antibiotic prescription.

The risk threshold of 10% in the intervention trial was chosen according to previous literature.[8,9,12] An appropriate threshold should balance the potential harm of undertreating bacterial LRTIs and the benefit of reducing unnecessary antibiotic prescriptions. Physicians may consider accepting a higher risk threshold of 15% if adequate safety-netting is provided.

The Feverkidstool is broadly validated for all paediatric age groups.[9,11] Since viral infections have higher incidence in younger children, the intervention trial was performed in children <5 years. Although the safety of withholding antibiotic prescriptions has not yet been established in children >5 years at low/intermediate risk for suspected LRTIs, our study shows that implementation of the Feverkidstool has the potential to reduce antibiotic prescriptions in this group. Future studies should be performed in older children to address safety and actual effect on antibiotic prescription.

Differences between European EDs, including acceptance of CRP measurement, should be taken into account when implementing a new strategy for antibiotic reduction in Europe.[21,22] Furthermore, a clinical decision rule could also aid in guiding decisions regarding appropriateness of antibiotic agents and prescription mode. Future research should focus on identifying local facilitators and barriers for the implementation of this clinical decision rule to achieve maximal uptake. In addition, the Feverkidstool should be validated in children with comorbidity.

Conclusions

Based on routine clinical data, we modelled the potential effect of implementation of the Feverkidstool, a clinical decision rule advising physicians whether or not to start antibiotic treatment in children with suspected LRTIs. Our simulation study showed that the Feverkidstool has the potential to reduce antibiotic prescription from 33.5% to 24.1% at European EDs. Both usage and compliance with the treatment advice influence the potential effect on antibiotic prescription. In addition, simulation predicted a significant reduction of antibiotics at nine participating EDs. EDs with both higher antibiotic prescription rates and many low/intermediate-risk patients are likely to benefit more from this decision rule. Therefore, the Feverkidstool could contribute to reducing antibiotic prescriptions for LRTIs in Europe.

Supplementary data

Available as online web appendix from the website of Journal of antimicrobial chemotherapy

Table S1. Variable descriptions of the Feverkidstool
Table S5. Subgroup analyses per ED

Table S2. Included patients per ED

	Patients in MOFICHE database, N=38480	Study population, N=4938	Patients with CRP measurement, N=2409
EDs	N (% of patients in MOFICHE)	N (% of patients per ED)	N (% of study population per ED)
Austria, Graz	2241 (5.8)	313 (14.0%)	298 (95.2%)
Germany, Munich	1173 (3.0)	151 (12.9%)	83 (55.0%)
Greece, Athens	4548 (11.8)	315 (6.9%)	149 (47.3%)
Latvia, Riga	9000 (23.4)	926 (10.3%)	724 (78.2%)
NL, Rotterdam	1683 (4.4)	117 (7.0%)	73 (62.4%)
NL, Nijmegen, RadboudUMC	677 (1.8)	44 (6.5%)	12 (27.3%)
NL, Nijmegen, Canisius	423 (1.1)	95 (22.5%)	29 (30.5%)
Slovenia, Ljubljana	3667 (9.5)	758 (20.7%)	712 (93.9%)
Spain, Santiago	3877 (10.1)	557 (14.4%)	50 (9.0%)
UK, Liverpool	1623 (4.2)	296 (18.2%)	71 (24.0%)
UK, London	5714 (14.8)	780 (13.7%)	132 (16.9%)
UK, Newcastle	3854 (10.0)	586 (15.2%)	76 (13.0%)

CRP, c-reactive protein; ED, emergency department; NL, the Netherlands; UK, United Kingdom

Table S3. Descriptive characteristics of patients with complete cases for C-reactive protein level and for patients with missing C-reactive protein level

	Population with complete cases for C-reactive protein level N=2409 N (%)	Missing Values N (%)	**Population with missing C-reactive protein level N=2529 N (%)**	Missing Values N (%)
Female	1053 (43.7)		1069 (42.3)	
Age in years, median [IQR]	1.8 (1.0-2.8)		1.7 (0.9-2.9)	
Simple comorbidity	282 (11.7)	17 (0.7)	329 (13.0)	53 (2.1)
Way of referral:		81 (3.4)		14 (0.6)
- Self-referral	914 (37.9)		1596 (63.1)	
- General practitioner or private paediatrician	872 (36.2)		332 (13.1)	
- Emergency medical Service	432 (17.9)		252 (9.9)	
- Other healthcare professional	110 (4.6)		335 (13.2)	
High triage urgency[a]	993 (41.2)	80 (3.3)	978 (38.7)	88 (3.5)
Clinical symptoms				
Ill appearance	769 (31.9)	78 (3.2)	203 (8.0)	193 (7.6)
Coughing	2305 (95.7)	46 (1.9)	2380 (94.1)	85 (3.4)
Fever duration in days, median [IQR]	1.5 [0.5-4]	105 (4.4)	1.5 [0.5-3]	290 (11.5)
Temperature °C, median [IQR]	37.8 [37.0-38.5]	98 (4.1)	37.5 [36.9-38.3]	205 (8.1)
Increased work of breathing	692 (28.7)	207 (8.6)	981 (38.8)	187 (7.4)
Tachypnea	745 (30.9)	554 (23.0)	1013 (40.1)	407 (16.1)
Tachycardia	991 (41.1)	88 (3.7)	917 (36.3)	239 (9.5)
Capillary refill time ≥ 3sec	68 (2.8)	381 (15.8)	19 (0.8)	233 (9.2)
Hypoxia <94%	227 (9.4)	261 (10.8)	187 (7.4)	279 (11.0)
Management				
Chest X-ray performed	1233 (51.2)	1 (0.0)	485 (19.2)	2 (0.0)
C-reactive protein level in mg/L, median [IQR]	19 [5 -52]		NA	
Oxygen therapy	304 (12.6)	11 (0.5)	155 (6.1)	11 (0.4)
Airway/breathing lifesaving interventions[b]	63 (2.6)		50 (1.9)	
Hemodynamic interventions[c]	33 (1.4)		4 (0.2)	
Admission to ward	1429 (59.3)	1 (0.0)	1920 (75.9)	5 (0.2)
Admission to intensive care unit	26 (1.1)		3 (0.1)	
Antibiotics prescribed	1010 (41.9)		646 (25.5)	

Variables are displayed as N (%) or median [IQR (interquartile range)

[a] High triage urgency included patients with urgency levels: "immediate", "very urgent" and "urgent"

[b] Airway/breathing lifesaving interventions are defined as the need for a non-rebreathing mask, non-invasive ventilation, intubation or ventilation.

[c] Hemodynamic lifesaving interventions are defined as the need for intravenous or intra-ossal fluid resuscitation, intra-ossal access or blood transfusion

Table S4. Main analysis: effect of simulating the implementation of the Feverkidstool based on pooled analysis of 12 EDs (n=6346)

(A) effect estimates on antibiotic prescription from the intervention trial, (B) varying usage and compliance rates. Risk difference and risk ratio of observed antibiotic prescription rate and simulated prescription rates based on pooled analysis of 12 Emergency Departments (n=4938)

A: Effect estimates of the intervention trial[a]	*Pooled Risk Difference (95% CI)*	*Pooled Risk Ratio (95% CI)*
Intervention trial	9.4% (5.7-13.1)	0.71 (0.63-0.80)
B: Varying usage rates (UR) and compliance rates (CR)		
100% UR, 100% CR	23.6% (19.2 – 28.0)	0.28 (0.22 – 0.36)
100% UR, 90% CR	15.8% (11.7 - 19.9)	0.52 (0.45 - 0.61)
100% UR, 80% CR	7.0% (2.4 - 11.6)	0.79 (0.68 - 0.92)
100% UR, 70% CR	-2.1% (-6.8 - 2.6)	1.07 (0.93 - 1.23)
90% UR, 100% CR	21.1% (17.0 - 25.1)	0.36 (0.30 - 0.43)
90% UR, 90% CR	14.0% (10.1 - 18.0)	0.58 (0.51 - 0.66)
90% UR, 80% CR	6.2% (1.8 - 10.5)	0.82 (0.71 - 0.94)
90% UR, 70% CR	-2.4% (-7.0 - 2.2)	1.08 (0.94 - 1.24)
80% UR, 100% CR	19.2% (15.6 - 22.8)	0.42 (0.37 - 0.47)
80% UR, 90% CR	13.1% (9.8 - 16.4)	0.60 (0.55 - 0.66)
80% UR, 80% CR	6.1% (2.3 - 9.8)	0.82 (0.73 - 0.92)
80% UR, 70% CR	-1.6% (-5.3 - 2.1)	1.05 (0.94 - 1.17)
70% UR, 100% CR	16.9% (14.0 - 19.7)	0.49 (0.44 - 0.53)
70% UR, 90% CR	11.6% (8.8 - 14.3)	0.65 (0.60 - 0.70)
70% UR, 80% CR	5.3% (2.2 - 8.4)	0.84 (0.77 - 0.92)
70% UR, 70% CR	-1.7% (-4.8 - 1.3)	1.05 (0.96 - 1.15)
60% UR, 100% CR	14.2% (11.8 - 16.7)	0.57 (0.53 - 0.61)
60% UR, 90% CR	10.0% (7.5 - 12.4)	0.69 (0.66 - 0.73)
60% UR, 80% CR	4.5% (1.7 - 7.3)	0.86 (0.80 - 0.93)
60% UR, 70% CR	-1.5% (-4.1 - 1.1)	1.04 (0.97 - 1.13)
50% UR, 100% CR	11.8% (9.6 - 14.0)	0.64 (0.61 - 0.67)
50% UR, 90% CR	8.3% (6.1 - 10.4)	0.74 (0.71 - 0.77)
50% UR, 80% CR	3.7% (1.1 - 6.3)	0.89 (0.83 - 0.95)
50% UR, 70% CR	-1.1% (-3.5 - 1.2)	1.03 (0.97 - 1.11)

CI, confidence interval; CR, compliance rate; UR, usage rate

[a]Effect estimates on antibiotic prescription from the intervention trial: low/intermediate-risk group: 0.31 (0.12-0.81]; high-risk group: 2.28 (0.84-6.17].

Figure S1 - Correlation between proportion low/intermediate-risk patients and risk ratios for observed vs simulated antibiotic prescription presented per ED

ED, emergency department; RR, risk ratio

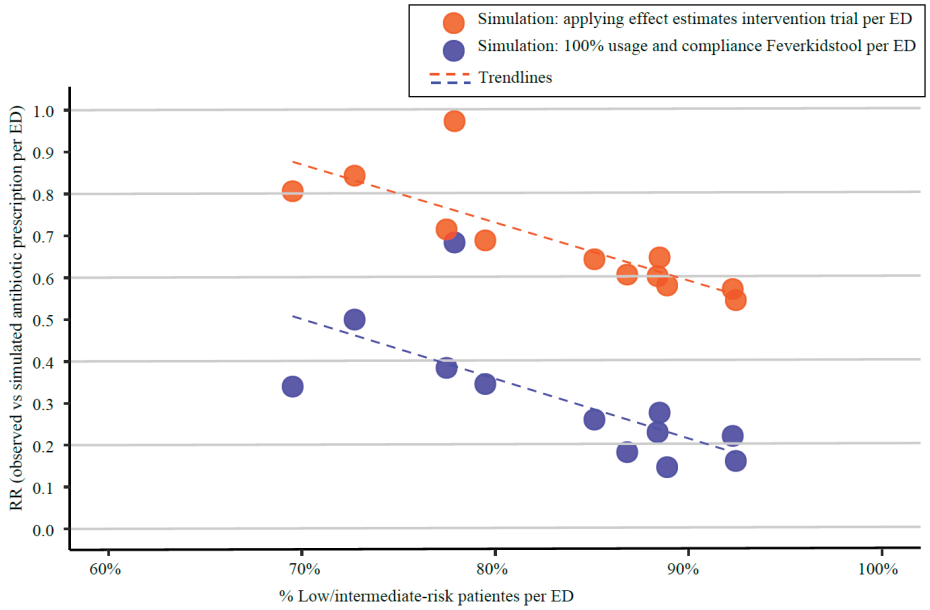


Table S6. Transferability of Feverkidstool's effect to older age groups

Subgroup analysis per age group

	N (% of age group)	Risk Score Feverkidstool for bacterial LRTI, median [IQR]	Real-life antibiotic prescription, N (% per risk group)	Difference of observed antibiotic prescription rates with the simulated prescription rate by applying effect estimates of the intervention trial ±	Difference of observed antibiotic prescription rates with the simulated prescription rate assuming 100% usage and compliance to the Feverkidstool
	1 month - 5 years				
low/intermediate-risk	4209 (85.2%)	2.5% [1.4-4.3]	1247 (29.6%)		
high-risk	729 (14.8%)	16.1% [12.3-24.3]	409 (56.1%)		
Total	4938	1.8% [0.9-3.7]	1656 (33.5%)		
			Pooled Risk Difference (95%CI)	9.4% (5.7-13.1)	23.6% (19.2 – 28.0)
			Pooled Risk Ratio (95% CI)	0.72 (0.63-0.81)	0.28 (0.22-0.36)
	5 – 12 years £				
low/intermediate-risk	915 (86.6%)	2.6% [1.6-4.4]	279 (30.5%)		
high-risk	141 (13.4%)	17.3% [11.8-26.8]	98 (69.5%)		
Total	1056	2.9% [1.7-6.0]	377 (35.7%)		
			Risk Difference (95%CI)*	13.4% (2.0-20.8)	26.4% (23.8 - 29.1)
			Risk Ratio (95% CI)*	0.62 (0.42-0.94)	0.26 (0.22 - 0.31)
	≥12 years old £				
low/intermediate-risk	271 (88.6%)	3.0% [1.8-4.9]	109 (40.2%)		
high-risk	35 (11.4%)	13.5% [11.6-22.4]	25 (71.4%)		
Total	306	3.4% [1.9-5.8]	134 (43.8%)		
			Risk Difference (95%CI)*	17.9% (4.0-27.6)	35.6% (30.2 - 41.0)
			Risk Ratio (95% CI)*	0.59 (0.37-0.91)	0.19 (0.13-0.27)

Data is displayed as antibiotic prescription: N (% within subgroup);

± For simulation of the intervention trial effect estimates, the following odds ratios were used: low/intermediate-risk group: 0.31 (0.12-0.81]; high-risk group: 2.28 (0.84-6.17].

*Due to limited cases per ED, based on data of total ED population in this age group

£ Inclusion criteria of the intervention trial were age 1 month - 5 years.

Table S7. Sensitivity analysis: study population with complete cases for CRP measurement (n=2409)

(A) effect estimates on antibiotic prescription from the intervention trial, (B) varying usage and compliance rates. Risk difference and risk ratio of observed antibiotic prescription rate and simulated prescription rates based on pooled analysis of 12 Emergency Departments

A: Effect estimates of the intervention trial[a]	Pooled Risk Difference (95% CI)	Pooled Risk Ratio (95% CI)
Intervention Trial[±]	13.5% (10.0 - 17.1)	0.73 (0.67 - 0.80)
B: Varying usage rates (UR) and compliance rates (CR)		
100% UR, 100% CR	34.6% (26.8 - 42.4)	0.31 (0.25 - 0.39)
100% UR, 90% CR	27.9% (20.3 - 35.5)	0.46 (0.40 - 0.53)
100% UR, 80% CR	19.8% (11.9 - 27.7)	0.63 (0.54 - 0.74)
100% UR, 70% CR	11.7% (3.6 - 19.7)	0.80 (0.67 - 0.94)
100% UR, 60% CR	2.3% (-5.9 - 10.5)	0.98 (0.83 - 1.17)
90% UR, 100% CR	31.1% (23.6 - 38.6)	0.39 (0.33 - 0.46)
90% UR, 90% CR	25.2% (17.8 - 32.5)	0.52 (0.46 - 0.60)
90% UR, 80% CR	17.9% (10.4 - 25.4)	0.67 (0.58 - 0.78)
90% UR, 70% CR	10.3% (2.7 - 17.9)	0.82 (0.70 - 0.96)
90% UR, 60% CR	1.6% (-6.0 - 9.3)	1.00 (0.85 - 1.17)
80% UR, 100% CR	28.0% (21.6 - 34.4)	0.45 (0.40 - 0.50)
80% UR, 90% CR	22.5% (16.3 - 28.6)	0.57 (0.51 - 0.63)
80% UR, 80% CR	16.0% (9.7 - 22.4)	0.70 (0.62 - 0.79)
80% UR, 70% CR	9.1% (2.6 - 15.7)	0.84 (0.74 - 0.96)
80% UR, 60% CR	1.4% (-5.3 - 8.2)	1.00 (0.87 - 1.15)
70% UR, 100% CR	24.4% (19.1 - 29.8)	0.51 (0.47 - 0.56)
70% UR, 90% CR	19.6% (14.2 - 24.9)	0.62 (0.57 - 0.67)
70% UR, 80% CR	13.9% (8.4 - 19.4)	0.74 (0.67 - 0.81)
70% UR, 70% CR	7.2% (1.6 - 12.8)	0.87 (0.78 - 0.98)
70% UR, 60% CR	0.8% (-5.0 - 6.5)	1.01 (0.89 - 1.14)
60% UR, 100% CR	20.4% (15.7 - 25.1)	0.59 (0.55 - 0.64)
60% UR, 90% CR	16.4% (11.9 - 20.9)	0.68 (0.64 - 0.72)
60% UR, 80% CR	11.6% (6.8 - 16.3)	0.78 (0.72 - 0.84)
60% UR, 70% CR	5.9% (1.0 - 10.9)	0.90 (0.82 - 0.99)
60% UR, 60% CR	0.3% (-5.2 - 5.8)	1.01 (0.91 - 1.13)
50% UR, 100% CR	16.7% (12.9 - 20.5)	0.66 (0.62 - 0.69)
50% UR, 90% CR	13.4% (9.7 - 17.1)	0.73 (0.70 - 0.76)
50% UR, 80% CR	9.3% (5.3 - 13.3)	0.83 (0.77 - 0.90)
50% UR, 70% CR	5.0% (0.8 - 9.2)	0.92 (0.85 - 0.99)
50% UR, 60% CR	0.3% (-4.1 - 4.7)	1.01 (0.92 - 1.11)
40% UR, 100% CR	13.1% (10.2 - 16.0)	0.72 (0.69 - 0.75)
40% UR, 90% CR	10.5% (7.6 - 13.4)	0.78 (0.75 - 0.81)
40% UR, 80% CR	7.5% (4.4 - 10.6)	0.86 (0.81 - 0.92)
40% UR, 70% CR	4.2% (0.9 - 7.6)	0.93 (0.88 - 0.99)
40% UR, 60% CR	0.5% (-2.8 - 3.8)	1.00 (0.94 - 1.07)

CI, confidence interval; CR, compliance rate; UR, usage rate

* Non-significant decrease

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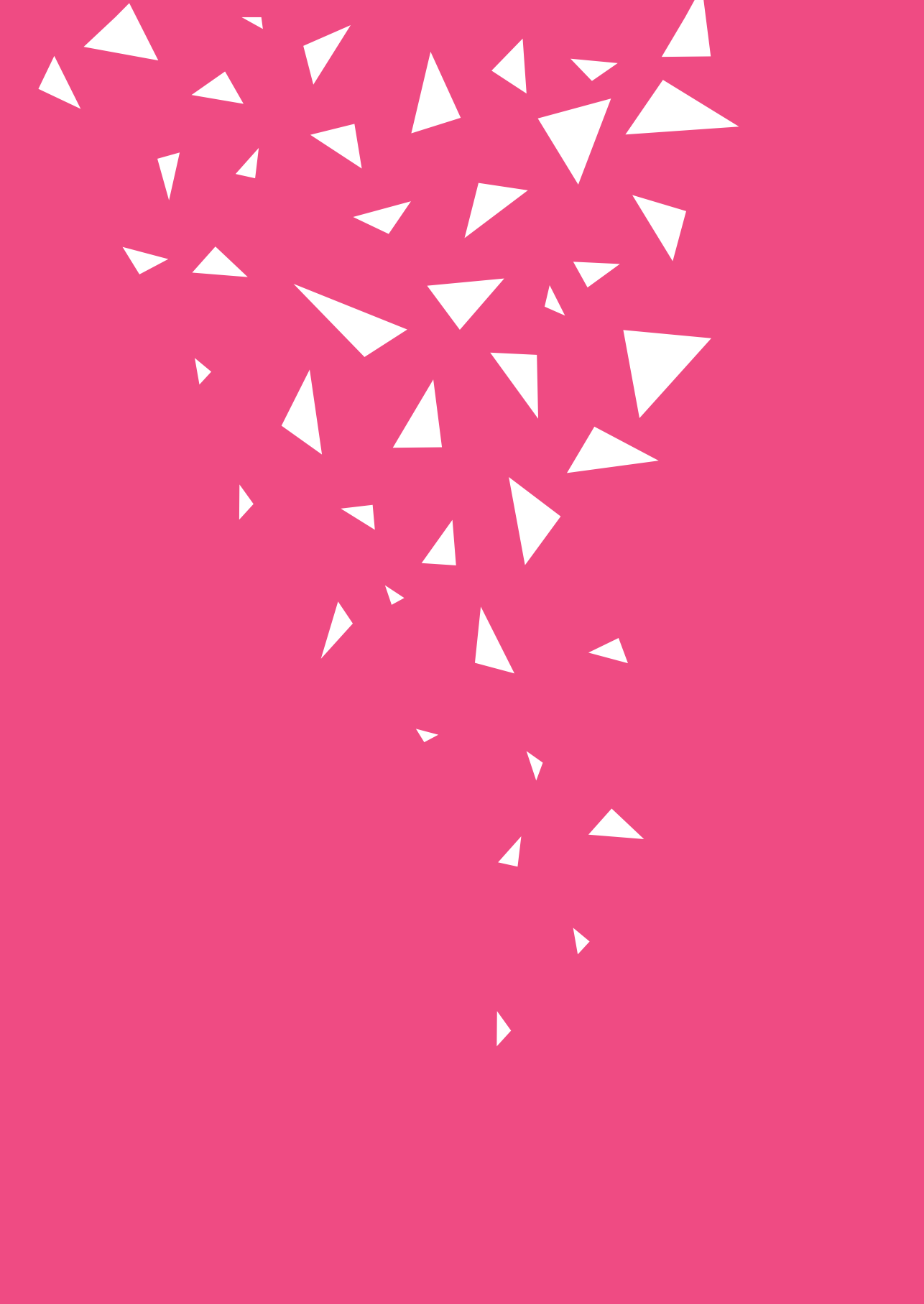
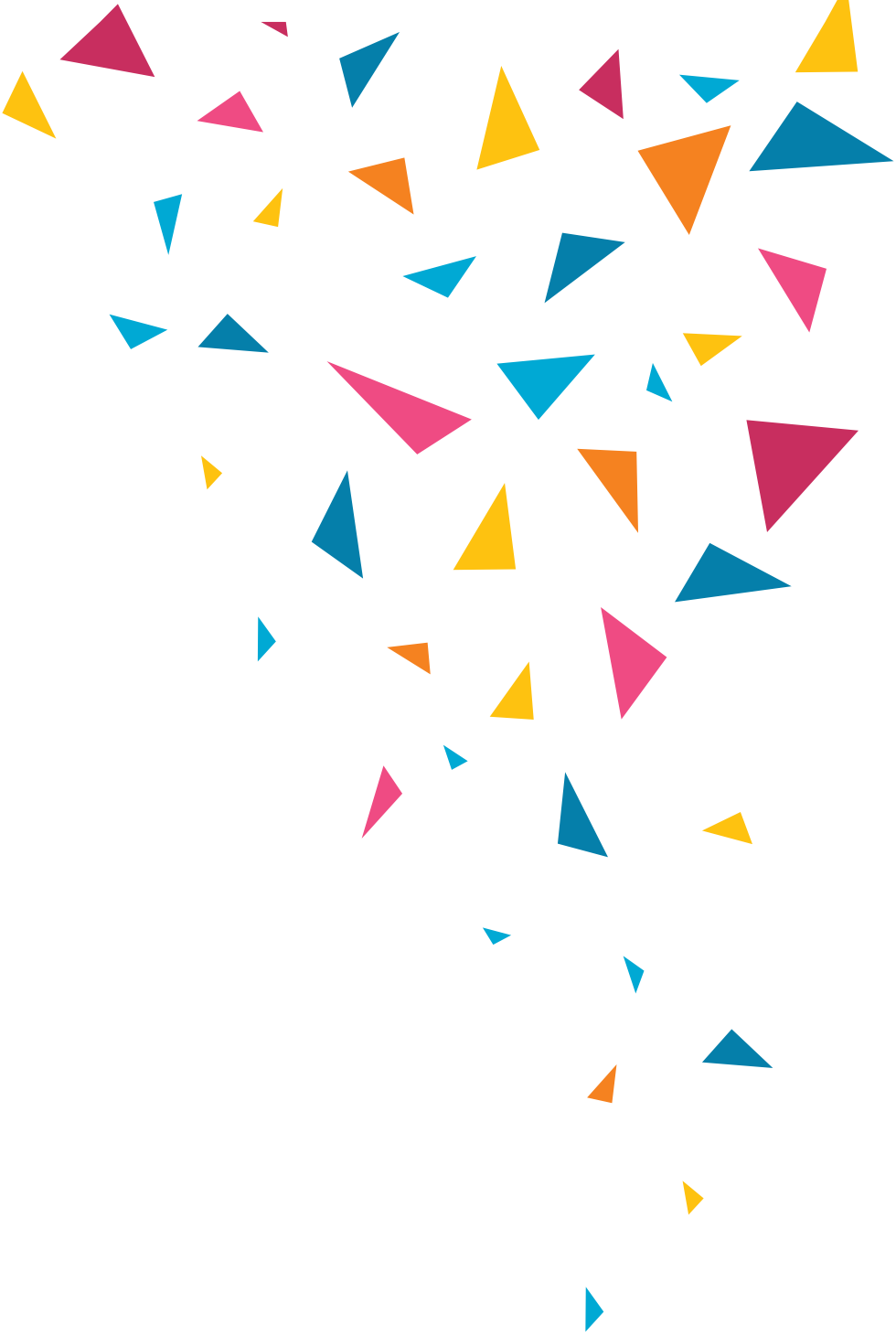


Part V

Discussion and Summary



10

General discussion

Research questions

The overarching aim of this thesis is to improve diagnostic strategies and antibiotic treatment of febrile children. First, we studied the value of routine blood pressure measurement in the emergency department (ED). Secondly, we developed and validated a clinical prediction model to identify invasive bacterial infections and risk-groups who might benefit from new biomarkers. Thirdly, we studied the prognostic value of host response biomarkers for adverse outcome in critically ill children with serious infections. Lastly, we identified strategies to improve antibiotic prescription in febrile children at European EDs.

The value of blood pressure measurement in the paediatric ED

In the ED, it is important to early identify patients with serious illness who need interventions but also to rule-out serious illness in order to safely discharge children. Currently, no consensus exist on the value of routine blood pressure measurement at the ED.[1] In this thesis, we studied the value of blood pressure measurement at the ED. First, we evaluated references values for low blood pressure in healthy children and compared those with cut-off values for low blood pressure in clinical guidelines. Existing cut-off values for low blood pressure applied in emergency care showed wide variation and had variable agreement with reference values for low blood pressure in healthy children. Next, we assessed the value of hypotension, according to three clinical cut-off values, for serious illness. This single-center study showed that hypotension was associated with serious illness, but the sensitivity of hypotension for serious illness was poor.

Another way to use blood pressure in the assessment of children is the Shock Index, the ratio of heart rate to systolic blood pressure. We provide reference values of the Shock Index for febrile children at the ED. Similar to hypotension, high Shock Index was associated with serious illness, but its rule-out value was insufficient for febrile children at the ED.

Since the rule-out value of both hypotension and high Shock Index is limited, we advise that blood pressure measurement should not be routinely performed in children at the ED. Accurate blood pressure measurement in children is time-consuming for ED nurses. Other circulatory parameters as heart rate and capillary refill are easier and quicker to assess and are earlier signs of clinical deterioration. Blood pressure measurement, however, should be performed on specific indications such as children with abnormal heart rate or prolonged capillary refill, for monitoring disease course, treatment effect or detection of hypertension.

Blood pressure is often incorporated in paediatric early warning scores and several scoring systems for sepsis.[2-4] The low rate of blood pressure measurement at paediatric EDs, leading to missing blood pressure values, negatively affects the potential performance of scoring systems that include blood pressure measurement. Recently, two scoring systems have been developed and validated for paediatric emergency care which do not have incorporated blood pressure: the ED-PEWS (Emergency Department paediatric early warning score) and LqSOFA (Liverpool quick Sequential Organ Failure Assessment).[5,6] The ED-PEWS aids in the prioritization of high-urgency patients at three levels of urgency and LqSOFA has been developed to predict critical care admission for febrile children which includes heart rate, respiratory rate, capillary refill and level of consciousness. These scoring systems without blood pressure values will facilitate higher acceptability and use in clinical practice.

Accurate reference values for vital signs are essential to identify children at risk for serious illness. Simplified age-adjusted reference values have the advantage to be intuitive and are essential for the first quick assessment. For example, the Advanced Paediatric Life Support (APLS) provides easy to use reference values for vital signs in practice. Some limitations, however, may exist. First, these reference values for children used in the ED are often based on population-based centiles in healthy children which may not be applicable in children attending the ED who have fever, pain or are in distress. Second, we compared guidelines for hypotension with population-based centiles for low blood pressure. We found that the APLS values were above the normal range for children > 12 years which could lead to over classification of hypotension in this group. To facilitate use of these reference values in clinical care, reference values and scoring systems should be integrated in electronic health care records, and preferably at monitoring systems at the bedside where the patient is evaluated.

Diagnostic uncertainty for febrile children

In febrile children, correctly discriminating the child with an invasive bacterial infection from those with self-limiting illness is challenging as typical alarming signs usually occur late in the disease course. In addition, the majority of febrile children have viral infections whereas invasive bacterial infections such as sepsis, bacteraemia and bacterial meningitis occur infrequently at the ED (<1%).[7,8] A small proportion of children presenting to the ED with a febrile illness have a confirmed serious bacterial infection (SBI), and of these a smaller number require admission to hospital or paediatric intensive care unit (PICU), as shown in the pyramid as a percentage of the total number of febrile children in EDs (Figure 1).[9] PICU admissions of patients with SBI was 25% of total PICU admis-

sions (39/158) and hospital admissions of patients with SBI was 20% (1947/9893) of total hospital admissions. Serious bacterial infections are thus a small proportion of PICU and hospital admissions of febrile children. Admissions in febrile children can also be due to serious viral illnesses (e.g. bronchiolitis, viral meningitis) which are not classified as SBI. In addition, in many febrile children no pathogen can be identified.[10]

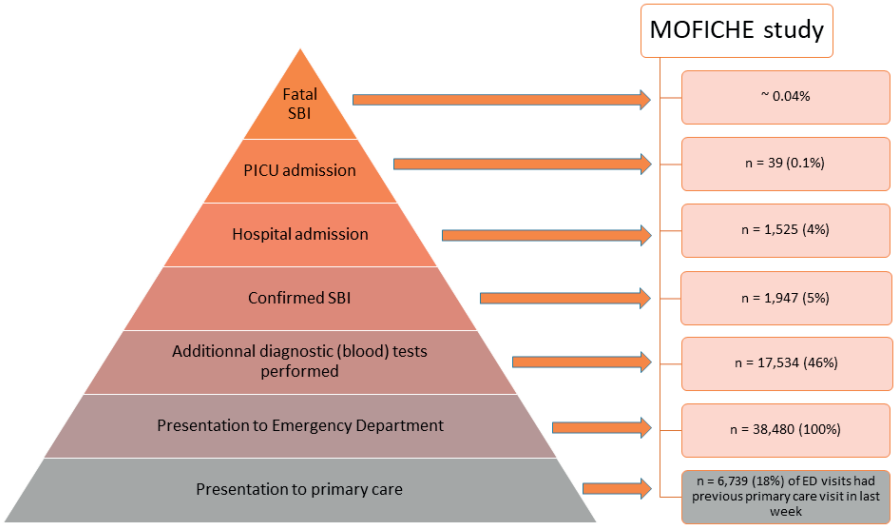


Figure 1 | Outcomes of serious bacterial infections in febrile children (adapted from Nijman et al.[9])
Legend: The data were collected in the MOFICHE study (Management and Outcome of Fever in Children in Europe), n=38,480). We estimated mortality due to bacterial infections to be 0.04% (based on the PERFORM data of febrile children with 28-day follow-up). Abbreviations: SBI, serious bacterial infection; PICU, paediatric intensive care unit

Diagnostics performed at the ED often include urinalysis or additional blood tests. In blood, CRP or procalcitonin can be measured which are increased in bacterial infections and used at the ED to improve diagnosis of bacterial infection. High CRP and procalcitonin levels, however, represent a non-specific inflammation response.

In the MOFICHE study, we provide insight in the likely cause of infectious disease in febrile children at the ED. The presumed cause of infection was categorized using a flowchart based on clinical sings, diagnostics including CRP level, and microbiological results. Overall, a definite sterile-site bacterial infection was present in 4.1% of febrile children, from which the majority were urinary tract infections (Figure 2). Presumed viral infections comprised the largest group in respiratory tract infections, gastro-intestinal infections and undifferentiated fever. Overall, the proportion of unknown bacterial/viral infections was 14.6% in which the proportion of antibiotic prescription was 45%. The proportion of unknown bacterial/viral varied from 3.7% to 24.6% per focus group and was highest in undifferentiated fever and lower respiratory tract infections. Clinicians

make their decision on antibiotic prescription by weighing risks and potential harms. In general, the risk of withholding antibiotic treatment for a bacterial infection is larger than the potential harm of unnecessary antibiotic treatment for the individual patient. More targeted antibiotic treatment can be achieved by improved diagnosis for both bacterial infections and viral infections. For example, rapid viral tests have shown to reduce antibiotic prescriptions when test results were positive, although an overall antibiotic reduction was not observed in two systematic reviews. [11,12]

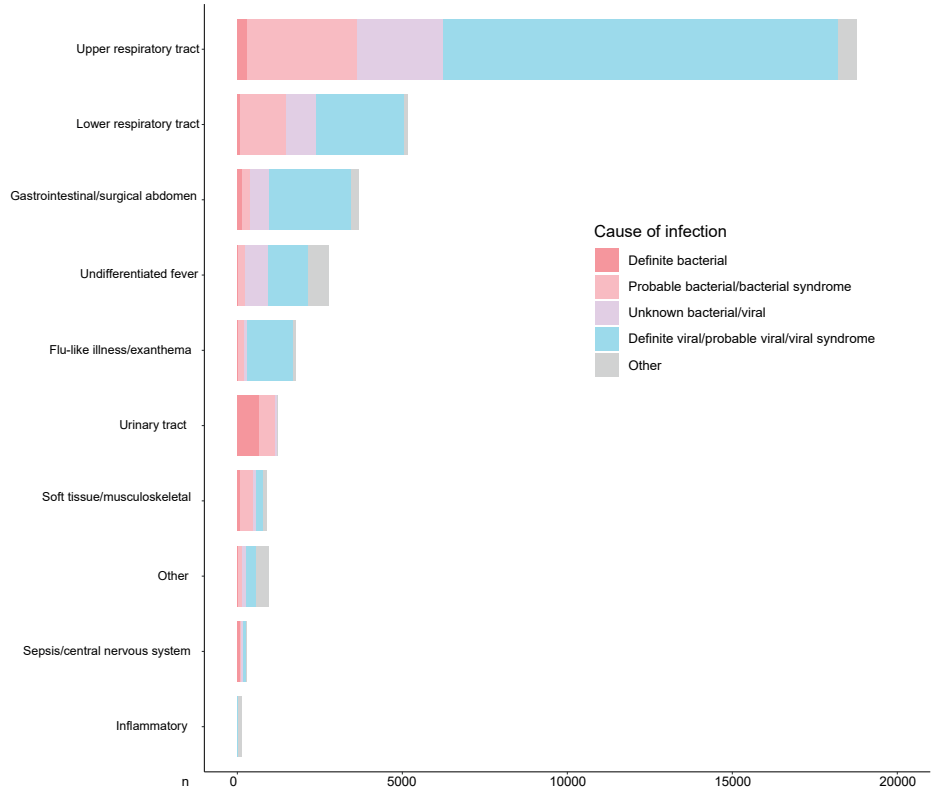


Figure 2 | Bar chart of cause of infection stratified for focus of infection based on MOFICHE study

The potential of host response biomarkers in children with fever

Vital signs and alarming signs are used for assessment of SBI in febrile children. Although alarming signs can be used to rule-out serious bacterial infection [13-16], they lead to a large number of false-positives. In one study of febrile children attending the ED, 41% of children had one or more warning signs and only 7% of those had a serious bacterial

infection.[7] For children <3 months, low-risk criteria as the Rochester or Philadelphia criteria aid to identify children at low-risk for serious bacterial infection. These criteria, however, are based on expert opinion and were developed in the era of absence of immunisation to several bacterial infections.[17-23] Clinical prediction models, which include both clinical parameters and biomarkers, could further improve early diagnosis of bacterial infection in febrile children.[24] Existing clinical prediction models which predict bacterial infections have mainly focused on young children (Table 1). Clinical prediction models that focus on older children include the Feverkidstool by Nijman *et al.* [24] and the model from Irwin *et al*. [25](Table 1)

Besides combining biomarkers with signs and symptoms in a clinical prediction model, recent studies such as the PERFORM project aim to find new discriminators for bacterial and viral infections by applying transcriptomic, proteomic and metabolomics approaches.[31-34] It is yet unclear which children will benefit most from new biomarkers. Clinical prediction models could aid in selection of children who will have diagnostic advantage from new biomarkers.

In this thesis, we derived and cross-validated a clinical prediction model based on the Feverkidstool (C reactive protein, and important clinical predictors) to early recognise invasive bacterial infections using a large observational European-wide cohort of febrile children. The rule-out threshold of our prediction model could reduce antibiotic treatment while the rule-in threshold could be used to target treatment in febrile children at the ED. In more than half of patients at intermediate risk, sensitive biomarkers could improve identification of invasive bacterial infections (Figure 3). In practice, clinical prediction models can be used to identify children with diagnostic uncertainty in whom additional diagnostic testing by novel biomarkers is warranted. Patient groups in which additional testing may be particular useful include children <3 months, children with undifferentiated fever, children with comorbidity and immunocompromised patients. For instance, children <3 months have a low, but important risk for invasive bacterial infections. In general, the diagnostic work-up for this group include collection of blood culture (lumbar puncture on indication) and intravenous antibiotic treatment awaiting blood culture results. Lefebvre *et al.* found that only 1.5% of blood cultures were positive for a pathogenic bacteria in children <3 months. Consequently, a large number of children, without having a bacterial infection, often endure numerous invasive procedures and are admitted to the hospital.[22,35,36]

Dupuy *et al.* evaluated the role of biomarkers in the management of infections and reported that although over 100 biomarkers have been studied, only a few are used in clinical practice.[37,38] An ideal biomarker for acute infections should either be a diagnostic

Table 1 | Overview of clinical prediction models for bacterial infections in febrile children which include biomarkers published between 2010 and 2020

Models with at least one laboratory variable and at least one clinical variable.

Author, year	Population	Outcome	Variables in prediction model	Model performance
Aronson, 2019 [26]	Febrile <60 days. Excluded: ill-appearing, complex chronic conditions	IBI	*New model:* age <21d, temperature, urinalysis, ANC	AUC: 0.83 (95% CI 0.79-0.86) Sens: 98.8% (95% CI: 95.7-99.9) Spec: 31.3% (95% CI: 26.3-36.6)
Aronson, 2018 [27]	Febrile <60 days. Excluded: ill appearance, Complex chronic conditions	IBI	*Rochester criteria:* previously healthy, well appearing, urinalysis, WBC, ABC, *modified Philadelphia*: age >28d, previously healthy, urinalysis, WBC	*Rochester*: Sens: 81.5% Spec: 59.8% *Modified Philadelphia*: Sens: 91.9% Spec: 34.5%
Gomez, 2016 [28]	Febrile without source <90 days, normal physical examination and no resp signs/symptoms or diarrhea. Previously healthy	IBI	*Step-by-step:* well appearing, age <21 d, leukocyturia, PCT, CRP, ANC *Rochester criteria*	*Step-by-step:* Sens: 92.0% (95% CI: 84.3-96.0) Spec: 46.9 (95% CI: 44.8-49.0) *Rochester:* Sens: 81.6% (95% CI: 72.2-88.4) Spec: 44.5% (95% CI: 42.4-46.6)
Irwin, 2017 [25]	Febrile <16 years, with blood tests Excluded: primary immunodeficiencies.	Pneumonia, other SBI	*New model:* respiratory rate, normal air entry, CRP, PCT, resistin *Feverkidstool* extended with PCT and resistin	*New model:* C-stat. pneumonia: 0.84 (95% CI: 0.78-0.90) C-stat. other SBI: 0.77 (95% CI: 0.71-0.83) *Recalibrated Feverkidstool:* C-stat. Pneumoniae: 0.88 C-stat. Other SBI : 0.82
Leroy, 2018 [29]	<3 years, with fever without source. Exclusion: previous antibiotic use immunodeficiencies etc.	SBI	*Refined lab-score:* PCT, CRP, urine dipstick and age	AUC: 0.94 (95% CI: 0.93-0.96) Sens: 96% (95% CI 92-98) Spec: 73% (95% CI: 70-77)
Nijman, 2013 [24]	Febrile 1m-16y Exclusion: comorbidity and clear focus of upper respiratory infection	Pneumonia, other SBI	*Feverkidstool:* age, duration of fever, tachycardia, temperature, tachypnea, ill appearance, chest wall retractions, prolonged capillary refill time, oxygen saturation <94%, CRP	C-stat. pneumonia: 0.81 (95% CI: 0.73-0.88) C-stat. other SBI: 0.86 (95% CI: 0.79-0.92)
Nijman, 2018 [30]	Febrile 1m-16y Exclusion: comorbidity and clear focus of upper respiratory infection	Pneumonia, other SBI	*Feverkidstool*, and update with PCT	C-stat. pneumonia: 0.86 (95% CI: 0.77-0.94) C-stat. other SBI: 0.83 (95% CI 0.75-0.91)

ABC, absolute band count; ANC, absolute neutrophil count; AUC, area under the receiver operating curve; C-stat., concordance statistic; CRP, c-reactive protein; IBI, invasive bacterial infection; PCT, procalcitonin; SBI, serious bacterial infection; sens, sensitivity; spec, specificity; WBC, white blood cell count;

or prognostic test and easily accessible and acceptable for patients and clinicians (Table 2). Importantly, biomarkers should be studied at the level of care where decisions are made regarding admission or antibiotic treatment such as the emergency department. For instance, the biomarker neutrophil CD64 showed good performance in paediatric intensive care setting but had poor performance in the population of febrile children at the ED.[39]

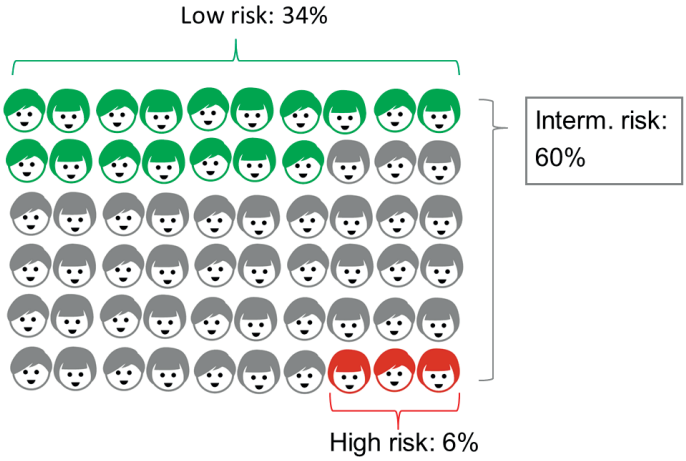


Figure 3 | Risk groups for invasive bacterial infection estimated by the clinical prediction model: Proportion identified as low-risk, intermediate-risk and high-risk for invasive bacterial infection.

Table 2 | Important characteristics of biomarkers for acute infections, adapted from Dupuy et al. [37]

Criteria for use	Characteristics
Diagnostic test	- General: known preanalytic and analytic (accuracy, reproducibility) as well as physiological (intra and interindividual) variability, integrated in the interpretation of assay results - High sensitivity/specificity - Ability to differentiate acute viral from bacterial infection independent from disease severity
Prognostic test	- Early detection of patients at risk of a complicated disease course (e.g. hospitalisation, ICU admission) - Levels associated with the inflammatory response (i.e. correlated to the severity of presentation and/or to organ dysfunctions)
Accessibility	- Routinely available - Good acceptability to patients - Rapid turnaround time - Low cost
Validation	- Evaluation of clinical utility in different studies: phase 1-4 trials [40] - Broad validation in different settings with different prevalence of bacterial infections

In our cohort of 38,480 febrile children attending European EDs, testing of procalcitonin was rarely performed (n=622, 1.6%) compared to testing with CRP (n=17213, 44.7%). Procalcitonin might be a better marker for bacterial infection than CRP as procalcitonin levels have an earlier peak in disease course. Reasons for limited routine procalcitonin measurements might include limited availability [41], higher costs [42], and longer duration of procalcitonin results in laboratory testing compared to CRP testing. Point-of-care testing for procalcitonin is currently possible and has also been studied in febrile children to be feasible and with good diagnostic accuracy for bacterial infections. [43-45]

Next to measurement of single biomarkers, multiple biomarkers can also be combined in one assay. For example, the protein assay ImmunoXpert, combination of TRAIL (tumour necrosis factor-related apoptosis-inducing ligand), IP-10 (interferon-gamma-induced protein 10) and CRP, has shown good diagnostic performance to distinguish bacterial from viral infections in children with lower respiratory tract infections or fever without source.[33] An advantage of this assay is the combination of biomarkers which are upregulated in viral infections (TRAIL, IP-10) as well as in bacterial infections (CRP). One study updated the Feverkidstool with ImmunoXpert which showed improved diagnostic accuracy for pneumoniae and other SBIs in febrile children.[46]

Another available viral marker in blood is MxA (myxovirus resistance protein A). Previous studies have shown that MxA levels are higher in children with viral respiratory tract infections [47-50] Future studies should focus on finding the best combination of viral and bacterial host-response biomarkers and combining clinical prediction models with biomarkers.

Besides providing information on the risk of bacterial infections, biomarkers can also predict disease severity or adverse outcomes. In critically ill children admitted to the intensive care, we studied the association of mHLA-DR expression with acquisition of secondary infections and found no association. In critically ill children, mHLA-DR expression was low suggesting an impaired immune response in this group. Immunostimulating therapies such as GM-CSF and interferon gamma have shown to be able to restore mHLA-DR levels and to improve outcomes.[51-56] It remains yet unclear which children would benefit from immunostimulating therapies.

In this thesis, we also studied the variation of haemostasis proteins across paediatric invasive bacterial infections due to *Neisseria meningitidis, Streptococcus pneumoniae, Staphylococcus aureus* and *Group A streptococcus* and described haemostasis protein levels and their relation with mortality and disease severity. We found that haemostasis disturbances were not limited to meningococcal infections, but also occurred in non-

meningococcal sepsis. In addition, we confirmed that high thrombomodulin levels, high ADAMTS-3 levels and low fibronectin levels were associated with mortality and that thrombomodulin had good discriminative ability for mortality. Although these haemostatic disturbances will not necessarily lead to disseminated intravascular coagulation (DIC), these results should raise awareness for haemostatic derangements in paediatric sepsis.

Strategies to improve antibiotic prescription

We studied current practice of antibiotic prescribing in febrile children attending European EDs. First, we observed wide variation between European EDs in prescription of overall antibiotics and broad-spectrum antibiotics in febrile children when adjusted for patient population, diagnostics and focus and cause of infection. Moreover, we found that one-third of antibiotic prescriptions were of inappropriate (presumed viral) or inconclusive (unknown bacterial/viral) indication. Therefore, antibiotic prescription can be improved for febrile children at the ED. Antimicrobial stewardship programs and guideline implementation are important to improve antibiotic prescription. Guidelines are especially helpful in recommendations regarding type of antibiotic agent and antibiotic duration, but often lack individual precision. In uncomplicated respiratory tract infections, antibiotic prescriptions were not according to guidelines in 22% of prescriptions. To optimise antibiotic prescription, ED specific antimicrobial stewardship programs are needed and should also include reviews of prescriptions and subsequent feedback to the individual physician.[57]

How can we further improve antibiotic prescription at the ED? The largest group of febrile children at the ED comprise respiratory tract infections, which are mainly caused by viruses. Clinical decision-rules can impact antibiotic prescription at the individual level by classifying patients at low-risk for bacterial infections. The Feverkidstool is a clinical decision rule, which estimates the risk of having bacterial pneumonia and advices withholding of antibiotics in patients at low/intermediate-risk for bacterial pneumonia.[24,25,58] In this thesis, we studied the impact of the Feverkidstool in febrile children visiting European EDs. Our simulation study showed that implementation of the Feverkidstool could reduce antibiotic prescription at European EDs. Although this study was a simulation study and only provides us with estimates of potential results, we show that the potential effect on antibiotic prescription is influenced by patient case-mix and baseline antibiotic prescription rate, and usage and compliance to a clinical decision rule. Since the Feverkidstool has shown to be cost-effective [59], the Feverkidstool should be implemented in European EDs, especially those with high baseline prescription rates

and many low/intermediate-risk patients. Insight of the potential performance of the Feverkidstool at the local level could be an important step in improving antibiotic prescription. Local facilitators and barriers should be evaluated to optimise implementation in the participating EDs of the MOFICHE study.

Our study shows that antibiotic prescriptions can be optimized in European EDs. Nevertheless, antibiotics prescribed at the ED are a small proportion of the total amount of antibiotics prescribed in the community. The largest group of febrile children is treated by a GP in the community (Figure 1). One study compared antibiotic prescription rates for febrile children with respiratory tract infections in three levels of care in the Netherlands: GP, general hospital and university hospital.[60] Antibiotic prescription rates were comparable in these three levels of health care indicating that primary care, where more children are managed for respiratory tract infections, accounts for a large amount of antibiotic prescriptions.

Other factors that influence antibiotic prescriptions include parental expectations, and physicians' expectations towards these, availability of an adequate safety net and diagnostic uncertainty.[61-63] Physicians might be more inclined to prescribe antibiotics to patients who they think expect a prescription. Parental or patient satisfaction, however, does not depend on antibiotic prescription but is mainly related to improved understanding of the illness.[62,64] This emphasizes the need for communication skills and explaining harms and benefits. Studies in adult primary care showed that point of care CRP testing in combination with communication training reduced antibiotic prescription without influencing disease course or satisfaction.[65] Implementation of point-of-care CRP should be combined with individual risk-prediction for bacterial infections and clear guidelines when to withhold or prescribe antibiotics.

Methodological issues: strengths and lessons learnt

In the MOFICHE study, which was part of the PERFORM project, we collected routine observational data of febrile children attending 12 European EDs. International collaboration is essential since the prevalence of severe disease such as paediatric ICU admissions and invasive bacterial are rare (<1%). For MOFICHE, we obtained a waiver for informed consent as collected data were anonymous. In general, it is not feasible to obtain informed consent for all febrile children attending the ED leading to frequently missed patients. The waiver for informed consent enabled us to collect data on all febrile children who attended the ED. Instead of performing an export of electronic health care data ('data dump') and harmonizing the data, we decided to manually enter the

data in an online platform (Redcap) [66] in order to obtain detailed and high-quality data. Although this is time-consuming and requests a lot of effort from the local study sites, it has provided us with high quality and in-depth data. The number of yearly paediatric visits ranged widely in the participating EDs. For the large hospitals, it was not feasible to include all visits of febrile children in the study period. Therefore, some hospitals sampled a period per month to ensure a year round data collections including all seasons.

Quality control

In platforms as Redcap [66], automated quality checks can be integrated so outliers and missing values are reduced. In the process of study design, it is essential to prioritize variables, define a minimal data set which are essential for the research question and minimize free-text variables. Quality of data collection for febrile children was optimized by using standardised data collection and optimized clinical assessment using a training module for the local clinical and research teams. This training module included clarification of the individual alarming signs and examples of classification of common diagnoses. To further improve data quality, entry guidelines for the eCRF were available, monthly teleconferences and biannual meetings were organised, and quarterly reports of data quality for each ED were discussed. These consortium teleconferences also included discussion of difficult cases. Furthermore, starting preliminary analysis in an early phase aids to improve collected data, to detect potential problems and to understand the data.

Challenges

Challenges in the study included technical problems with Redcap due to the large number of entries which were solved by opening an additional second dataset. Furthermore, since we used routine observational data, missing data are inevitable. In analyzing the data and depending on the research question, different strategies can be performed to handle missing data which all have their advantages and disadvantages. To perform multiple imputation and estimate missing data reliably, detailed variables and a representative sample of collected variables are needed.[67,68] A complete case analysis reflects clinical practice. However, the population in which CRP or blood pressure are measured might be selected and more severely ill and therefore generalizability is limited. In addition, both a complete case analysis and an analysis based on multiple imputation can be compared.

Evaluating appropriateness of antibiotic prescription should be performed in reference to local guidelines. Early collection of local guidelines for empiric antibiotic prescription is therefore essential in correct interpretation of the data. Furthermore, antibiotic

prescription should be evaluated taking in to account local antimicrobial resistance patterns and differences in immunisation programs.

Future perspectives

The value of a large, epidemiological dataset of febrile children

The MOFICHE study included over 38,000 visits of febrile children attending 12 EDs in 8 European countries. We were able to develop a clinical prediction model for the rare outcome of invasive bacterial infections. In addition, this large database can answer future research questions and could provide descriptions of uncommon populations such as adolescents, children <3 months, patients with petechiae, patients with chronic conditions and other specific diagnosis. To further improve diagnosis and treatment in these patient groups, description of these populations might be a first step for further in-depth studies.

Improving antibiotic treatment and identification of bacterial infections

Decision tools as the Feverkidstool aid in reducing antibiotic prescription for children with lower respiratory tract infections whereas our updated model aids in prediction of IBI. Both these tools will need CRP measurement and therefore CRP measurement should be available, preferably at the point-of-care. Furthermore, in children with comorbidity no clinical decision tool exists for prediction of bacterial lower respiratory tract infections. Since the proportion of children with comorbidity increases at the ED, clinical decision models are needed for this specific group. Therefore, the Feverkidstool will need to be validated in children with comorbidity and, when model performance is adequate; its safety to guide antibiotic treatment will need to be established in a new trial.

In the future, we will need to incorporate early warning scores and clinical decision tools in electronic health record data. These tools can help the clinician in estimating the risk of serious illness or bacterial infection. Furthermore, CE (Conformité Européen)- certification and translation to multiple languages would be an important next step for broad implementation of the Feverkidstool at EDs.

New biomarkers

International collaboration remains essential in improving diagnostic strategies for febrile children. Large international multicenter studies such as the PERFORM project are needed to recruit sufficient numbers of children representing the wide range of clinical presentations including a range of pathogens and disease severity. In the PERFORM

study, new discriminators for viral and bacterial infection for children are studied using transcriptomic and proteomic approaches.

Different strategies can be followed in order to identify the best biomarkers that discriminate viral from bacterial infections. On the one hand, the use of transcriptomic, proteomic and metabolomic approaches ('omics') could lead to discovery of novel biomarkers. On the other hand, one could focus on validation of promising biomarkers already described in the literature and finding the best combination of biomarkers for accurate diagnosis. Furthermore, novel biomarkers need to be compared with existing biomarkers as CRP and procalcitonin. In addition, results of these novel biomarkers need to be timely available, preferably at the point-of-care. To ensure clinical application of these novel biomarkers in the future, the allocation of resources need to be questioned. Who will benefit from these new diagnostics and are these cost-effective? Future studies will need to evaluate which groups will benefit most such as patients at higher risk of diagnostic uncertainty including young infants and children with chronic conditions. Lastly, to implement new biomarkers and clinical prediction models in clinical practice it is essential to perform cost-effectiveness analysis using statistical modelling and simulation studies to estimate the potential effect on antibiotic prescriptions, hospital admissions and misdiagnosis.

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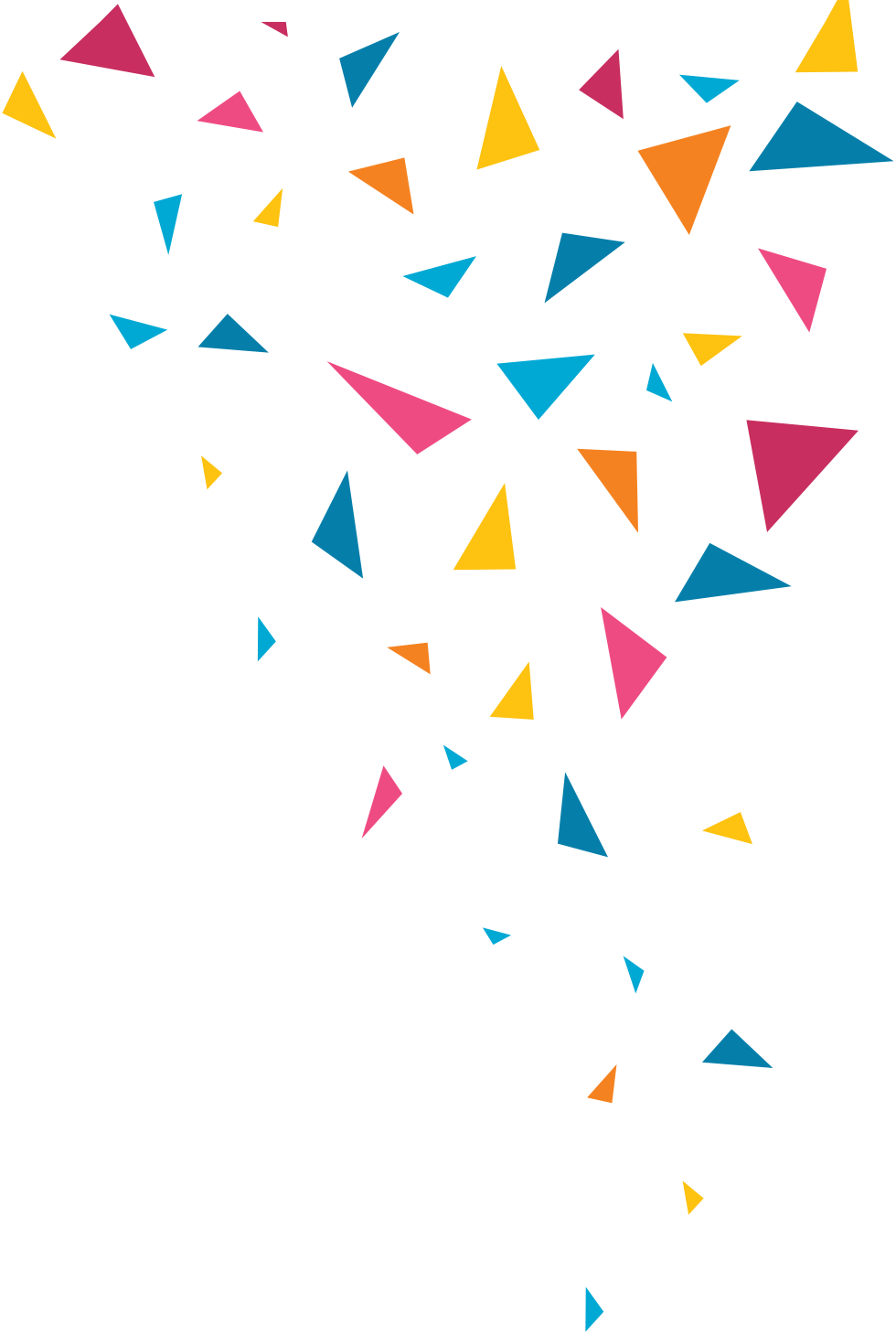

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Summary

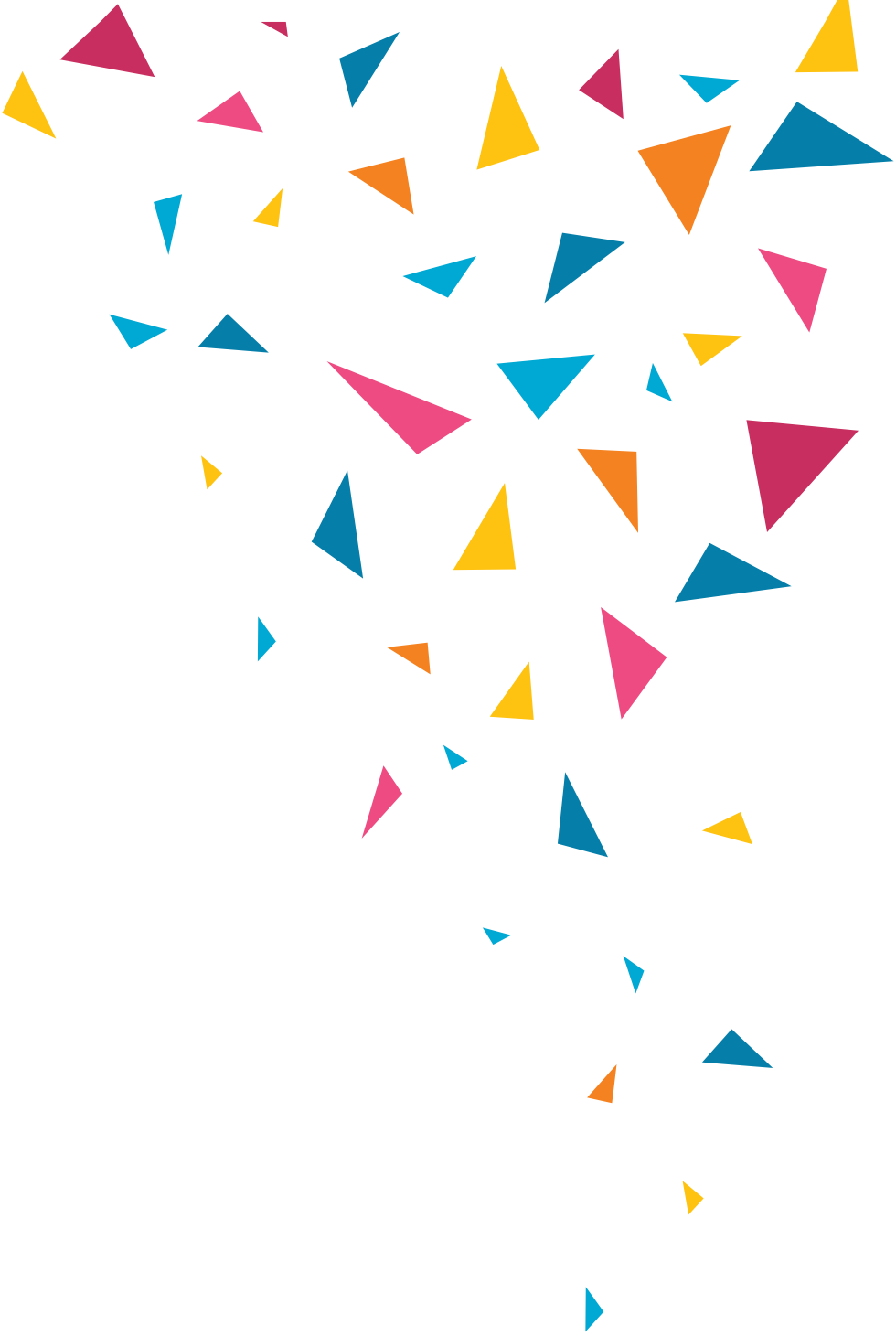
Chapter 1 includes the general introduction and description of the aims of this thesis. Fever in children is a frequent reason for visit to the Emergency Department (ED). The majority of children have viral respiratory infections that are self-limiting in nature. A small proportion of children with fever have a serious bacterial infection that requires early treatment with antibiotics to prevent morbidity and mortality in children. It is therefore essential to early recognize serious bacterial infections in the ED. Physicians use alarming symptoms and vital signs in their assessment of the febrile child. Distinguishing children with bacterial infections from those with self-limiting viral infections, however, is challenging due to similarities of presenting clinical symptoms. Physicians prescribe antibiotics in order not to miss one child with invasive bacterial infection which leads to overuse of antibiotics. Emergency care of febrile children can be improved by focusing on 1) improved diagnosis by the use of host response biomarkers and 2) improvement of antibiotic prescription. The overarching aim of this thesis is to improve diagnostic strategies and antibiotic treatment of febrile children.

In **Part II**, we study the value of routine blood pressure measurement in the ED. In **chapter 2**, we identified evidence-based reference values for low blood pressure and compared those with clinical guidelines for hypotension. We showed that clinical guidelines for hypotension show large variability and that they have low to moderate agreement with population-based lower centiles for blood pressure in children. In **chapter 3**, we studied children attending an university ED in the Netherlands and showed that hypotension, adjusted for tachycardia, was associated with serious illness, although its sensitivity was limited. High Shock Index (ratio heart rate/systolic blood pressure) showed an association with serious illness, but no acceptable cut-off value could be identified. In **chapter 4**, we provide reference values for Shock Index in febrile children. In addition, we observed that high Shock Index was associated with serious illness in febrile children. However, its rule-out value is insufficient which makes the Shock Index not valuable as screening tool for febrile children at the ED.

In **Part III**, we study host response biomarkers. In **chapter 5**, we derived and cross-validated a clinical prediction model based on the Feverkidstool (clinical symptoms, C reactive protein) and important clinical predictors to early recognise invasive bacterial infections (IBI) using a large observational European-wide cohort of febrile children. The rule- out threshold of this prediction model could reduce antibiotic treatment while the rule- in threshold could be used to target treatment in febrile children at the ED. In more than half of patients at intermediate risk, sensitive biomarkers could improve identification of IBI. In **chapter 6**, we studied mHLA-DR expression in children. We showed that critically ill children have lower mHLA-DR expression than controls. In infectious critically ill children, mHLA-DR had no prognostic value for acquisition of secondary

infections. In **chapter 7**, we described the variation of haemostasis proteins in children with infections due to meningococcus, pneumococcus, *Staphylococcus aureus* and Group A streptococcus (GAS). Haemostatic disturbances in paediatric bacterial infections were not limited to meningococcal sepsis, but occur with a comparable frequency and severity across infections with different pathogens. In addition, thrombomodulin, ADAMTS-13 and fibronectin were associated with mortality.

In **Part IV**, we studied antibiotic prescription in febrile children across Europe. In **chapter 8**, we observed wide variation between European EDs in prescriptions of antibiotics and broad-spectrum antibiotics in febrile children. Overall, one-third of prescriptions were inappropriate or inconclusive, with marked variation between EDs. In **chapter 9**, we simulated the potential impact of the Feverkidstool on antibiotic prescription in febrile children with suspected lower respiratory tract infections in different European EDs. Implementation of the Feverkidstool could reduce antibiotic prescriptions in children with suspected LRTIs in European EDs. The largest reduction of antibiotic prescriptions was simulated in EDs with high baseline antibiotic prescription rates or predominantly (>85%) low/intermediate-risk children.



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Nederlandse samenvatting

Hoofdstuk 1 is een algemene introductie met een beschrijving van de doelen van dit proefschrift. Koorts is een veelvoorkomende reden voor kinderen om de Spoedeisende hulp (SEH) te bezoeken. De meerderheid van de kinderen heeft een virale luchtweginfectie die zelflimiterend van aard is. Een klein deel van kinderen met koorts heeft een ernstige bacteriële infectie waarvoor vroegtijdige behandeling met antibiotica nodig is om morbiditeit en mortaliteit te voorkomen. Het is daarom belangrijk om ernstige bacteriële infecties vroegtijdig te herkennen op de SEH. In de beoordeling van het kind met koorts worden alarmsymptomen en vitale kenmerken gebruikt. Het onderscheiden van kinderen met een bacteriële infectie van virale infecties is echter lastig op basis van klinische kenmerken. Vaak wordt antibiotica voorgeschreven om het missen van een bacteriële infectie te voorkomen. Dit kan leiden tot overmatig gebruik van antibiotica. De zorg van kinderen met koorts op de SEH kan verbeterd worden met optimaliseren van de diagnose met behulp van host-response biomarkers en verbetering van voorschrijven van antibiotica. Het overkoepelende doel van dit proefschrift is het verbeteren van diagnostische strategieën en antibiotische behandeling van kinderen met koorts. In het bijzonder beoogt dit proefschrift antwoord te geven op de volgende onderzoeksvragen:

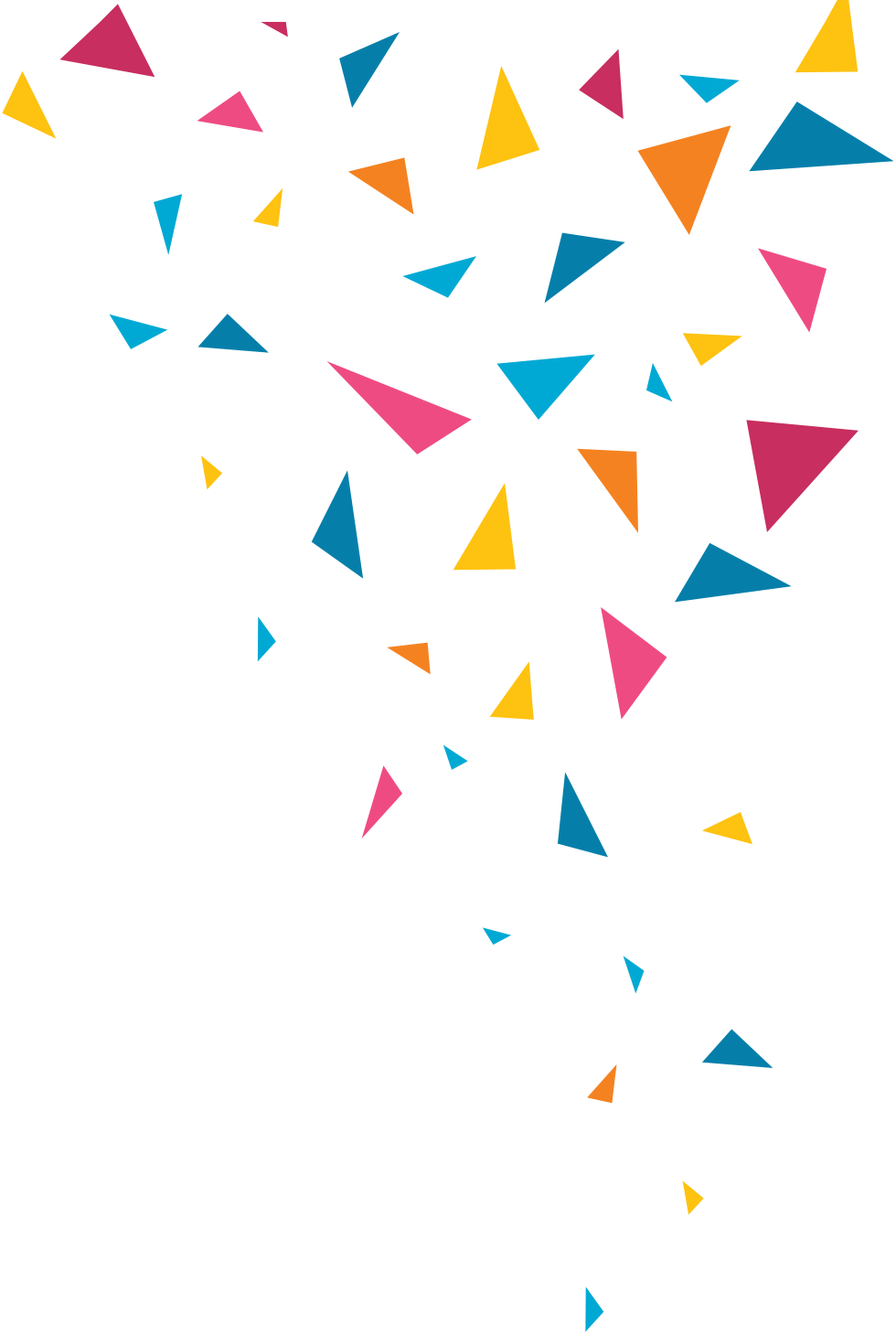
- Kan systolische bloeddruk de herkenning verbeteren van ernstige ziekte bij kinderen op de SEH?
- Kunnen we een klinisch voorspelmodel ontwikkelen om invasieve bacteriële infecties te identificeren en om kinderen te identificeren die baat kunnen hebben bij nieuwe biomarkers ter verbetering van de diagnose?
- Kunnen host-response biomarkers nadelige gevolgen van ernstig zieke kinderen met invasieve infecties beter voorspellen?
- Kunnen we strategieën identificeren om het voorschrijven van antibiotica bij kinderen met koorts op Europese SEH's te verbeteren?

In **deel II** onderzoeken we de waarde van routinematige bloeddrukmeting op de SEH. In **hoofdstuk 2** hebben we evidence-based referentiewaarden voor lage bloeddruk geïdentificeerd en deze vergeleken met klinische richtlijnen voor hypotensie. Klinische richtlijnen voor hypotensie laten grote variabiliteit zien en hebben lage tot matige overeenstemming met lagere centielen voor bloeddruk bij kinderen die op populaties gebaseerd zijn. In **hoofdstuk 3** bestuderen we kinderen die zich presenteerden bij een universitaire SEH in Nederland en tonen aan dat hypotensie, gecorrigeerd voor tachycardie, geassocieerd is met ernstige ziekte, hoewel de sensitiviteit ervan beperkt is. De combinatie van hartslag en bloeddruk in de Shock Index (ratio hartfrequentie/systolische bloeddruk) toont een associatie met ernstige ziekte, hoewel geen acceptabele afkapwaarde kan worden bepaald. In **hoofdstuk 4** presenteren we referentiewaarden voor de Shock Index bij kinderen met koorts. Daarnaast tonen we aan dat hoge Shock

Index geassocieerd is met ernstige ziekte bij kinderen met koorts. De uitsluitingswaarde van de Shock Index is echter onvoldoende waardoor de Shock Index niet waardevol is als screeningsinstrument voor kinderen met koorts op de SEH.

In **deel III** onderzoeken we host-response biomarkers. In **hoofdstuk 5** hebben we met behulp van een groot Europees observationeel cohort van kinderen met koorts een klinisch voorspelmodel ontwikkeld en kruis-gevalideerd welke gebaseerd was op de Feverkidstool (klinische symptomen, C-reactief proteïne) en belangrijke klinische voorspellers voor vroege herkenning van invasieve bacteriële infecties (IBI). De laag-risico drempelwaarde van dit model kan kinderen met IBI's uitsluiten en daarmee antibiotica gebruik verminderen. Aan de andere kant kan de hoog-risico drempelwaarde gebruikt worden om kinderen met IBI's aan te tonen en vroegtijdig behandeling te starten. Bij meer dan de helft van de patiënten met een gemiddeld risico zouden gevoelige biomarkers de identificatie van IBI kunnen verbeteren. In **hoofdstuk 6** hebben we mHLA-DR-expressie bij kinderen bestudeerd. We tonen aan dat ernstig zieke kinderen een lagere mHLA-DR-expressie hebben dan controles. Bij infectieuze ernstig zieke kinderen heeft mHLA-DR geen prognostische waarde voor het verwerven van secundaire infecties. In **hoofdstuk 7** hebben we de variatie van hemostase-eiwitten beschreven bij kinderen met infecties die veroorzaakt werden door meningokokken, pneumokokken, Staphylococcus aureus en Groep A streptokokken (GAS). Hemostatische stoornissen bij bacteriële infecties bij kinderen waren niet beperkt tot meningokokkensepsis, maar komen met een vergelijkbare frequentie en ernst voor bij infecties met verschillende pathogenen. Daarnaast zijn trombomoduline, ADAMTS-13 en fibronectine geassocieerd met mortaliteit.

In **deel IV** onderzoeken we het gebruik van antibiotica bij kinderen met koorts in Europa. In **hoofdstuk 8** beschrijven we een grote variatie tussen Europese SEH's in het voorschrijven van antibiotica en breedspectrum antibiotica bij kinderen met koorts. Een derde van de antibiotica voorschriften was ongeschikt of inconclusief voor de indicatie waarbij er duidelijke verschillen waren tussen SEH's. In **hoofdstuk 9** simuleren we de mogelijke impact van de Feverkidstool in verschillende Europese SEH's op het voorschrijven van antibiotica bij kinderen met koorts met lagere luchtweginfecties. Implementatie van de Feverkidstool zou het voorschrijven van antibiotica bij kinderen met lage luchtweginfecties in Europese SEH's kunnen verminderen. De grootste afname van het aantal antibioticavoorschriften werd gesimuleerd op SEH's met hoge percentages voor antibioticavoorschrift of met een groot aandeel (>85%) kinderen met een laag/gemiddeld risico.



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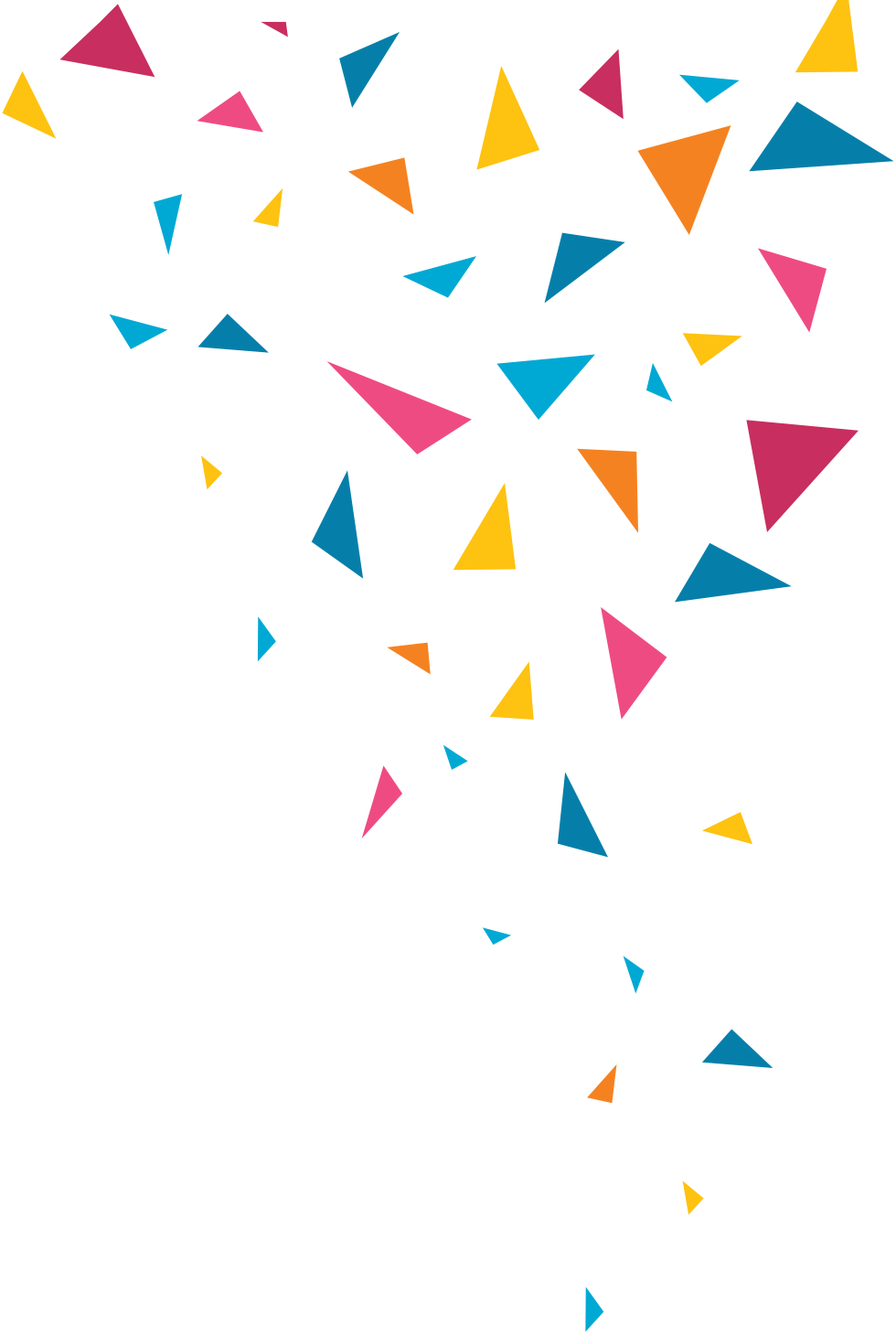

Appendix II

List of publications

This thesis

Hagedoorn NN, Zachariasse JM, and Moll HA. A comparison of clinical paediatric guidelines for hypotension with populationbased lower centiles: a systematic review
Critical Care; 2019, 23(1): 380. doi: 10.1186/s13054-019-2653-9.

Hagedoorn NN, Zachariasse JM, and Moll HA. Association between hypotension and serious illness in the emergency department: an observational study
Archives of Disease in Childhood; 2020, 105(6): 545-551. archdischild-2018-316231.

Hagedoorn NN, Zachariasse JM, Borensztajn D, Adriaansens E, von Both U, Carrol ED, Eleftheriou I, Emonts M, van der Flier M, de Groot R, Herberg JA, Kohlmaier B, Lim E, Maconochie I, Martinón-Torres F, Nijman RG, Pokorn M, Rivero-Calle I, Tsolia M, Zavadska D, Zenz W, Levin M, Vermont C, Moll HA, and PERFORM consortium
Shock Index in the early assessment of febrile children at the emergency department: a prospective multicentre study.
Archives of Disease in Childhood; 2021 Jun 22. archdischild-2020-320992.

Hagedoorn NN, Borensztajn D, Nijman RG, Nieboer D, Herberg JA, Balode A, von Both U, Carrol E, Eleftheriou I, Emonts M, van der Flier M, de Groot R, Kohlmaier B, Lim E, Maconochie I, Martinón-Torres F, Pokorn M, Strle F, Tsolia M, Zavadska D, Zenz W, Levin M, Vermont C, and Moll HA
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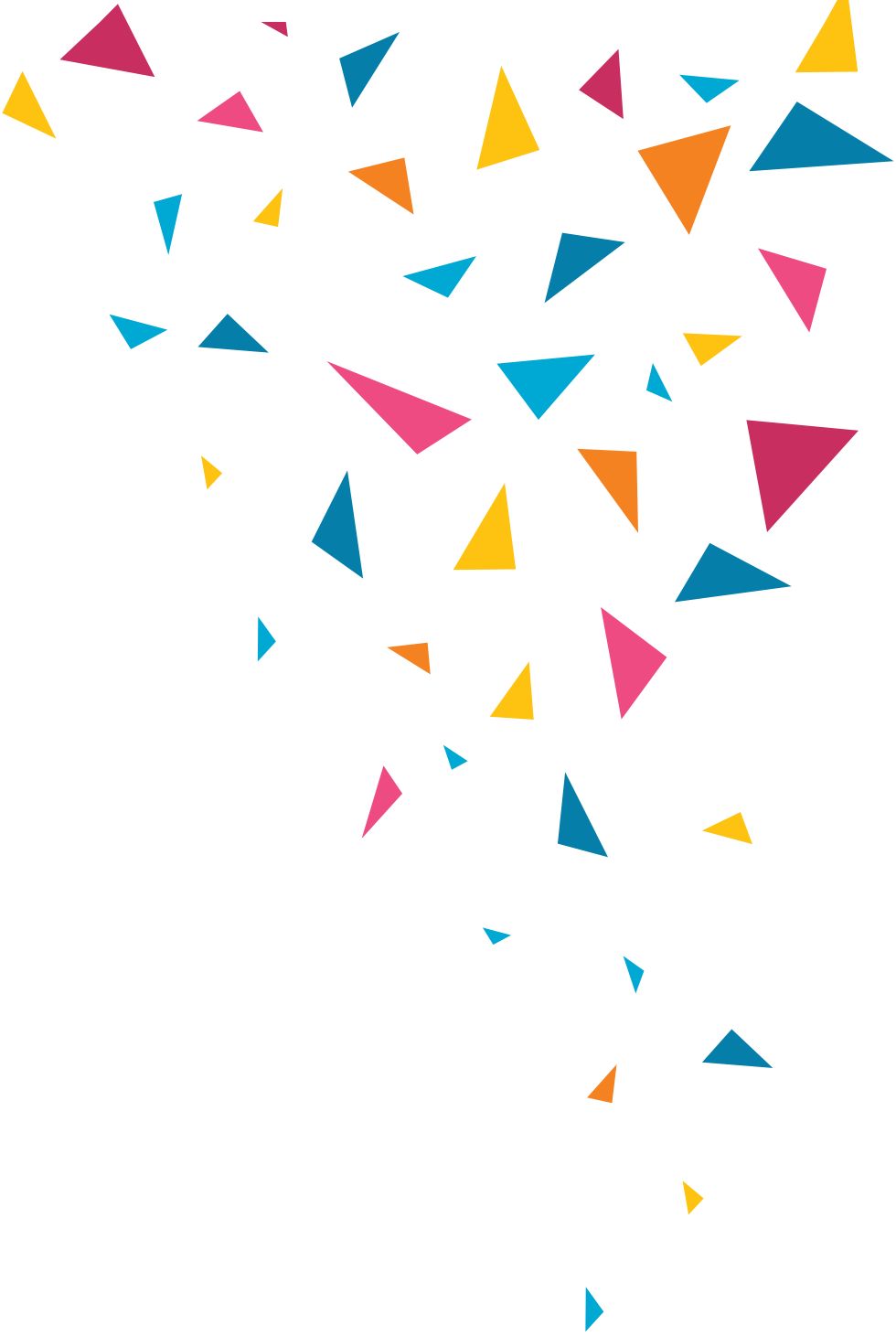


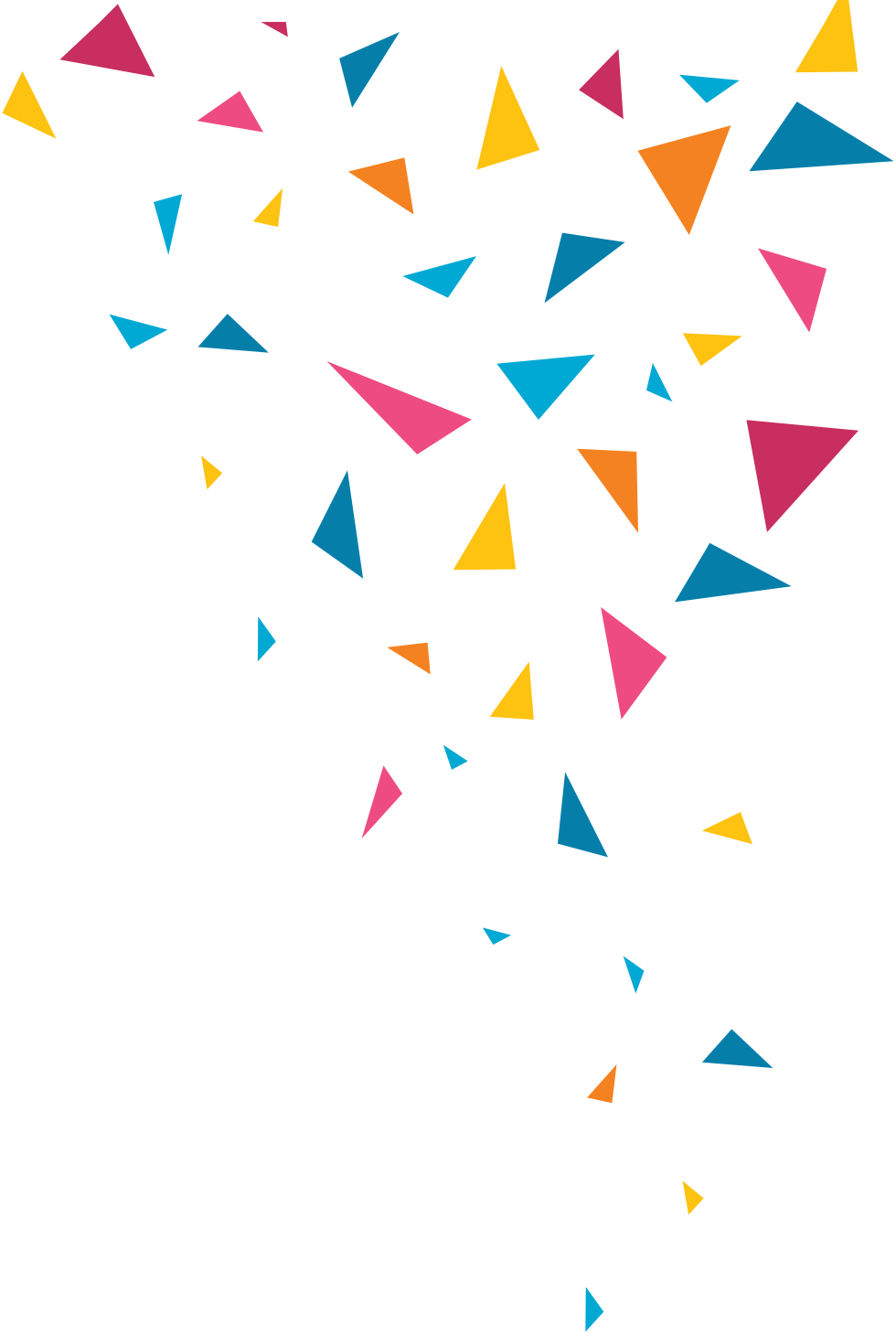
Appendix III

About the author

Nienke Nekesa Hagedoorn was born in Mumias, Kenya on 12th September 1990. As a baby, her family moved back to the Netherlands and lived in Nijmegen and Velp. After graduation from Stedelijk Gymnasium Arnhem (2008), she went on a 'gap' year to Chichester College, England. In 2009 she started her medical education in Leiden University Medical Centre (LUMC), the Netherlands. She did an elective rotation for emergency medicine in Paramaribo, Surinam. Nienke did her master thesis under supervision of Prof. dr. Moll at the department of general paediatrics in Erasmus MC-Sophia.

After obtaining her medical degree in 2016, Nienke worked as a clinical resident on the department of paediatrics in the Haga-Juliana children's hospital in the Hague. In 2017, she started her PhD research in the Erasmus MC-Sophia in Rotterdam under supervision of prof. dr. Moll and dr. Vermont in the department of general paediatrics and department of paediatrics infectious diseases and immunology. During this PhD research, she obtained the Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences (NIHES). Her master thesis on variation on antibiotic prescriptions in European Emergency Departments was awarded with the best thesis award.

In 2021, Nienke worked temporarily in source and contact tracing for COVID-19 while finishing her PhD. In March 2021, she started working in the department of internal medicine at the IJsselland hospital in Capelle a/d IJssel. Next year, she will move to Christchurch, New Zealand, together with her husband Pieter Hebly.



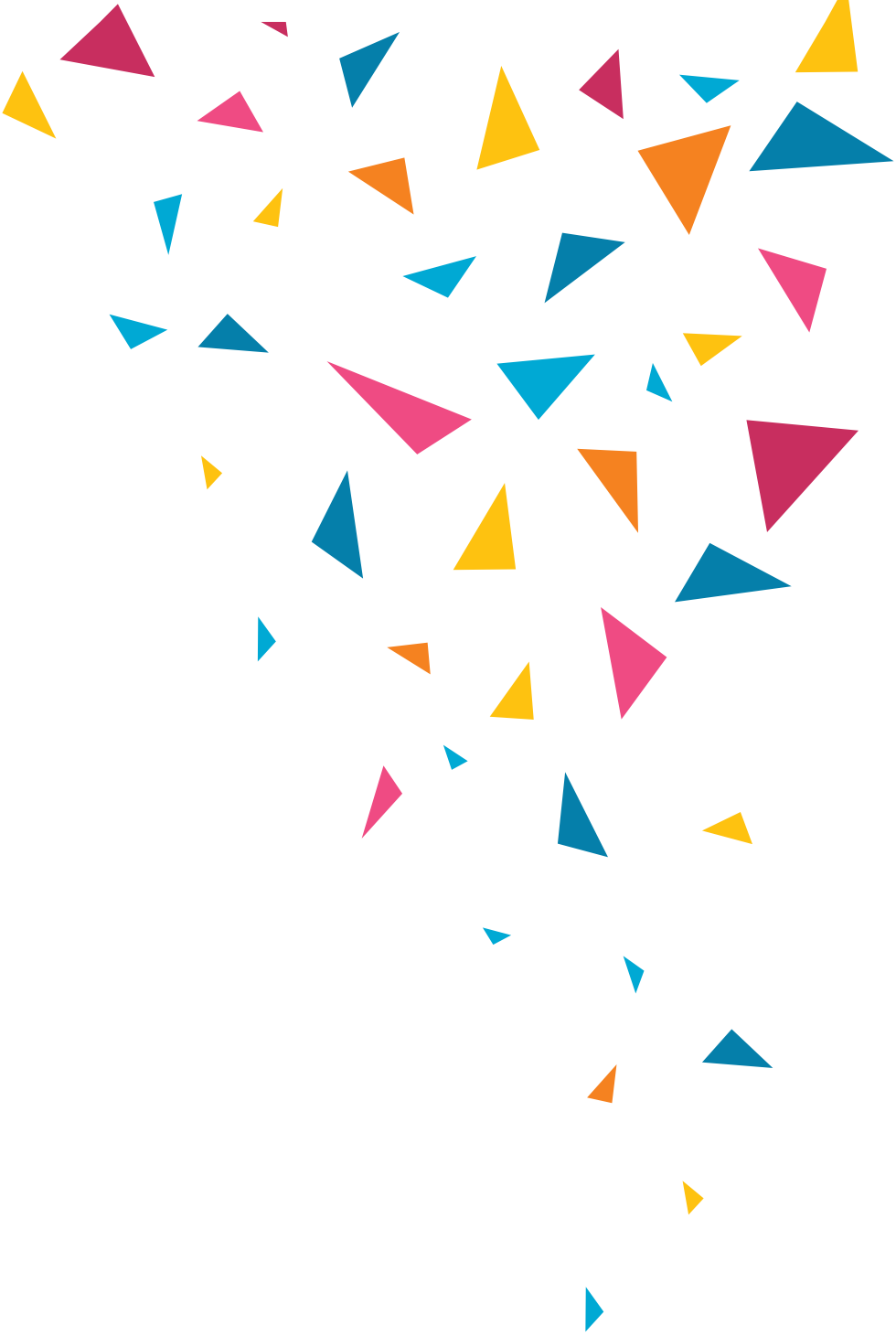
Appendix IV

PhD Portfolio

Erasmus MC Department	General Paediatrics
Research School	Netherlands Institute for Health Sciences
PhD Period:	January 2017 - December 2020

1. PhD Training	Year	Workload (ECTS)
Research skills		
BROK Course	2017 and 2020	1
Integrity in research	2017	0.3
Internal research meetings, Department of General Paediatrics, Erasmus MC	2017-2020	1
Master of Scienc in Health Science, specialisation Clinical Epidemiology	2017-2019	36
Core curriculum		
Study Design		
Biostatistical methods I: basic principles		
Biostatistical methods II: classical regression models		
Principles of Research in Medicine and Epidemiology		
Introduction to medical writing		
Specialisation		
Clinical Translation of Epidemiology		
Clinical Epidemiology		
Repeated Measurements in Clinical Studies		
Principles in Causal Inference		
Methods of Public Health Research		
Clinical Trials		
Health Economics		
The practice of epidemiologic analysis		
Fundamentals of medical decision making		
Elective courses		
Intermediate course in R		
Missing values in clinical research		
Markers and Prognostic research		
Erasmus Summer Lectures		
Logistics regression		
Introduction to Bayesian methods in Clinical research		
Conferences		
Sophia Research Days, Erasmus MC (Oral presentation)	2017	0.3
Symposium paediatric infectious diseases, Dutch Paediatric society	2019	0.5
Oral presentation		
Congress of European Academy of Paediatric Societies (EAPS)	2018, 2020	1
Paris (Oral presentation), Barcelona, (Oral presentation)		
Congress of European Society for Paediatric infectious diseases (ESPID)	2018, 2019	0.6

Ljubljana (poster presentation, Malmo (poster presentation)		
Congress of European Society for Emergency Medicine (EUSEM),	2017, 2019	
Prague (Oral presentation), Athens (Oral presentation)		
Pediatric Rescuscitation and Emergency Medicine (PREM)	2019	0.6
Ghent (Oral presentation)		
TULIPS Young investigators day	2017, 2018	0.6
Teaching		
Supervising Master students, medical students (n=6), nursing science (n=1)	2017-2020	10.5



Appendix V

Dankwoord

Gelijktijdig bij het afronden van mijn promotie onderzoek, eindigt hierbij ook mijn periode in Rotterdam waar ik met erg veel plezier gewoond heb. Uiteraard heb ik dit promotietraject niet kunnen voltooien zonder de hulp van velen. Graag zou ik in het bijzonder de volgende bedanken:

Ten eerste dank aan mijn promotor prof. dr. Moll en co-promotor dr. Vermont.

Lieve Henriette, het is alweer een tijd terug (2015..) dat ik bij je op gesprek kwam om masteronderzoek te doen bij de kindergeneeskunde. Ik was meteen enthousiast over epidemiologisch onderzoek op de algemene kindergeneeskunde en ben erg blij dat ik hier ook mijn promotie onderzoek ben gaan doen. Veel dank voor de intensieve begeleiding, alle kansen en mogelijkheden die ik gekregen heb en ook je aandacht voor persoonlijke ontwikkeling. Je enthousiasme en energie voor onderzoek in de kindergeneeskunde is inspirerend en ik had me geen betere begeleider kunnen wensen…! Met name onze congresbezoeken en PERFORM meetings waren een mooie ervaring. Naast het leren van onderzoek in brede zin en het vertalen van onderzoek naar de praktijk ('de gewone dokter moet het ook begrijpen'), heb ik ook mijn diplomatieke voelsprieten kunnen ontwikkelen.

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Members of the PERFORM consortium, this whole study and thesis could not have occurred without this wonderful project. Thanks for the opportunity to participate. I very much enjoyed the ESPID meetings, annual meetings and of course the social events (London, Athens, Riga, Lyon etc.). All MOFICHE co-authors, in particular Ruud Nijman, prof. dr. Carrol and prof. dr. Emonts: thank you for all the feedback and input on the manuscripts.

Onderzoeksgroep van de algemene kindergeneeskunde, Rianne Oostenbrink en Dorien Geurts: dank voor jullie input tijdens de researchbesprekingen. Dorine Borensztajn, dank voor de fijne samenwerking en onze gesprekken tijdens het verplichte lekkere

kopje koffie. Wat onwijs knap hoe jij je klinische taken combineert met onderzoek en altijd dicht bij je zelf blijft.

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SP-onderzoekers: Maartje, Myrthe, Joany, Josephine, Fabienne, Britt, Chantal en Doesjka, wat was het heerlijk om een promotie-traject samen met elkaar te beleven!

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Linda, dank ook voor al je hulp bij de biva-studies en gezelligheid daarbuiten. Benieuwd wat de toekomst je gaat brengen!

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