OPEN ACCESS

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

Nienke N Hagedoorn ,[1] Dorine Borensztajn ,[1] Ruud Gerard Nijman ,[2] Daan Nieboer,[3] Jethro Adam Herberg ,[2] Anda Balode,[4] Ulrich von Both ,[5,6] Enitan Carrol,[7,8] Irini Eleftheriou,[9] Marieke Emonts,[10,11] Michiel van der Flier,[12,13] Ronald de Groot,[12] Benno Kohlmaier,[14] Emma Lim,[10,11] Ian Maconochie,[15] Federico Martinón-Torres,[16] Marko Pokorn,[17] Franc Strle,[17] Maria Tsolia,[9] Dace Zavadska,[4] Werner Zenz,[14] Michael Levin,[2] Clementien Vermont,[18] Henriette A Moll [1]

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/archdischild-2020-319794).

For numbered affiliations see end of article.

**Correspondence to**
Professor Henriette A Moll, Paediatrics, Erasmus MC Sophia Children's Hospital, Rotterdam 3015 GJ, The Netherlands; h.a.moll@erasmusmc.nl

Received 22 July 2020
Revised 25 October 2020
Accepted 27 October 2020
Published Online First 18 November 2020



**To cite:** Hagedoorn NN, Borensztajn D, Nijman RG, *et al*. *Arch Dis Child* 2021;**106**:641–647.

## 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 0.84 (95% CI 0.81 to 0.88) and 0.78 (95% CI 0.74 to 0.82) 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 8.4 (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 LR 1.9–4.8) and comprised 9784 patients (60%).

**Conclusions** The rule-out threshold of this model has potential to 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 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 aged 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 immunological 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 risk 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 aged <16 years, predicts risks for pneumonia and other serious bacterial infections which besides IBIs also includes bacterial infections of the urinary tract, gastrointestinal 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 neurological 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 multicentre study, which is part of PERFORM (PErsonalized Risk assessment in Febrile illness to Optimise Real-life Management across the European Union) (www.perform2020.org).

Children aged from 0 to 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 UK (n=3).[19] Data were collected for at least 1 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 to 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. Furthermore, 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 10 587 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, tachypnoea and tachycardia defined by Advanced Paediatric 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 aged <1 year and >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 and 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 V.3.6.

## 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 patients with IBI, resulting in 16 268 patients. Of those, most common infections were the upper respiratory tract (45%), lower respiratory tract (18%), gastrointestinal 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 patients with IBI vs 6% of patients without IBI. IBI incidence varied from 0.1% to 5.6% of patients per ED (online supplemental appendix 4).

Patients with IBI were similar in age and sex compared with 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: 0.45–0.81, range intercept −1.2 to 1.0) (online supplemental appendix 8). Apparent calibration was improved by adding an ED-specific variable for high (>2%) versus low (<2%) incidence of IBI (online supplemental appendix 9).

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

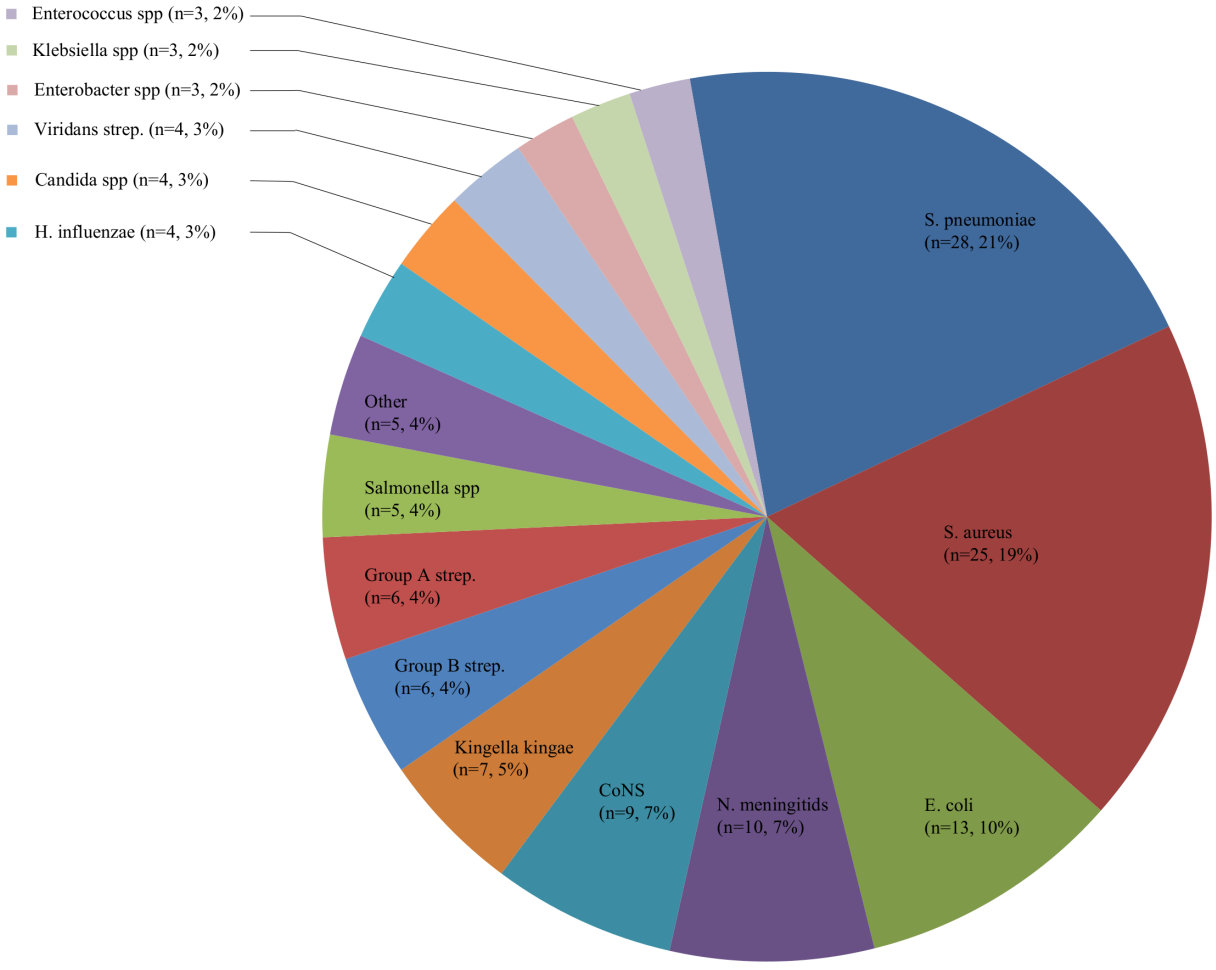



**Figure 1** Identified pathogens for invasive bacterial infections (n=135). CoNS, coagulase-negative staphylococci; spp, species.

**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=16 133) | |
|---|---|---|---|---|
| | 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 |
| Tachypnoea (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 haemodynamic 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; LSI, life-saving intervention; NICE, National Institute for Health and Care Excellence.

**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 |
| | Tachypnoea | −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 |

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

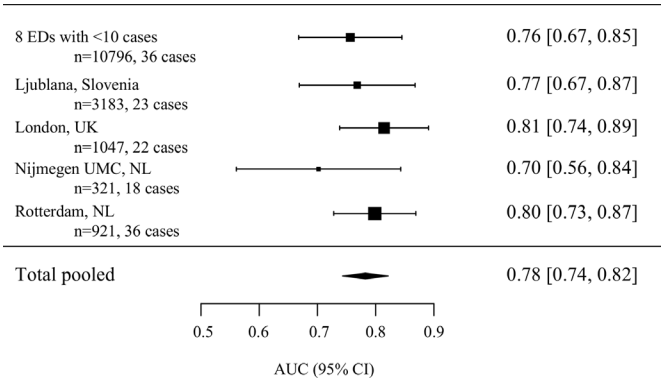



**Figure 2** Discriminative value of the prediction model for invasive bacterial infection for five internal-external cross-validations. The model was repeatedly derived on four ED groups, and validated on the fifth ED group which was left out from the derivation. The five cross-validations were pooled using a random-effects model. More details are provided in figure A in online supplemental appendix 3. AUC, area under the receiver operating curve; ED, emergency department; NL, The Netherlands; UK, United Kingdom; UMC, University Medical Centre.

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 | 5495 (33.8) | 10 773 (66.2) | 0.97 (0.93 to 0.99) | 0.09 (0.03 to 0.23) | 0.34 (0.33 to 0.35) | 1.5 (1.4 to 1.5) |
| 0.2 | 8461 (52.0) | 7807 (48.0) | 0.93 (0.87 to 0.96) | 0.14 (0.08 to 0.26) | 0.52 (0.52 to 0.53) | 1.9 (1.9 to 2.1) |
| 0.25 | 9416 (57.9) | 6852 (42.1) | 0.90 (0.84 to 0.95) | 0.17 (0.10 to 0.28) | 0.58 (0.58 to 0.59) | 2.2 (2.0 to 2.3) |
| 0.5 | 12 200 (75.0) | 4068 (25.0) | 0.76 (0.67 to 0.83) | 0.32 (0.24 to 0.44) | 0.75 (0.75 to 0.76) | 3.1 (2.8 to 3.4) |
| 1.0 | 14 224 (87.4) | 2044 (12.6) | 0.59 (0.50 to 0.67) | 0.47 (0.39 to 0.58) | 0.88 (0.87 to 0.88) | 4.8 (4.1 to 5.6) |
| 2.0 | 15 279 (93.9) | 989 (6.1) | 0.48 (0.39 to 0.57) | 0.55 (0.47 to 0.65) | 0.94 (0.94 to 0.95) | 8.4 (6.9 to 10) |
| 5 | 15 831 (97.3) | 437 (2.7) | 0.36 (0.37 to 0.45) | 0.65 (0.57 to 0.74) | 0.98 (0.97 to 0.98) | 15 (12 to 19) |

LR, likelihood ratio.

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 0.14–0.47, 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 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=37 093, 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, while 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 minimised. 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: 0.78 (95% CI 0.74 to 0.82) was better compared with 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.

**Author affiliations**
[1]General Paediatrics, Erasmus MC Sophia Children's Hospital, Rotterdam, Zuid-Holland, The Netherlands
[2]Section of Paediatric Infectious Diseases, Imperial College London, London, UK
[3]Public Health, Erasmus MC, Rotterdam, Zuid-Holland, The Netherlands
[4]Paediatrics, Children clinical university hospital, Rigas Stradinas Universitate, Riga, Latvia
[5]Division of Paediatric Infectious Diseases, Dr von Haunersches Kinderspital Kinderklinik und Kinderpoliklinik der Ludwig Maximilian Universitat Munchen, Munchen, Bayern, Germany
[6]Partner site Munich, German Centre for Infection Research, Braunschweig, Niedersachsen, Germany
[7]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK
[8]Alder Hey Children's NHS Foundation Trust, Liverpool, UK
[9]Second Department of Paediatrics, P & A Kyriakou Children's Hospital, National and Kapodistrian University of Athens, Athinon, Greece
[10]Paediatric Immunology, Infectious Diseases & Allergy, Great North Children's Hospital, Newcastle upon Tyne, UK
[11]Newcastle upon Tyne Hospital NHS Trust and Newcastle University, NIHR Newcastle Biomedical Research Centre, Newcastle upon Tyne, UK
[12]Paediatric Infectious Diseases and Immunology, Amalia Children's Hospital, Radboudumc, Nijmegen, Gelderland, The Netherlands
[13]Wilhelmina Children's Hospital, Paediatric Infectious Diseases and Immunology, UMC Utrecht, Utrecht, The Netherlands
[14]Department of General Paediatrics, Medical University of Graz, Graz, Steiermark, Austria
[15]Paediatric Emergency Medicine, Imperial College Healthcare NHS Trust, London, UK
[16]Genetics, Vaccines, Infections and Paediatrics Research group (GENVIP), Hospital Clinico Universitario de Santiago de Compostela, Santiago de Compostela, Galicia, Spain
[17]Department of Infectious Diseases and Faculty of Medicine, Ljubljanski Univerzitetni klinicni center, Ljubljana, Slovenia
[18]Department of Paediatric Infectious Diseases and Immunology, Erasmus MC Sophia Children's Hospital, Rotterdam, Nederland, The Netherlands

**Correction notice** This paper has been amended since it was published online. The data sharing statement has been updated.

**Twitter** Ruud Gerard Nijman @rgnijman and Enitan Carrol @CarrolEnitan

**Acknowledgements** The authors would like to thank the members of the PERFORM consortium as listed in online supplemental appendix 13.

**Contributors** Conceptualisation: NNH, DB, RGN, DN, JAH, AB, UvB, EC, IE, ME, MvdF, RdG, BK, EL, IM, FM-T, MP, FS, MT, DZ, WZ, ML, CV, HAM. Data curation: NNH, DB, RGN, JAH, AB, UvB, EC, IE, ME, MvdF, RdG, JAH, BK, EL, IM, FM-T, MP, FS, MT, DZ, WZ, ML, CV, HAM. Formal analysis: NNH, DN. Methodology: NNH, DB, RGN, DN, JAH, AB, UvB, EC, IE, ME, MvdF, RdG, BK, EL, FM-T, DN, MP, MT, DZ, CV, WZ, ML, HAM. Supervision: CV, HAM. Writing—original draft: NNH. Writing—review and editing: NNH, DB, RN, DN, JAH, AB, UvB, EC, IE, ME, MvdF, RdG, BK, EL, IM, FM-T, MP, FS, MT, DZ, WZ, ML, CV, HAM.

**Funding** This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 668303. The research was supported by the National Institute for Health Research Biomedical Research Centres at Imperial College London, Newcastle Hospitals NHS Foundation Trust and Newcastle University.

**Disclaimer** The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

**Competing interests** None declared.

**Patient and public involvement statement** The design of the study did not involve patients. The study results will be disseminated to members of the public, patients and experts through social media and networks.

**Patient consent for publication** Not required.

**Ethics approval** The study was approved by all the participating hospitals. No informed consent was needed for this study. 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), UK (Ethics Committee, ID: 16/LO/1684, IRAS application no. 209035, confidentiality advisory group reference: 16/CAG/0136).

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** An anonymized data set containing individual participant data is available in a public data repository: https://data.hpc.imperial.ac.uk/resolve/?doi=7549. DOI: 10.14469/hpc/7549. For inquiries to obtain the full dataset, please contact the data manager of the PERFORM consortium (Tisham.de08@imperial.ac. uk).



**ORCID iDs**
Nienke N Hagedoorn http://orcid.org/0000-0001-9237-4904
Dorine Borensztajn http://orcid.org/0000-0002-2437-0757
Ruud Gerard Nijman http://orcid.org/0000-0001-9671-8161
Jethro Adam Herberg http://orcid.org/0000-0001-6941-6491
Ulrich von Both http://orcid.org/0000-0001-8411-1071
Henriette A Moll http://orcid.org/0000-0001-9304-3322

## REFERENCES

1 Wolfe I, Cass H, Thompson MJ, *et al*. Improving child health services in the UK: insights from Europe and their implications for the NHS reforms. *BMJ* 2011;342:d1277.

2 Liu L, Oza S, Hogan D, *et al*. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet* 2015;385:430–40.
3 Pruitt CM, Neuman MI, Shah SS, *et al*. Factors associated with adverse outcomes among febrile young infants with invasive bacterial infections. *J Pediatr* 2019;204:177–82.
4 van de Maat J, van de Voort E, Mintegi S, *et al*. Antibiotic prescription for febrile children in European emergency departments: a cross-sectional, observational study. *Lancet Infect Dis* 2019;19:382–91.
5 Herberg JA, Kaforou M, Wright VJ, *et al*. Diagnostic test accuracy of a 2-Transcript host RNA signature for discriminating bacterial vs viral infection in febrile children. *JAMA* 2016;316:835–45.
6 Oved K, Cohen A, Boico O, *et al*. A novel host-proteome signature for distinguishing between acute bacterial and viral infections. *PLoS One* 2015;10:e0120012.
7 van Houten CB, de Groot JAH, Klein A, *et al*. A host-protein based assay to differentiate between bacterial and viral infections in preschool children (opportunity): a double-blind, multicentre, validation study. *Lancet Infect Dis* 2017;17:431–40.
8 Gómez-Carballa A, Cebey-López M, Pardo-Seco J, *et al*. A qPCR expression assay of IFI44L gene differentiates viral from bacterial infections in febrile children. *Sci Rep* 2019;9:11780.
9 Nijman RG, Vergouwe Y, Thompson M, *et al*. Clinical prediction model to aid emergency doctors managing febrile children at risk of serious bacterial infections: diagnostic study. *BMJ* 2013;346:f1706.
10 Kuppermann N, Dayan PS, Levine DA, *et al*. A clinical prediction rule to identify febrile infants 60 days and younger at low risk for serious bacterial infections. *JAMA Pediatr* 2019;173:342–51.
11 Irwin AD, Grant A, Williams R, *et al*. Predicting risk of serious bacterial infections in febrile children in the emergency department. *Pediatrics* 2017;140. doi:10.1542/peds.2016-2853. [Epub ahead of print: 05 Jul 2017].
12 Gomez B, Mintegi S, Bressan S, *et al*. Validation of the "Step-by-Step" Approach in the Management of Young Febrile Infants. *Pediatrics* 2016;138. doi:10.1542/peds.2015-4381. [Epub ahead of print: 05 Jul 2016].
13 Vos-Kerkhof Ede, Gomez B, Milcent K, *et al*. Clinical prediction models for young febrile infants at the emergency department: an international validation study. *Arch Dis Child* 2018;103:1033–41.
14 Leroy S, Bressan S, Lacroix L, *et al*. Refined Lab-score, a risk score predicting serious bacterial infection in febrile children less than 3 years of age. *Pediatr Infect Dis J* 2018;37:387–93.
15 Aronson PL, Shabanova V, Shapiro ED, *et al*. A prediction model to identify febrile infants ≤60 days at low risk of invasive bacterial infection. *Pediatrics* 2019;144. doi:10.1542/peds.2018-3604. [Epub ahead of print: 05 Jun 2019].
16 Burman C, Serra L, Nuttens C, *et al*. Meningococcal disease in adolescents and young adults: a review of the rationale for prevention through vaccination. *Hum Vaccin Immunother* 2019;15:459–69.
17 Watson RS, Carcillo JA, Linde-Zwirble WT, *et al*. The epidemiology of severe sepsis in children in the United States. *Am J Respir Crit Care Med* 2003;167:695–701.
18 van de Maat JS, Peeters D, Nieboer D, *et al*. Evaluation of a clinical decision rule to guide antibiotic prescription in children with suspected lower respiratory tract infection in the Netherlands: a stepped-wedge cluster randomised trial. *PLoS Med* 2020;17:e1003034.
19 Borensztajn D, Yeung S, Hagedoorn NN, *et al*. Diversity in the emergency care for febrile children in Europe: a questionnaire study. *BMJ Paediatr Open* 2019;3:e000456.
20 Hagedoorn NN, Borensztajn DM, Nijman R, *et al*. Variation in antibiotic prescription rates in febrile children presenting to emergency departments across Europe (MOFICHE): A multicentre observational study. *PLoS Med* 2020;17:e1003208.
21 , Roberts KB, Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;128:595–610.
22 Simon TD, Cawthon ML, Stanford S, *et al*. Pediatric medical complexity algorithm: a new method to stratify children by medical complexity. *Pediatrics* 2014;133:e1647–54.
23 The National Institute for Health and Care Excellence (NICE). Fever in under 5S: assessment and initial managment CG160 may 2013, 2013. Available: https://www.nice.org.uk/guidance/cg160 [Accessed Aug 2017].
24 Pneumonia Etiology Research for Child Health (PERCH) Study Group. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet* 2019;394:757–79.
25 Riley RD, Snell KI, Ensor J, *et al*. Minimum sample size for developing a multivariable prediction model: PART II - binary and time-to-event outcomes. *Stat Med* 2019;38:1276–96.
26 Su T-L, Jaki T, Hickey GL, *et al*. A review of statistical updating methods for clinical prediction models. *Stat Methods Med Res* 2018;27:185–97.
27 Steyerberg EW, Borsboom GJJM, van Houwelingen HC, *et al*. Validation and updating of predictive logistic regression models: a study on sample size and shrinkage. *Stat Med* 2004;23:2567–86.
28 Advanced Life Support Group. *Advanced paediatric life support: the practical approach*. 5th edn. Wiley, 2011.
29 Friedman J, Hastie T, Tibshirani R. Regularization paths for generalized linear models via coordinate descent. *J Stat Softw* 2010;33:1–22.
30 Tibshirani R. Regression shrinkage and selection via the LASSO. *J R Stat Soc Series B* 1996;58:267–88.
31 Steyerberg EW, Harrell FE. Prediction models need appropriate internal, internal-external, and external validation. *J Clin Epidemiol* 2016;69:245–7.
32 Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. *Med Decis Making* 2006;26:565–74.
33 Van Calster B, Nieboer D, Vergouwe Y, *et al*. A calibration hierarchy for risk models was defined: from utopia to empirical data. *J Clin Epidemiol* 2016;74:167–76.
34 Collins GS, Ogundimu EO, Altman DG. Sample size considerations for the external validation of a multivariable prognostic model: a resampling study. *Stat Med* 2016;35:214–26.
35 Craig JC, Williams GJ, Jones M, *et al*. The accuracy of clinical symptoms and signs for the diagnosis of serious bacterial infection in young febrile children: prospective cohort study of 15 781 febrile illnesses. *BMJ* 2010;340:c1594.
36 Wasserman A, Karov R, Shenhar-Tsarfaty S, *et al*. Septic patients presenting with apparently normal C-reactive protein: a point of caution for the ER physician. *Medicine* 2019;98:e13989.

# Supplemental information

## Appendix 1: Statistical analysis plan

# Statistical Analysis Plan

Prediction of invasive bacterial infections in febrile children presenting to Emergency Departments in Europe

SAP version 1.0 date 14th July 2019

## Background

Still today children die on treatable infectious diseases due to delayed or missed diagnosis presented at the Emergency Department (ED) or primary care.(1-3) On the other hand, antibiotics are prescribed for viral infections and infection with an unknown bacterial or viral cause in order not to miss one child with an invasive bacterial infection.(4)

The distinction between invasive bacterial infections and viral infections on only clinical signs and symptoms is difficult. Biomarkers as C-reactive protein and procalcitonin are currently used in febrile children to detect bacterial infections and to target appropriate antibiotic prescribing. However, these markers measure non-specific inflammation and immunologic responses. Recent research focuses on finding new discriminators of bacterial and viral infections using novel, sophisticated techniques (genomic, proteomic and transcriptomic approaches).(5-7) It is yet unclear which patients would benefit from potential new biomarkers. It is not feasible to apply new biomarkers to all febrile children. Therefore, decision models need to be developed which can identify these patients.

We searched PUBMED from 1st January 2009 to 1st July 2019 for published studies covering clinical prediction models for bacterial infections in children using keywords “child”, “fever”, “bacterial infection” and “clinical prediction” and checked references for relevant articles. The existing literature on clinical prediction models for bacterial infections focuses on young infants (< 3 months) and healthy children in particular. For older children, the Feverkidstool (Nijman et al.) is an extensively validated clinical prediction model for prediction of pneumonia and other serious bacterial infections which includes bacteraemia and meningitis but also infections of the urinary tract, gastro-intestinal tract and soft tissue. We could not identify a clinical prediction model for the outcome invasive bacterial infections including older children or children with chronic conditions.

## Objectives

1. To update an existing clinical prediction model to identify invasive bacterial infections in febrile children at the ED
2. Can we target patients who can benefit from a new biomarker based on risk-prediction by this model?

## Methods

**Study design:**

Prospective observational study

This study is a prospectively planned analysis in the MOFICHE study (Management and Outcome of Febrile Illness in Children) which is part of the PERFORM project. MOFICHE is a prospective observational study using routine data. The need for informed consent was waived.

**Setting:**

12 Emergency Departments (EDs) in 8 countries

**Population:**

Children 0-18 years with fever (temperature >38.0 C) measured at ED or history of fever (<72 hours) before ED visit. For this analysis, we will exclude children with working diagnosis of urinary tract infections after ED visit. For diagnosis of urinary tract infections, easy available diagnostics are already available at the ED. Therefore, a clinical prediction model has limited additional value in this group. Furthermore, we will focus our analysis on patients with CRP measurement since these are patients with diagnostic uncertainty after initial assessment by the physician.

**Inclusion period:**

1 January 2017 – 1 April 2018, at least 12 months per study site.

**Primary outcomes:**
Invasive bacterial infections (IBI): bacteraemia, bacterial meningitis and bacterial bone and join infections. Infections were defined positive growth of a single pathogenic bacterium in blood, cerebrospinal fluid or synovial fluid from cultures collected at ED visit or the first 24 hours from hospital admission.

Cultures growing contaminants (coagulase-negative staphylococci, alpha-haemolytic streptococci, *Micrococcus* species or *Propionibacterium* species are defined negative (8)

In children who are immunocompromised, malignancies or with a central line, these contaminants are still relevant invasive bacterial infections that need antibiotic treatment. In these patient groups, cultures with a single contaminant are defined positive.

All patients were entered in the electronic case record form (eCRF) by the local team. We will check all the positive cultures to ensure consistency and validity of coding.

**Missing data**
For this analysis, we will exclude patients with no CRP value and exclude patients with working diagnosis of urinary tract infection. We will use multiple imputation by chained equations using the MICE package in R to impute all missing predictor variables. We will assume the variables to be 'missing at random' where missingness can be explained by other variables in the data. We will incorporate hospital, all predictor variables, outcome measures and other auxiliary variables in the imputation model.
Multiple imputation will be performed on all patients (n=38480).

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 | Capillary refill time | Temperature | Urinalysis categories | Inhalation medication | Focus of infection |
| Referral type (self / GP / emergency services / other) | Ill appearance | Oxygen saturation | Blood culture performed | Antibiotic prescription type | |
| Previous medical care (yes, primary care / yes, this ED / yes other secondary care) | Work of breathing | | Cerebrospinal fluid performed | Antibiotic prescription mode | |
| Season | Meningeal signs | | | Previous antibiotic treatment | |
| Arrival hours (morning / evening / night) | Focal neurology | | | | |
| Comorbidity | Non-blanching rash | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Dehydration | | | | |
| | Seizures | | | | |

**Descriptive analysis**
We will perform descriptive analysis for children with and without IBI. We will use frequencies, mean and standard deviation for normally distributed data, median and interquartile range for normally distributed data. In addition, we will compare patients with CRP measurement and patients without CRP measurement.

**Predictor variables**
We will include predictor variables chosen a-priori that have predictive value for bacterial infection. We will perform univariate logistic regression analysis for these predictor variables:

*Predictor variables included in the Feverkidstool (9):*
- Age
- Sex
- Temperature
- Fever duration in days
- Tachypnea: defined by Advanced Paediatric Life Support (10)
- Tachycardia: defined by Advanced Paediatric Life Support (10)
- Hypoxia: oxygen saturation <94%
- Prolonged capillary refill time: >3 seconds
- Increased work of breathing: chest wall retractions, nasal flaring, grunting or apnoea
- Ill appearance: ill, moderately ill, irritable or uncomfortable
- C-reactive protein value

*NICE red warning signs for serious illness (11):*
- Abnormal consciousness: responsive to verbal stimulation, responsive to pain or unresponsive
- Presence of meningeal signs: presence of Kernig, Brudzinski, tripod phenomenon, neck stiffness or bulging fontanelle
- Focal neurological signs
- Status epilepticus: seizures for >=30 minutes
- Non-blanching rash: petechiae or other non-blanching rash

*Complex chronic condition (12)*
- Chronic condition in ≥2 body systems that is expected to last at least 1 year or malignancy or immunocompromised

We will use 10 events per variable to include predictor variables in model development. If not enough events are available, we will combine abnormal consciousness, presence of meningeal signs and focal neurological signs in a composite variable.
Linearity of continuous variables will be assessed using restricted cubic splines. Outliers for continuous variables will be truncated at the 0.01 percentile and the 0.99 centile.

**Model development**
We will perform variable selection by least absolute shrinkage and selection operator (LASSO). Using LASSO, we perform variable selection and reduce degree of overfitting by shrinking large regression coefficients.(13) We will estimate the lambda using 10 times 10-fold-cross validation. To note, variable selection will not be based on significance in univariate logistic regression analysis.

**Model validation**
The model will be validated using internal-external cross-validation. In this method, the model is repeatedly derived on all EDs except one, and validated on the remaining ED.(14, 15)

**Model performance**
Model performance will be assessed by

- Discrimination of the model by concordance (c)-statistic.
- Calibration, the agreement between predicted risks and observed outcome will be visualized using calibration plots.(16)
- Diagnostic performance at different risk-threshold for the probability of IBI using sensitivity, specificity and negative and positive likelihood ratios. We will focus on cut-offs that can be used to rule-out (negative LR <0.2) or rule-in IBI (positive LR>5).(17)

**Sensitivity analysis**

A sensitivity analysis will be performed in the population where missing CRP values will be imputed.

---

Drafted by: Nienke N. Hagedoorn
Statistician: Daan Nieboer
Supervision: Dr. Clementien Vermont, Prof. Henriette A. Moll

---

## Appendix 2: Definition of contaminants

| **Appendix 3: 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**

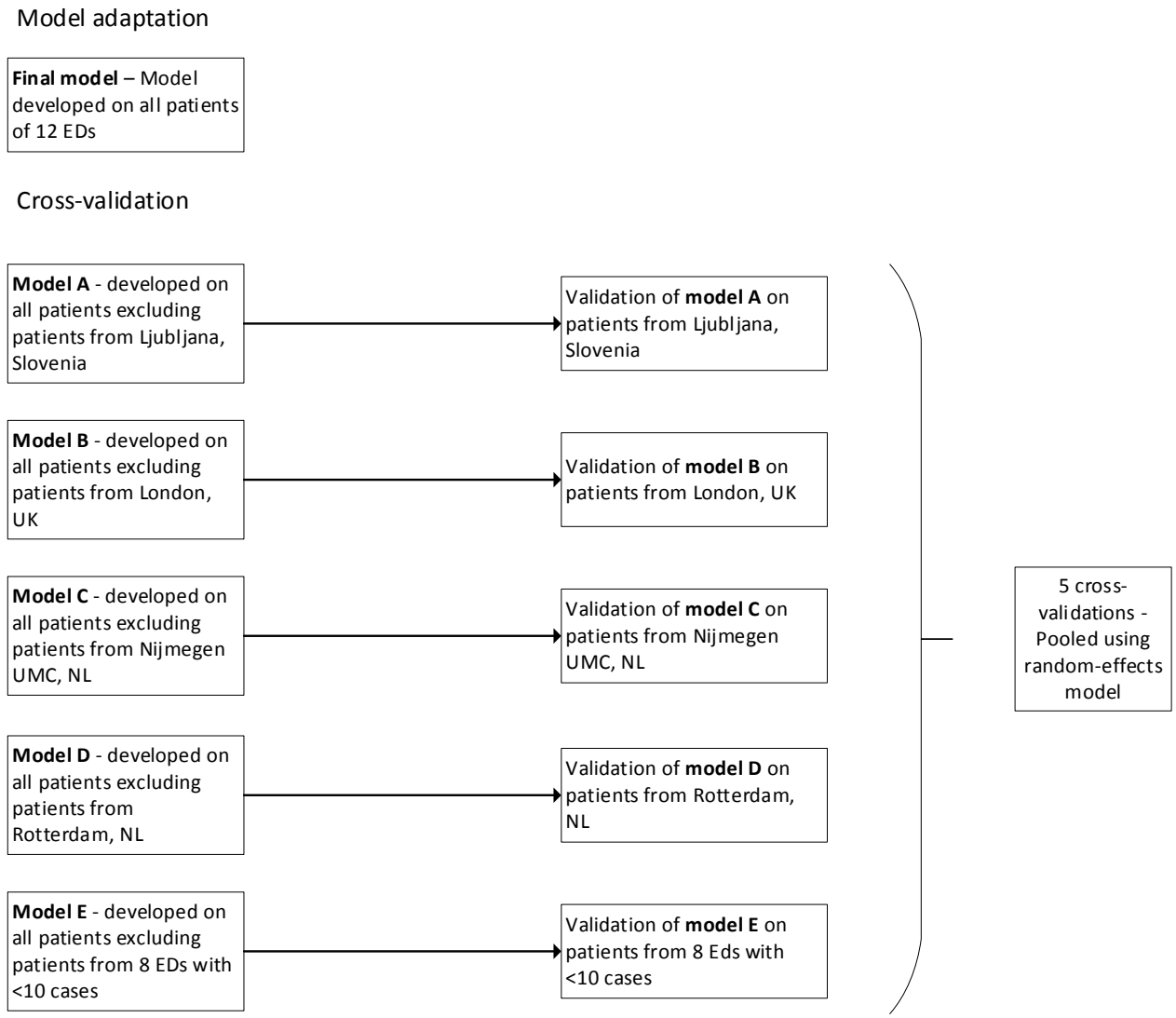
Missing data were multiple imputed using the MICE package in R v3.4. The imputation model included the outcome variable IBI, all considered predictors, ED and other auxiliary variables related to casemix and disease severity (specific details of the multiple imputation model are proved in the Statistical 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.(18) The LASSO was applied to a stacked dataset containing all imputed data.(19) 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 (20, 21), 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(10), 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)(11) and 3) complex chronic condition (condition in ≥2 body systems, malignancy or immunocompromised).(12) 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).(13, 22) This approach aims to reduce the degree of overfitting by shrinking large regression coefficients and performs variable selection.(13) 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).(14) 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.(14, 15) 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).(17) 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 5: Patient characteristics of patients with CRP measurement and patients without CRP measurement

| | CRP measured (n=17,213) | | | No CRP measured (n=21267) | | |
|---|---|---|---|---|---|---|
| | n (%) | Range EDs | Missing | n (%) | Range EDs | Missing |
| **General characteristics** | | | | | | |
| **Age in years,** median (IQR) | 2.77 (1.29-6.02) | | | 2.74 (1.31-5.28) | | |
| **Male** | 9305 (54.1) | 49.6-62.0 | | 11805 (55.5) | 52.4-62.4 | 1 |
| **Previous chronic condition** | | | 97 | | | 273 |
| Any | 3332 (19.4) | 7.8-71.8 | | 3162 (14.9) | 3.9-61.6 | |
| Complex | 1138 (6.6) | 1.1-41.3 | | 729 (3.4) | 0.0-32.6 | |
| **Referred** | 9287 (53.9) | 6.9-99.2 | 980 | 6789 (31.9) | 3.9-99.3 | 185 |
| **Triage urgency** | | | 529 | | | 647 |
| Low: standard, non-urgent | 9794 (56.9) | 10.9-86.5 | | 14291 (67.2) | 8.8-93.9 | |
| High: immediate, very urgent, intermediate | 6890 (40.0) | 13.5-86.8 | | 6329 (29.8) | 6.1-89.9 | |
| **Feverkidstool** | | | | | | |
| Temperature in °C, median (IQR) | 37.8 (37-38.5) | | 809 | 37.7 (36.9-38.4) | | 2211 |
| Fever duration in days, median (IQR) | 1.5 (0.5-3) | | 875 | 1.5 (0.5-3.0) | | 1900 |
| Tachypnea (APLS) | 3585 (20.8) | 5.9-45.8 | 4186 | 4942 (23.2) | 2.4-48.3 | 4607 |
| Tachycardia (APLS) | 6001 (34.9) | 11.0-54.9 | 887 | 6854 (32.2) | 11.4-49.4 | 2620 |
| Hypoxia <95% | 762 (4.4) | 1.3-9.2 | 2538 | 733 (3.4) | 0.3-12.9 | 3043 |
| Prolonged capillary refill (>3 sec) | 339 (1.9) | 0.2-7.0 | 2503 | 84 (0.4) | 0.0-2.6 | 1928 |
| Work of breathing | 913 (5.3) | 0.5-13.2 | 2315 | 1732 (8.1) | 0.0-35.6 | 3176 |
| Ill appearance | 4742 (27.5) | 1.9-52.6 | 664 | 1265 (5.9) | 0.4-43.3 | 1057 |
| CRP in mg/L, median (IQR) | 17 (5-49) | | 7 | NA | | |
| **NICE Warning signs** | | | | | | |
| Decreased consciousness | 148 (0.9) | 0.1-5.4 | 150 | 53 (0.2) | 0.0-1.8 | 240 |
| Meningeal signs | 126 (0.7) | 0.1-3.7 | 943 | 11 (0.1) | 0.0-0.1 | 1101 |
| Focal neurology | 102 (0.6) | 0.0-3.7 | 1376 | 31 (0.1) | 0.0-1.8 | 1081 |
| Status epilepticus | 51 (0.3) | 0.0-2.3 | 940 | 15 (0.1) | 0.0-1.2 | 201 |
| Rash: petechiae/non blanching | 664 (3.9) | 1.1-18.0 | 1307 | 448 (2.1) | 0.4-4.1 | 3106 |
| **Blood cultures performed** | 3478 (20.2) | 0.5-73.7 | | 88 (0.4) | 0.0-2.0 | |
| **CSF performed** | 444 (2.6) | 0.2-13.5 | | 8 (0.0) | 0.0-0.2 | |
| **Admission to the ward >24 hours** | 6590 (38.3) | 18.0-63.8 | 175 | 668 (3.1) | 0.9-29.6 | 347 |
| **Admission to the ICU** | 135 (0.8) | 0.3-5.4 | 21 | 23 (0.1) | 0.0-2.4 | 35 |
| **Antibiotic treatment following ED visit** | 6795 (39.5) | 27.9-70.8 | 211 | 5504 (25.9) | 16.9-43.0 | 273 |
| **Lifesaving interventions:** airway, breathing or hemodynamic support | 371 (2.2) | 0.0-11.5 | | 112 (0.5) | 0.0-3.0 | |
| **Urinary tract infection** | 935 (5.4) | 3.2-9.7 | 4 | 418 (1.9) | 0.9-3.8 | 23 |

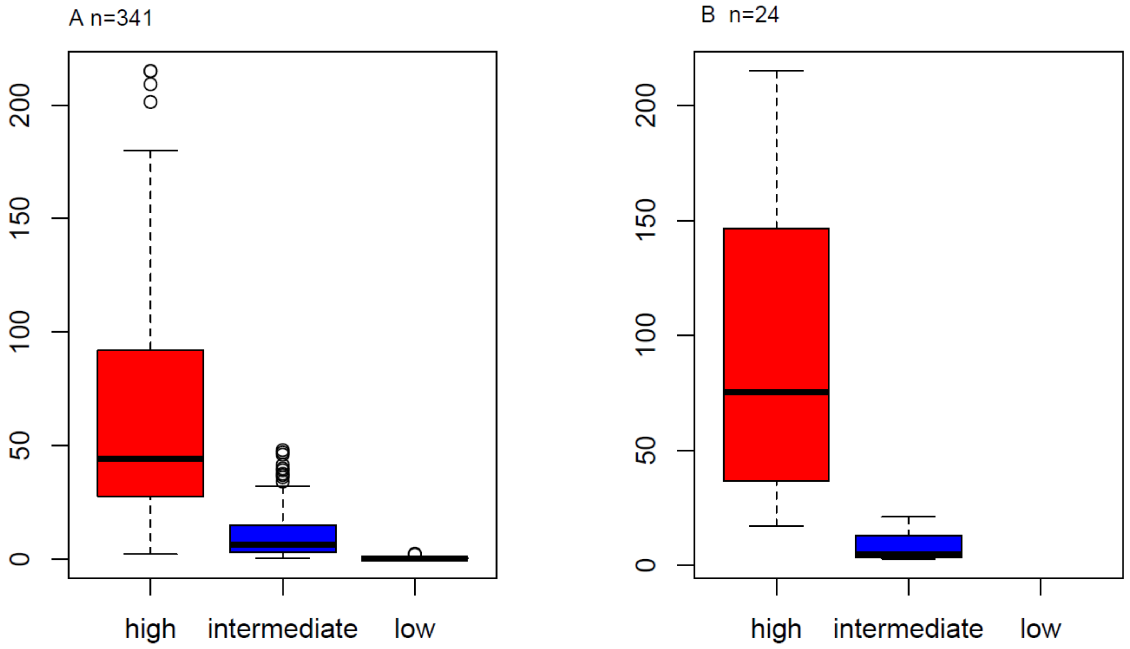
APLS, advanced paediatric life support; CRP, C-reactive protein; CSF, cerebrospinal fluid; ED, emergency department; ICU, intensive care unit; IQR, interquartile range; NA, not applicable

## Appendix 6: Details of patients with complex chronic conditions

Identified pathogen stratified for complex chronic comorbidity

| Identified pathogen | No complex chronic condition , n=85<br>n (%) | Complex chronic condition , n=50<br>n (%) |
|---|---|---|
| Strep. pneumoniae | 23 (27.1%) | 5 (10%) |
| Staph. aureus | 15 (17.6%) | 10 (20%) |
| E. coli | 9 (10.6%) | 4 (8%) |
| Neisseria meningitidis | 9 (10.6%) | 1 (2%) |
| Kingella kingae | 7 (8.2%) | 0 (0%) |
| Group B streptococcus | 6 (7.1%) | 0 (0%) |
| Group A streptococcus | 5 (5.9%) | 1 (2%) |
| Salmonella spp | 4 (4.7%) | 1 (2%) |
| Haemophilus influenzae | 4 (4.7%) | 0 (0%) |
| Enterobacter spp | 2 (2.4%) | 1 (2%) |
| Coagulase-negative staphylococci (CoNS) | | 9 (18%) |
| Candida species | | 4 (8%) |
| Viridans streptococci | | 4 (8%) |
| Klebsiella spp | | 3 (6%) |
| Enterococcus spp | | 3 (6%) |
| Moraxella spp | | 1 (2%) |
| Other | 1 (1.2%) | 3 (6%) |

C-reactive protein level in immunocompromised patients for no IBI (A) vs IBI (B) for IBI risk categories



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

**Supplementary file 5:**
**Univariate logistic regression analysis for invasive bacterial infection.**
**N=16268, IBI cases N=135**

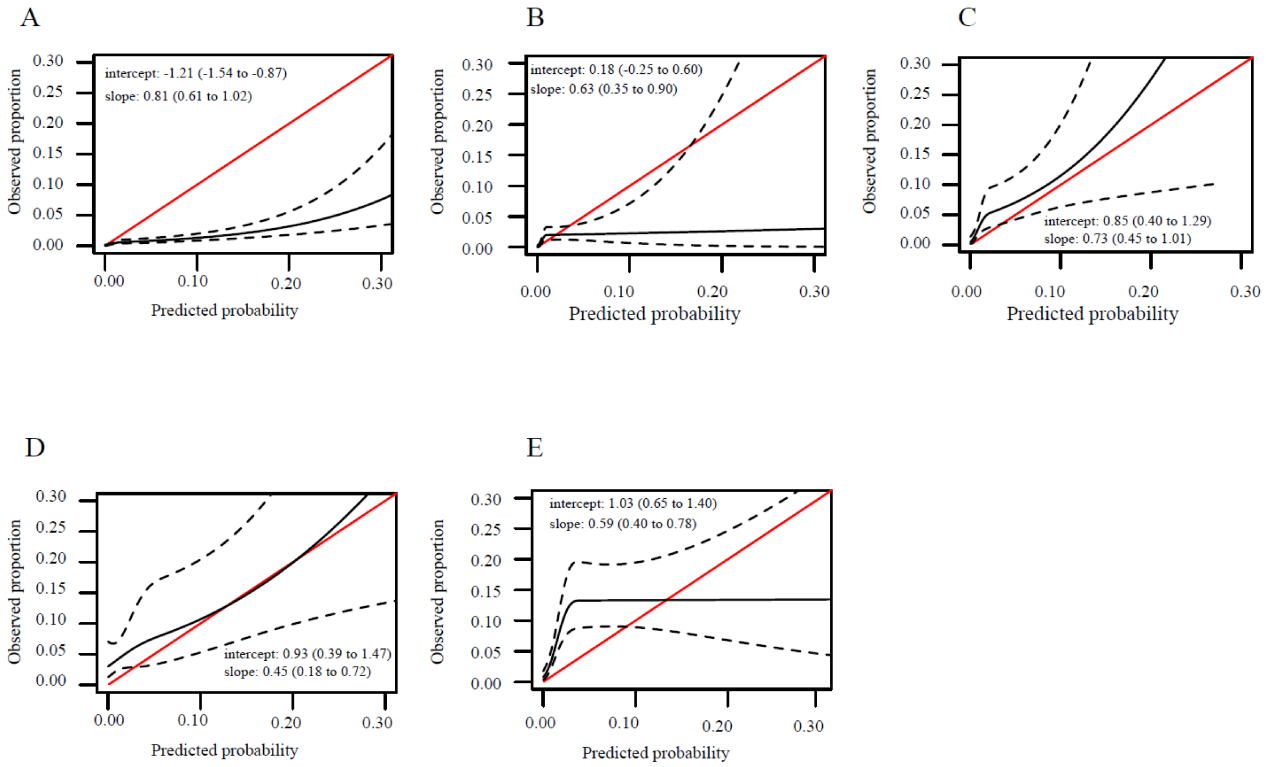
| Variables | OR (95% CI)* |
|---|---|
| **Feverkidstool** | |
| Male | 1.04 (0.74-1.46) |
| Age <1 year± | 0.25 (0.14-0.43)* |
| Age >1 year± | 1.01 (0.97-1.05) |
| Temperature in °C | 1.34 (1.13-1.59)* |
| Fever duration in days | 0.89 (0.80-0.99)* |
| Tachypnea (APLS) | 1.50 (1.03-2.18)* |
| Tachycardia (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: $\beta_{(age <1 year)}$×age in years.
The risk of children aged >1 years was calculated with: $\beta_{(age <1 year)}\times 1+\beta_{(age \geq 1 year)}\times$(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: $\beta_{(age <1 year)}$× age in years.
The risk of children age ≥ 1 year was calculated: $\beta_{(age <1 year)} \times 1 + (\text{age in years}-1) \times \beta_{(age \geq 1 \text{ in years})}$.

CRP, C-reactive protein; IBI, invasive bacterial infection; ln, natural log

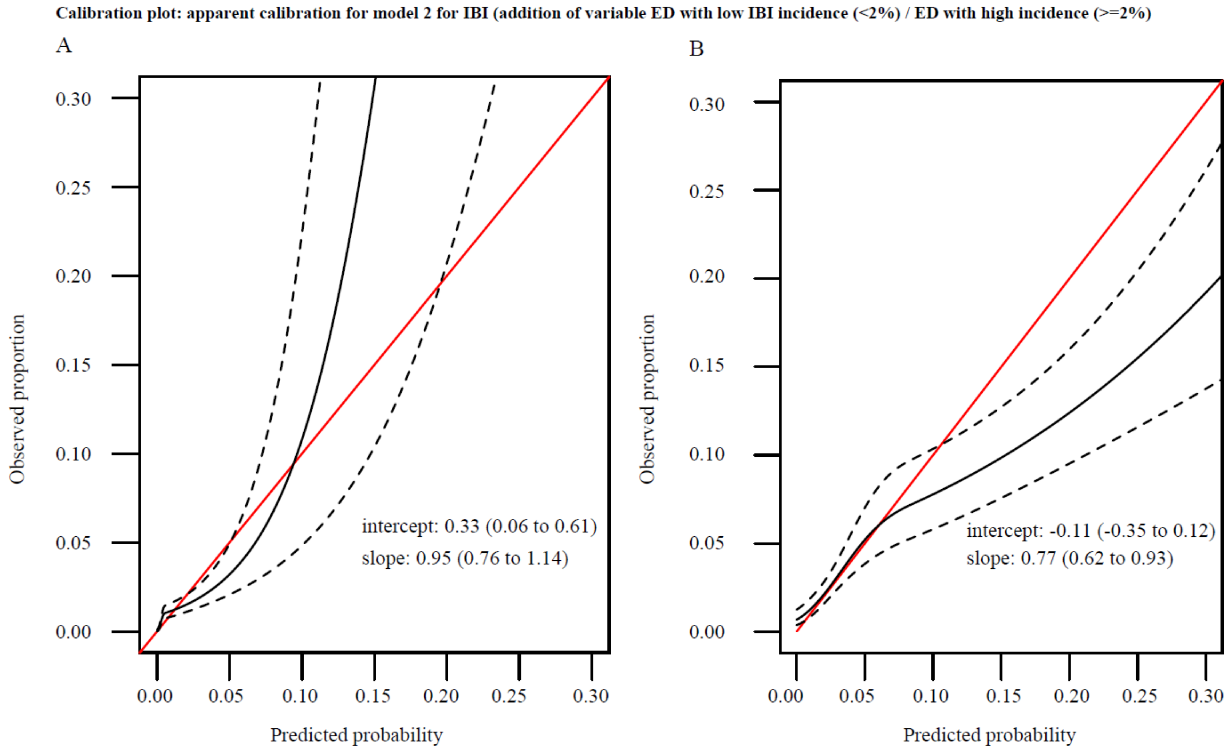
## Model 2 - performance

**Discrimination:**

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

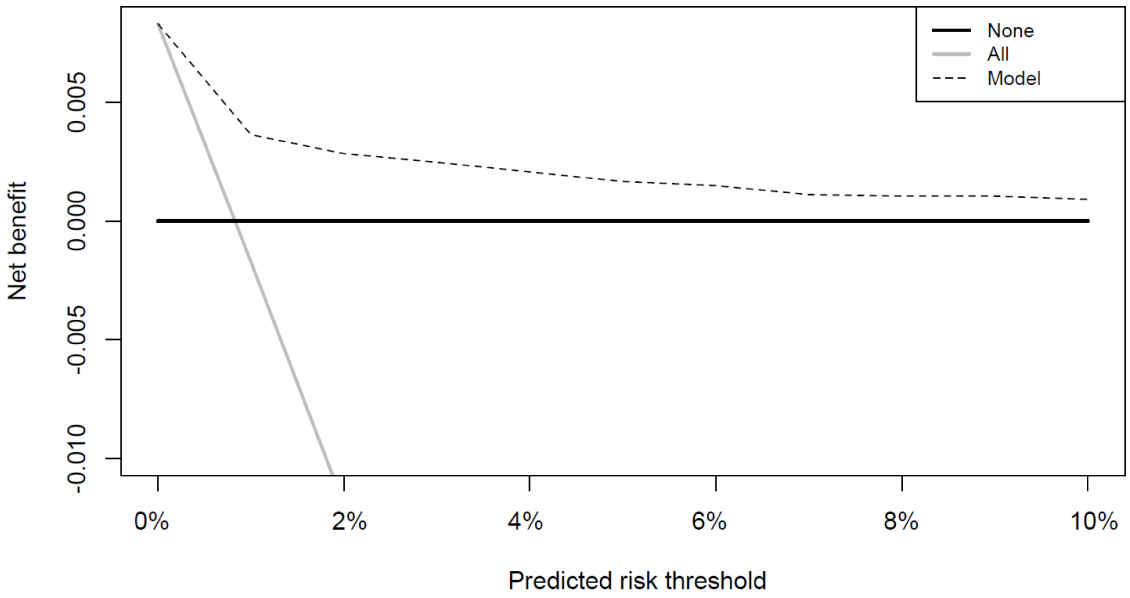
**Calibration:**

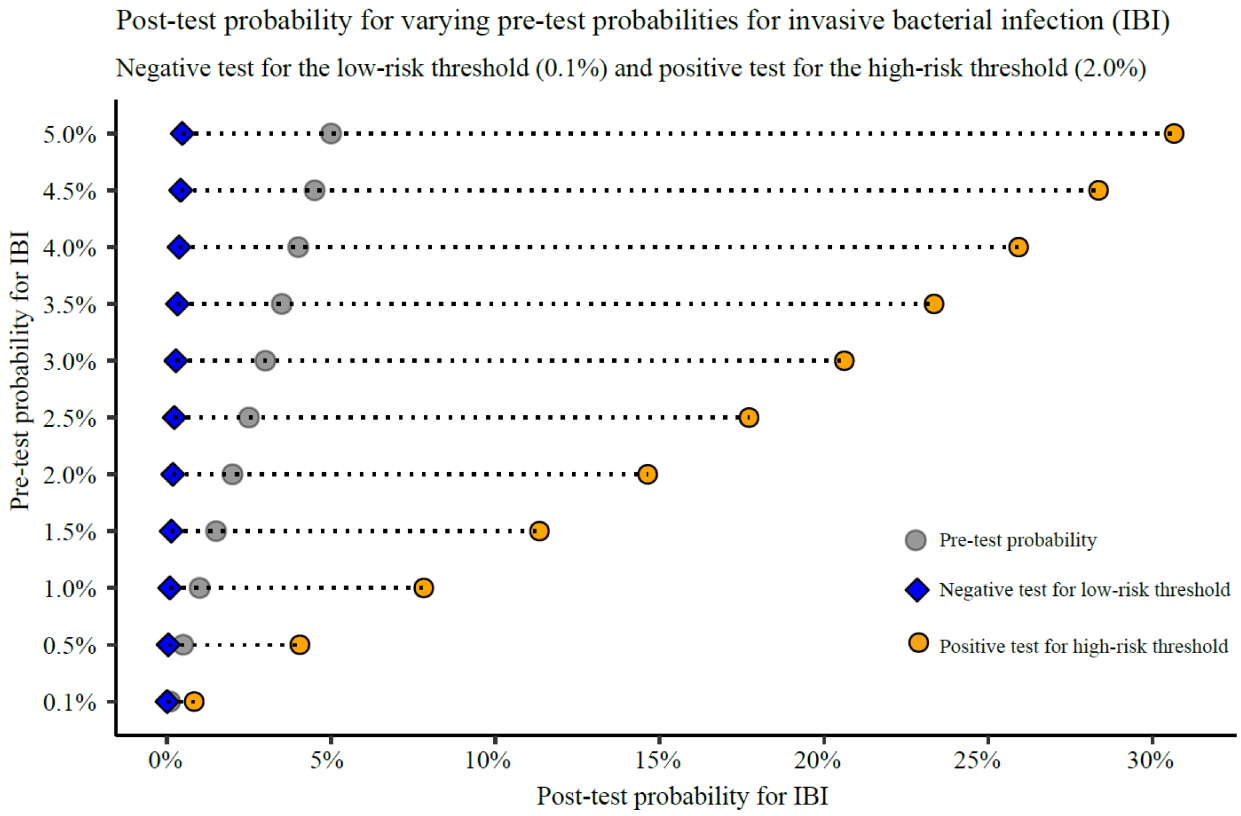
Apparent calibration for model 2 for IBI (addition of variable ED with low IBI incidence (<2%) / ED with high IBI incidence (>=2%)). Risk predictions are calculated on the developed model using all data (n=16268). These risk predictions are calibrated in the two groups: EDs with low IBI incidence (A)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



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

**Model specification of multivariate logistic model for IBI based on population with imputed CRP-level (n=37093)**

| | | Coefficients | OR |
|---|---|---|---|
| | (Intercept) | -9.67 | 0.00 |
| Feverkidstool | Male | -0.19 | 0.83 |
| | Age < 1 year* | -2.58 | 0.08 |
| | Age >1 year* | 0.00 | 1.00 |
| | Temperature | -0.05 | 0.95 |
| | Fever duration in days | -0.15 | 0.86 |
| | Tachypnea | -0.43 | 0.65 |
| | Tachycardia | 0.71 | 2.03 |
| | Hypoxia | -0.86 | 0.42 |
| | Prolonged capillary refill | 0.02 | 1.02 |
| | Increased work of breathing | -0.34 | 0.71 |
| | Ill appearance | 0.94 | 2.55 |
| | Ln CRP | 0.78 | 2.17 |
| NICE warning signs | Abnormal neurology | 1.54 | 4.66 |
| | Non-blanching rash | 1.40 | 4.04 |
| Comorbidity | Complex chronic condition | 2.43 | 11.3 |

*The risk of children aged < 1 year was calculated: $\beta_{(age <1 year)}$×age in years. 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).

CRP, C-reactive protein; ln, natural log

## Appendix 12: Clinical case examples

**Case 1:**
A previously healthy, 4 year old boy presents with fever since 1.5 day.
At the ED he has a temperature of 38.9 degrees, heart rate of 160/min, respiratory rate of 45/min, oxygen saturation of 99% and normal capillary refill time. He is ill-appearing, has increased work of breathing and a normal neurological exam.
CRP-level = 10 mg/L.

Risk-prediction:
The patient is at intermediate-risk (>0.1% and <2%) for an invasive bacterial infection.

**Case 2:**
A previously healthy neonate of 2 months presents with fever since 12 hours.
She has temperature of 38.8 degrees, heart rate of 170/min, respiratory rate of 35/min, normal oxygen saturation and normal capillary refill time. She is ill-appearing and has no increased work of breathing. Neurological exam is normal.
CRP-level = 5 mg/L.

Risk-prediction:
The patient is at high-risk (>2%) for an invasive bacterial infection.

## References

**References**

1. Wolfe I, Cass H, Thompson MJ, *et al.* Improving child health services in the UK: insights from Europe and their implications for the NHS reforms. *Bmj*. 2011;**342**:d1277 Online.
2. Liu L, Oza S, Hogan D, *et al.* Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet*. 2015;**385**:430-40 Online.
3. Pruitt CM, Neuman MI, Shah SS, *et al.* Factors Associated with Adverse Outcomes among Febrile Young Infants with Invasive Bacterial Infections. *J Pediatr*. 2019;**204**:177-82 e1 Online.
4. van de Maat J, van de Voort E, Mintegi S, *et al.* Antibiotic prescription for febrile children in European emergency departments: a cross-sectional, observational study. *Lancet Infect Dis*. 2019; Online.
5. Herberg JA, Kaforou M, Wright VJ, *et al.* Diagnostic Test Accuracy of a 2-Transcript Host RNA Signature for Discriminating Bacterial vs Viral Infection in Febrile Children. *JAMA*. 2016;**316**:835-45 Online.
6. Oved K, Cohen A, Boico O, *et al.* A novel host-proteome signature for distinguishing between acute bacterial and viral infections. *PLoS One*. 2015;**10**:e0120012 Online.
7. van Houten CB, de Groot JAH, Klein A, *et al.* A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): a double-blind, multicentre, validation study. *Lancet Infect Dis*. 2017;**17**:431-40 Online.
8. Pneumonia Etiology Research for Child Health Study G. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet*. 2019; Online.
9. Nijman RG, Vergouwe Y, Thompson M, *et al.* Clinical prediction model to aid emergency doctors managing febrile children at risk of serious bacterial infections: diagnostic study. *BMJ*. 2013;**346**:f1706 Online.
10. Advanced Life Support Group. Advanced Paediatric Life Support: The Practical Approach. 5 ed: Wiley; 2011.
11. The National Institute for Health and Care Excellence (NICE). Fever in under 5s: assessment and initial managment CG160 May 2013. 2013.
12. Simon TD, Cawthon ML, Stanford S, *et al.* Pediatric medical complexity algorithm: a new method to stratify children by medical complexity. *Pediatrics*. 2014;**133**:e1647-54 Online.
13. Tibshirani R. Regression Shrinkage and Selection Via the Lasso. *Journal of the Royal Statistical Society: Series B (Methodological)*. 1996;**58**:267-88 doi: 10.1111/j.2517-6161.1996.tb02080.x [published Online.
14. Steyerberg EW, Harrell FE, Jr. Prediction models need appropriate internal, internal-external, and external validation. *J Clin Epidemiol*. 2016;**69**:245-7 Online.
15. Vos-Kerkhof E, Gomez B, Milcent K, *et al.* Clinical prediction models for young febrile infants at the emergency department: an international validation study. *Arch Dis Child*. 2018;**103**:1033-41 Online.
16. Van Calster B, Nieboer D, Vergouwe Y, De Cock B, Pencina MJ, Steyerberg EW. A calibration hierarchy for risk models was defined: from utopia to empirical data. *J Clin Epidemiol*. 2016;**74**:167-76 Online.
17. Van den Bruel A, Haj-Hassan T, Thompson M, Buntinx F, Mant D, European Research Network on Recognising Serious Infection i. Diagnostic value of clinical features at presentation to identify serious infection in children in developed countries: a systematic review. *Lancet*. 2010;**375**:834-45 Online.
18. Donders AR, van der Heijden GJ, Stijnen T, Moons KG. Review: a gentle introduction to imputation of missing values. *J Clin Epidemiol*. 2006;**59**:1087-91 Online.
19. Zhao Y, Long Q. Variable selection in the presence of missing data: imputation-based methods. *WIREs Computational Statistics*. 2017;**9**:e1402 doi: 10.1002/wics.1402 [published Online.
20. Su TL, Jaki T, Hickey GL, Buchan I, Sperrin M. A review of statistical updating methods for clinical prediction models. *Stat Methods Med Res*. 2018;**27**:185-97 Online.

21. Steyerberg EW, Borsboom GJ, van Houwelingen HC, Eijkemans MJ, Habbema JD. Validation and updating of predictive logistic regression models: a study on sample size and shrinkage. *Stat Med*. 2004;**23**:2567-86 Online.
22. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *J Stat Softw*. 2010;**33**:1-22 Online.

Version: 6.1 September 2020

**PARTNER: IMPERIAL COLLEGE (UK)**
Chief investigator/PERFORM coordinator:

Michael Levin

Principal and co-investigators; work package leads (alphabetical order)

Aubrey Cunnington (grant application)

Tisham De (work package lead)

Jethro Herberg (Principle Investigator, Deputy Coordinator, grant application)

Myrsini Kaforou (grant application, work package lead)

Victoria Wright (grant application, Scientific Coordinator)

Research Group (alphabetical order)

Lucas Baumard; Evangelos Bellos; Giselle D'Souza; Rachel Galassini; Dominic Habgood-Coote; Shea Hamilton; Clive Hoggart; Sara Hourmat; Heather Jackson; Ian Maconochie; Stephanie Menikou; Naomi Lin; Samuel Nichols; Ruud Nijman; Ivonne Pena Paz; Priyen Shah; Ching-Fen Shen; Ortensia Vito; Clare Wilson

Clinical recruitment at Imperial College Healthcare NHS Trust (alphabetical order))

Amina Abdulla; Ladan Ali; Sarah Darnell; Rikke Jorgensen; Sobia Mustafa; Salina Persand

Imperial College Faculty of Engineering

Molly Stevens (co-investigator), Eunjung Kim (research group); Benjamin Pierce (research group)

Version: 6.1 September 2020

Clinical recruitment at Brighton and Sussex University Hospitals

Katy Fidler (Principle Investigator)

Julia Dudley (Clinical Research Registrar)

Research nurses: Vivien Richmond, Emma Tavliavini

Clinical recruitment at National Cheng Kung University Hospital

Ching-Fen Shen (Principal Investigator); Ching-Chuan Liu (Co-investigator); Shih-Min Wang (Co-investigator), funded by the Center of Clinical Medicine Research, National Cheng Kung University

**SERGAS Partner (Spain)**

Principal Investigators

Federico Martinón-Torres[1]
Antonio Salas[1,2]

GENVIP RESEARCH GROUP (in alphabetical order):

Fernando Álvez González[1], Cristina Balo Farto[1], Ruth Barral-Arca[1,2], María Barreiro Castro[1], Xabier Bello[1,2], Mirian Ben García[1], Sandra Carnota[1], Miriam Cebey-López[1], María José Curras-Tuala[1,2], Carlos Durán Suárez[1], Luisa García Vicente[1], Alberto Gómez-Carballa[1,2], Jose Gómez Rial[1], Pilar Leboráns Iglesias[1], Federico Martinón-Torres[1], Nazareth Martinón-Torres[1], José María Martinón Sánchez[1], Belén Mosquera Pérez[1], Jacobo Pardo-Seco[1,2], Lidia Piñeiro Rodríguez[1], Sara Pischedda[1,2], Sara Rey Vázquez[1], Irene Rivero Calle[1], Carmen Rodríguez-Tenreiro[1], Lorenzo Redondo-Collazo[1], Miguel Sadiki Ora[1], Antonio Salas[1,2], Sonia Serén Fernández[1], Cristina Serén Trasorras[1], Marisol Vilas Iglesias[1].

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

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

Version: 6.1 September 2020

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

**RSU Partner (Latvia)**

Principal Investigator

Dace Zavadska[1,2]

Other RSU group authors (in alphabetical order):

Anda Balode[1,2], Arta Bārzdiņa[1,2], Dārta Deksne[1,2], Dace Gardovska[1,2], Dagne Grāvele[2], Ilze Grope[1,2], Anija Meiere[1,2], Ieva Nokalna[1,2], Jana Pavāre[1,2], Zanda Pučuka[1,2], Katrīna Selecka[1,2], Aleksandra Sidorova[1,2], Dace Svile[2], Urzula Nora Urbāne[1,2].

[1] Riga Stradins university, Riga, Latvia.
[2] Children clinical university hospital, Riga, Latvia.

**Medical Research Council Unit The Gambia (MRCG) at LSHTM Partner**

**Principal Investigator**

Effua Usuf

**Additional Investigators**

Kalifa Bojang
Syed M. A. Zaman
Fatou Secka
Suzanne Anderson
Anna RocaIsatou Sarr
Momodou Saidykhan
Saffiatou Darboe
Samba Ceesay
Umberto D'alessandro

Medical Research Council Unit The Gambia at LSHTM
P O Box 273,

Version: 6.1 September 2020

Fajara, The Gambia

**ERASMUS MC-Sophia Children's Hospital**

Principal Investigator

Henriëtte A. Moll[1]

Research group

Dorine M. Borensztajn[1], Nienke N. Hagedoorn, Chantal Tan [1], [1], Clementien L. Vermont[2], Joany Zachariasse [1]

Additional investigator

W Dik [3]

[1] Erasmus MC-Sophia Children's Hospital, Department of General Paediatrics, Rotterdam, the Netherlands

[2] Erasmus MC-Sophia Children's Hospital, Department of Paediatric Infectious Diseases & Immunology, Rotterdam, the Netherlands

[3] Erasmus MC, Department of immunology, Rotterdam, the Netherlands

***Swiss Pediatric Sepsis Study***

**Principal Investigators*:***

Philipp Agyeman, MD [1] (ORCID 0000-0002-8339-5444), Luregn J Schlapbach, MD, FCICM [2,3] (ORCID 0000-0003-2281-2598)

**Clinical recruitment at University Children's Hospital Bern for PERFORM:**

Christoph Aebi [1], Verena Wyss [1], Mariama Usman [1]

**Principal and co-investigators for the Swiss Pediatric Sepsis Study:**

Philipp Agyeman, MD [1], Luregn J Schlapbach, MD, FCICM [2,3], Eric Giannoni, MD [4,5], Martin Stocker, MD [6], Klara M Posfay-Barbe, MD [7], Ulrich Heininger, MD [8], Sara Bernhard-Stirnemann, MD [9], Anita Niederer-Loher, MD [10], Christian Kahlert, MD [10], Giancarlo Natalucci, MD [11], Christa Relly, MD [12], Thomas Riedel, MD [13], Christoph Aebi, MD [1], Christoph Berger, MD [12] **for the Swiss Pediatric Sepsis Study**

**Affiliations:**

[1] Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland

Version: 6.1 September 2020

[2] Neonatal and Pediatric Intensive Care Unit, Children's Research Center, University Children's Hospital Zurich, University of Zurich, Zurich, Switzerland

[3]Child Health Research Centre, University of Queensland, and Queensland Children`s Hospital, Brisbane, Australia

[4] Clinic of Neonatology, Department Mother-Woman-Child, Lausanne University Hospital and University of Lausanne, Switzerland

[5] Infectious Diseases Service, Department of Medicine, Lausanne University Hospital and University of Lausanne, Switzerland

[6] Department of Pediatrics, Children's Hospital Lucerne, Lucerne, Switzerland

[7] Pediatric Infectious Diseases Unit, Children's Hospital of Geneva, University Hospitals of Geneva, Geneva, Switzerland

[8] Infectious Diseases and Vaccinology, University of Basel Children's Hospital, Basel, Switzerland

[9] Children's Hospital Aarau, Aarau, Switzerland

[10] Division of Infectious Diseases and Hospital Epidemiology, Children's Hospital of Eastern Switzerland St. Gallen, St. Gallen, Switzerland

[11] Department of Neonatology, University Hospital Zurich, Zurich, Switzerland

[12] Division of Infectious Diseases and Hospital Epidemiology, and Children's Research Center, University Children's Hospital Zurich, Switzerland

[13] Children's Hospital Chur, Chur, Switzerland

***Liverpool Partner***

Principal Investigators

Enitan D Carrol[1,2,3]

Stéphane Paulus [1,]

Research Group (in alphabetical order):

Elizabeth Cocklin[1], Rebecca Jennings[4], Joanne Johnston[4], Simon Leigh[1], Karen Newall[4], Sam Romaine[1]

Version: 6.1 September 2020

[1] Department of Clinical Infection, Microbiology and Immunology, University of Liverpool Institute of Infection and Global Health , Liverpool, England
[2] Alder Hey Children's Hospital, Department of Infectious Diseases, Eaton Road, Liverpool, L12 2AP
[3] Liverpool Health Partners, 1st Floor, Liverpool Science Park, 131 Mount Pleasant, Liverpool, L3 5TF

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

**NKUA Partner (Greece)**

Principal investigator: Professor **Maria Tsolia** (all activities)

Investigator/Research fellow: **Irini Eleftheriou** (all activities)

Additional investigators:

Recruitment: Maria Tambouratzi

Lab: Antonis Marmarinos (Quality Manager)

Lab: Marietta Xagorari

Kelly Syggelou

2nd Department of Pediatrics, National and Kapodistrian University of Athens,

"P. and A. Kyriakou" Children's Hospital

Thivon and Levadias

Goudi, Athens

**Micropathology Ltd :**

Principal Investigator:

Professor Colin Fink[1], Clinical Microbiologist

Additional investigators

Dr Marie Voice[1], Post doc scientist

Version: 6.1 September 2020

Dr. Leo Calvo-Bado[1], Post doc scientist

[1] Micropathology Ltd, The Venture Center, University of Warwick Science Park, Sir William Lyons Road, Coventry, CV4 7EZ.

**Medical University of Graz, Austria (MUG)**

Principal Investigator:

Werner Zenz[1] (all activities)

Co-investigators (in alphabetical order)

Benno Kohlmaier[1] (all activities)

Nina A. Schweintzger[1] (all activities)

Manfred G. Sagmeister[1] (study design, consortium wide sample management)

Research team

Daniela S. Kohlfürst[1] (study design)

Christoph Zurl[1] (BIVA PIC)

Alexander Binder[1] (grant application)

Recruitment team, data managers, (in alphabetical order):

Susanne Hösele[1], Manuel Leitner[1], Lena Pölz[1], Glorija Rajic[1],

Clinical recruitment partners (in alphabetical order):

Sebastian Bauchinger[1], Hinrich Baumgart[4], Martin Benesch[3], Astrid Ceolotto[1], Ernst Eber[2], Siegfried Gallistl[1], Gunther Gores[5], Harald Haidl[1], Almuthe Hauer[1], Christa Hude[1], Markus Keldorfer[5], Larissa Krenn[4], Heidemarie Pilch[5], Andreas Pfleger[2], Klaus Pfurtscheller[4], Gudrun

Version: 6.1 September 2020

Nordberg[5], Tobias Niedrist[8], Siegfried Rödl[4], Andrea Skrabl-Baumgartner[1], Matthias Sperl[7], Laura Stampfer[5], Volker Strenger[3], Holger Till[6], Andreas Trobisch[5], Sabine Löffler[5]

Author Affiliations:

[1] Department of Pediatrics and Adolescent Medicine, Division of General Pediatrics, Medical University of Graz, Graz, Austria

[2]Department of Pediatric Pulmonology, Medical University of Graz, Graz, Austria

[3]Department of Pediatric Hematooncoloy, Medical University of Graz, Graz, Austria

[4]Paediatric Intensive Care Unit, Medical University of Graz, Graz, Austria

[5]University Clinic of Paediatrics and Adolescent Medicine Graz, Medical University Graz, Graz,Austria

[6]Department of Paediatric and Adolescence Surgery, Medical University Graz, Graz, Austria

[7]Department of Pediatric Orthopedics, Medical University Graz, Graz, Austria

[8]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz, Graz, Austria

**London School of Hygiene and Tropical Medicine**

**WP 1 WP2, WP5**

Principal Investigator:

Dr Shunmay Yeung[1,2 3] PhD, MBBS, FRCPCH, MRCP, DTM&H

Research Group

Dr Juan Emmanuel Dewez[1] MD, DTM&H, MSc

Prof Martin Hibberd [1] BSc, PhD

Mr David Bath[2] MSc, MAppFin, BA(Hons)

Dr Alec Miners[2] BA(Hons), MSc, PhD

Dr Ruud Nijman[3] PhD MSc MD MRCPCH

Dr Catherine Wedderburn[1] BA, MBChB, DTM&H, MSc, MRCPCH

Version: 6.1 September 2020

Ms Anne Meierford[1] MSc, BMedSc, BMBS

Dr Baptiste Leurent[4], PhD, MSc

1. Faculty of Infectious and Tropical Disease, London School of Hygiene and Tropical Medicine, London, UK
2. Faculty of Public Health and Policy, London School of Hygiene and Tropical Medicine, London, UK
3. Department of Paediatrics, St. Mary's Hospital Imperial College Hospital, London, UK
4. Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK

**Radboud University Medical Center (RUMC), The Netherlands**

Principal Investigators:
Ronald de Groot [1], Michiel van der Flier [1,2,3], Marien I. de Jonge[1]

Co-investigators Radboud University Medical Center (in alphabetical order):
Koen van Aerde[1,2], Wynand Alkema[1], Bryan van den Broek[1], Jolein Gloerich[1], Alain J. van Gool[1], Stefanie Henriet[1,2], Martijn Huijnen[1], Ria Philipsen[1], Esther Willems[1]

Investigators PeDBIG PERFORM DUTCH CLINICAL NETWORK (in alphabetical order):
G.P.J.M. Gerrits[8], M. van Leur[8,] J. Heidema [4],L. de Haan[1,2] C.J. Miedema [5], C. Neeleman [1] C.C. Obihara [6], G.A. Tramper-Stranders7[6]

1. Radboud University Medical Center, Nijmegen, The Netherlands
2. Amalia Children's Hospital, Nijmegen, The Netherlands
3. Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands
4. St. Antonius Hospital, Nieuwegein, The Netherlands
5. Catharina Hospital, Eindhoven, The Netherlands
6. ETZ Elisabeth, Tilburg, The Netherlands
7. Franciscus Gasthuis, Rotterdam, The Netherlands
8. Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

**Oxford team (UK)**

Version: 6.1 September 2020

Principal Investigators

Andrew J. Pollard[1,2], Rama Kandasamy[1,2], Stéphane Paulus [1,2]

Additional Investigators

Michael J. Carter[1,2], Daniel O'Connor[1,2], Sagida Bibi[1,2], Dominic F. Kelly[1,2], Meeru Gurung[3], Stephen Thorson[3], Imran Ansari[3], David R. Murdoch[4], Shrijana Shrestha[3], Zoe Oliver[5]

Author Affiliations:

[1]Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, United Kingdom.

[2]NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom.

[3]Paediatric Research Unit, Patan Academy of Health Sciences, Kathmandu, Nepal.

[4]Department of Pathology, University of Otago, Christchurch, New Zealand.

[5] Department of Paediatrics, University of Oxford.

**Newcastle University, Newcastle upon Tyne, (UK)**

Principal Investigator:

Marieke Emonts [1,2,3] (all activities)

Co-investigators

Emma Lim[2,3,7] (all activities)

Lucille Valentine[4]

Recruitment team (alphabetical), data-managers, and GNCH Research unit:

Version: 6.1 September 2020

Karen Allen[5], Kathryn Bell[5], Adora Chan[5], Stephen Crulley[5], Kirsty Devine[5], Daniel Fabian[5], Sharon King[5], Paul McAlinden[5], Sam McDonald[5], Anne McDonnell2,[5], Ailsa Pickering[2,5], Evelyn Thomson[5], Amanda Wood[5], Diane Wallia[5], Phil Woodsford[5],

Sample processing: Frances Baxter[5], Ashley Bell[5], Mathew Rhodes[5]

PICU recruitment

Rachel Agbeko[8]

Christine Mackerness[8]

Students MOFICHE

Bryan Baas[2], Lieke Kloosterhuis[2], Wilma Oosthoek[2]

Students/medical staff PERFORM

Tasnim Arif[6], Joshua Bennet[2], Kalvin Collings[2], Ilona van der Giessen[2], Alex Martin[2], Aqeela Rashid[6], Emily Rowlands[2], Gabriella de Vries[2], Fabian van der Velden[2]

Engagement work/ethics/cost effectiveness

Lucille Valentine [4], Mike Martin[9], Ravi Mistry[2], Lucille Valentine[4]

Author Affiliations:

[1] Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne UK

[2]Great North Children's Hospital, Paediatric Immunology, Infectious Diseases & Allergy, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.

[3]NIHR Newcastle Biomedical Research Centre based at Newcastle upon Tyne Hospitals NHS Trust and Newcastle University, Westgate Rd, Newcastle upon Tyne NE4 5PL, United Kingdom

[4]Newcastle University Business School, Centre for Knowledge, Innovation, Technology and Enterprise (KITE), Newcastle upon Tyne, United Kingdom

[5]Great North Children's Hospital, Research Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.

[6]Great North Children's Hospital, Paediatric Oncology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.

Version: 6.1 September 2020

[7]Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne, UK

[8]Great North Children's Hospital, Paediatric Intensive Care Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.

[9]Northumbria University, Newcastle upon Tyne, United Kingdom.

**LMU Munich Partner (Germany)**

Principal Investigator:

Ulrich von Both[1,2] MD, FRCPCH (all activities)

Research group:

Laura Kolberg[1] MSc (all activities)

Manuela Zwerenz[1] MSc, Judith Buschbeck[1] PhD

Clinical recruitment partners (in alphabetical order):

Christoph Bidlingmaier[3], Vera Binder[4], Katharina Danhauser[5], Nikolaus Haas[10], Matthias Griese[6], Tobias Feuchtinger[4], Julia Keil[9], Matthias Kappler[6], Eberhard Lurz[7], Georg Muench[8], Karl Reiter[9], Carola Schoen[9]

Author Affiliations:

[1]Div. Paediatric Infectious Diseases, Hauner Children's Hospital, University Hospital, Ludwig Maximilians University (LMU), Munich, Germany

[2]German Center for Infection Research (DZIF), Partner Site Munich, Munich, Germany

[3]Div. of General Paediatrics, [4]Div. Paediatric Haematology & Oncology, [5]Div. of Paediatric Rheumatology, [6]Div. of Paediatric Pulmonology, [7]Div. of Paediatric Gastroenterology, [8]Neonatal Intensive Care Unit, [9]Paediatric Intensive Care Unit Hauner Children's Hospital, University

Version: 6.1 September 2020

Hospital, Ludwig Maximilians University (LMU), Munich, Germany, [10]Department Pediatric Cardiology and Pediatric Intensive Care, University Hospital, Ludwig Maximilians University (LMU), Munich, Germany

**bioMérieux, France**

Principal Investigator:
François Mallet[1,2, 3]

Research Group:
Karen Brengel-Pesce[1,2, 3]
Alexandre Pachot[1]
Marine Mommert[1,2]

*[1]Open Innovation & Partnerships (OIP), bioMérieux S.A., Marcy l'Etoile, France*
*[2] Joint research unit Hospice Civils de Lyon - bioMérieux, Centre Hospitalier Lyon Sud, 165 Chemin du Grand Revoyet, 69310 Pierre-Bénite, France*
*[3] EA 7426 Pathophysiology of Injury-induced Immunosuppression, University of Lyon1-Hospices Civils de Lyon-bioMérieux, Hôpital Edouard Herriot, 5 Place d'Arsonval, 69437 Lyon Cedex 3, France*

**Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia**

Principal Investigator:

Marko Pokorn[1,2,3] MD, PhD

Research Group:

Mojca Kolnik[1] MD, Katarina Vincek[1] MD, Tina Plankar Srovin[1] MD, PhD, Natalija Bahovec[1] MD, Petra Prunk[1] MD, Veronika Osterman[1] MD, Tanja Avramoska[1] MD

Affiliations:

[1]Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva 2, SI-1525 Ljubljana, Slovenia

[2]University Childrens' Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

[3]Department of Infectious Diseases and Epidemiology, Faculty of Medicine, University of Ljubljana, Slovenia

Version: 6.1 September 2020

**Amsterdam, Academic Medical Hospital & Sanquin Research Institute (NL)**

Principal Investigator:

Taco Kuijpers [1,2]

Co-investigators

Ilse Jongerius [2]

Recruitment team (EUCLIDS, PERFORM):

J.M. van den Berg[1], D. Schonenberg[1], A.M. Barendregt[1], D. Pajkrt[1], M. van der Kuip[1,3], A.M. van Furth[1,3]

Students PERFORM

Evelien Sprenkeler [2], Judith Zandstra [2],

Technical support PERFORM

G. van Mierlo [2], J. Geissler [2]

Author Affiliations:

[1] Amsterdam University Medical Center (Amsterdam UMC), location Academic Medical Center (AMC), Dept of Pediatric Immunology, Rheumatology and Infectious Diseases, University of Amsterdam, Amsterdam, the Netherlands

[2] Sanquin Research Institute, & Landsteiner Laboratory at the AMC, University of Amsterdam, Amsterdam, the Netherlands.

[3] Amsterdam University Medical Center (Amsterdam UMC), location Vrije Universiteit Medical Center (VUMC), Dept of Pediatric Infectious Diseases and Immunology, Free University (VU), Amsterdam, the Netherlands (former affiliation)

# Supplemental information

## Appendix 1: Statistical analysis plan

# Statistical Analysis Plan

Prediction of invasive bacterial infections in febrile children presenting to Emergency Departments in Europe

SAP version 1.0 date 14th July 2019

## Background

Still today children die on treatable infectious diseases due to delayed or missed diagnosis presented at the Emergency Department (ED) or primary care.(1-3) On the other hand, antibiotics are prescribed for viral infections and infection with an unknown bacterial or viral cause in order not to miss one child with an invasive bacterial infection.(4)

The distinction between invasive bacterial infections and viral infections on only clinical signs and symptoms is difficult. Biomarkers as C-reactive protein and procalcitonin are currently used in febrile children to detect bacterial infections and to target appropriate antibiotic prescribing. However, these markers measure non-specific inflammation and immunologic responses. Recent research focuses on finding new discriminators of bacterial and viral infections using novel, sophisticated techniques (genomic, proteomic and transcriptomic approaches).(5-7) It is yet unclear which patients would benefit from potential new biomarkers. It is not feasible to apply new biomarkers to all febrile children. Therefore, decision models need to be developed which can identify these patients.

We searched PUBMED from 1st January 2009 to 1st July 2019 for published studies covering clinical prediction models for bacterial infections in children using keywords “child”, “fever”, “bacterial infection” and “clinical prediction” and checked references for relevant articles. The existing literature on clinical prediction models for bacterial infections focuses on young infants (< 3 months) and healthy children in particular. For older children, the Feverkidstool (Nijman et al.) is an extensively validated clinical prediction model for prediction of pneumonia and other serious bacterial infections which includes bacteraemia and meningitis but also infections of the urinary tract, gastro-intestinal tract and soft tissue. We could not identify a clinical prediction model for the outcome invasive bacterial infections including older children or children with chronic conditions.

## Objectives

1. To update an existing clinical prediction model to identify invasive bacterial infections in febrile children at the ED
2. Can we target patients who can benefit from a new biomarker based on risk-prediction by this model?

## Methods

**Study design:**

Prospective observational study

This study is a prospectively planned analysis in the MOFICHE study (Management and Outcome of Febrile Illness in Children) which is part of the PERFORM project. MOFICHE is a prospective observational study using routine data. The need for informed consent was waived.

**Setting:**

12 Emergency Departments (EDs) in 8 countries

**Population:**

Children 0-18 years with fever (temperature >38.0 C) measured at ED or history of fever (<72 hours) before ED visit. For this analysis, we will exclude children with working diagnosis of urinary tract infections after ED visit. For diagnosis of urinary tract infections, easy available diagnostics are already available at the ED. Therefore, a clinical prediction model has limited additional value in this group. Furthermore, we will focus our analysis on patients with CRP measurement since these are patients with diagnostic uncertainty after initial assessment by the physician.

**Inclusion period:**

1 January 2017 – 1 April 2018, at least 12 months per study site.

**Primary outcomes:**
Invasive bacterial infections (IBI): bacteraemia, bacterial meningitis and bacterial bone and join infections. Infections were defined positive growth of a single pathogenic bacterium in blood, cerebrospinal fluid or synovial fluid from cultures collected at ED visit or the first 24 hours from hospital admission.

Cultures growing contaminants (coagulase-negative staphylococci, alpha-haemolytic streptococci, *Micrococcus* species or *Propionibacterium* species are defined negative (8)

In children who are immunocompromised, malignancies or with a central line, these contaminants are still relevant invasive bacterial infections that need antibiotic treatment. In these patient groups, cultures with a single contaminant are defined positive.

All patients were entered in the electronic case record form (eCRF) by the local team. We will check all the positive cultures to ensure consistency and validity of coding.

**Missing data**
For this analysis, we will exclude patients with no CRP value and exclude patients with working diagnosis of urinary tract infection. We will use multiple imputation by chained equations using the MICE package in R to impute all missing predictor variables. We will assume the variables to be 'missing at random' where missingness can be explained by other variables in the data. We will incorporate hospital, all predictor variables, outcome measures and other auxiliary variables in the imputation model.
Multiple imputation will be performed on all patients (n=38480).

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 | Capillary refill time | Temperature | Urinalysis categories | Inhalation medication | Focus of infection |
| Referral type (self / GP / emergency services / other) | Ill appearance | Oxygen saturation | Blood culture performed | Antibiotic prescription type | |
| Previous medical care (yes, primary care / yes, this ED / yes other secondary care) | Work of breathing | | Cerebrospinal fluid performed | Antibiotic prescription mode | |
| Season | Meningeal signs | | | Previous antibiotic treatment | |
| Arrival hours (morning / evening / night) | Focal neurology | | | | |
| Comorbidity | Non-blanching rash | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | Dehydration | | | | |
| | Seizures | | | | |

**Descriptive analysis**
We will perform descriptive analysis for children with and without IBI. We will use frequencies, mean and standard deviation for normally distributed data, median and interquartile range for normally distributed data. In addition, we will compare patients with CRP measurement and patients without CRP measurement.

**Predictor variables**
We will include predictor variables chosen a-priori that have predictive value for bacterial infection. We will perform univariate logistic regression analysis for these predictor variables:

*Predictor variables included in the Feverkidstool (9):*
- Age
- Sex
- Temperature
- Fever duration in days
- Tachypnea: defined by Advanced Paediatric Life Support (10)
- Tachycardia: defined by Advanced Paediatric Life Support (10)
- Hypoxia: oxygen saturation <94%
- Prolonged capillary refill time: >3 seconds
- Increased work of breathing: chest wall retractions, nasal flaring, grunting or apnoea
- Ill appearance: ill, moderately ill, irritable or uncomfortable
- C-reactive protein value

*NICE red warning signs for serious illness (11):*
- Abnormal consciousness: responsive to verbal stimulation, responsive to pain or unresponsive
- Presence of meningeal signs: presence of Kernig, Brudzinski, tripod phenomenon, neck stiffness or bulging fontanelle
- Focal neurological signs
- Status epilepticus: seizures for >=30 minutes
- Non-blanching rash: petechiae or other non-blanching rash

*Complex chronic condition (12)*
- Chronic condition in ≥2 body systems that is expected to last at least 1 year or malignancy or immunocompromised

We will use 10 events per variable to include predictor variables in model development. If not enough events are available, we will combine abnormal consciousness, presence of meningeal signs and focal neurological signs in a composite variable.
Linearity of continuous variables will be assessed using restricted cubic splines. Outliers for continuous variables will be truncated at the 0.01 percentile and the 0.99 centile.

**Model development**
We will perform variable selection by least absolute shrinkage and selection operator (LASSO). Using LASSO, we perform variable selection and reduce degree of overfitting by shrinking large regression coefficients.(13) We will estimate the lambda using 10 times 10-fold-cross validation. To note, variable selection will not be based on significance in univariate logistic regression analysis.

**Model validation**
The model will be validated using internal-external cross-validation. In this method, the model is repeatedly derived on all EDs except one, and validated on the remaining ED.(14, 15)

**Model performance**
Model performance will be assessed by

- Discrimination of the model by concordance (c)-statistic.
- Calibration, the agreement between predicted risks and observed outcome will be visualized using calibration plots.(16)
- Diagnostic performance at different risk-threshold for the probability of IBI using sensitivity, specificity and negative and positive likelihood ratios. We will focus on cut-offs that can be used to rule-out (negative LR <0.2) or rule-in IBI (positive LR>5).(17)

**Sensitivity analysis**

A sensitivity analysis will be performed in the population where missing CRP values will be imputed.

---

Drafted by: Nienke N. Hagedoorn
Statistician: Daan Nieboer
Supervision: Dr. Clementien Vermont, Prof. Henriette A. Moll

---

## Appendix 2: Definition of contaminants

| **Appendix 3: 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**

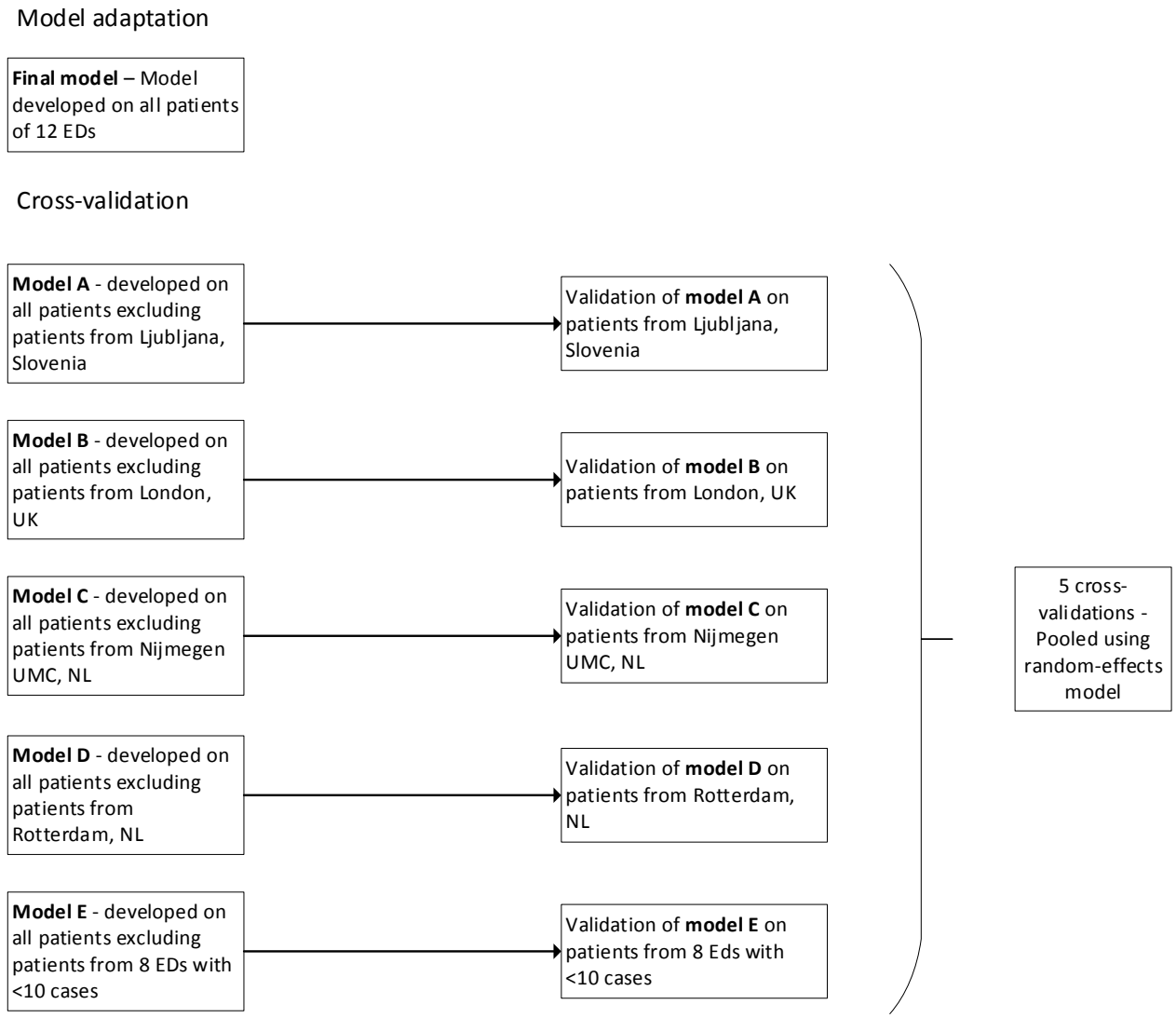
Missing data were multiple imputed using the MICE package in R v3.4. The imputation model included the outcome variable IBI, all considered predictors, ED and other auxiliary variables related to casemix and disease severity (specific details of the multiple imputation model are proved in the Statistical 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.(18) The LASSO was applied to a stacked dataset containing all imputed data.(19) 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 (20, 21), 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(10), 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)(11) and 3) complex chronic condition (condition in ≥2 body systems, malignancy or immunocompromised).(12) 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).(13, 22) This approach aims to reduce the degree of overfitting by shrinking large regression coefficients and performs variable selection.(13) 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).(14) 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.(14, 15) 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).(17) 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 5: Patient characteristics of patients with CRP measurement and patients without CRP measurement

| | CRP measured (n=17,213) | | | No CRP measured (n=21267) | | |
|---|---|---|---|---|---|---|
| | n (%) | Range EDs | Missing | n (%) | Range EDs | Missing |
| **General characteristics** | | | | | | |
| **Age in years,** median (IQR) | 2.77 (1.29-6.02) | | | 2.74 (1.31-5.28) | | |
| **Male** | 9305 (54.1) | 49.6-62.0 | | 11805 (55.5) | 52.4-62.4 | 1 |
| **Previous chronic condition** | | | 97 | | | 273 |
| Any | 3332 (19.4) | 7.8-71.8 | | 3162 (14.9) | 3.9-61.6 | |
| Complex | 1138 (6.6) | 1.1-41.3 | | 729 (3.4) | 0.0-32.6 | |
| **Referred** | 9287 (53.9) | 6.9-99.2 | 980 | 6789 (31.9) | 3.9-99.3 | 185 |
| **Triage urgency** | | | 529 | | | 647 |
| Low: standard, non-urgent | 9794 (56.9) | 10.9-86.5 | | 14291 (67.2) | 8.8-93.9 | |
| High: immediate, very urgent, intermediate | 6890 (40.0) | 13.5-86.8 | | 6329 (29.8) | 6.1-89.9 | |
| **Feverkidstool** | | | | | | |
| Temperature in °C, median (IQR) | 37.8 (37-38.5) | | 809 | 37.7 (36.9-38.4) | | 2211 |
| Fever duration in days, median (IQR) | 1.5 (0.5-3) | | 875 | 1.5 (0.5-3.0) | | 1900 |
| Tachypnea (APLS) | 3585 (20.8) | 5.9-45.8 | 4186 | 4942 (23.2) | 2.4-48.3 | 4607 |
| Tachycardia (APLS) | 6001 (34.9) | 11.0-54.9 | 887 | 6854 (32.2) | 11.4-49.4 | 2620 |
| Hypoxia <95% | 762 (4.4) | 1.3-9.2 | 2538 | 733 (3.4) | 0.3-12.9 | 3043 |
| Prolonged capillary refill (>3 sec) | 339 (1.9) | 0.2-7.0 | 2503 | 84 (0.4) | 0.0-2.6 | 1928 |
| Work of breathing | 913 (5.3) | 0.5-13.2 | 2315 | 1732 (8.1) | 0.0-35.6 | 3176 |
| Ill appearance | 4742 (27.5) | 1.9-52.6 | 664 | 1265 (5.9) | 0.4-43.3 | 1057 |
| CRP in mg/L, median (IQR) | 17 (5-49) | | 7 | NA | | |
| **NICE Warning signs** | | | | | | |
| Decreased consciousness | 148 (0.9) | 0.1-5.4 | 150 | 53 (0.2) | 0.0-1.8 | 240 |
| Meningeal signs | 126 (0.7) | 0.1-3.7 | 943 | 11 (0.1) | 0.0-0.1 | 1101 |
| Focal neurology | 102 (0.6) | 0.0-3.7 | 1376 | 31 (0.1) | 0.0-1.8 | 1081 |
| Status epilepticus | 51 (0.3) | 0.0-2.3 | 940 | 15 (0.1) | 0.0-1.2 | 201 |
| Rash: petechiae/non blanching | 664 (3.9) | 1.1-18.0 | 1307 | 448 (2.1) | 0.4-4.1 | 3106 |
| **Blood cultures performed** | 3478 (20.2) | 0.5-73.7 | | 88 (0.4) | 0.0-2.0 | |
| **CSF performed** | 444 (2.6) | 0.2-13.5 | | 8 (0.0) | 0.0-0.2 | |
| **Admission to the ward >24 hours** | 6590 (38.3) | 18.0-63.8 | 175 | 668 (3.1) | 0.9-29.6 | 347 |
| **Admission to the ICU** | 135 (0.8) | 0.3-5.4 | 21 | 23 (0.1) | 0.0-2.4 | 35 |
| **Antibiotic treatment following ED visit** | 6795 (39.5) | 27.9-70.8 | 211 | 5504 (25.9) | 16.9-43.0 | 273 |
| **Lifesaving interventions:** airway, breathing or hemodynamic support | 371 (2.2) | 0.0-11.5 | | 112 (0.5) | 0.0-3.0 | |
| **Urinary tract infection** | 935 (5.4) | 3.2-9.7 | 4 | 418 (1.9) | 0.9-3.8 | 23 |

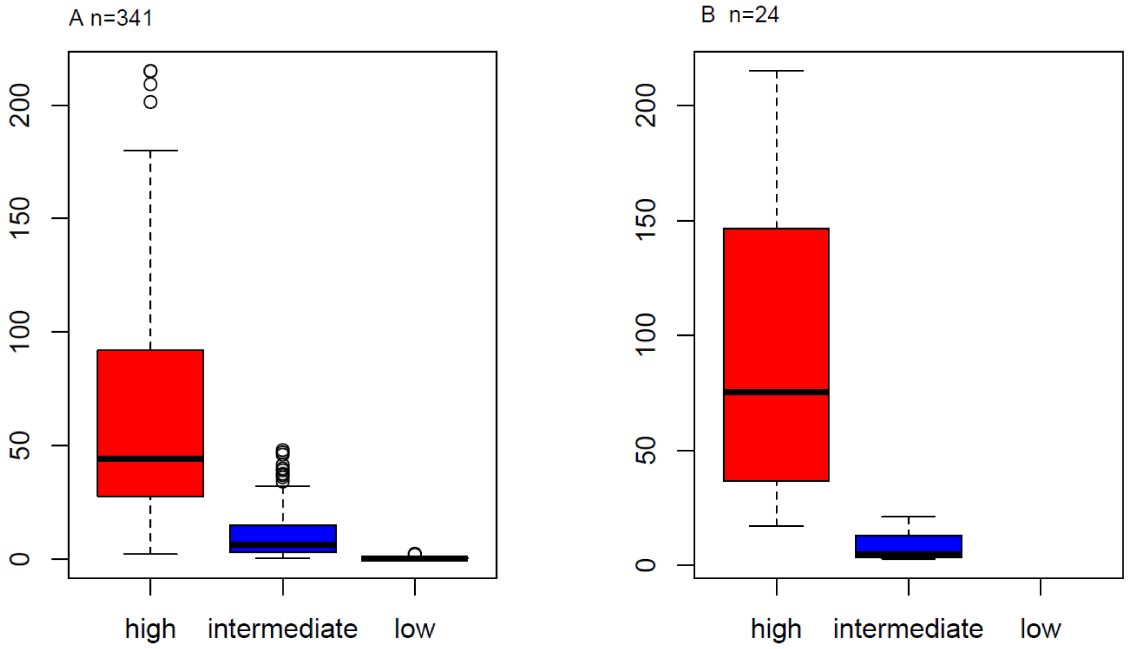
APLS, advanced paediatric life support; CRP, C-reactive protein; CSF, cerebrospinal fluid; ED, emergency department; ICU, intensive care unit; IQR, interquartile range; NA, not applicable

## Appendix 6: Details of patients with complex chronic conditions

Identified pathogen stratified for complex chronic comorbidity

| Identified pathogen | No complex chronic condition , n=85<br>n (%) | Complex chronic condition , n=50<br>n (%) |
|---|---|---|
| Strep. pneumoniae | 23 (27.1%) | 5 (10%) |
| Staph. aureus | 15 (17.6%) | 10 (20%) |
| E. coli | 9 (10.6%) | 4 (8%) |
| Neisseria meningitidis | 9 (10.6%) | 1 (2%) |
| Kingella kingae | 7 (8.2%) | 0 (0%) |
| Group B streptococcus | 6 (7.1%) | 0 (0%) |
| Group A streptococcus | 5 (5.9%) | 1 (2%) |
| Salmonella spp | 4 (4.7%) | 1 (2%) |
| Haemophilus influenzae | 4 (4.7%) | 0 (0%) |
| Enterobacter spp | 2 (2.4%) | 1 (2%) |
| Coagulase-negative staphylococci (CoNS) | | 9 (18%) |
| Candida species | | 4 (8%) |
| Viridans streptococci | | 4 (8%) |
| Klebsiella spp | | 3 (6%) |
| Enterococcus spp | | 3 (6%) |
| Moraxella spp | | 1 (2%) |
| Other | 1 (1.2%) | 3 (6%) |

C-reactive protein level in immunocompromised patients for no IBI (A) vs IBI (B) for IBI risk categories



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

**Supplementary file 5:**
**Univariate logistic regression analysis for invasive bacterial infection.**
**N=16268, IBI cases N=135**

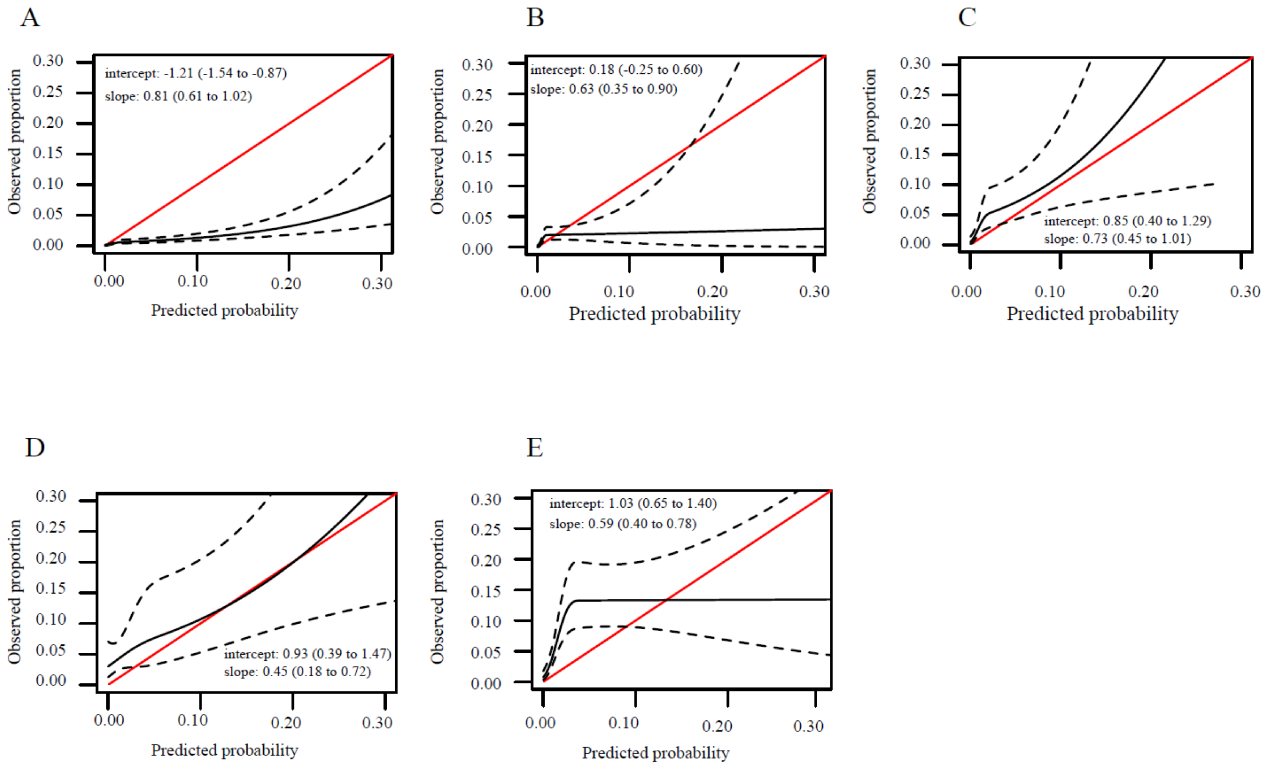
| Variables | OR (95% CI)* |
|---|---|
| **Feverkidstool** | |
| Male | 1.04 (0.74-1.46) |
| Age <1 year± | 0.25 (0.14-0.43)* |
| Age >1 year± | 1.01 (0.97-1.05) |
| Temperature in °C | 1.34 (1.13-1.59)* |
| Fever duration in days | 0.89 (0.80-0.99)* |
| Tachypnea (APLS) | 1.50 (1.03-2.18)* |
| Tachycardia (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: $\beta_{(age <1 year)}$×age in years.
The risk of children aged >1 years was calculated with: $\beta_{(age <1 year)}\times 1+\beta_{(age \geq 1 year)}\times$(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: $\beta_{(age <1 year)}$× age in years.
The risk of children age ≥ 1 year was calculated: $\beta_{(age <1 year)} \times 1 + (\text{age in years}-1) \times \beta_{(age \geq 1 \text{ in years})}$.

CRP, C-reactive protein; IBI, invasive bacterial infection; ln, natural log

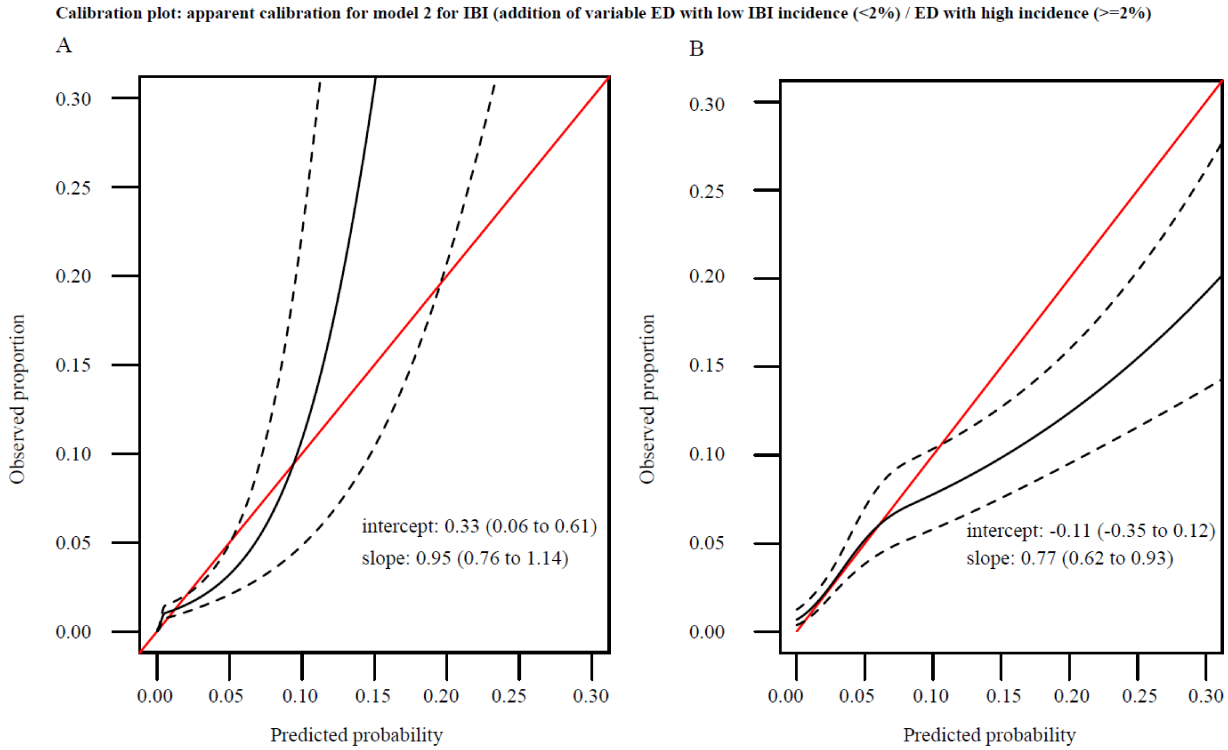
## Model 2 - performance

**Discrimination:**

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

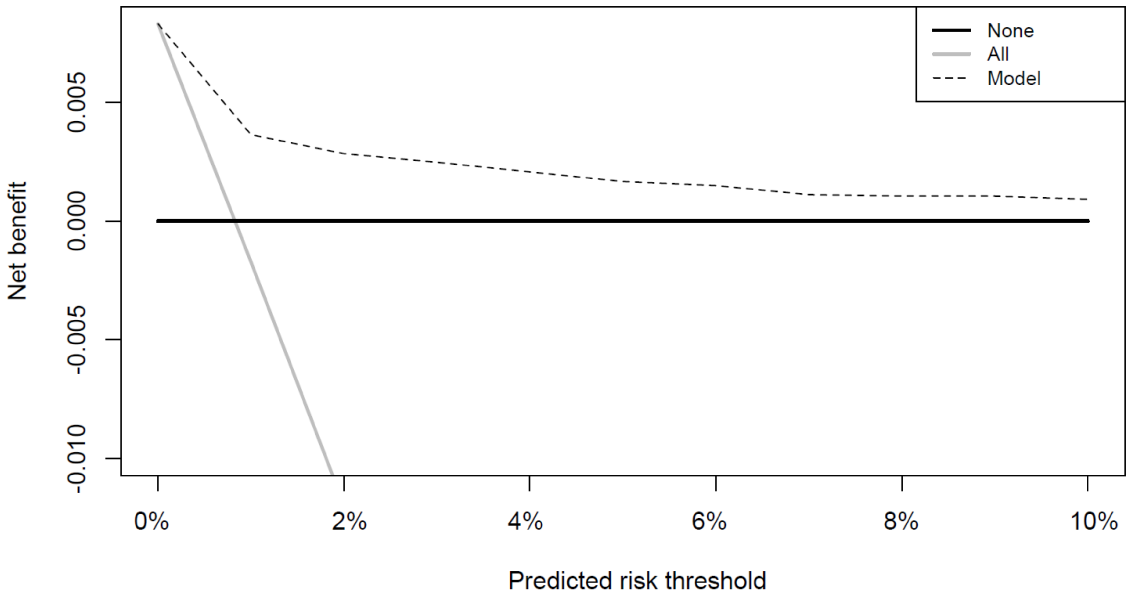
**Calibration:**

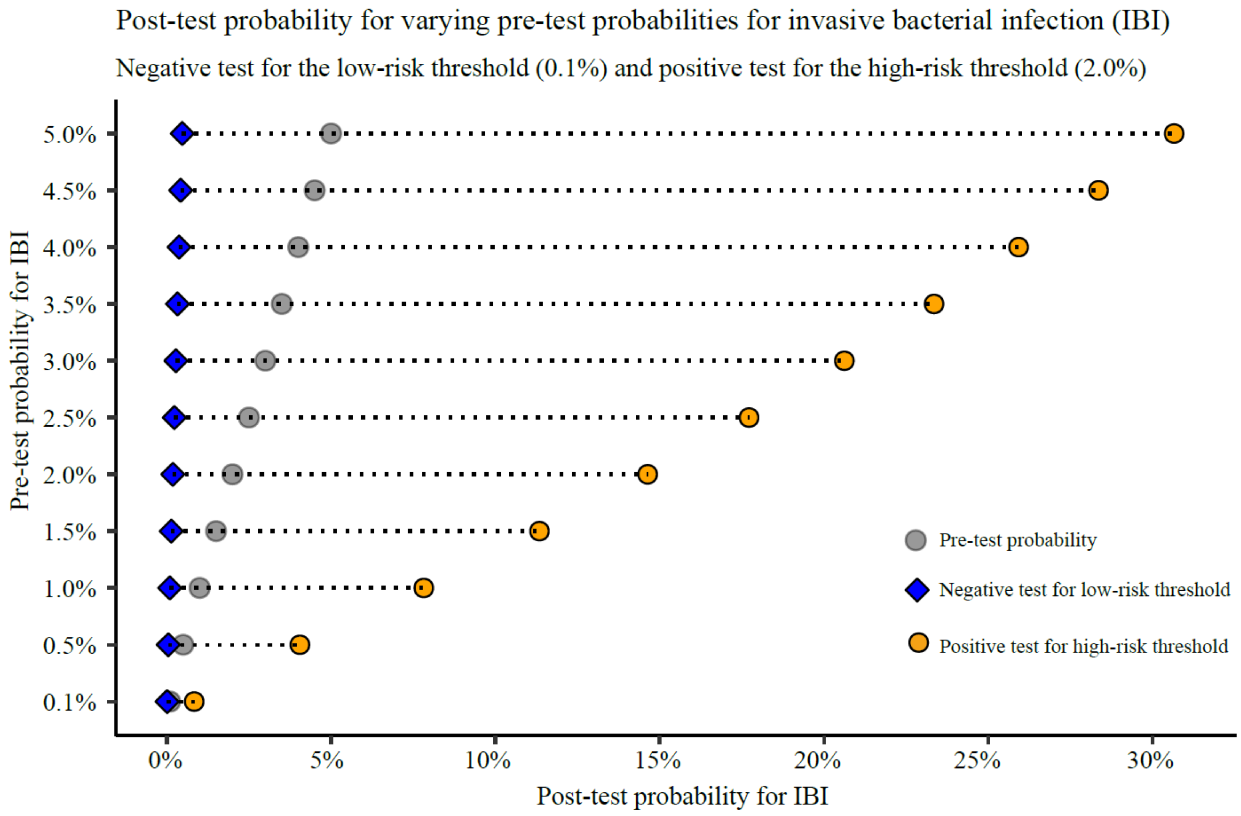
Apparent calibration for model 2 for IBI (addition of variable ED with low IBI incidence (<2%) / ED with high IBI incidence (>=2%)). Risk predictions are calculated on the developed model using all data (n=16268). These risk predictions are calibrated in the two groups: EDs with low IBI incidence (A)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



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

**Model specification of multivariate logistic model for IBI based on population with imputed CRP-level (n=37093)**

| | | Coefficients | OR |
|---|---|---|---|
| | (Intercept) | -9.67 | 0.00 |
| Feverkidstool | Male | -0.19 | 0.83 |
| | Age < 1 year* | -2.58 | 0.08 |
| | Age >1 year* | 0.00 | 1.00 |
| | Temperature | -0.05 | 0.95 |
| | Fever duration in days | -0.15 | 0.86 |
| | Tachypnea | -0.43 | 0.65 |
| | Tachycardia | 0.71 | 2.03 |
| | Hypoxia | -0.86 | 0.42 |
| | Prolonged capillary refill | 0.02 | 1.02 |
| | Increased work of breathing | -0.34 | 0.71 |
| | Ill appearance | 0.94 | 2.55 |
| | Ln CRP | 0.78 | 2.17 |
| NICE warning signs | Abnormal neurology | 1.54 | 4.66 |
| | Non-blanching rash | 1.40 | 4.04 |
| Comorbidity | Complex chronic condition | 2.43 | 11.3 |

*The risk of children aged < 1 year was calculated: $\beta_{(age <1 year)}$×age in years. 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).

CRP, C-reactive protein; ln, natural log

## Appendix 12: Clinical case examples

**Case 1:**
A previously healthy, 4 year old boy presents with fever since 1.5 day.
At the ED he has a temperature of 38.9 degrees, heart rate of 160/min, respiratory rate of 45/min, oxygen saturation of 99% and normal capillary refill time. He is ill-appearing, has increased work of breathing and a normal neurological exam.
CRP-level = 10 mg/L.

Risk-prediction:
The patient is at intermediate-risk (>0.1% and <2%) for an invasive bacterial infection.

**Case 2:**
A previously healthy neonate of 2 months presents with fever since 12 hours.
She has temperature of 38.8 degrees, heart rate of 170/min, respiratory rate of 35/min, normal oxygen saturation and normal capillary refill time. She is ill-appearing and has no increased work of breathing. Neurological exam is normal.
CRP-level = 5 mg/L.

Risk-prediction:
The patient is at high-risk (>2%) for an invasive bacterial infection.

## References

**References**

1. Wolfe I, Cass H, Thompson MJ, *et al.* Improving child health services in the UK: insights from Europe and their implications for the NHS reforms. *Bmj*. 2011;**342**:d1277 Online.
2. Liu L, Oza S, Hogan D, *et al.* Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet*. 2015;**385**:430-40 Online.
3. Pruitt CM, Neuman MI, Shah SS, *et al.* Factors Associated with Adverse Outcomes among Febrile Young Infants with Invasive Bacterial Infections. *J Pediatr*. 2019;**204**:177-82 e1 Online.
4. van de Maat J, van de Voort E, Mintegi S, *et al.* Antibiotic prescription for febrile children in European emergency departments: a cross-sectional, observational study. *Lancet Infect Dis*. 2019; Online.
5. Herberg JA, Kaforou M, Wright VJ, *et al.* Diagnostic Test Accuracy of a 2-Transcript Host RNA Signature for Discriminating Bacterial vs Viral Infection in Febrile Children. *JAMA*. 2016;**316**:835-45 Online.
6. Oved K, Cohen A, Boico O, *et al.* A novel host-proteome signature for distinguishing between acute bacterial and viral infections. *PLoS One*. 2015;**10**:e0120012 Online.
7. van Houten CB, de Groot JAH, Klein A, *et al.* A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): a double-blind, multicentre, validation study. *Lancet Infect Dis*. 2017;**17**:431-40 Online.
8. Pneumonia Etiology Research for Child Health Study G. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet*. 2019; Online.
9. Nijman RG, Vergouwe Y, Thompson M, *et al.* Clinical prediction model to aid emergency doctors managing febrile children at risk of serious bacterial infections: diagnostic study. *BMJ*. 2013;**346**:f1706 Online.
10. Advanced Life Support Group. Advanced Paediatric Life Support: The Practical Approach. 5 ed: Wiley; 2011.
11. The National Institute for Health and Care Excellence (NICE). Fever in under 5s: assessment and initial managment CG160 May 2013. 2013.
12. Simon TD, Cawthon ML, Stanford S, *et al.* Pediatric medical complexity algorithm: a new method to stratify children by medical complexity. *Pediatrics*. 2014;**133**:e1647-54 Online.
13. Tibshirani R. Regression Shrinkage and Selection Via the Lasso. *Journal of the Royal Statistical Society: Series B (Methodological)*. 1996;**58**:267-88 doi: 10.1111/j.2517-6161.1996.tb02080.x [published Online.
14. Steyerberg EW, Harrell FE, Jr. Prediction models need appropriate internal, internal-external, and external validation. *J Clin Epidemiol*. 2016;**69**:245-7 Online.
15. Vos-Kerkhof E, Gomez B, Milcent K, *et al.* Clinical prediction models for young febrile infants at the emergency department: an international validation study. *Arch Dis Child*. 2018;**103**:1033-41 Online.
16. Van Calster B, Nieboer D, Vergouwe Y, De Cock B, Pencina MJ, Steyerberg EW. A calibration hierarchy for risk models was defined: from utopia to empirical data. *J Clin Epidemiol*. 2016;**74**:167-76 Online.
17. Van den Bruel A, Haj-Hassan T, Thompson M, Buntinx F, Mant D, European Research Network on Recognising Serious Infection i. Diagnostic value of clinical features at presentation to identify serious infection in children in developed countries: a systematic review. *Lancet*. 2010;**375**:834-45 Online.
18. Donders AR, van der Heijden GJ, Stijnen T, Moons KG. Review: a gentle introduction to imputation of missing values. *J Clin Epidemiol*. 2006;**59**:1087-91 Online.
19. Zhao Y, Long Q. Variable selection in the presence of missing data: imputation-based methods. *WIREs Computational Statistics*. 2017;**9**:e1402 doi: 10.1002/wics.1402 [published Online.
20. Su TL, Jaki T, Hickey GL, Buchan I, Sperrin M. A review of statistical updating methods for clinical prediction models. *Stat Methods Med Res*. 2018;**27**:185-97 Online.

21. Steyerberg EW, Borsboom GJ, van Houwelingen HC, Eijkemans MJ, Habbema JD. Validation and updating of predictive logistic regression models: a study on sample size and shrinkage. *Stat Med*. 2004;**23**:2567-86 Online.
22. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *J Stat Softw*. 2010;**33**:1-22 Online.

Version: 6.1 September 2020

**PARTNER: IMPERIAL COLLEGE (UK)**
Chief investigator/PERFORM coordinator:

Michael Levin

Principal and co-investigators; work package leads (alphabetical order)

Aubrey Cunnington (grant application)

Tisham De (work package lead)

Jethro Herberg (Principle Investigator, Deputy Coordinator, grant application)

Myrsini Kaforou (grant application, work package lead)

Victoria Wright (grant application, Scientific Coordinator)

Research Group (alphabetical order)

Lucas Baumard; Evangelos Bellos; Giselle D'Souza; Rachel Galassini; Dominic Habgood-Coote; Shea Hamilton; Clive Hoggart; Sara Hourmat; Heather Jackson; Ian Maconochie; Stephanie Menikou; Naomi Lin; Samuel Nichols; Ruud Nijman; Ivonne Pena Paz; Priyen Shah; Ching-Fen Shen; Ortensia Vito; Clare Wilson

Clinical recruitment at Imperial College Healthcare NHS Trust (alphabetical order))

Amina Abdulla; Ladan Ali; Sarah Darnell; Rikke Jorgensen; Sobia Mustafa; Salina Persand

Imperial College Faculty of Engineering

Molly Stevens (co-investigator), Eunjung Kim (research group); Benjamin Pierce (research group)

Version: 6.1 September 2020

Clinical recruitment at Brighton and Sussex University Hospitals

Katy Fidler (Principle Investigator)

Julia Dudley (Clinical Research Registrar)

Research nurses: Vivien Richmond, Emma Tavliavini

Clinical recruitment at National Cheng Kung University Hospital

Ching-Fen Shen (Principal Investigator); Ching-Chuan Liu (Co-investigator); Shih-Min Wang (Co-investigator), funded by the Center of Clinical Medicine Research, National Cheng Kung University

**SERGAS Partner (Spain)**

Principal Investigators

Federico Martinón-Torres[1]
Antonio Salas[1,2]

GENVIP RESEARCH GROUP (in alphabetical order):

Fernando Álvez González[1], Cristina Balo Farto[1], Ruth Barral-Arca[1,2], María Barreiro Castro[1], Xabier Bello[1,2], Mirian Ben García[1], Sandra Carnota[1], Miriam Cebey-López[1], María José Curras-Tuala[1,2], Carlos Durán Suárez[1], Luisa García Vicente[1], Alberto Gómez-Carballa[1,2], Jose Gómez Rial[1], Pilar Leboráns Iglesias[1], Federico Martinón-Torres[1], Nazareth Martinón-Torres[1], José María Martinón Sánchez[1], Belén Mosquera Pérez[1], Jacobo Pardo-Seco[1,2], Lidia Piñeiro Rodríguez[1], Sara Pischedda[1,2], Sara Rey Vázquez[1], Irene Rivero Calle[1], Carmen Rodríguez-Tenreiro[1], Lorenzo Redondo-Collazo[1], Miguel Sadiki Ora[1], Antonio Salas[1,2], Sonia Serén Fernández[1], Cristina Serén Trasorras[1], Marisol Vilas Iglesias[1].

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

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

Version: 6.1 September 2020

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

**RSU Partner (Latvia)**

Principal Investigator

Dace Zavadska[1,2]

Other RSU group authors (in alphabetical order):

Anda Balode[1,2], Arta Bārzdiņa[1,2], Dārta Deksne[1,2], Dace Gardovska[1,2], Dagne Grāvele[2], Ilze Grope[1,2], Anija Meiere[1,2], Ieva Nokalna[1,2], Jana Pavāre[1,2], Zanda Pučuka[1,2], Katrīna Selecka[1,2], Aleksandra Sidorova[1,2], Dace Svile[2], Urzula Nora Urbāne[1,2].

[1] Riga Stradins university, Riga, Latvia.
[2] Children clinical university hospital, Riga, Latvia.

**Medical Research Council Unit The Gambia (MRCG) at LSHTM Partner**

**Principal Investigator**

Effua Usuf

**Additional Investigators**

Kalifa Bojang
Syed M. A. Zaman
Fatou Secka
Suzanne Anderson
Anna RocaIsatou Sarr
Momodou Saidykhan
Saffiatou Darboe
Samba Ceesay
Umberto D'alessandro

Medical Research Council Unit The Gambia at LSHTM
P O Box 273,

Version: 6.1 September 2020

Fajara, The Gambia

**ERASMUS MC-Sophia Children's Hospital**

Principal Investigator

Henriëtte A. Moll[1]

Research group

Dorine M. Borensztajn[1], Nienke N. Hagedoorn, Chantal Tan [1], [1], Clementien L. Vermont[2], Joany Zachariasse [1]

Additional investigator

W Dik [3]

[1] Erasmus MC-Sophia Children's Hospital, Department of General Paediatrics, Rotterdam, the Netherlands

[2] Erasmus MC-Sophia Children's Hospital, Department of Paediatric Infectious Diseases & Immunology, Rotterdam, the Netherlands

[3] Erasmus MC, Department of immunology, Rotterdam, the Netherlands

***Swiss Pediatric Sepsis Study***

**Principal Investigators*:***

Philipp Agyeman, MD [1] (ORCID 0000-0002-8339-5444), Luregn J Schlapbach, MD, FCICM [2,3] (ORCID 0000-0003-2281-2598)

**Clinical recruitment at University Children's Hospital Bern for PERFORM:**

Christoph Aebi [1], Verena Wyss [1], Mariama Usman [1]

**Principal and co-investigators for the Swiss Pediatric Sepsis Study:**

Philipp Agyeman, MD [1], Luregn J Schlapbach, MD, FCICM [2,3], Eric Giannoni, MD [4,5], Martin Stocker, MD [6], Klara M Posfay-Barbe, MD [7], Ulrich Heininger, MD [8], Sara Bernhard-Stirnemann, MD [9], Anita Niederer-Loher, MD [10], Christian Kahlert, MD [10], Giancarlo Natalucci, MD [11], Christa Relly, MD [12], Thomas Riedel, MD [13], Christoph Aebi, MD [1], Christoph Berger, MD [12] **for the Swiss Pediatric Sepsis Study**

**Affiliations:**

[1] Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland

Version: 6.1 September 2020

[2] Neonatal and Pediatric Intensive Care Unit, Children's Research Center, University Children's Hospital Zurich, University of Zurich, Zurich, Switzerland

[3]Child Health Research Centre, University of Queensland, and Queensland Children`s Hospital, Brisbane, Australia

[4] Clinic of Neonatology, Department Mother-Woman-Child, Lausanne University Hospital and University of Lausanne, Switzerland

[5] Infectious Diseases Service, Department of Medicine, Lausanne University Hospital and University of Lausanne, Switzerland

[6] Department of Pediatrics, Children's Hospital Lucerne, Lucerne, Switzerland

[7] Pediatric Infectious Diseases Unit, Children's Hospital of Geneva, University Hospitals of Geneva, Geneva, Switzerland

[8] Infectious Diseases and Vaccinology, University of Basel Children's Hospital, Basel, Switzerland

[9] Children's Hospital Aarau, Aarau, Switzerland

[10] Division of Infectious Diseases and Hospital Epidemiology, Children's Hospital of Eastern Switzerland St. Gallen, St. Gallen, Switzerland

[11] Department of Neonatology, University Hospital Zurich, Zurich, Switzerland

[12] Division of Infectious Diseases and Hospital Epidemiology, and Children's Research Center, University Children's Hospital Zurich, Switzerland

[13] Children's Hospital Chur, Chur, Switzerland

***Liverpool Partner***

Principal Investigators

Enitan D Carrol[1,2,3]

Stéphane Paulus [1,]

Research Group (in alphabetical order):

Elizabeth Cocklin[1], Rebecca Jennings[4], Joanne Johnston[4], Simon Leigh[1], Karen Newall[4], Sam Romaine[1]

Version: 6.1 September 2020

[1] Department of Clinical Infection, Microbiology and Immunology, University of Liverpool Institute of Infection and Global Health , Liverpool, England
[2] Alder Hey Children's Hospital, Department of Infectious Diseases, Eaton Road, Liverpool, L12 2AP
[3] Liverpool Health Partners, 1st Floor, Liverpool Science Park, 131 Mount Pleasant, Liverpool, L3 5TF

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

**NKUA Partner (Greece)**

Principal investigator: Professor **Maria Tsolia** (all activities)

Investigator/Research fellow: **Irini Eleftheriou** (all activities)

Additional investigators:

Recruitment: Maria Tambouratzi

Lab: Antonis Marmarinos (Quality Manager)

Lab: Marietta Xagorari

Kelly Syggelou

2nd Department of Pediatrics, National and Kapodistrian University of Athens,

"P. and A. Kyriakou" Children's Hospital

Thivon and Levadias

Goudi, Athens

**Micropathology Ltd :**

Principal Investigator:

Professor Colin Fink[1], Clinical Microbiologist

Additional investigators

Dr Marie Voice[1], Post doc scientist

Version: 6.1 September 2020

Dr. Leo Calvo-Bado[1], Post doc scientist

[1] Micropathology Ltd, The Venture Center, University of Warwick Science Park, Sir William Lyons Road, Coventry, CV4 7EZ.

**Medical University of Graz, Austria (MUG)**

Principal Investigator:

Werner Zenz[1] (all activities)

Co-investigators (in alphabetical order)

Benno Kohlmaier[1] (all activities)

Nina A. Schweintzger[1] (all activities)

Manfred G. Sagmeister[1] (study design, consortium wide sample management)

Research team

Daniela S. Kohlfürst[1] (study design)

Christoph Zurl[1] (BIVA PIC)

Alexander Binder[1] (grant application)

Recruitment team, data managers, (in alphabetical order):

Susanne Hösele[1], Manuel Leitner[1], Lena Pölz[1], Glorija Rajic[1],

Clinical recruitment partners (in alphabetical order):

Sebastian Bauchinger[1], Hinrich Baumgart[4], Martin Benesch[3], Astrid Ceolotto[1], Ernst Eber[2], Siegfried Gallistl[1], Gunther Gores[5], Harald Haidl[1], Almuthe Hauer[1], Christa Hude[1], Markus Keldorfer[5], Larissa Krenn[4], Heidemarie Pilch[5], Andreas Pfleger[2], Klaus Pfurtscheller[4], Gudrun

Version: 6.1 September 2020

Nordberg[5], Tobias Niedrist[8], Siegfried Rödl[4], Andrea Skrabl-Baumgartner[1], Matthias Sperl[7], Laura Stampfer[5], Volker Strenger[3], Holger Till[6], Andreas Trobisch[5], Sabine Löffler[5]

Author Affiliations:

[1] Department of Pediatrics and Adolescent Medicine, Division of General Pediatrics, Medical University of Graz, Graz, Austria

[2]Department of Pediatric Pulmonology, Medical University of Graz, Graz, Austria

[3]Department of Pediatric Hematooncoloy, Medical University of Graz, Graz, Austria

[4]Paediatric Intensive Care Unit, Medical University of Graz, Graz, Austria

[5]University Clinic of Paediatrics and Adolescent Medicine Graz, Medical University Graz, Graz,Austria

[6]Department of Paediatric and Adolescence Surgery, Medical University Graz, Graz, Austria

[7]Department of Pediatric Orthopedics, Medical University Graz, Graz, Austria

[8]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Graz, Graz, Austria

**London School of Hygiene and Tropical Medicine**

**WP 1 WP2, WP5**

Principal Investigator:

Dr Shunmay Yeung[1,2 3] PhD, MBBS, FRCPCH, MRCP, DTM&H

Research Group

Dr Juan Emmanuel Dewez[1] MD, DTM&H, MSc

Prof Martin Hibberd [1] BSc, PhD

Mr David Bath[2] MSc, MAppFin, BA(Hons)

Dr Alec Miners[2] BA(Hons), MSc, PhD

Dr Ruud Nijman[3] PhD MSc MD MRCPCH

Dr Catherine Wedderburn[1] BA, MBChB, DTM&H, MSc, MRCPCH

Version: 6.1 September 2020

Ms Anne Meierford[1] MSc, BMedSc, BMBS

Dr Baptiste Leurent[4], PhD, MSc

1. Faculty of Infectious and Tropical Disease, London School of Hygiene and Tropical Medicine, London, UK
2. Faculty of Public Health and Policy, London School of Hygiene and Tropical Medicine, London, UK
3. Department of Paediatrics, St. Mary's Hospital Imperial College Hospital, London, UK
4. Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK

**Radboud University Medical Center (RUMC), The Netherlands**

Principal Investigators:
Ronald de Groot [1], Michiel van der Flier [1,2,3], Marien I. de Jonge[1]

Co-investigators Radboud University Medical Center (in alphabetical order):
Koen van Aerde[1,2], Wynand Alkema[1], Bryan van den Broek[1], Jolein Gloerich[1], Alain J. van Gool[1], Stefanie Henriet[1,2], Martijn Huijnen[1], Ria Philipsen[1], Esther Willems[1]

Investigators PeDBIG PERFORM DUTCH CLINICAL NETWORK (in alphabetical order):
G.P.J.M. Gerrits[8], M. van Leur[8,] J. Heidema [4],L. de Haan[1,2] C.J. Miedema [5], C. Neeleman [1] C.C. Obihara [6], G.A. Tramper-Stranders7[6]

1. Radboud University Medical Center, Nijmegen, The Netherlands
2. Amalia Children's Hospital, Nijmegen, The Netherlands
3. Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands
4. St. Antonius Hospital, Nieuwegein, The Netherlands
5. Catharina Hospital, Eindhoven, The Netherlands
6. ETZ Elisabeth, Tilburg, The Netherlands
7. Franciscus Gasthuis, Rotterdam, The Netherlands
8. Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

**Oxford team (UK)**

Version: 6.1 September 2020

Principal Investigators

Andrew J. Pollard[1,2], Rama Kandasamy[1,2], Stéphane Paulus [1,2]

Additional Investigators

Michael J. Carter[1,2], Daniel O'Connor[1,2], Sagida Bibi[1,2], Dominic F. Kelly[1,2], Meeru Gurung[3], Stephen Thorson[3], Imran Ansari[3], David R. Murdoch[4], Shrijana Shrestha[3], Zoe Oliver[5]

Author Affiliations:

[1]Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, United Kingdom.

[2]NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom.

[3]Paediatric Research Unit, Patan Academy of Health Sciences, Kathmandu, Nepal.

[4]Department of Pathology, University of Otago, Christchurch, New Zealand.

[5] Department of Paediatrics, University of Oxford.

**Newcastle University, Newcastle upon Tyne, (UK)**

Principal Investigator:

Marieke Emonts [1,2,3] (all activities)

Co-investigators

Emma Lim[2,3,7] (all activities)

Lucille Valentine[4]

Recruitment team (alphabetical), data-managers, and GNCH Research unit:

Version: 6.1 September 2020

Karen Allen[5], Kathryn Bell[5], Adora Chan[5], Stephen Crulley[5], Kirsty Devine[5], Daniel Fabian[5], Sharon King[5], Paul McAlinden[5], Sam McDonald[5], Anne McDonnell2,[5], Ailsa Pickering[2,5], Evelyn Thomson[5], Amanda Wood[5], Diane Wallia[5], Phil Woodsford[5],

Sample processing: Frances Baxter[5], Ashley Bell[5], Mathew Rhodes[5]

PICU recruitment

Rachel Agbeko[8]

Christine Mackerness[8]

Students MOFICHE

Bryan Baas[2], Lieke Kloosterhuis[2], Wilma Oosthoek[2]

Students/medical staff PERFORM

Tasnim Arif[6], Joshua Bennet[2], Kalvin Collings[2], Ilona van der Giessen[2], Alex Martin[2], Aqeela Rashid[6], Emily Rowlands[2], Gabriella de Vries[2], Fabian van der Velden[2]

Engagement work/ethics/cost effectiveness

Lucille Valentine [4], Mike Martin[9], Ravi Mistry[2], Lucille Valentine[4]

Author Affiliations:

[1] Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne UK

[2]Great North Children's Hospital, Paediatric Immunology, Infectious Diseases & Allergy, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.

[3]NIHR Newcastle Biomedical Research Centre based at Newcastle upon Tyne Hospitals NHS Trust and Newcastle University, Westgate Rd, Newcastle upon Tyne NE4 5PL, United Kingdom

[4]Newcastle University Business School, Centre for Knowledge, Innovation, Technology and Enterprise (KITE), Newcastle upon Tyne, United Kingdom

[5]Great North Children's Hospital, Research Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.

[6]Great North Children's Hospital, Paediatric Oncology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.

Version: 6.1 September 2020

[7]Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne, UK

[8]Great North Children's Hospital, Paediatric Intensive Care Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.

[9]Northumbria University, Newcastle upon Tyne, United Kingdom.

**LMU Munich Partner (Germany)**

Principal Investigator:

Ulrich von Both[1,2] MD, FRCPCH (all activities)

Research group:

Laura Kolberg[1] MSc (all activities)

Manuela Zwerenz[1] MSc, Judith Buschbeck[1] PhD

Clinical recruitment partners (in alphabetical order):

Christoph Bidlingmaier[3], Vera Binder[4], Katharina Danhauser[5], Nikolaus Haas[10], Matthias Griese[6], Tobias Feuchtinger[4], Julia Keil[9], Matthias Kappler[6], Eberhard Lurz[7], Georg Muench[8], Karl Reiter[9], Carola Schoen[9]

Author Affiliations:

[1]Div. Paediatric Infectious Diseases, Hauner Children's Hospital, University Hospital, Ludwig Maximilians University (LMU), Munich, Germany

[2]German Center for Infection Research (DZIF), Partner Site Munich, Munich, Germany

[3]Div. of General Paediatrics, [4]Div. Paediatric Haematology & Oncology, [5]Div. of Paediatric Rheumatology, [6]Div. of Paediatric Pulmonology, [7]Div. of Paediatric Gastroenterology, [8]Neonatal Intensive Care Unit, [9]Paediatric Intensive Care Unit Hauner Children's Hospital, University

Version: 6.1 September 2020

Hospital, Ludwig Maximilians University (LMU), Munich, Germany, [10]Department Pediatric Cardiology and Pediatric Intensive Care, University Hospital, Ludwig Maximilians University (LMU), Munich, Germany

**bioMérieux, France**

Principal Investigator:
François Mallet[1,2, 3]

Research Group:
Karen Brengel-Pesce[1,2, 3]
Alexandre Pachot[1]
Marine Mommert[1,2]

*[1]Open Innovation & Partnerships (OIP), bioMérieux S.A., Marcy l'Etoile, France*
*[2] Joint research unit Hospice Civils de Lyon - bioMérieux, Centre Hospitalier Lyon Sud, 165 Chemin du Grand Revoyet, 69310 Pierre-Bénite, France*
*[3] EA 7426 Pathophysiology of Injury-induced Immunosuppression, University of Lyon1-Hospices Civils de Lyon-bioMérieux, Hôpital Edouard Herriot, 5 Place d'Arsonval, 69437 Lyon Cedex 3, France*

**Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia**

Principal Investigator:

Marko Pokorn[1,2,3] MD, PhD

Research Group:

Mojca Kolnik[1] MD, Katarina Vincek[1] MD, Tina Plankar Srovin[1] MD, PhD, Natalija Bahovec[1] MD, Petra Prunk[1] MD, Veronika Osterman[1] MD, Tanja Avramoska[1] MD

Affiliations:

[1]Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva 2, SI-1525 Ljubljana, Slovenia

[2]University Childrens' Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

[3]Department of Infectious Diseases and Epidemiology, Faculty of Medicine, University of Ljubljana, Slovenia

Version: 6.1 September 2020

**Amsterdam, Academic Medical Hospital & Sanquin Research Institute (NL)**

Principal Investigator:

Taco Kuijpers [1,2]

Co-investigators

Ilse Jongerius [2]

Recruitment team (EUCLIDS, PERFORM):

J.M. van den Berg[1], D. Schonenberg[1], A.M. Barendregt[1], D. Pajkrt[1], M. van der Kuip[1,3], A.M. van Furth[1,3]

Students PERFORM

Evelien Sprenkeler [2], Judith Zandstra [2],

Technical support PERFORM

G. van Mierlo [2], J. Geissler [2]

Author Affiliations:

[1] Amsterdam University Medical Center (Amsterdam UMC), location Academic Medical Center (AMC), Dept of Pediatric Immunology, Rheumatology and Infectious Diseases, University of Amsterdam, Amsterdam, the Netherlands

[2] Sanquin Research Institute, & Landsteiner Laboratory at the AMC, University of Amsterdam, Amsterdam, the Netherlands.

[3] Amsterdam University Medical Center (Amsterdam UMC), location Vrije Universiteit Medical Center (VUMC), Dept of Pediatric Infectious Diseases and Immunology, Free University (VU), Amsterdam, the Netherlands (former affiliation)