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, Dorine Borensztajn, Ruud Gerard Nijman, Daan Nieboer, Jethro Adam Herberg, Anda Balode, Ulrich von Both, Enitan Carrol, Irini Eleftheriou, Marieke Emonts, Michiel van der Flier, Ronald de Groot, Benno Kohlmaier, Emma Lim, Ian Maconochie, Federico Martinón-Torres, Marko Pokorn, Franc Strle, Maria Tsolia, Dace Zavadska, Werner Zenz, Michael Levin, Clementien Vermont, Henriette A Moll

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.

INTRODUCTION

Children presenting at the emergency department (ED) still die from treatable invasive bacterial infections (IBI) due to delayed or missed diagnosis. For not missing one child with IBI, antibiotics are prescribed in children with self-limiting viral infections.
Original research

Infections. The distinction between bacterial and viral infections is made based solely on clinical signs and symptoms; it 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. 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 the treatment of febrile children, 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 exist for older children who are also at risk for IBI. 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 exist for prediction of bacterial pneumonia and the impact of the original Feverkidstool on antibiotic use in respiratory tract infections is proven. 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 cancer 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 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). Data were collected for at least 1 year from January 2017 to April 2018. Details of the study design have been described previously.

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. 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), warning signs for identifying risk of serious illness (National Institute for Health and Care Excellence (NICE)) (consciousness, ill appearance, work of breathing, meningeval 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 bacteria 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). 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. Assuming 16 predictors, a prevalence of 0.8% and an expected R² of 0.0135 (15% of maximum achievable R²), a sample size of 10 587 with 85 cases would be sufficient. For model development, we considered predefined variables with predictive value for IBI: (1) variables in the Feverkidstool (age, sex, temperature, fever duration, tachypnoea and tachycardia defined by Advanced Paediatric Life Support, oxygen saturation <94%, capillary refill ≥3 s, work of breathing, ill appearance and CRP value), (2) NICE warnings signs (consciousness, meningeval signs, focal neurology, status epilepticus, non-blanching rash) and (3) complex chronic condition (≥2 body systems, malignancy or immunocompromised). Sensitivity, meningeval 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). 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. 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. We assessed the

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

|                                | Invasive bacterial infection (n=135) | No invasive bacterial infection (n=16133) |
|--------------------------------|-------------------------------------|----------------------------------------|
|                                | n (%)                               | Missing                                | n (%)                               | Missing                                |
| Age in years, median (IQR)     | 3.2 (0.8–6.0)                       | 2.8 (1.4–6.0)                           |
| Female                         | 76 (56.2)                           | 8932 (55.4)                             |
| Underlying chronic condition   |                                     |                                        |                                     |
| Any                            | 68 (50.4)                           | 3005 (18.6)                             |
| Complex                        | 50 (37.0)                           | 1008 (6.2)                              |
| Referred                       | 96 (71.1)                           | 8633 (53.5)                             |
| Triage urgency                 |                                     |                                        |                                     |
| Low: standard, non-urgent      | 41 (30.4)                           | 9242 (57.3)                             |
| High: immediate, very urgent, intermediate | 89 (65.9)                        | 6414 (39.8)                             |
| Fever kidstool                 |                                     |                                        |                                     |
| Temperature in °C, median (IQR)| 38.0 (37.4–38.7)                    | 37.8 (37.0–38.5)                       | 764                                  |
| Fever duration in days, median (IQR)| 0.5 (0.5–3)                  | 1.5 (0.5–3)                             | 817                                  |
| Tachypnoea (APLS)              | 38 (28.1)                           | 3345 (20.7)                             | 3919                                  |
| Tachycardia (APLS)             | 81 (60.0)                           | 5578 (34.6)                             | 821                                  |
| Hypoxia <95%                   | 4 (2.9)                             | 749 (4.6)                               | 2373                                  |
| Prolonged capillary refill (>3s)| 8 (5.9)                             | 305 (1.9)                               | 2111                                  |
| Increased work of breathing    | 11 (8.1)                            | 887 (5.5)                               | 2136                                  |
| Ill appearance                 | 60 (44.4)                           | 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 (8.0)                               | 141                                  |
| Focal neurology                | 2 (1.5)                             | 95 (0.6)                                | 1249                                  |
| Status epilepticus             | 0 (0.0)                             | 49 (0.3)                                | 887                                  |
| Rash: petechiae/non-blanching  | 10 (7.4)                            | 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)                          | 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 |
| Fever kidstool                 | −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 |
| In CRP                         | 0.76         | 2.14 |
| NICE warning signs             | 1.54         | 4.66 |
| Abnormal neurology             | 1.38         | 3.96 |
| Non-blanching rash             | 2.41         | 11.1 |

The risk of children aged <1 year was calculated: \( \beta \times \text{age in years} \).

The risk of children aged ≥1 year was calculated: \( \beta \times (\text{age in years}−1) \times \beta \).

*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.
Second, diagnostic tests were ordered according to usual care. If on imputed CRP levels. Therefore, model performance was activity analysis, predictors were similar in the model developed to inclusion of all eligible IBIs in the main analysis. In our sensibility. However, the CRP group reflect patients with diagnostic involvement more severe illness than patients without CRP measurement on patients who had CRP measurement on indication. This web appendix 12) and, to help physicians to use this model in practice, a web-based clinical case examples of the model (online supplemental model involves accessible predictors as clinical symptoms and present infection. A prediction model exclusively for IBI.9–11 Furthermore, our while previous studies did not have sufficient cases to define heterogeneity between EDs, and improves the generalisability of 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).31 32 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 procalsitonin 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 infections18 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

| 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)           | 10773 (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                 | 12200 (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                 | 14224 (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                 | 15279 (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                   | 15831 (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.
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 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 Stradiņš University, Riga, Latvia
5. Division of Paediatric Infectious Diseases, Dr von Haunersches Kinderspital Kinderklinik und Kinderpoliklinik der Ludwig Maximilian University Munich, Munich, 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, Athens, 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 Clínico Universitario de Santiago de Compostela, Santiago de Compostela, Galicia, Spain
17. Department of Infectious Diseases and Faculty of Medicine, Ljubljanski klinični center, Ljubljana, Slovenia
18. Department of Paediatric Infectious Diseases and Immunology, Erasmus MC Sophia Children’s Hospital, Rotterdam, Nederland, The Netherlands

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Twitter Ruud Gerard Nijman @rgnijman and Enitan Carroll @CarrollEnitan

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

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ORCID iDs

Nieneke N Hagedoorn http://orcid.org/0000-0001-9237-4904
Dorine Borenstajn 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

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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:
I 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           | Ill appearance              | Oxygen saturation | Blood culture performed | Antibiotic prescription type |
| (self / GP / emergency services / other) | | | | |
| 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         | | | |
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.
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 casemix 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

| Study | CRP measured (n=17,213) | No CRP measured (n=21267) |
|-------|------------------------|--------------------------|
| **General characteristics** | | |
| Age in years, median (IQR) | 2.77 (1.29-6.02) | 2.74 (1.31-5.28) |
| Male | 9305 (54.1) | 11805 (55.5) |
| Previous chronic condition | | |
| Any | 3332 (19.4) | 3162 (14.9) |
| Complex | 1138 (6.6) | 729 (3.4) |
| Referred | 9287 (53.9) | 6789 (31.9) |
| **Triage urgency** | | |
| Low: standard, non-urgent | 9794 (56.9) | 14291 (67.2) |
| High: immediate, very urgent, intermediate | 6890 (40.0) | 6329 (29.8) |
| **Fever/illness/ill appearance** | | |
| Temperature in °C, median (IQR) | 37.8 (37-38.5) | 37.7 (36.9-38.4) |
| Fever duration in days, median (IQR) | 1.5 (0.5-3) | 1.5 (0.5-3) |
| Tachypnea (APLS) | 3585 (20.8) | 4942 (23.2) |
| Hypoxia <95% | 762 (4.4) | 733 (3.4) |
| Prolonged capillary refill (>3 sec) | 339 (1.9) | 84 (0.4) |
| Work of breathing | 913 (5.3) | 1732 (8.1) |
| Ill appearance | 4742 (27.5) | 1265 (5.9) |
| CRP in mg/L, median (IQR) | 17 (5-49) | NA |
| **NICE Warning signs** | | |
| Decreased consciousness | 148 (0.9) | 53 (0.2) |
| Meningeal signs | 126 (0.7) | 11 (0.1) |
| Focal neurology | 102 (0.6) | 31 (0.1) |
| Status epilepticus | 51 (0.3) | 15 (0.1) |
| Rash: petechiae/non blanching | 664 (3.9) | 448 (2.1) |
| **Blood cultures performed** | | |
| CSF performed | 444 (2.6) | 118 (0.5) |
| Admission to the ward >24 hours | 6590 (38.3) | 668 (3.1) |
| Admission to the ICU | 135 (0.8) | 23 (0.1) |
| Antibiotic treatment following ED visit | 6795 (39.5) | 5504 (25.9) |
| Lifesaving interventions: airway, breathing or hemodynamic support | 371 (2.2) | 112 (0.5) |
| Urinary tract infection | 935 (5.4) | 418 (1.9) |

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 | Complex chronic condition, n=50 |
|-----------------------------------|------------------------------------|---------------------------------|
| 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

A n=341

B n=24

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Hagedoorn NN, *et al.* Arch Dis Child 2020;0:1–7. doi: 10.1136/archdischild-2020-319794
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: $\beta_{(\text{age <1 year})} \times \text{age in years}.$

The risk of children aged >1 years was calculated with: $\beta_{(\text{age <1 year})} \times 1 + \beta_{(\text{age ≥1 year})} \times (\text{age in years}-1).$

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 |
| 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 |
| Abnormal neurology                                            | 1.10         | 3.01 |
| Non-blanching rash                                            | 1.06         | 2.89 |
| Complex chronic condition                                     | 1.56         | 4.78 |
| 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: $\beta_{\text{age <1 year}} \times \text{age in years}$. The risk of children age ≥ 1 year was calculated: $\beta_{\text{age <1 year}} \times 1 + (\text{age in years -1}) \times \beta_{\text{age ≥ 1 year}}$. 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

![Calibration plot: apparent calibration for model 2 for IBI (addition of variable ED with low IBI incidence (<2%) / ED with high incidence (≥2%))](image)
Appendix 10: Performance of the prediction model (model 1)

Decision curve analysis

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

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

Pre-test probability for IBI

Post-test probability for IBI

Pre-test probability

Negative test for low-risk threshold

Positive test for high-risk threshold

0.1% 0.5% 1.0% 1.5% 2.0% 2.5% 3.0% 3.5% 4.0% 4.5% 5.0% 0% 5% 10% 15% 20% 25% 30%
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 |
| 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 |
| Abnormal neurology                  | 1.54 4.66 |
| Non-blanching rash                  | 1.40 4.04 |
| Complex chronic condition           | 2.43 11.3 |

Feverkidstool

NICE warning signs

Comorbidity

*The risk of children aged < 1 year was calculated: \( \beta_{(\text{age }<1 \text{ year})} \times \text{age in years} \). The risk of children aged < 1 year was calculated: \( \beta_{(\text{age }<1 \text{ year})} \times \text{age in years} \). The risk of children aged >1 years was calculated with: \( \beta_{(\text{age }<1 \text{ year})} \times 1 + \beta_{(\text{age }\geq1 \text{ year})} \times (\text{age in years}−1) \).

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 days.
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.
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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.

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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)
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-Torres¹
Antonio Salas¹,²

GENVIP RESEARCH GROUP (in alphabetical order):
Fernando Álvez González¹, Cristina Balo Farto¹, Ruth Barral-Arca¹,², María Barreiro Castro¹, Xabier Bello¹,², Mirian Ben García¹, Sandra Carnota¹, Miriam Cebeý-López¹, María José Curras-Tualá¹,², Carlos Durán Suárez¹, Luisa García Vicente¹, Alberto Gómez-Carballa¹,², Jose Gómez Rial¹, Pilar Leboráns Iglesias¹, Federico Martinón-Torres¹, Nazareth Martinón-Torres¹, José Maria Martinón Sánchez¹, Belén Mosquera Pérez¹, Jacobo Pardo-Seco¹,², Lidia Piñeiro Rodríguez¹, Sara Pischedda¹,², Sara Rey Vázquez¹, Irene Rivero Calle¹, Carmen Rodríguez-Tenreiro¹, Lorenzo Redondo-Collazo¹, Miguel Sadiki Ora¹, Antonio Salas¹,², Sonia Serén Fernández¹, Cristina Serén Trasorras¹, Marisol Vilas Iglesias¹.

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 Patológica 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 Bārzdīna\(^1,2\), 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,
Fajara, The Gambia

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

Principal Investigator
Henriëtte A. Moll¹

Research group
Dorine M. Borensztajn¹, Nienke N. Hagedoorn, Chantal Tan¹,¹, Clementien L. Vermont², Joany Zachariasse¹

Additional investigator
W Dik³

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, MD¹ (ORCID 0000-0002-8339-5444), Luregn J Schlapbach, MD, FCICM²,³ (ORCID 0000-0003-2281-2598)

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

Christoph Aebi¹, Verena Wyss¹, Mariama Usman¹

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

Philipp Agyeman, MD¹, Luregn J Schlapbach, MD, FCICM²,³, Eric Giannoni, MD⁴,⁵, Martin Stocker, MD⁶, Klara M Postay-Barbe, MD⁷, Ulrich Heininger, MD⁸, Sara Bernhard-Stirnemann, MD⁹, Anita Niederer-Loher, MD¹⁰, Christian Kahlert, MD¹⁰, Giancarlo Natalucci, MD¹¹, Christa Relly, MD¹², Thomas Riedel, MD¹³, Christoph Aebi, MD¹, Christoph Berger, MD¹² **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

Stéphane Paulus

Research Group (in alphabetical order):

Elizabeth Cocklin, Rebecca Jennings, Joanne Johnston, Simon Leigh, Karen Newall, Sam Romaine
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 Fink¹, Clinical Microbiologist
Additional investigators
Dr Marie Voice¹, Post doc scientist
Dr. Leo Calvo-Bado\textsuperscript{1}, Post doc scientist

\textsuperscript{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\textsuperscript{1} (all activities)

Co-investigators (in alphabetical order)

Benno Kohlmaier\textsuperscript{1} (all activities)

Nina A. Schweintzger\textsuperscript{1} (all activities)

Manfred G. Sagmeister\textsuperscript{1} (study design, consortium wide sample management)

Research team

Daniela S. Kohlfürst\textsuperscript{1} (study design)

Christoph Zurl\textsuperscript{1} (BIVA PIC)

Alexander Binder\textsuperscript{1} (grant application)

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

Susanne Hösele\textsuperscript{1}, Manuel Leitner\textsuperscript{1}, Lena Pölz\textsuperscript{1}, Glorija Rajic\textsuperscript{1},

Clinical recruitment partners (in alphabetical order):

Sebastian Bauchinger\textsuperscript{1}, Hinrich Baumgart\textsuperscript{4}, Martin Benesch\textsuperscript{3}, Astrid Ceolotto\textsuperscript{1}, Ernst Eber\textsuperscript{2}, Siegfried Gallistl\textsuperscript{1}, Gunther Gores\textsuperscript{5}, Harald Haidl\textsuperscript{1}, Almuthe Hauer\textsuperscript{1}, Christa Hude\textsuperscript{3}, Markus Keldorfer\textsuperscript{5}, Larissa Krenn\textsuperscript{4}, Heidemarie Pilch\textsuperscript{5}, Andreas Pfleger\textsuperscript{2}, Klaus Pfurtscheller\textsuperscript{4}, Gudrun
Nordberg\textsuperscript{5}, Tobias Niedrist\textsuperscript{8}, Siegfried Rödl\textsuperscript{4}, Andrea Skrabl-Baumgartner\textsuperscript{1}, Matthias Sperl\textsuperscript{7}, Laura Stampfer\textsuperscript{5}, Volker Strenger\textsuperscript{3}, Holger Till\textsuperscript{6}, Andreas Trobisch\textsuperscript{5}, Sabine Löffler\textsuperscript{5}

Author Affiliations:

\textsuperscript{1} Department of Pediatrics and Adolescent Medicine, Division of General Pediatrics, Medical University of Graz, Graz, Austria
\textsuperscript{2} Department of Pediatric Pulmonology, Medical University of Graz, Graz, Austria
\textsuperscript{3} Department of Pediatric Hematooncology, Medical University of Graz, Graz, Austria
\textsuperscript{4} Paediatric Intensive Care Unit, Medical University of Graz, Graz, Austria
\textsuperscript{5} University Clinic of Paediatrics and Adolescent Medicine Graz, Medical University Graz, Graz, Austria
\textsuperscript{6} Department of Paediatric and Adolescence Surgery, Medical University Graz, Graz, Austria
\textsuperscript{7} Department of Pediatric Orthopedics, Medical University Graz, Graz, Austria
\textsuperscript{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\textsuperscript{1,2,3} PhD, MBBS, FRCPCH, MRCP, DTM&H

Research Group

Dr Juan Emmanuel Dewez\textsuperscript{1} MD, DTM&H, MSc
Prof Martin Hibberd\textsuperscript{1} BSc, PhD
Mr David Bath\textsuperscript{2} MSc, MAppFin, BA(Hons)
Dr Alec Miners\textsuperscript{2} BA(Hons), MSc, PhD
Dr Ruud Nijman\textsuperscript{3} PhD MSc MD MRCPCCH
Dr Catherine Wedderburn\textsuperscript{2} BA, MBChB, DTM&H, MSc, MRCPCCH
Ms Anne Meierford\textsuperscript{1} MSc, BMedSc, BMBS

Dr Baptiste Leurent\textsuperscript{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\textsuperscript{1}, Michiel van der Flier\textsuperscript{1,2,3}, Marien I. de Jonge\textsuperscript{1}

**Co-investigators Radboud University Medical Center (in alphabetical order):**
Koen van Aerde\textsuperscript{1,2}, Wynand Alkema\textsuperscript{1}, Bryan van den Broek\textsuperscript{1}, Jolein Gloerich\textsuperscript{1}, Alain J. van Gool\textsuperscript{5}, Stefanie Henriet\textsuperscript{1,2}, Martijn Huijnen\textsuperscript{1}, Ria Philipsen\textsuperscript{1}, Esther Willems\textsuperscript{1}

**Investigators PeDBiG PERFORM DUTCH CLINICAL NETWORK (in alphabetical order):**
G.P.J.M. Gerrits\textsuperscript{8}, M. van Leur\textsuperscript{8}, J. Heidema\textsuperscript{4}, L. de Haan\textsuperscript{1,2}, C.J. Miedema\textsuperscript{5}, C. Neeleman\textsuperscript{1} C.C. Obihara\textsuperscript{6}, G.A. Tramper-Stranders\textsuperscript{7}

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)**
Principal Investigators

Andrew J. Pollard\textsuperscript{1,2}, Rama Kandasamy\textsuperscript{1,2}, Stéphane Paulus \textsuperscript{1,2}

Additional Investigators

Michael J. Carter\textsuperscript{1,2}, Daniel O‘Connor\textsuperscript{1,2}, Sagida Bibi\textsuperscript{1,2}, Dominic F. Kelly\textsuperscript{1,2}, Meeru Gurung\textsuperscript{3}, Stephen Thorson\textsuperscript{3}, Imran Ansari\textsuperscript{3}, David R. Murdoch\textsuperscript{4}, Shrijana Shrestha\textsuperscript{3}, Zoe Oliver\textsuperscript{5}

Author Affiliations:

\textsuperscript{1}Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, United Kingdom.

\textsuperscript{2}NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom.

\textsuperscript{3}Paediatric Research Unit, Patan Academy of Health Sciences, Kathmandu, Nepal.

\textsuperscript{4}Department of Pathology, University of Otago, Christchurch, New Zealand.

\textsuperscript{5}Department of Paediatrics, University of Oxford.

Newcastle University, Newcastle upon Tyne, (UK)

Principal Investigator:

Marieke Emonts \textsuperscript{1,2,3} (all activities)

Co-investigators

Emma Lim\textsuperscript{2,3,7} (all activities)

Lucille Valentine\textsuperscript{4}

Recruitment team (alphabetical), data-managers, and GNCH Research unit:
Karen Allen⁵, Kathryn Bell⁵, Adora Chan⁵, Stephen Crulley⁵, Kirsty Devine⁵, Daniel Fabian⁵, Sharon King⁵, Paul McAlinden⁵, Sam McDonald⁵, Anne McDonnell²,⁵, Ailsa Pickering²,⁵, Evelyn Thomson⁵, Amanda Wood⁵, Diane Wallia⁵, Phil Woodsford⁵,

Sample processing: Frances Baxter⁵, Ashley Bell⁵, Mathew Rhodes⁵

PICU recruitment

Rachel Agbeko⁸

Christine Mackerness⁸

Students MOFICHE

Bryan Baas², Lieke Kloosterhuis², Wilma Oosthoek²

Students/medical staff PERFORM

Tasnim Arif⁶, Joshua Bennet², Kalvin Collings², Ilona van der Giessen², Alex Martin², Aqeela Rashid⁶, Emily Rowlands², Gabriella de Vries², Fabian van der Velden²

Engagement work/ethics/cost effectiveness

Lucille Valentine⁴, Mike Martin⁹, Ravi Mistry², Lucille Valentine⁴

Author Affiliations:

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

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

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

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

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

⁶Great North Children’s Hospital, Paediatric Oncology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.
LMU Munich Partner (Germany)

Principal Investigator:
Ulrich von Both¹,² MD, FRCPCH (all activities)

Research group:
Laura Kolberg¹ MSc (all activities)
Manuela Zwerenz¹ MSc, Judith Buschbeck¹ PhD

Clinical recruitment partners (in alphabetical order):
Christoph Bidlingmaier³, Vera Binder⁴, Katharina Danhauser⁵, Nikolaus Haas¹⁰, Matthias Griese⁶, Tobias Feuchtinger⁴, Julia Keil⁹, Matthias Kappler⁶, Eberhard Lurz⁷, Georg Muench⁸, Karl Reiter⁹, Carola Schoen⁹

Author Affiliations:
¹Div. Paediatric Infectious Diseases, Hauner Children’s Hospital, University Hospital, Ludwig Maximilians University (LMU), Munich, Germany
²German Center for Infection Research (DZIF), Partner Site Munich, Munich, Germany
³Div. of General Paediatrics, ⁴Div. Paediatric Haematology & Oncology, ⁵Div. of Paediatric Rheumatology, ⁶Div. of Paediatric Pulmonology, ⁷Div. of Paediatric Gastroenterology, ⁸Neonatal Intensive Care Unit, ⁹Paediatric Intensive Care Unit Hauner Children’s Hospital, University
Hospital, Ludwig Maximilians University (LMU), Munich, Germany, Department Pediatric Cardiology and Pediatric Intensive Care, University Hospital, Ludwig Maximilians University (LMU), Munich, Germany

bioMérieux, France

Principal Investigator:
François Mallet

Research Group:
Karen Brengel-Pesce
Alexandre Pachot
Marine Mommert

1Open Innovation & Partnerships (OIP), bioMérieux S.A., Marcy l’Etoile, France
2Joint research unit Hospice Civils de Lyon - bioMérieux, Centre Hospitalier Lyon Sud, 165 Chemin du Grand Revoyet, 69310 Pierre-Bénite, France
3EA 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

Research Group:
Mojca Kolnik, Katarina Vincek, Tina Plankar Srovin, PhD, Natalija Bahovec, MD, Petra Prunk, MD, Veronika Osterman, MD, Tanja Avramoska, MD

Affiliations:
1Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva 2, SI-1525 Ljubljana, Slovenia
2University Childrens' Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia
3Department of Infectious Diseases and Epidemiology, Faculty of Medicine, University of Ljubljana, Slovenia
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 joint infections. Infections were defined as 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           | Ill appearance              | Oxygen saturation | Blood culture performed | Antibiotic prescription type |         |
| (self / GP / emergency services / other) | | | | | |
| Previous medical care   | Work of breathing           | Cerebrospinal fluid performed | Antibiotic prescription mode |                     |         |
| (yes, primary care / yes, this ED / yes other secondary care) | | | | | |
| Season                  | Meningeal signs             |             | Previous antibiotic treatment |                     |         |
| Arrival hours           | Focal neurology             |             |             |                     |         |
| (morning / evening / night) | | | | | |
| Comorbidity             | Non-blanching rash          |             |             |                     |         |
### 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 retraction, 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

| Dehydration | Seizures |
|-------------|----------|
- 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 | IBI 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) | CRP measured (n=17,213) | No CRP measured (n=21,267) | No CRP measured (n=21,267) |
|-------------------------|-------------------------|-----------------------------|-----------------------------|
| n (%)                   | Range (IQR)             | n (%)                       | Range (IQR)                |
| **General characteristics** |                         |                             |                            |
| Age in years, median (IQR) | 2.77 (1.29-6.02)        | 2.74 (1.31-5.28)            | 5.28                        |
| Male                     |                         |                             |                            |
| Previous chronic condition |                         |                             |                            |
| Any                      | 3332 (19.4)             | 3162 (14.9)                 | 273                         |
| Complex                  | 1138 (6.6)              | 729 (3.4)                   | 185                         |
| Referred                 | 9287 (53.9)             | 6789 (31.9)                 | 185                         |
| Triage urgency           |                         |                             |                            |
| Low: standard, non-urgent | 9794 (56.9)             | 14291 (67.2)                | 14291 (67.2)                |
| High: immediate, very urgent, intermediate | 6890 (40.0) | 9806789 (31.9) | 6329 (29.8) |
| **Feverkidstool**        |                         |                             |                            |
| Temperature in °C, median (IQR) | 37.8 (37.5-38.5)        | 37.7 (36.9-38.4)            | 2211                        |
| Fever duration in days, median (IQR) | 1.5 (0.5-3) | 1.5 (0.5-3) | 1900                        |
| Tachypnea (APLS)         | 3585 (20.8)             | 4942 (23.2)                 | 4607                        |
| Tachycardia (APLS)       | 6001 (34.9)             | 6854 (32.2)                 | 2620                        |
| Hypoxia <95%             | 762 (4.4)               | 733 (3.4)                   | 3043                        |
| Prolonged capillary refill (>3 sec) | 339 (1.9) | 84 (0.4) | 1928                        |
| Work of breathing        | 913 (5.3)               | 1732 (8.1)                  | 3176                        |
| Ill appearance           | 4742 (27.5)             | 1265 (5.9)                  | 1057                        |
| CRP in mg/L, median (IQR) | 17 (5.49) | 17 NA | 17 NA                       |
| **NICE Warning signs**   |                         |                             |                            |
| Decreased consciousness  | 148 (0.9)               | 53 (0.2)                    | 240                         |
| Meningeal signs          | 126 (0.7)               | 11 (0.1)                    | 1101                        |
| Focal neurology          | 102 (0.6)               | 31 (0.1)                    | 1081                        |
| Status epilepticus       | 51 (0.3)                | 15 (0.1)                    | 201                         |
| Rash: petechiae/non blanching | 664 (3.9) | 448 (2.1) | 3106                        |
| **Blood cultures performed** |                       |                             |                            |
| CSF performed            | 444 (2.6)               | 8 (0.0)                     | 0.0-2.6                     |
| Admission to the ward >24 hours | 6590 (38.3) | 668 (3.1) | 0.0-29.6 | 347 |
| Admission to the ICU     | 135 (0.8)               | 23 (0.1)                    | 35                          |
| Antibiotic treatment following ED visit | 6795 (39.5) | 5504 (25.9) | 16.9-273 |
| Lifesaving interventions: airway, breathing or hemodynamic support | 371 (2.2) | 112 (0.5) | 0.0-3.0 |
| Urinary tract infection  | 935 (5.4)               | 418 (1.9)                   | 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 | Complex chronic condition, n=50 |
|--------------------------------------|----------------------------------|----------------------------------|
| 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%)                       | 4 (8%)                           |
| Candida species                      | 4 (8%)                           | 4 (8%)                           |
| Viridans streptococci                | 4 (8%)                           | 3 (6%)                           |
| Klebsiella spp                       | 3 (6%)                           | 1 (2%)                           |
| Enterococcus spp                     | 3 (6%)                           | 1 (2%)                           |
| 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

A n=341

B n=24
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**

| Variables                          | OR (95% CI)* |
|-----------------------------------|--------------|
| 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**

| Variable                           | OR (95% CI)* |
|-----------------------------------|--------------|
| Complex chronic condition         | 8.83 (6.19-12.59)* |

*Significant, p<0.05

±The risk of children aged < 1 year was calculated: \( \beta_\text{age <1 year} \times \text{age in years} \).
The risk of children aged >1 years was calculated with: \( \beta_\text{age <1 year} \times 1 + \beta_\text{age ≥1 year} \times (\text{age in years} - 1) \).

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|
| 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: \(\beta_{(age <1 \text{ year})} \times \text{age in years}\).
The risk of children age ≥ 1 year was calculated: \(\beta_{(age <1 \text{ year})} \times 1 + (\text{age in years}-1) \times \beta_{(age ≥ 1 \text{ in years})}\).

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

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

Negative test for the low-risk threshold (0.1%) and positive test for the high-risk threshold (2.0%)
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|
| 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|
| Abnormal neurology | 1.54| 4.66|
| Non-blanching rash | 1.40| 4.04|
| Complex chronic condition | 2.43| 11.3|

*The risk of children aged < 1 year was calculated: \( \beta_{\text{age < 1 year}} \times \text{age in years} \). The risk of children aged < 1 year was calculated: \( \beta_{\text{age < 1 year}} \times \text{age in years} \). The risk of children aged >1 years was calculated with: \( \beta_{\text{age < 1 year}} \times 1 + \beta_{\text{age >1 year}} \times (\text{age in years} - 1) \).

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.
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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)
Clinical recruitment at Brighton and Sussex University Hospitals

Katy Fidler (Principal 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
Antonio Salas

GENVIP RESEARCH GROUP (in alphabetical order):
Fernando Álvez González, Cristina Balo Farto, Ruth Barral-Arca, María Barreiro Castro, Xabier Bello, Mirian Ben García, Sandra Carnota, Miriam Cehey-López, María José Curra-Tualal, Carlos Durán Suárez, Luisa García Vicente, Alberto Gómez-Carballa, Jose Gómez Rial, Pilar Leboráns Iglesias, Federico Martinón-Torres, Nazareth Martinón-Torres, José María Martinón Sánchez, Belén Mosquera Pérez, Jacobo Pardo-Seco, Lidia Piñeiro Rodríguez, Sara Pishedda, Sara Rey Vázquez, Irene Rivero Calle, Carmen Rodríguez-Tenreiro, Lorenzo Redondo-Collazo, Miguel Sadiki Orá, Antonio Salas, Sonia Serén Fernández, Cristina Serén Trasorras, Marisol Vilas Iglesias.

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.
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 Bārzdīna\(^1,2\), Dārta Dekšne\(^1,2\), Dace Gardovska\(^1,2\), Dagne Grāve\(^2\), 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 Svilē\(^2\), 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 Roca Isatou Sarr
Momodou Saidykhan
Saffiatou Darboe
Samba Ceesay
Umberto D’alessandro

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

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

**Principal Investigator**
Henriëtte A. Moll¹

**Research group**
Dorine M. Borensztajn¹, Nienke N. Hagedoorn, Chantal Tan¹,¹, Clementien L. Vermont², Joany Zachariasse¹

**Additional investigator**
W Dik³

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, MD¹ (ORCID 0000-0002-8339-5444), Luregn J Schlapbach, MD, FCICM²,³ (ORCID 0000-0003-2281-2598)

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

Christoph Aebi¹, Verena Wyss¹, Mariama Usman¹

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

Philipp Agyeman, MD¹, Luregn J Schlapbach, MD, FCICM²,³, Eric Giannoni, MD⁴,⁵, Martin Stocker, MD⁶, Klara M Posfay-Barbe, MD⁷, Ulrich Heininger, MD⁸, Sara Bernhard-Stirnemann, MD⁹, Anita Niederer-Loher, MD¹⁰, Christian Kahlert, MD¹⁰, Giancarlo Natalucci, MD¹¹, Christa Relly, MD¹², Thomas Riedel, MD¹³, Christoph Aebi, MD¹, Christoph Berger, MD¹² for the Swiss Pediatric Sepsis Study

**Affiliations:**

¹ Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland
Neonatal and Pediatric Intensive Care Unit, Children’s Research Center, University Children’s Hospital Zurich, University of Zurich, Zurich, Switzerland

Child Health Research Centre, University of Queensland, and Queensland Children’s Hospital, Brisbane, Australia

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

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

Department of Pediatrics, Children’s Hospital Lucerne, Lucerne, Switzerland

Pediatric Infectious Diseases Unit, Children’s Hospital of Geneva, University Hospitals of Geneva, Geneva, Switzerland

Infectious Diseases and Vaccinology, University of Basel Children’s Hospital, Basel, Switzerland

Children’s Hospital Aarau, Aarau, Switzerland

Division of Infectious Diseases and Hospital Epidemiology, Children’s Hospital of Eastern Switzerland St. Gallen, St. Gallen, Switzerland

Department of Neonatology, University Hospital Zurich, Zurich, Switzerland

Division of Infectious Diseases and Hospital Epidemiology, and Children’s Research Center, University Children’s Hospital Zurich, Switzerland

Children’s Hospital Chur, Chur, Switzerland

**Liverpool Partner**

**Principal Investigators**

Enitan D Carroll\(^1,2,3\)

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, Clinical Microbiologist
Additional investigators
Dr Marie Voice, Post doc scientist
Dr. Leo Calvo-Bado¹, 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 Zenz¹ (all activities)

Co-investigators (in alphabetical order)

Benno Kohlmaier¹ (all activities)

Nina A. Schweintzger¹ (all activities)

Manfred G. Sagmeister¹ (study design, consortium wide sample management)

Research team

Daniela S. Kohlfürst¹ (study design)

Christoph Zurl¹ (BIVA PIC)

Alexander Binder¹ (grant application)

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

Susanne Hösele¹, Manuel Leitner³, Lena Pölz¹, Glorija Rajic¹,

Clinical recruitment partners (in alphabetical order):

Sebastian Bauchinger¹, Hinrich Baumgart⁴, Martin Benesch³, Astrid Ceolotto¹, Ernst Eber², Siegfried Gallistl¹, Gunther Gores⁵, Harald Haidl¹, Almuthe Hauer¹, Christa Hude³, Markus Keldorfer⁵, Larissa Krenn⁴, Heidemarie Pilch³, Andreas Pfleger², Klaus Pfurtscheller⁴, Gudrun
Nordberg\(^5\), Tobias Niedrist\(^8\), Siegfried Rödl\(^6\), 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\(^5\) BA, MBChB, DTM&H, MSc, MRCPCH
Ms Anne Meierford MSc, BMedSc, BMBS

Dr Baptiste Leurent, 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 8, L. de Haan 1,2, C.J. Miedema 5, C. Neeleman 1 C.C. Obihara 6, G.A. Tramper-Stranders 7

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)
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:
Author Affiliations:

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

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

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

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

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

6Great North Children’s Hospital, Paediatric Oncology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom.
Version: 6.1 September 2020

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

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

9Northumbria University, Newcastle upon Tyne, United Kingdom.

LMU Munich Partner (Germany)

Principal Investigator:

Ulrich von Both1,2 MD, FRCPCH (all activities)

Research group:

Laura Kolberg¹ MSc (all activities)

Manuela Zwerenz¹ MSc, Judith Buschbeck¹ PhD

Clinical recruitment partners (in alphabetical order):

Christoph Bidlingmaier3, Vera Binder4, Katharina Danhauser5, Nikolaus Haas10, Matthias Griese6, Tobias Feuchtinger4, Julia Keil9, Matthias Kappler6, Eberhard Lurz7, Georg Muench8, Karl Reiter9, Carola Schoen9

Author Affiliations:

¹Div. Paediatric Infectious Diseases, Hauner Children’s Hospital, University Hospital, Ludwig Maximillians University (LMU), Munich, Germany

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

³Div. of General Paediatrics, ⁴Div. Paediatric Haematology & Oncology, ⁵Div. of Paediatric Rheumatology, ⁶Div. of Paediatric Pulmonology, ⁷Div. of Paediatric Gastroenterology, ⁸Neonatal Intensive Care Unit, ⁹Paediatric Intensive Care Unit Hauner Children’s Hospital, University
Hospital, Ludwig Maximilians University (LMU), Munich, Germany, 10Department Pediatric Cardiology and Pediatric Intensive Care, University Hospital, Ludwig Maximilians University (LMU), Munich, Germany

bioMérieux, France

Principal Investigator:
François Mallet1,2,3

Research Group:
Karen Brengel-Pesce1,2,3
Alexandre Pachot1
Marine Mommert1,2

1Open Innovation & Partnerships (OIP), bioMérieux S.A., Marcy l’Etoile, France
2Joint research unit Hospice Civils de Lyon - bioMérieux, Centre Hospitalier Lyon Sud, 165 Chemin du Grand Revoyet, 69310 Pierre-Bénite, France
3EA 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 Pokorn1,2,3 MD, PhD

Research Group:
Mojca Kolnik1 MD, Katarina Vincek1 MD, Tina Plankar Srovin1 MD, PhD, Natalija Bahovec1 MD, Petra Prunk1 MD, Veronika Osterman1 MD, Tanja Avramoska1 MD

Affiliations:
1Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva 2, SI-1525 Ljubljana, Slovenia
2University Childrens’ Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia
3Department of Infectious Diseases and Epidemiology, Faculty of Medicine, University of Ljubljana, Slovenia
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 Berg1, D. Schonenberg1, A.M. Barendregt1, D. Pajkrt1, M. van der Kuip1,3, A.M. van Furth1,3

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)