CLINICAL PROTOCOL

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Project full title: **Pe**rsonalised **R**isk assessment in **F**ebrile illness to **O**ptimise **R**eal-life **M**anagement across the European Union (PERFORM)

Project acronym: PERFORM

Project type of funding scheme: Large scale integrating collaborative project

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Sponsor: Imperial College London

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CONTENTS

1. LIST OF ABREVIATIONS

ATC	Anatomical Therapeutic Chemical
BIVA	Biomarker Validation studies
BIVABI	Biomarkers of Viral and Bacterial Infection
BIVA-ED	Biomarker Validation in Emergency Department
BIVA-HR	Biomarker Validation in High Risk patients
BIVA-INF	Biomarker Validation in Inflammatory patients
BIVA-PIC	Biomarker Validation in Paediatric Intensive Care
CRF	Case Report Form
CRP	C-reactive protein
ED	Emergency Department
eQTL	expression Quantitative Trait Locus
EUCLIDS	EU Childhood Life-threatening Infectious Disease Study
FBC	Full blood count
GCP	Good Clinical Practice
GENDRES Project	Genetic influence and Vitamin D levels on the individual susceptibility and prognostic to Influenza Virus, Respiratory Syncytial Virus and other Respiratory viral infections
GP	General Practitioner
ICED	Infection in the Emergency Department
ICU	Intensive Care Unit
IRIS	Immunopathology of Respiratory Infection Study
MOFICHE	Management and Outcome of Fever in children in Europe
NPV	Negative Predictive Value
PERFORM	Personalised Risk assessment in Febrile illness to Optimise Real-life Management across the European Union
PICU	Paediatric Intensive Care Unit
SBI	Serious Bacterial Infection
SOP	Standard Operational Procedure
TB	Tuberculosis

2. GENERAL INFORMATION

2.1. STUDY ORGANIGRAM

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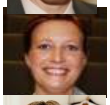
Deputy BIVA-PIC	GRAZ (AUS)	Werner Zenz	
Deputy BIVA-HR	NEWCASTLE (UK)	Marieke Emonts	
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2.2. PARTICIPATING SITES AND PRINCIPAL INVESTIGATORS

Name of participating site	Address, and local Principal Investigator
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2.3. CLINICAL NETWORK CONFIGURATION

The PERFORM clinical network will be organised into nodes, as shown in the figure below (Fig. 1).

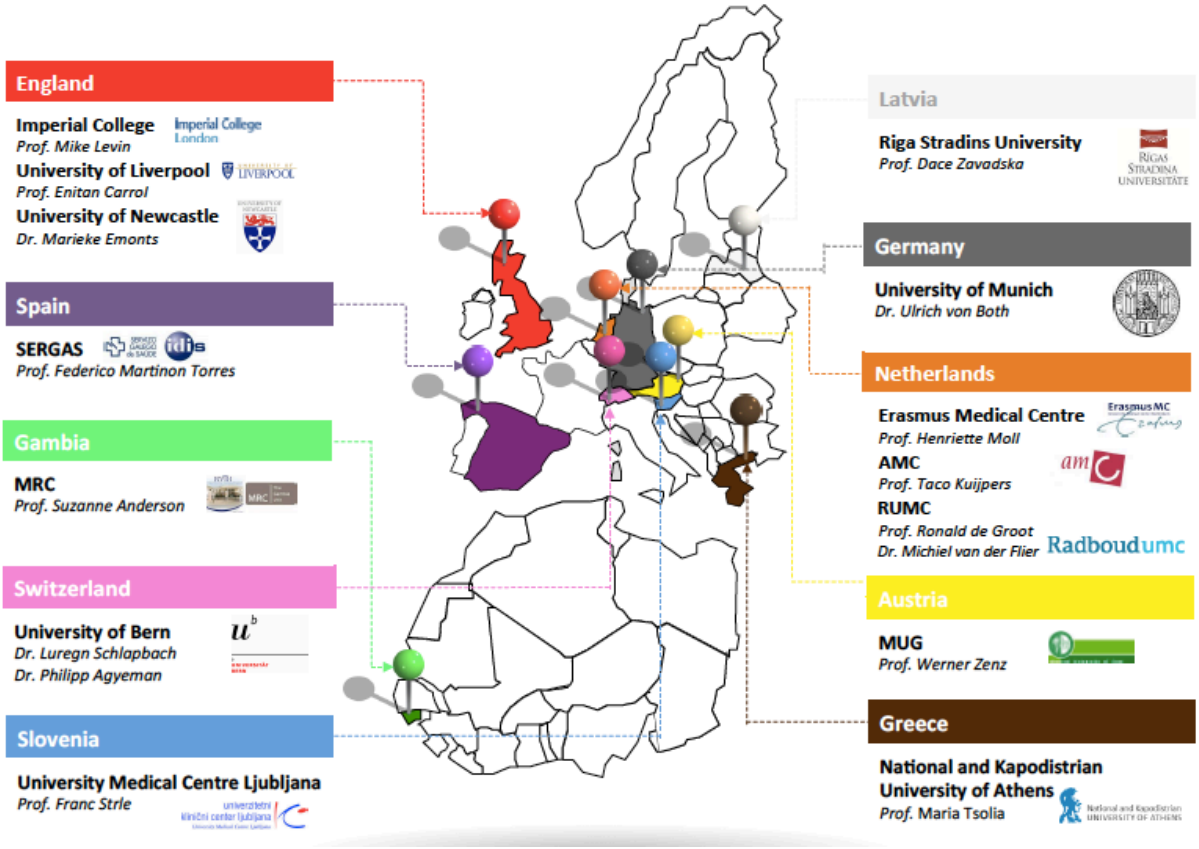

Figure 1.- Clinical network in PERFORM study

The PERFORM clinical network nodes will recruit prospective patients into one or more biomarker studies. To achieve this, clinical nodes will work with existing or new local networks of collaborating centres and investigators. The Principal Investigator in each node will take responsibility for local networks including budget and local ethics.

3. OBJECTIVES OF PERFORM

This study aims to improve the diagnosis and management of febrile children across Europe and West Africa through the development of simple diagnostic tests to discriminate febrile illnesses in children, including bacterial and viral infection and inflammatory diseases. The project will:

- Assess current management of febrile illness across Europe and West Africa using quantitative and qualitative methods (including cost-effectiveness analysis).
- Identify personalized discriminators of bacterial and viral infection and inflammatory diseases, and diagnostic signatures that distinguish severe and mild disease, using a combination of clinical phenotypic markers, host genetic markers, and biomarkers derived using transcriptomic, proteomic, and bioinformatic approaches.
- Develop simple proof-of-concept personalized tests for application at the point of care.
- Model the impact of the introduction of optimised management strategies for febrile children, in the varied healthcare settings of Europe, thus providing the evidence necessary for European and international health systems to adopt new management strategies for febrile children.

The PERFORM international clinical network constitutes a key factor for the PERFORM project as a whole and for each of the different participating countries in Europe and West Africa (Fig. 1). The network will provide all necessary patient cohorts for all working packages (Fig. 2) as needed (discovery and validation studies).

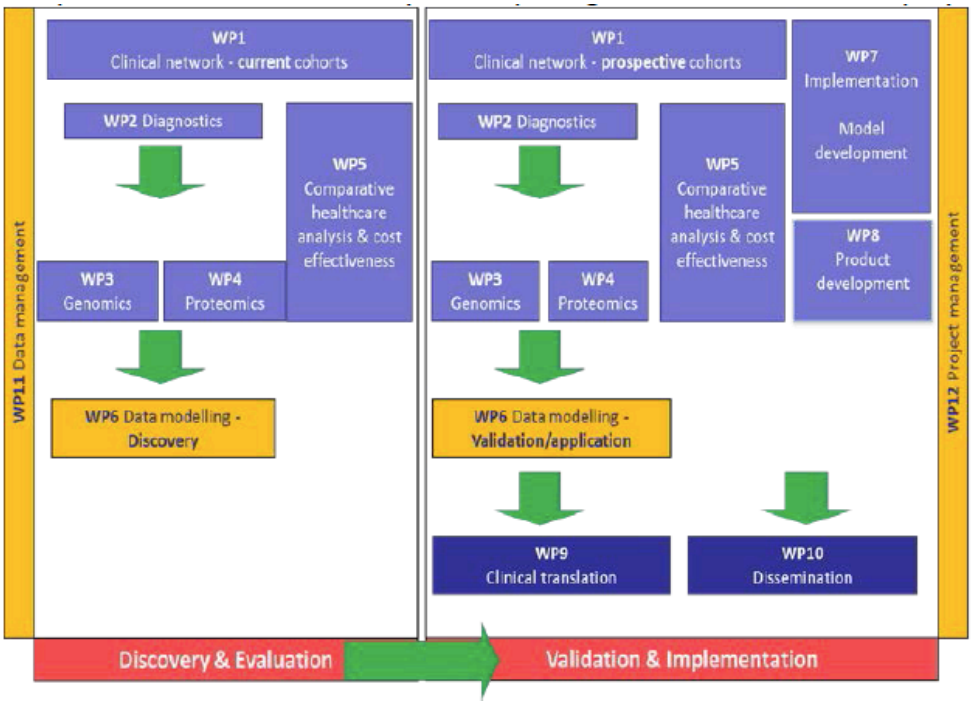

Figure 2.- Strategy and general description of the project

An established Europe-wide network of researchers in infectious diseases, molecular and cellular biology, informatics and public health will combine their expertise to improve the diagnosis and treatment of febrile patients across Europe through the application and evaluation of sophisticated new personalized medicinal, molecular, transcriptomic and proteomic methods.

Clinical data and sample banks from previous EU-funded or national funded studies undertaken by members of the consortium will be combined with samples recruited by PERFORM to create a comprehensive and well-characterised biobank.

4. ACTIONS IN PERFORM

PERFORM will achieve its objectives through the following actions:

- Detection of novel transcriptomic, proteomic and phenotypic clinical markers in existing patient cohorts with bacterial and viral infections and inflammatory diseases.
- Correlation of these biomarkers with genetic polymorphisms known to influence phenotypes of infectious disease identified in the patients' genetic sequence, including genetic changes discovered as part of the FP7-funded EUCLIDS study (www.euclids-project.eu), using expression Quantitative Trait Locus (eQTL) mapping and other approaches
- Collection of data from different European countries on current practice in management of febrile children, including investigation, hospitalization and antibiotic use in emergency departments, wards and intensive care units (ICU) in hospitals, and analysis of current costs of management.
- Evaluation of the performance of novel transcriptomic, proteomic and phenotypic markers that distinguish bacterial infection from viral infection, first in patients with "gold standard" confirmed bacterial or viral infections, and then patients with presumed bacterial or viral infections in whom existing culture based diagnostic methods have failed to assign accurate diagnosis.
- Prospective validation of the most promising transcriptomic, proteomic and phenotypic markers in cohorts of febrile patients attending primary and secondary care settings in different European countries as well as in West Africa.
- Evaluation and validation of the diagnostic biomarkers in diagnostically challenging groups of patients with immune-suppression, inflammatory diseases, hospital acquired infections and patients admitted to paediatric intensive care units (PICU).
- Development of a management algorithm for febrile children including use of the novel diagnostic markers and evaluation of its performance in the settings of different European countries.
- Cost-effectiveness analysis of the model of care for febrile patients including its effects on antibiotic use, reduction in hospital admissions and prevention of misdiagnosis.
- Initiate product development and implementation of the novel diagnostic approaches identified by our program in collaboration with our industrial and SME partners.
- Dissemination of the findings at government level to healthcare systems in different European countries for inclusion in the management of febrile children across Europe.

5. SUMMARY OF STUDIES INCLUDED IN THIS PROTOCOL

This protocol will describe three components of the patient and sample groups used in PERFORM:

1. **Biomarker discovery using pre-existing sample collections (Biomarkers of Viral and Bacterial Infection (BIVABI))**

   The BIVABI study will use transcriptomic, proteomic and metabolomic approaches to discover biomarkers that distinguish the different phenotypes of childhood febrile illness, including children with infection (viral, bacterial, mycobacterial, other) and inflammation (including Kawasaki disease, juvenile idiopathic arthritis).

2. **Observational study of patient management and outcome, using large, aggregated datasets and not involving sample collection (Management and Outcome of Fever in children in Europe (MOFICHE))**

   MOFICHE is an observational study assessing the management and outcome of children presenting to Emergency Departments (ED) with fever across Europe. This study will use large departmental datasets to collect information on nearly 50,000 febrile episodes in 12 ED's in 9 European countries. **This study will use large-scale, pseudo-anonymized departmental data to safeguard identity of participants and will not involve consented patient recruitment; nor will it use patient samples**. Data included in MOFICHE will be based on that collected as part of routine clinical care. No tissue samples will be taken for research use, and informed consent will not be needed for the MOFICHE study.

3. **Biomarker validation using prospectively recruited patients and patient samples (Biomarker Validation studies (BIVA))**

   Prospective, observational studies will recruit a validation group of children with infectious and inflammatory conditions. Research blood samples will be taken and analysed in order to validate biomarker findings identified in the BIVABI discovery study. There are 4 related prospective studies. The BIVA-ED study will recruit the majority of the children. The other studies will target more challenging groups who will not be adequately recruited in an ED study, in order to increase numbers. The studies are:

   - Biomarker Validation in Emergency Department (BIVA-ED)
   - Biomarker Validation in Paediatric Intensive Care (BIVA-PIC)
   - Biomarker Validation in High Risk patients (BIVA-HR)
   - Biomarker Validation in Inflammatory patients (BIVA-INF)

6. DETAILED STUDY PROTOCOLS

a. Biomarkers of Viral and Bacterial Infection (BIVABI) – For biomarker discovery

SUMMARY

The BIVABI study will use transcriptomic, proteomic and metabolomic approaches to discover biomarkers that distinguish the different phenotypes of childhood febrile illness, including children with infection (viral, bacterial, mycobacterial, other) and inflammation (including Kawasaki disease, juvenile idiopathic arthritis).

PATIENT GROUPS

Study	Lead country	Target diseases	DNA	metabolomics	transcriptomic	proteomics
EU action for diseases of poverty	UK	Tuberculosis, other infections, latent TB infection, HIV		✓	✓	✓
EU Childhood Life-threatening Infectious Disease Study (EUCLIDS)	UK	Bacterial and viral illness	✓	✓	✓	✓
ESPID/IKDGC Kawasaki Disease study Consortium	UK	Kawasaki disease	✓	✓	✓	✓
ESPID Meningococcal study Consortium	UK	Meningococcal meningitis and sepsis	✓	✓	✓	✓
International IKDGC collaborative study	NL	Rheumatological and inflammatory diseases	✓	✓	✓	✓
GENDRES	Spain	Respiratory viral infections	✓	✓	✓	✓
UCAN	Canada	Childhood Arthritis and Rheumatic Diseases	✓	✓	✓	✓
VIRGO	NL	Respiratory Virgo viral infections	✓	✓	✓	✓
Immunopathology of Respiratory Infection Study (IRIS)	UK	All febrile illness	✓	✓	✓	✓
Infection in the Emergency Department (ICED)	UK	All febrile illness	✓	✓	✓	✓
Biomarkers of Acute Severe Infection Consortium (BASIC)	UK	All critical illness	✓	✓	✓	✓
Swiss Pediatric Sepsis Study	CH	Bacteremia and systemic inflammatory response syndrome	✓	✓	✓	✓
IPD study (pneumococcal disease) Nepal	Malawi UK	Pneumonia and meningitis Pneumonia	✓ ✓	 ✓	 ✓	 ✓
Oxford Vaccine Studies	UK	Paediatric vaccine recipients	✓	✓	✓	✓

Table 1.- Studies which will support BIVABI study

Clinical data and stored samples from previous EU-funded or national funded studies undertaken by members of the consortium will be combined to form a discovery set that will be used to identify transcriptomic, proteomic, metabolomic and clinical markers that distinguish the different causes of febrile illness, including bacterial and viral infection and inflammatory disease. **This study will use only those samples that have been collected as part of an ethically-approved study, in which written consent has already been obtained which permits their future use in ethically-approved studies.** No new patients will be recruited for the BIVABI study.
The retrospective collections used in BIVABI will be primarily drawn from samples collected in the studies summarized in Table 1.

STUDY DESIGN

The discovery group will include patients from across the spectrum of febrile disease, including bacterial infection, viral infection, inflammatory (non-infectious) disease, and children with febrile illness of unclear aetiology. The discovery group will also include control samples from children without infection, taken as part of ethically approved studies. We will apply phenotyping algorithms for patient selection and categorization, using defined clinical criteria, and application of both conventional microbial diagnostics and molecular diagnostic approaches to detect bacterial and viral genomes (Fig. 3). We will categorise patients into phenotypic groups, including bacterial infection, viral infection and inflammatory diseases, from which stored samples will be drawn for biomarker discovery. After analysis of RNA expression by microarray and RNAseq, and proteomic and metabolomic analysis by multiplex immunoassays and mass spectrometry respectively, bioinformatics analysis will be undertaken to identify small diagnostic signatures that accurately identify bacterial infection, viral infection and inflammatory diseases. We will identify diagnostic signatures that distinguish severe and mild disease.

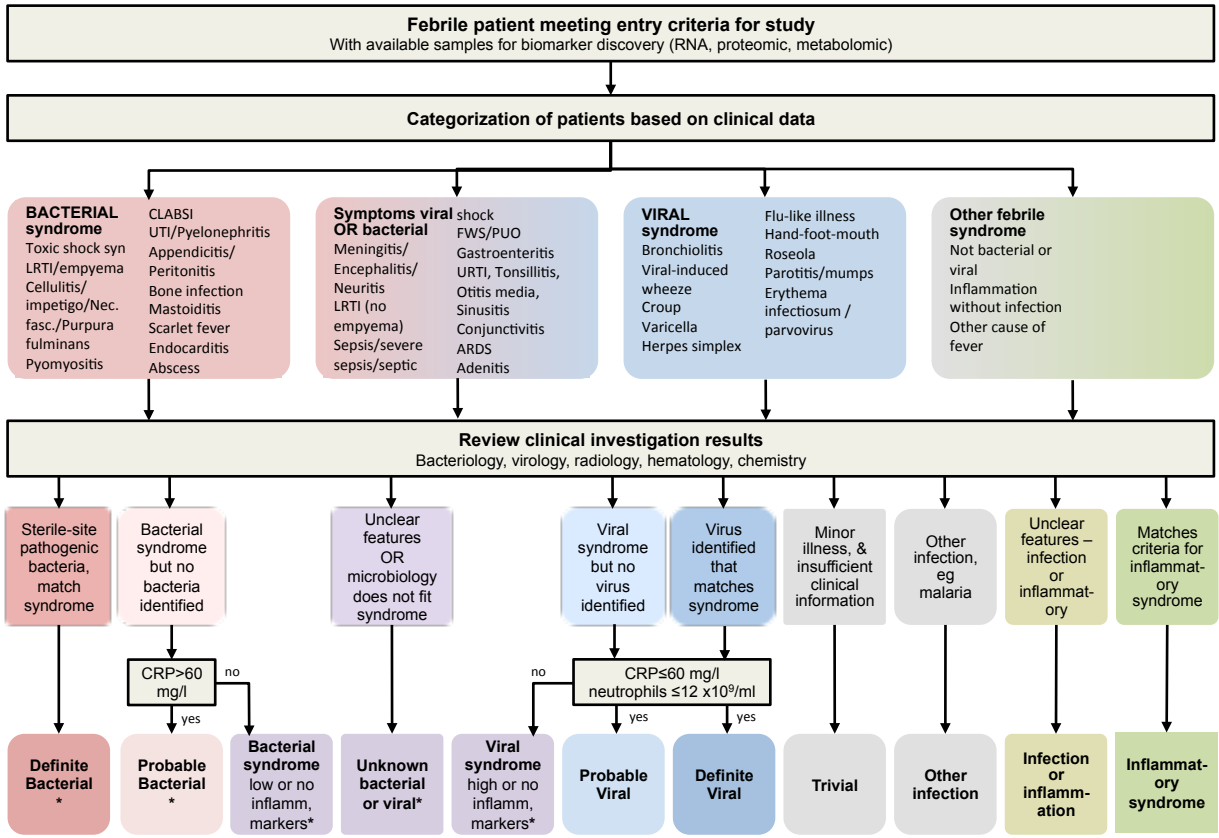

Fig. 3.- Phenotyping flow chart for febrile patients in the BIVABI study

b. Protocol for the Management and Outcome of Fever in children in Europe (MOFICHE)

SUMMARY

This is an observational study assessing the management and outcome of children presenting to Emergency Departments (ED) with fever across Europe. This study will use large departmental datasets to collect information on nearly 50,000 febrile episodes in 12 ED's in 9 European countries. **This study will use large-scale, pseudo-anonymized departmental data, and will not involve consented patient recruitment; nor will it use patient samples**. Data included in MOFICHE will be based on that collected as part of routine clinical care. No tissue samples will be taken for research use, and informed consent will not be needed for the MOFICHE study.

BACKGROUND

The organisation of Emergency care varies between European countries. In several European countries acute medical care is delivered by general practitioners (who can act as gatekeepers), primary care paediatricians, emergency departments and out of office GP services. The number of children presenting to the ED varies across Europe, influenced by the number and availability of GPs primary care paediatricians and the preferences of parents[1,2] . Differences in morbidity and mortality have been described [3,4].

Fever is the most frequent reason for a child to attend the paediatric emergency department (20-40%). The majority of febrile children suffer from self-limiting illness, with serious bacterial infections (SBI) ranging from 5% to 30% [5,6]. Pneumonia and urinary tract infection constitute the majority of serious infections, and septicaemia/meningitis are currently rare [7]. Current guidelines state that there is no indication for oral antibiotics in children with fever without a clear diagnosis of bacterial infection [6]. Nevertheless, antibiotic prescription rates in febrile children remain high [8, 9] and there is marked variation between countries [10].

Furthermore the diagnostic work-up and the number of febrile children hospitalised varies between settings [11]. This study will investigate the nature and cause of variations in management practices and outcomes for febrile children in the ED in 9 European countries.

OBJECTIVES

- To evaluate the management of febrile children visiting the ED in different European countries.
- To study the determinants of diversity between countries in hospital admission, antibiotic prescription and investigations related to clinical signs and symptoms in febrile children.
- To provide data for comparative health evaluation and cost effectiveness modelling

STUDY DESIGN

An observational study on febrile children aged 0- 18 years visiting the emergency department of 12 different hospitals in 9 European countries.

STUDY POPULATION

Inclusion criteria:

Febrile children aged 0- 18 years visiting the emergency care.

Febrile child: fever measured at ED or history of (measured) fever less than 3 consecutive days prior to presentation at emergency department,

In the participating hospitals more than 50.000 febrile children visit the ED annually (1200-29.000 per emergency setting) (Table 2).

Participant	Name of institution	City	Country	Children to ED/year	Number with fever (%)	Number admitted (%)
1-IMPERIAL	Imperial College Healthcare NHS Trust	London	UK	26,000	8,666 (33%)	2,340 (12%)
2-LIV	Alder Hey Children's NHS Foundation Trust	Liverpool	UK	60,000	8,600 (14%)	554 (6%)
3- UNEW	Great North Children's Hospital	Newcastle-upon-Tyne	UK	30,000	4,500 (15%)	700-1000 (2-3%)
4-EMC	Erasmus Medical Centre	Rotterdam	Netherlands	7,000	1,400 (20%)	1,050 (15%)
5-AMC	Amsterdam Medical Centre	Amsterdam	Netherlands	6,000	1,200 (20%)	550 (9%)
6- NKUA	National and Kapodistrian University of Athens	Athens	Greece	40,000	2,600 (6%)	6,500 (16%)
7-RUMC	Nijmegen and PedBIG sites	Nijmegen	Netherlands	12,000	3,600 (30%)	1,500 (12%)
8- SEGAS	Hospital Universitario de Santiago de Compostela	Santiago de Compostela	Spain	33,000	19,080 (57,8%)	1,172 (3,6%)
9-MUG	Medical University of Graz	Graz	Austria	18,000	6,000 (33%)	4,000 (22%)
10- UKCL	University Klinicini Centre Ljubljana	Ljubljana	Slovenia			
11-RSU	Riga Stradins University	Riga	Latvia	42,750	29,900(69,9%)	4,865 (11.4%)
12-UBERN	Medizinische Kinderklinik, University of Bern	Bern	Switzerland	20,000	6,000 (25%)	1,800 (9%)
13-LMU	Ludwig Maximilian University Munich	Munich	Germany	20,000	6,400 (33%)	2,200 (11%)

Table 2.- Data of febrile children which visit ED in the participating sites per year

STUDY PROCEDURES

Data of all febrile children will be flagged at triage and a standardised dataset will be collected. ED data will be extracted from the standardized clinical notes or electronic records of the individual febrile child. This will include data on age, gender, temperature, vital signs, triage category, clinical alarming signs, grade of doctor/under supervision of consultant, admission or discharge and the following outcome measures: investigations (blood, cerebrospinal fluid, nasopharyngeal aspirates

/throat swabs, stools or urine tests and result), antibiotics prescribed and final diagnosis/working diagnosis at discharge.

Data protection

This study will use large-scale, pseudo-anonymized departmental data. Each febrile patient will be identified with a subject code in the ED (3 digit study center code- 5 digit individual patient code (starting at P00001 for each center)). Data analysis in MOFICHE will differ from an audit in that there will not be complete anonymisation of data analysed, in order that we can identify patients that make repeated ED visits, and match patients in MOFICHE with those in other studies. We will maintain pseudo-anonymization of the MOFICHE data (birthdate will be included), so that patients will not be fully anonymised.

We will not seek informed consent from individual patients in the MOFICHE study, as the data will consist of routinely-collected departmental-level data on all febrile patients seen in that location. We will collect data for the MOFICHE study only at sites that have ethics committee and local clearance for this protocol, for the analysis of the pseudo-anonymized data.

Working diagnosis and infectious focus

Each patient will be coded in the following areas, using an electronic questionnaire filled in at the ED [***Appendix 1/A***]:

- working diagnosis of the likely cause of the febrile illness (including definite/probable bacterial, definite/probable viral, inflammatory causes, unknown (see figure 3))
- description of the focus and syndrome (for instance upper/lower respiratory infection, urinary tract infection, enteric infection, cutaneous infection, septicaemia/meningitis, osteomyelitis, other, no clear focus or spot diagnosis)
- treatment received (including details of antibiotics used and duration)
- *follow-up* at discharge of the ED (ambulant, hospitalization, intensive care, no follow-up, ED readmission within 5 days)

Data quality

Each individual WP1 participant will carry the responsibility to collect the required data in his/her ED setting. Moreover, each WP1 participant will develop a procedure to monitor the completeness of the data collection and the quality of the data. Furthermore, all centers will have a limited number of key parameters checked (sex, age, working diagnosis category; in 5% of inclusion in MOFICHE) 100% in other substudies) by an independent trained investigator. For publication of the results of the study, it is important that the process of data collection and quality assurance is documented by each WP1 participant.

Description of the setting (to be completed once for each setting)

In addition to individual patient-level data, we will collect general information on each setting for the comparative health service study including information on the organisation, management and delivery of acute and emergency paediatric services; human resources; summary data on paediatric attendances (where available); contextual information (rural/ inner city, university/ teaching/ non-

teaching hospital, catchment area, country, indications for hospitalization/short-stay). [***Appendix 1/B***].

OUTCOMES

Primary outcomes: Antibiotic prescription, hospitalization and number/type of investigations. Number of children re-attending within 5 days of the first hospital presentation.

The antibiotic prescription rate is defined as the number of children that receive antibiotics among the number of febrile children. We will use the Anatomical Therapeutic Chemical (ATC) classification system to group antibiotics into narrow and broad spectrum [12]. Broad spectrum antibiotics include broad spectrum penicillins, cephalosporins, and macrolides (J01CR, J01DC, J01DD and J01F (without J01FA01). Narrow spectrum antibiotics include beta lactamase sensitive penicillins (J01CE), first generation cephalosporins (J01DB) and macrolides (J01FA01).

Investigations are defined as routine (FBC, CRP, urine analyses) and extended diagnostic tests (Chest X-Ray, culture of blood or liquor, viral tests)

Secondary outcome: number of prescriptions of broad spectrum antibiotics versus the number of prescription of narrow spectrum antibiotics (dose in 24 hours). Clinical and general characteristics of antibiotic prescription, hospitalization duration of stay and investigations.

ANALYSIS

We will describe clinical and general characteristics of the patients at the different ED's. We will define the number of children with alarming signs for serious infections [6, 13], quantify a risk assessment for serious infections for each child, using the feverkidstool (http://www.erasmusmc.nl/formulieren/feverkidstool/?lang=en [13]) and describe the number of investigations and hospitalizations. We will quantify antibiotic prescription duration, antibiotic prescription rate, defined as the number of children with antibiotics among the number of febrile children and the number of prescription of broad spectrum antibiotics versus the number of prescription of narrow spectrum antibiotics with 95% confidence interval in different settings and for different working diagnoses. For antibiotic prescription, diagnostic tests and hospitalization we will use logistic regression analyses to evaluate influencing factors as age, triage urgency, clinical symptoms/alarming signs, CRP and other diagnostic tests, working diagnosis, season and setting.

SAMPLE SIZE ESTIMATION

We expect to include nearly 50,000 febrile children in the study, ranging from 6,000 in Austria to 50,000 in the UK, with at most 5% of missing data. Pilot data indicate an overall antibiotic prescription rate of 20%, a hospitalization rate of 10%, a CRP rate of 15% and a blood culture rate of 5%. Applying a commonly used rule of thumb of 10 events per variable, we expect that the study sample is large enough to analyse 120 determinants for the most prevalent outcome of antibiotic prescription and 25 determinants (0.1 determinants per 5% of 6000 events) for the least prevalent outcome of blood culture with sufficient accuracy [14, 15].

c. Overview of Biomarker Validation Studies (BIVA):

a) Overview of approach

We will use prospective, observational studies to recruit children with infectious and inflammatory diseases. Research samples, including blood, urine, stool (in the case of gastroenteritis), nasopharyngeal/throat swab will be taken for detailed pathogen analysis, and for host DNA, RNA, protein and metabolomics analysis at the earliest time point possible as well as 48 hours later and at follow-up in order to assess how the biomarkers change with illness progression and/or treatment. We will use a variety of RNA and protein estimation techniques to validate biomarkers identified in the BIVABI study as predictive of bacterial infection, viral infection or inflammatory disease. We will also use sequencing techniques to validate the predictive value of host genetic changes in predisposition to illness.

We envisage that a new diagnostic test that can discriminate between bacterial, viral and mycobacterial infections will play a key role in the management of febrile children in Emergency Deparments. In addition, a diagnostic marker that is able to discriminate between bacterial, viral and mycobacterial infections will be particularly useful in specific groups of children in whom clinical diagnosis is particularly difficult or identification of bactieral infection is particularly important to make. This includes children on intensive care units who often develop fevers and end up on 2nd or 3rd line antibiotics with no positive microbiological diagnosis; immunocompromised children who develop fevers often without localising signs; and children with a range of inflammatory diagnoses such as Kawasaki Disease and Juvenile Idiopathic Arthritis, whose initial presentation is difficult to discriminate from bacterial infection. We will conduct separate sub-studies specifically aimed at targeting these children:

There are 4 related prospective studies. The studies are:

- Biomarker Validation in Emergency Department (BIVA-ED)
- Biomarker Validation in Paediatric Intensive Care (BIVA-PIC)
- Biomarker Validation in High Risk patients (BIVA-HR)
- Biomarker Validation in Inflammatory patients (BIVA-INF)

The potential performance of the identified biomarkers (in the ED setting) will be interpreted using data on numbers and types of presentations established by the MOFICHE study, to better model the potential impact of the biomarkers on management of febrile children.

b) Analysis approach

We will use data from the biomarker validation studies to calculate sensitivity and specificity of the new biomarkers to discriminate viral and bacterial infections and inflammatory diseases. Using comparative methods, the added value of new biomarkers to usual diagnostic work-up will be estimated. Additionally, the MOFICHE study data will allow us to calculate positive and negative predictive values of the biomarkers in different healthcare settings, using information on the numbers of febrile children presenting, and the proportion with bacterial, viral and inflammatory conditions.

c) Sample sizes

We will recruit a minimum of 3,000 children to the BIVA-ED study, in order to capture sufficient children with confirmed bacterial infection.

In order to increase the number of children with less common but important febrile illnesses who will not be adequately recruited in an ED study, we will recruit harder-to-reach groups using targeted studies as follows:

- We will recruit 500 additional critically ill children - Biomarker Validation in Paediatric Intensive Care (BIVA-PIC)
- We will recruit 200 additional children at high-risk of bacterial illness through primary or secondary immunodeficiency - Biomarker Validation in High Risk patients (BIVA-HR)
- We will recruit 150 additional children with a range of inflammatory diagnoses, whose initial presentation is difficult to discriminate from bacterial infection - Biomarker Validation in Inflammatory patients (BIVA-INF)

A sample size calculation is shown for each study below.

d) Laboratory methodologies

We will undertake molecular diagnostics to improve distinction between bacterial and viral diseases in both the discovery and prospective validation work packages. We will use PCR to detect a panel of respiratory viruses on nasopharyngeal or throat swabs. We will use PCR to detect a panel of viral and bacterial pathogens in blood. In addition, all patients will have blood culture performed. These molecular assays both extend and complement the routine microbiological investigations undertaken in the participating hospitals. We will also undertake validation of candidate protein biomarkers using multiplex immunoassay methodology in the validation stage of the project.

The proteomic analysis will receive plasma/urine samples from well-characterised patients and undertake shotgun and targeted proteomic analysis using LC-MS/MS in selected well defined groups of patients. Immunoassays will be developed against the protein biomarkers selected to finally test these in the validation phase.

Furthermore, we will undertake RNA transcriptomic analysis by microarray, RNASeq analysis and analysis of micro RNA and non-coding RNAs from the RNASeq data. We will identify diagnostic host transcriptional profiles, and validate selected sets of these RNA biomarkers using RT-PCR and nanotechnology. We will concentrate investigations on mRNA, but other RNA species discovered during the sequencing (such as micro RNA), that have the potential for translation will also be investigated.

Specific techniques will be:

- RT-PCR, microarray and RNA-seq for RNA
- Mass spectrometry-based metabolomics
- Sequencing to analyse host DNA using targeted and high-throughput (whole exome sequence WES) approaches
- Multiplex immunoassays once candidates have been identified

e) Detail of the Biomarker Validation Studies

1. Biomarker Validation in Emergency Department (BIVA-ED)

This study will recruit 3,000 febrile children attending the Emergency Department or primary care in selected centres in the PERFORM consortium. Participating sites will vary with the numbers of patients recruited and the prevalence of serious bacterial infection.

SUMMARY

The PERFORM consortium will recruit at least 3,000 febrile children attending ED, as part of an observational study. Patients will have a set of samples taken for research. We will take an approach to recruitment to ensure the study cohort is highly representative to the true population of children presenting with febrile illness. At each recruitment site, we will therefore recruit as comprehensively as possible all febrile children who attend the ED who are willing to participate.

ENDPOINTS

Children's involvement in the study will last for the duration of their illness episode, until discharge, and they will be followed up at 48-72 hours and at 28 days (+/- 2 days) post-admission to document any re-attendances or re-admissions. 48 hours and 28-day follow up will be by visit if clinically needed, telephone interview or text messaging depending on the preference of the parent/guardian.

OBJECTIVES

Evaluation and validation of the diagnostic biomarkers in diagnostically challenging groups of patients presenting to the ED suspected of infections or febrile children presenting to the ED. Development of a management algorithm for febrile children including use of the novel diagnostic marker and evaluation of its performance in the settings of different European countries.

STUDY DESIGN

A prospective observational study of febrile children attending the Emergency Department in selected centres in the PERFORM consortium.

STUDY POPULATION

All febrile children <18 years (16 years depending in the setting) of age presenting to Emergency Departments at participating sites:

Inclusion Criteria

- All children <18 years presenting to the Emergency department with fever >38ºC, or a history of fever (within 3 days), in whom the attending clinician determines the need for blood sampling or whom parents give consent for bloods taken for research purposes
- All children <18 years presenting to the Emergency department suspected of infection. We will include children with the full spectrum of disease severity, and will include children with co-morbidities, as these children are at higher risk of antibiotic treatment and are therefore a particular target for study.

- We will collect samples from afebrile control children who are having blood tests for reasons other than for investigation of infectious or inflammatory illness.

Exclusion Criteria

- Children from whom parent/legal guardian signed consent is not received
- For healthy control children only: febrile illness or vaccination within the last 3 weeks.

STUDY PROCEDURES

See 8.b ENROLLING AND CONSENTING PATIENTS INTO PROSPECTIVE STUDIES section.

A complete history and physical examination will be performed. Information on degree and duration of fever, previous antibiotic use and previous existing co-morbidities will be collected (including also all items for the febrile children in the MOFICHE study). If the attending paediatrician decides that a full sepsis work-up is indicated (full blood count, CRP, blood culture, urine, lumbar puncture), then a nasopharyngeal or throat swab and research samples will be taken in addition. Research samples will include blood, a sample for respiratory secretions (nasopharyngeal aspirate or throat swab) a urine sample, and in the case of gastroenteritis, a stool sample. A full description of the research samples taken is shown in section 8.c.

We will use a variety of recruitment approaches in order to achieve our aim of maximising the comprehensive recruitment of febrile children.

- Where febrile patients are having blood tests as part of their clinically directed assessment, we will align research blood sample collection with the same blood draw used for clinically indicated tests
- Only in the following situations will we approach patients and their families to consider research blood sampling that is not taken simultaneously with clinically indicated tests:
    - Patients with central access lines from which blood can be drawn painlessly, without needle puncture
    - Patients in whom antibiotics were prescribed following clinical assessment, but in whom no clinically indicated bloods were deemed necessary. It is important for the study to include these patients, in order to understand whether blood biomarkers might identify what proportion of these patients did actually need antibiotic treatment
- We will take written consent and assent from older children and their families prior to sample collection when there are personnel from the research team available to speak to families, in a non-emergency situation and where the research approach will not disrupt the clinical management of the patient.
- In circumstances where the child's clinical condition necessitates immediate investigation and management, we will use a 'deferred consent approach', such that blood tests are carried out before written consent is obtained, through collection of research samples at the same time as the first clinical samples are taken. Parents and children will be approached at the earliest opportunity after sample collection to seek consent and assent from the children and their families. If no consent is given, all samples taken will be destroyed.

Where written informed consent is obtained, our study of biomarkers will be carried out on blood left over from the initial clinical sample once all necessary clinical tests have been performed.

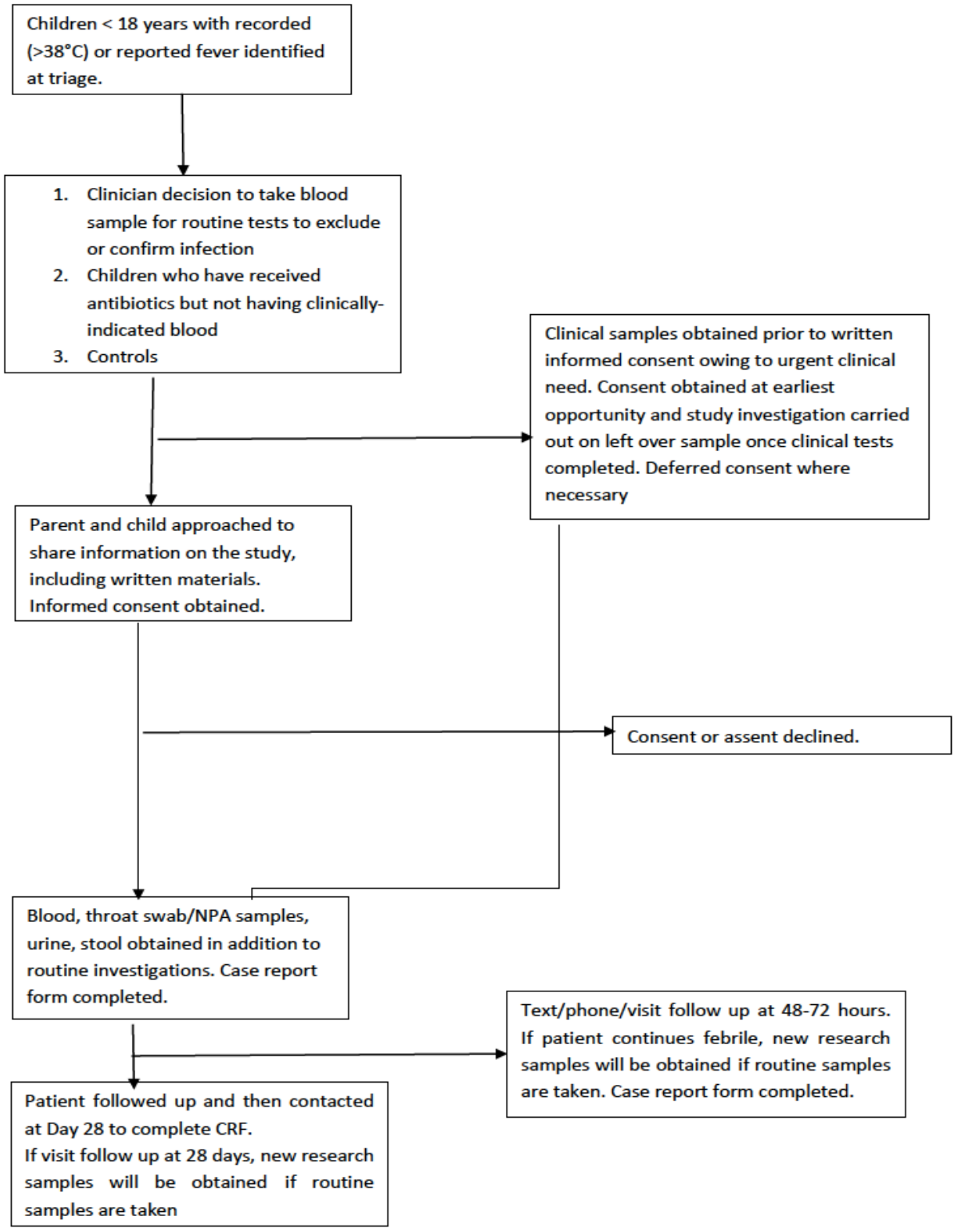

Figure 4.- Schematic of the flow chart of the study

TIME POINTS

Patients will be followed up to discharge to ensure collection of all relevant clinical and laboratory data. Furthermore, there will be a text/phone/visit 48-72 hours after discharge follow-up and an optional 28 days follow-up if the patient remains feverish or illness is on-going.

The majority of patients in the BIVA-ED study will have a single set of research samples, which will be collected at their point of entry to the study. Where children recruited to the BIVA-ED study are followed-up in convalescence, and are having clinically-indicated bloods taken, we will request permission to take a 2nd set of research samples at the time of follow up (Discharge/Day 28).

Patients may withdraw from the study at any stage and a withdrawal CRF should be completed. Data collected up to the time of their withdrawal from the study will be included in the analysis. Clinical samples for those patients who withdraw or do not give deferred consent will be destroyed.

OUTCOMES

Primary:

The validation of clinical, proteomic and transcriptomic biomarkers for diagnosis of febrile illness, including markers of bacterial and viral infection (confirmed by culture and/or molecular microbiology) and inflammatory conditions.

Secondary:

The identification of biomarkers predictive of disease severity in children with bacterial and viral infection, using a combination of clinical phenotypic markers and proteomic and transcriptomic biomarker signature discovery.

ANALYSIS

Sensitivity and specificity of the new biomarkers and the added value of new biomarkers to usual clinical and diagnostic work up to discriminate viral and bacterial infections.

SAMPLE SIZE ESTIMATION

Using pilot data on gene expression microarrays, we have calculated that transcript biomarkers can distinguish bacterial from viral illness with a sensitivity of 95% and a specificity of 95% (Herberg *et al*. JAMA submitted). Assuming that validation will be undertaken using the same platform, we have based sample size calculations on the performance (sensitivity and specificity) of the biomarkers in the pilot study.

We aimed to validate the performance of the candidate biomarker, with 95% confidence intervals of <5%. Assuming there will be similar biomarker performance in the pilot and validation datasets, a sample size of 150 bacterial patients and 150 viral patients would provide 95% confidence intervals spanning 3.8% for the point estimate of sensitivity and 3.8% of the point estimate for specificity, calculated using the binomial distribution.

As SBI is estimated to represent 5% (5-20%)[16] of illnesses in the group of febrile children (fever over 38 ºC), we would need to recruit 3000 febrile children, to study 150 children with SBI. Based on studies successfully completed at these sites, we expect to meet the target of 3000 recruited febrile children in 36 months, and this is our recruitment target for the BIVA-ED study.

Additionally, if we assume a prevalence of SBI of 5%, based on pilot data, we would expect a test with 95% specificity and sensitivity to have a negative predictive value (NPV) of 99%. In order to show with high probability (0.9) that NPV would not be lower than 99%, a total sample size of at least 1404 patients is required, with a prevalence of SBI of 0.05, assuming binomial sampling. This total sample size we expect to meet and exceed. We will recruit 3000 children in the BIVA-ED study as a minimum. Additional recruitment above 3000 children will improve stratified analyses based on clinical syndrome and disease severity.

2. Protocol for Biomarker Validation in Paediatric Intensive Care (BIVA-PIC)

This study will recruit children admitted to Intensive Care Units with severe infection. Selected centres in the PERFORM consortium will recruit 500 subjects.

SUMMARY

We will recruit at least 500 children admitted to Intensive Care Units with fever, suspected or confirmed infection. We will also recruit children suffering with healthcare-associated infections and non-infectious children (children after operative procedures) to use as an internal control group of critically ill children without infection. Written consent from parents will be taken prior to sample collection. In an emergency situation we will use deferred consent. Patients and parents will be approached at the earliest opportunity. Research samples will be taken immediately after the first clinical samples are taken.

BACKGROUND

Paediatric Intensive Care Units take care of the most severely ill children in hospital. Due to high suspicion or fear of severe bacterial infection in febrile PICU patients, broad spectrum antibiotics are given frequently, whilst awaiting the results of a sepsis work-up, as inadequate treatment of sepsis further endangers essential organs i.e the kidneys. On the other hand, unneccesary antibiotic treatment in febrile children with viral illness can be damaging to patients, for instance causing drug reactions, nephrotoxicity and propagating antibiotic resistance.

The routine sepsis work-up includes full blood count and other blood markers including CRP, blood culture, urine, throat swabs and often lumbar puncture to obtain cerebrospinal fluid. We plan to conduct an observational study and collect comprehensive information about clinical and laboratory data generated during routine care, treatment and follow-up of febrile children and children suspected of infection at the PICU.

These data will be used to validate biomarkers that distinguish severe bacterial infections from other life threatening causes of fever and could result in the development of a new diagnostic approach, which will reduce the quantity of antibiotic use, preserve organ functions and reduce antibiotic resistance.

OBJECTIVE

Identify discriminators of bacterial and viral infection in children with a suspected/confirmed severe infection presenting with febrile illness.

To evaluate the diagnostic value of Sepsis-3 Criteria in children compared to existing sepsis scores and to develop adjusted Sepsis-3 Criteria based on the needs of a paediatric population.

STUDY DESIGN

A prospective observational study of data and sample collection of children admitted to Intensive Care Units with fever, suspected or confirmed infection in selected centres in the PERFORM consortium

STUDY POPULATION

All febrile children or children with suspected infection aged from birth to 18 years of age admitted into the paediatric intensive care units (PICUs) at participating sites.

Inclusion Criteria

Any patient, aged birth to 18 years, who requires a blood test for clinical reasons or consents for research bloods to be taken, AND who has:

- **a)** Fever **and/or**
- **b)** Suspected viral or bacterial infection **and/or**
- **c)** Healthcare-associated infection **and/or**
- **d)** Inflammatory disease requiring PICU admission **and/or**
- **e)** Immunodeficiency (primary and secondary (steroids, chemotherapy, biological, post-transplant) **and**
- **f)** Already been admitted to intensive care unit **and/or**
- **g)** Illness of sufficient severity that would warrant admission to an intensive care unit and Informed consent in writing from parent(s) or legally-acceptable representative(s) and, informed assent from the subject (if age appropriate according to local requirements)
- **h)** Patients admitted for elective surgey will act as controls

Exclusion Criteria

- Patients who do not give consent or withdraw

STUDY PROCEDURES

See 8.b ENROLLING AND CONSENTING PATIENTS INTO PROSPECTIVE STUDIES section.

After written informed consent from parents/guardians and patients, when applicable, data of history and physical examination will be collected. Information on degree and duration of fever, previous antibiotic use, previous existing co-morbidities, analytic results as well as PICU specific data (severity, organ support, outcomes) information will be collected.

In circumstances where the child's clinical condition necessitates immediate investigation and management, such that blood tests are carried out before written consent is obtained (at sites where deferred consent is approved by local ethics boards), parents and children will be approached at the earliest opportunity. Where written informed consent is obtained, our study of biomarkers will be carried out on blood left over from the initial sample once all necessary clinical tests have been performed.

A 2nd set of research samples (as taken at first presentation) will be taken 48 hours after admission.

Children will be followed up for at least 28 days after recruitment or until 28 days post admission. If reasonable a clinical visit will be preferred, otherwise, the parents will be phoned. Outcome at that time will be recorded (readmissions, hospital acquired infection, ongoing disease, mortality). If

clinical samples are taken at this point, a 3rd set of research samples (as taken at first presentation) will be collected for research.

Patients may withdraw from the study at any stage and a withdrawal CRF should be completed. Data collected up to the time of their withdrawal from the study will be included in the analysis. Clinical samples for those patients who withdraw or do not give deferred consent will be destroyed.

OUTCOMES

The identification of serious bacterial or viral infection (confirmed by culture and/or molecular microbiology), or inflammatory disease.

ANALYSIS

We will calculate the sensitivity and specificity of the new biomarkers and the added value of new biomarkers to usual diagnostic work up (see case definitions for defined gold standards) to discriminate severe viral and bacterial infections in children admitted to Intensive Care Units.[17,18]

SAMPLE SIZE ESTIMATION

The BIVA-PIC study aims to evaluate the performance of our biomarkers in the group of patients that need admission to PICU. Our pilot transcript biomarkers had a sensitivity of 95% and a specificity of 95% (Herberg *et al*. JAMA submitted). However, we have based our sample size calculations for BIVA-PIC on a sensitivity of 90% and a specificity of 90%, to allow for inferior biomarker performance in severly ill patients, while assuming that validation will be undertaken using the same platform. We aimed to recruit a sample size which would allow validation of the sensitivity and specificity with <10% margin of error.

Basing calculations on the binomial distribution (α=0.05), for a biomarker with sensitivity of 90% and a specificity of 90%, we would require a sample size of 70 bacterial patients and 70 non-bacterial patients to be able to calculate sensitivity and specificity in the prospective dataset, with 95% confidence intervals spanning the point estimate + or - 7%.

As SBI is estimated to represent 14% of the patients admitted in PICU with disease due to an infectious agent (EUCLIDS data Graz 2015), we would need to recruit 500 febrile children, to study 70 children with SBI. Based on studies successfully completed at our sites, we expect to meet the target of 500 recruited children.

3. Protocol for Biomarker Validation in High Risk patients (BIVA-HR)

This study will recruit children who are immunocompromised. Selected centres in the PERFORM consortium will recruit 200 subjects

SUMMARY

The PERFORM consortium will recruit at least 200 episodes of febrile children who are immunocompromised attending ED or admitted on the appropriate wards, as part of an observational study. Patients will have a set of samples taken for research at presentation, at the same blood draw alongside their usual clinical tests; a 2nd set of research samples at 48 hours after admission and a 3rd set of research samples at least 28 days after recruitment or until 28 days post admission if clinical samples are taken at this point.

We will take an approach to recruitment that maximises the representation of the recruited sample to the true population of immunocompromised children presenting with febrile illness. At each recruitment site, we will therefore recruit as comprehensively as possible all febrile immunocompromised children having bloods taken for clinical reasons, using methods as described for BIVA-ED.

Patients can participate for multiple episodes. Consent will be taken with the first episode, to include future episodes with the same underlying condition, for the duration of the PERFORM project.

ENDPOINTS

Children will be followed up for 48-72 hours and at least 28 days after recruitment or until 28 days post admission. Outcome at that time will be recorded (cure, ongoing disease, mortality). When clinical samples will be taken at that point, we will also take a further set of research samples, as at enrollment.

BACKGROUND

Immunocompromised children (either primary or secondary) are at increased risk of infection with a variety of pathogens. These require a more vigilant approach than otherwise healthy individuals. Presenting symptoms can be minor and atypical. This results in frequent use of broad spectrum antibiotics and/or antifungals. Also antivirals are used, particularly in primary immunodeficient patients requiring haematopoietic stem cell transplant. While providing broad cover to treat potential infection, these drugs have toxic side effects. Challenges in diagnostics in this group include the use of prophylactic antibiotics making it difficult to identify a causative pathogen using conventional diagnostics. As these patients are more likely to experience several episodes with fever and to receive antimicrobial therapy, they are at increased risk of developing antimicrobial-resistant micro-organisms. Improved targeting of antimicrobial therapy might help prevent this. However, development of diagnostic biomarkers in this group is made more difficult by the changing dynamics in the immune system at different stages of treatment.

OBJECTIVE

Evaluation and validation of the diagnostic biomarkers in diagnostically challenging groups of immunocompromised patients with fever

Development of a management algorithm for febrile children including use of the novel diagnostic marker and evaluation of its performance in the settings of different European countries.

STUDY DESIGN

A prospective observational study of data and sample collection of immunocompromised in selected centres in the PERFORM consortium

STUDY POPULATION

All febrile immunocompromised children from birth to 18 years of age presenting to Emergency Departments or admitted to appropriate wards at participating sites.

Inclusion Criteria
All immunocompromised children from birth to 18 years presenting to the Emergency department or admitted in other appropriate wards or ICU, with fever, or a history of fever, or suspected of infection in whom the attending clinician determines the need for blood sampling or consent for research bloods to be taken has been achieved.

Exclusion Criteria

- Patients who withdraw or do not give consent

STUDY PROCEDURES

See 8.b ENROLLING AND CONSENTING PATIENTS INTO PROSPECTIVE STUDIES section.

After written informed parental consent, a complete history and physical examination will be performed. Information on degree and duration of fever, previous antibiotic use and previous existing co-morbidities will be collected. If the attending paediatrician decides that a full sepsis work-up is indicated (full blood count, CRP, blood culture, urine), then a nasopharyngeal swab/throat swab, urine and blood sample for other markers of SBI will also be performed.

In circumstances where the child's clinical condition necessitates immediate investigation and management, such that blood tests are carried out before written consent is obtained, parents and children will be approached at the earliest opportunity. Where written informed consent is obtained, our study of biomarkers will be carried out on blood left over from the initial sample once all necessary clinical tests have been performed.

Patients will be followed up to discharge to ensure collection of all relevant clinical and laboratory data.

Patients may withdraw from the study at any stage and a withdrawal CRF should be completed. Data collected up to the time of their withdrawal from the study will be included in the analysis. Clinical samples for those patients who withdraw or do not give deferred consent will be destroyed.

OUTCOMES

The identification of serious bacterial or viral infection (confirmed by culture and/or molecular microbiology).

ANALYSIS

Sensitivity and specificity of the new biomarkers and the added value of new biomarkers to usual clinical signs and diagnostic work up to discriminate viral and bacterial infections in diagnostically challenging groups of immunocompromised patients with fever

SAMPLE SIZE ESTIMATION

The BIVA-HR study aims to evaluate the performance of our biomarkers in the challenging group of immunocompromised patients. Our pilot transcript biomarkers have a sensitivity of 95% and a specificity of 95% for detection of bacterial infection (Herberg *et al*. JAMA submitted). However, we have based the sample size calculations for BIVA-HR on sensitivity of 90% and specificity of 90% to allow for inferior biomarker performance in immunocompromised patients, while assuming that validation will be undertaken using the same platform. We have considered the feasibility to recruit large numbers of patients in this hard-to-reach group, based on the number of children seen by the consortium members, and have considered the number needed for successful validation of sensitivity and specificity. Based on studies successfully completed at our sites, we expect to meet the target of 200 recruited children in 36 months.

As SBI is estimated to represent ~15% (literature reports 9-18%)[19-22] of febrile immunocompromised patients, therefore with 200 febrile children recruited, we would expect to study 30 children with SBI. Basing calculations on the binomial distribution (α=0.05), for a biomarker with sensitivity of 90% and a specificity of 90%, with a sample size of 30 bacterial patients and 30 non-bacterial patients, we would be able to calculate sensitivity and specificity in the prospective dataset, with 95% confidence intervals spanning the point estimate + or - 11%.

4. Protocol for Biomarker Validation in Inflammatory patients (BIVA-INF)

This study will recruit children who have inflammatory/rheumatological disorders that mimic infection. Selected centres within the PERFORM consortium as well as in centres of collaborating partners (IKDGC and UCAN) who have agreed to share data and materials will recruit 200 subjects.

SUMMARY

The PERFORM consortium will recruit at least 200 episodes of children who have a treatment-naïve presentation of inflammatory/rheumatological disorders attending ED or admitted on the appropriate wards, as part of an observational and/or local treatment study protocol. Patients will have a set of samples taken for research at presentation, at the same blood draw alongside their usual clinical tests; a 2nd set of research samples at 48 hours after admission and a 3rd set of research samples at least 28 days after recruitment or until 28 days post admission if clinical samples are taken at this point. Since some will have fever during admission and others a history of febrile episodes, the number of true febrile cases will be about 25%. The inflammatory markers and signatures will be required to consolidate the inflammatory signatures within the cohort (including children diagnosed with Kawasaki disease, systemic JIA or treatment-naïve JIA).

We will take an approach to recruitment that maximises the representation of the recruited sample to the true population of inflammatory/rheumatological disorders in children presenting with febrile illness, using methods as described for BIVA-ED.

Patients can participate for multiple episodes. Consent will be taken with the first episode, to include future episodes with the same underlying condition, for the duration of the PERFORM project.

ENDPOINTS

Children will be followed up for 48-72 hours and at least 28 days after recruitment or until 28 days post admission for the PERFORM study (telephone/text/visit if discharged). Outcome at that time will be recorded (cure, ongoing disease, mortality). When clinical samples will be taken at that point, we will also take samples for research.

BACKGROUND

Inflammatory/rheumatological disorders are a great mimic of infection. Several clinical and laboratory features are common to both inflammatory/rheumatological disorders and infection. Constitutional symptoms such as fever, malaise, arthralgia, myalgia and weight loss, and laboratory features such as normocytic normochromic anaemia, peripheral blood leucocytosis, thrombocytosis, raised erthyrocyte sedimentation rate (ESR) and C reactive protein (CRP) are encountered in both. Some patients, especially early in the course of their illness, present with isolated systemic manifestations posing a diagnostic challenge. Conversely, systemic inflammatory response is not seen in most patients with localised forms of inflammatory/rheumatological disorders.

The treatment of inflammatory/rheumatological disorders entails the use of immunosuppressive drugs, and the consequences of not recognising infection can be disastrous. Thus, it is mandatory to perform a full and appropriate infection screen in all patients with suspected

inflammatory/rheumatological disorders especially those who present with systemic inflammatory features.

OBJECTIVE

Evaluation and validation of the diagnostic biomarkers in diagnostically challenging groups of patients with inflammatory/rheumatological disorders presenting with fever

STUDY DESIGN

A prospective observational study of data and sample collection of patients with inflammatory/rheumatological disorders in selected centres in the PERFORM consortium

STUDY POPULATION

All febrile children from birth to 18 years of age with inflammatory/rheumatological disorders presenting to Emergency Departments or admitted on appropriate wards at participating sites.

Inclusion Criteria

All children with (or suspicion of) inflammatory/rheumatological disorders from birth to 18 years presenting to the Emergency department or admitted in other appropriate wards with fever, or a history of fever, in whom the attending clinician determines the need for blood sampling because of disease diagnosed or consent for research bloods to be taken has been achieved.

Exclusion Criteria

- Patients who withdraw or do not give consent

STUDY PROCEDURES

See 8.2 ENROLLING AND CONSENTING PATIENTS INTO PROSPECTIVE STUDIES section.

After written informed parental consent, a complete history and physical examination will be performed. Information on degree and duration of fever, previous antibiotic use and previous existing co-morbidities will be collected. If the attending paediatrician decides that a full sepsis work-up is indicated (full blood count, CRP, blood culture, urine), then a nasopharyngeal/throat swab, urine and blood sample for other markers of SBI will also be performed.

In circumstances where the child's clinical condition necessitates immediate investigation and management, such that blood tests are carried out before written consent is obtained, parents and children will be approached at the earliest opportunity. Where written informed consent is obtained, our study of biomarkers will be carried out on blood left over from the initial sample once all necessary clinical tests have been performed.

Patients will be followed up to discharge to ensure collection of all relevant clinical and laboratory data.

Patients may withdraw from the study at any stage and a withdrawal CRF should be completed. Data collected up to the time of their withdrawal from the study will be included in the analysis. Clinical samples for those patients who withdraw or do not give deferred consent will be destroyed.

OUTCOMES

The identification of inflammatory syndromes, consistent with international agreed definitions.

ANALYSIS

Sensitivity and specificity of the new biomarkers and the added value of new biomarkers to usual clinical signs and diagnostic work up to discriminate severe viral and bacterial infections in children admitted with inflammatory/rheumatological disorders presenting with fever.

SAMPLE SIZE ESTIMATION

The BIVA-INF study aims to evaluate the performance of our biomarkers in patients with a range of inflammatory/rheumatological disorders. Our pilot transcript biomarkers have a sensitivity of 90% and specificity for discrimination of bacterial infection from JIA and Henoch Schonlein Purpura, and 94% sensitivity and specificity for discrimination of bacterial infection and Systemic Lupus Erythematosus (using adjusted thresholds). We will carry out a case-control study, using targeted recruitment of children referred to tertiary rheumatological centres.

We have based the sample size calculations for BIVA-INF on the lower estimates of sensitivity and specificity (90%), whilst assuming that validation will be undertaken using the same platform. We have considered the feasibility to recruit large numbers of patients with inflammatory conditions, based on the number of children seen by the consortium members, and have considered the number needed for successful validation of sensitivity and specificity. We aim to recruit 150 recruited children in 36 months, to match the 150 children with bacterial infection recruited in the BIVA-ED study.

Basing calculations on the binomial distribution (α=0.05), for a biomarker with sensitivity of 90% and a specificity of 90%, with a sample size of 150 bacterial patients and 150 inflammatory patients, we would be able to calculate sensitivity and specificity in the prospective dataset, with 95% confidence intervals spanning the point estimate + or - 5%.

f) STUDY DATA BIVA studies

a. CLINICAL RECORD FORMS AND DATA COLLECTION

A unique PERFORM study number will be given to all patients included in the study. The study number and date of birth will be used to identify patients.

A patient identification log will be kept in the site file to allow future location of the patient. This information will be only accessible to the local clinical research team:

- Hospital number
- Patient name
- Date of birth
- Date of consent
- PERFORM study number

All necessary documents (informed consent forms, paper clinical record forms, protocol, etc) will be available with local R&D approval. A paper copy of the clinical record form will be used to collect prospective data. The fields will match those on the database.

b. INTERNET DATABASE

A web-based database (Redcap) will be developed that incorporates EU security requirements for data protection and storage.

Different levels of access will be established with personal login/password. The responsibilities for different personnel with respect to the database are as follows;

The *Principal investigator* of each node must sign off each recruit with respect to data entry including phenotype.

The *Principal investigator* and *subinvestigator* will also be able to see/modify/ his/her locally recruited patients and export their own anonymised existing dataset into a different programme (such as Excel)

The *Coordinator* will be allowed to see / modify / export all anonymised information from the node and will also be allowed to open/close the patient records and to generate queries regarding patient data.

The data entry (study nurses/datamangers) will also be able to see/modify/ and enter data for his/her locally recruited patients.

Administrator: allowed to see and modify all, generate queries, export anonymised global data

Study User: allowed to see all, generate queries, export anonymised global data

Pop-up menus with the definitions for each of the items requested in the electronic case report form will be available.

7. IMPLEMENTATION

The implementation stage of the study will run concurrently with the recruitment of the validation cohort although it is anticipated that implementation will extend beyond the recruitment stage. The potential healthcare benefits of the RNA signatures and protein biomarkers will be identified and their role and effect on patient care will be evaluated.

The guidelines developed by the EMA on clinical evaluation of diagnostic agents www.ema.europa.eu and the draft Guidance for industry FDA staff and clinical laboratories: Framework for Regulatory Oversight of Laboratory Developed Tests (LDTs) www.fda.gov will be followed for the implementation of diagnostic tests and clinical guidelines.

Public health management (including a cost effectiveness analysis) and the effect of introduction of the new diagnostic approach on healthcare in the diverse healthcare setting of Europe will also be evaluated.

Industrial and biotechnology partners will be engaged to develop a clinically applicable prototype test and model its effect on healthcare, to provide information to national and international agencies on roll out of an improved model of healthcare for febrile children across Europe.

8. REGULATORY AND ADMINISTRATIVE ISSUES

a. ETHICAL APPROVAL

The Chief Investigator will obtain approval from the National Research Ethics Committee (NRES). Each participating site will obtain written local Trust approval (SSA), a copy of which will be sent to the Chief Investigator before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Other sites from outside the UK (including West Africa) will obtain their own national ethical approval and local site agreements to implement the study protocol.

b. ENROLLING AND CONSENT

Patients will be enrolled from the Emergency Department, hospital wards and paediatric intensive care if they fulfil the study inclusion criteria for the biomarker group.

Consent for children to enter any of the biomarker studies will be sought from parents or guardians only after a full explanation has been given, an information sheet offered and time allowed for consideration and to make questions. Informed consent can be obtained by any member of the clinical research team.

Signed parental (or guardian) and (where appropriate) patient consent will be obtained for all children for agreement to take part in the study. The right of the participant and their parent/legal guardian to refuse to participate without giving reasons will be respected.
Once the child's parents or guardians have been informed of the study they will have time to discuss with each other and ask the research team any questions about the research. After having adequate time to consider the study and they are happy to take part, written documented consent will be obtained. A copy of the signed informed consent form will be given to parents/legal guardian.

We declare that:

1. Physicians, researchers and supporting staff involved in the research have the knowledge and skills to perform this research;
2. All staff contributing to this research have received sufficient information about the study;
3. The infrastructure at the 12 hospitals is suitable to perform the research adequately;
4. No other research projects will be hindered by this research; and
5. Enough patients can be included in the study

a) DEFERRED CONSENT

For optimal respect of patient's autonomy, seeking consent before study participation is preferable. However, in emergency research this is often not possible. Emergency research represents an exceptional situation in which the mechanisms of the consent process may need to be modified, but the social contract between researcher and research subject must be respected in order to provide a safeguard against unethical research. If data obtained in emergency situations without consent

cannot be used, and selection bias is thus introduced, study results may be less robust and future patients may not benefit. While this premise cannot provide an argument for including data when research subjects expressly deny consent, it does make an ethically valid case for including samples or data collected before consent has been taken, and where such explicit denial of consent does not exist.

There is a lack of significant evidence in many aspects of PICU and Emergency care management, due to paucity of randomised controlled trials in this setting. This means that the sickest children are subjected to evolved, anecdotal medical practice. It is essential that new research and therapeutic strategies are based on the most robust scientific understanding of critical illness, which is what our study aims to advance. In particular, our study uses state-of-the-art techniques to identify the pathogens causing critical illness. Without study samples taken before or close to the time of antibiotic treatment, pathogen identification is made difficult.

Our study also aims to define potential biomarkers associated with critical illness, and deriving a robust marker of critical illness requires that samples are collected before treatments (such as fluids, inotropes) are initiated which may affect the results. We aim to recruit patients as close to presentation as possible. As the blood sample volumes in this study are small and would not constitute a harmful intervention, we will implement a system of deferred consent in emergency admissions if parents are not initially present, or a critical situation makes discussion of research inappropriate. If parents do not agree to participate they only have to indicate it to the personnel of the study. In the same way if parents require additional information, this will be provided by research team and as consequence informed consent will be required. This is an approach which has been used successfully in several other UK observational and interventional studies of childhood critical illness in Emergency Departments and in paediatric intensive care units. This process has been successful in increasing the number of recruits.

We expect that our deferred consent approach will improve the representation of the full range of febrile illnesses in the study, and thus allow us to carry out a more meaningful validation of our bacterial and viral diagnostic biomarkers, discovered in the BIVABI study.

Refer to the findings from the CONNECT study:

https://www.liverpool.ac.uk/media/livacuk/instituteofpsychology/Research,without,prior,consent,in,trials,investigating,the,emergency,treatment,of,critically,ill,children,CONNECT,guidance,July,2015.pdf

Research or clinical staff trained in the project will collect baseline samples alongside clinical samples. No data or further sample would be collected until consent has been obtained. Parents or guardians will be approached for consent at the earliest appropriate opportunity during their hospital admission. If consent is given the child would be enrolled in the study, given a study number and subsequent research samples and data will be collected. If parents/guardians do not wish or are unable to consent, or study staff is unable to make contact with parents/guardians, the samples will be destroyed. Centres will take consent according to their country's legislation.

b) WAIVER OF INFORMED CONSENT FOR MOFICHE STUDY

We believe that the rules laid down in the Medical Research Involving Human Subjects Act do not apply to this research proposal because the research does not involve any analysis, intervention, strategy or any other act, beside routine care.

The MOFICHE study is an international observational study. It involves the creation of a large, multicenter database with routinely collected electronic patient information. During the study, patients will not undergo any intervention beside routine care at the ED. Data will be shared with the scientific coordinator of the project. The database will be pseudo-anonymised (no personal information of the patients is available beside a unique identifier code), but not fully anonymised, in order that we can identify patients that make repeated ED visits, and match patients in MOFICHE with those in other studies. The data in MOFICHE will be transferred through an encrypted file to ensure safety.

c) INFORMED CONSENT FOR FUTURE RESEARCH

The PERFORM study will use samples obtained in previous ethically approved studies or prospectively in the PERFORM study. Samples from earlier studies will be used only if written consent for future use of samples was obtained at the time of patient recruitment into previous studies.

We will request written consent for future research use of any leftover research samples for children prospectively recruited into PERFORM. All future such research will be approved by an ethics committee.

Standards of operating practices (SOPs) for sample and data management will be used to both safeguard subject's identity and to ensure samples are uniformally collected, processed and stored.

c. SAMPLE TYPES

Children taking part in the study will have the best available diagnostic assessment through a combination of clinical diagnostic laboratory tests and research tests. Tests performed in the clinical laboratory will be targeted towards the presenting syndrome, for instance, children with suspected urinary tract infection will have urine culture, children with lower respiratory tract infections will undergo chest radiographs. All febrile children included in the study will have blood cultures and inflammatory markers (including full blood count and CRP) performed by the clinical diagnostic laboratories. In addition we aim to collect the following research sample set on patients recruited to the biomarker validation studies:

- Blood culture
- Blood for RNA collected in RNA-preserving medium (1.0ml-2.5ml depending on body weight)
- EDTA blood, which will be divided into sub-aliquots as follows (1.0ml-2.5ml on body weight):
    - microbiological work-up using molecular approaches (whole blood) to identify bacterial and viral pathogens in blood (200µl aliquot)
    - disease biomarker studies based on host markers including proteins (plasma) (100µl aliquots after centrifugation)
    - correlation of clinical syndrome with host genetic markers using host DNA (extracted from the cell pellet)
- non-invasive sample for respiratory secretions (nasopharyngeal or throat swab)

- urine sample for host metabolomic biomarker studies (using left-over of clinical samples where possible)
- stool sample for pathogen detection (using left-over of clinical samples where possible)

d. BLOOD SAMPLING VOLUME RESTRICTIONS

Blood sampling volume restrictions will comply with the recommendations of the European Union for the blood loss associated with pediatric research (European Commission, 2008) and the World Health Organization guidelines "Blood Sample Volumes in Child Health Research: Review of Safe Limits" [23].

Volumes will be calculated based on patient's weight and only term neonates will be suitable for recruitment as follows [23]:

Body weight (in kg)	Volume of blood allowed for extraction
2.4 - ≤ 3.7	2ml
3.7 - ≤ 4.9	3ml
4.9 - ≤ 6.2	4ml
>6.2	5ml

e. CONFIDENTIALITY

The Principal Investigator will preserve the confidentiality of participants taking part in the study and will follow the Data Protection Act 1998.

PERFORM project will respect the subjects' rights to privacy and will ensure the confidentiality of their medical information in accordance with all applicable laws and regulations. The Declaration of Helsinki and the convention of the Council of Europe on Human Rights and Biomedicine, the ICH-GCP guidelines and national guidelines will be carefully respected.

All personal data and individual clinical information will be retained within the standard information system, and all analysis of clinical and outcome data will be conducted using codified identifiers and without reference to individual patient information. The identified code list of each project will be retained in the research centre with limited access to researchers involved in the project. Once each project has ended that codification list will be safeguarded by the principal investigator during legally required time.

All of the databases utilised by the study will be password protected and encrypted in conformance with current national and EU standards for data handling.

The confidentiality of records that could identify subjects will be protected at all times. To ensure the confidentiality of records they will be kept in secure storage areas.

All analysis performed on patient samples will be conducted on codified samples, identifiable only by a study number, and with all source data and clinical records held separately.

f. INDEMNITY

Imperial College London holds insurance policies which apply to the design and management of the study. The NHS indemnity scheme and professional indemnity will apply to the conduct of the study.

g. SPONSOR

Imperial College London will act as the main sponsor for this study.

h. FUNDING

European Commission (H2020)

Grant Number- 668303 **Pe**rsonalised **R**isk assessment in **F**ebrile illness to **O**ptimise **R**eal-life **M**anagement across the European Union (PERFORM)

i. DATA PROTECTION, HANDLING AND RETENTION

i. Data protection

Genetic analysis of patient samples will be conducted on pseudo-anonymised samples, identifiable only by a study number and with all source data and clinical records held separately. The link between patient and samples will only be known to the local study data management team. All personal data and individual clinical information will be retained within the standard information systems of each participating hospital, all analysis of clinical and outcome data will be conducted using subject codes and without reference to individual patient information. All the partner institutions will follow their own established procedures for data protection and this study will conform to the national and European standards required for each partner institution.

ii. Data handling

All databases utilised by the study will be password protected, encrypted and conform with current national and EU standards for data handling. Imperial College is the co-ordinating centre for data handling and all data will be handled in keeping with Imperial College's Data protection policy and codes of practice. The College Data Protection Officer has been informed about the data and measures to ensure that confidentiality of electronic data is maintained.

iii. Data retention

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study

j. AUDITS

The Chief Investigator of the study will delegate recruitment at other sites in the UK to local principal investigators. This may be delegated further at local sites to research nurses, data managers and sub-investigators. Any delegation will be recorded on delegation logs. It will be the responsibility of local PI's to ensure individuals are trained adequately. In addition to the protocol, standard operating

procedures will be created for specific tasks including sample collection, processing and shipment. Source documents (hospital record or Electronic Patient Record) will be kept at local sites as described previously. Data input will be monitored at each site and globally between sites to ensure accuracy. Documented audits will be performed regularly by a member of the research team.

In addition, The Joint Research Office, part of Imperial Academic Health Science Centre, audits the studies it sponsors ensure adherence to GCP and compliance with the NHS Research Governance Framework for Health and Social Care (2nd edition).

10. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through the Chief Investigator. Local issues will be managed by PIs.

11. PUBLICATION POLICY

All publications and presentations relating to the study will be authorised by the Chief Investigator and according to the publication policy of Imperial College and of the other universities involved. If there are named authors, these will include at least the trial's Chief Investigator and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy. Authorship of parallel studies initiated outside of the main research team will be according to the individuals involved in the project but must acknowledge the contribution of any of the main study researchers involved.

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APPENDIX 1A – MOFICHE and common information for biomarker studies CRF

*** Green only apply for BIVA-ED and other biomarker studies

Signed consent/assent Date___/___/___ Time: __:__ Number of parents signed: ___

Study samples

- PAXgene RNA volume: ...ml	1st sample Date of collection	___/___/___Time: __:__ Tª:__ºC
	2nd sample Date of collection	___/___/___Time: __:__ Tª:__ºC
	3rd sample Date of collection	___/___/___Time: __:__ Tª:__ºC
- EDTA (volume: ...ml)	1st sample Date of collection	___/___/___Time: __:__ Tª:__ºC
☐ finger prick	2nd sample Date of collection	___/___/___Time: __:__ Tª:__ºC
	3rd sample Date of collection	___/___/___Time: __:__ Tª:__ºC
- Nasopharyngeal/throat swab	1st sample Date of collection	___/___/___Time: __:__ Tª:__ºC
	2nd sample Date of collection	___/___/___Time: __:__ Tª:__ºC
	3rd sample Date of collection	___/___/___Time: __:__ Tª:__ºC
- Urine	1st sample Date of collection	___/___/___Time:__:__ Tª:__ºC
	2nd sample Date of collection	___/___/___Time: __:__ Tª:__ºC
	3rd sample Date of collection	___/___/___Time: __:__ Tª:__ºC
- Stools	1st sample Date of collection	___/___/___Time:__:__ Tª:__ºC
	2nd sample Date of collection	___/___/___Time: __:__ Tª:__ºC

General questions

- Code of hospital, name and country* (automatically inserted)
- Subject number and episode number.
- Date of registration ddmmyyyy, automatically inserted
- Date of visit ddmmyyyy
- Sought medical/nurse care in previous 48 hours no/yes/unknown-not stated
- Date of previous ED visit for the same complaint in the previous 5 days no visit/[date]/unknown-not stated
- Gender child [**required field]** male/female
- Date of birth [**required field]** ddmmyyyy
- Referral GP/self/emergency medical service/other hospital or specialist/ unknown-not stated, if other, please specify
- Comorbidity**none/cardiac/pulmonary/immunodeficiency/malignancy/neurologic/psychomot or retardation/prematurity [gestational age]/unknown or not stated/other.
  Please specify comorbidity when present…….***Comorbidity as stated in the ED charts or previous history form .***
  Defined as a chronic underlying condition that is expected to last at least 1 year. Adapted from Simon et al., Pediatrics, 2014.
- Triage code immediate, very urgent, urgent, standard, non-urgent/unknown-not stated

***SMH, London, UK**
LIV, Liverpool, UK
UNEW, Newcastle, UK
EMC, Rotterdam, the Netherlands
NKUA, Athens, Greece
RUMC, Nijmegen, the Netherlands
SERGAS, Santiago de Compostela, Spain
MUG, Graz, Austria
RSU, Riga, Latvia
AMC, Amsterdam, the Netherlands
LMU, München, Germany
UKCL, Ljubljana, Slovenia

Specific disease questions (history based)

- Antibiotic use in the last 7 days? no/yes/unknown-not stated
- Duration of fever: < 24 hours, 24-48, longer than 48 hours [specify in hours/days] unknown-not stated
- Duration of fever if less then 48 hours:…..hours:
- Parental concern no/yes/unknown-not stated
- Rigors no/yes/unknown-not stated
- Myalgia no/yes/unknown-not stated
- Pallor according to caregiver no/yes/unknown-not stated
- Decreased intake no/yes/unknown-not stated
- Wheeze no/yes/unknown-not stated
- Cough no/yes/unknown-not stated
- Difficulty breathing no/yes/unknown-not stated
- Sore throat no/yes/unknown-not stated
- Ear ache no/yes/unknown-not stated
- Chest pain no/yes/unknown-not stated
- Abdominal pain no/yes/unknown-not stated
- Vomiting in the last 48 hours no/yes/unknown-not stated
- Diarrhea in the last 48 hours no/yes/unknown-not stated
- Dysuria no/yes/unknown-not stated
- Seizures no/yes/focal/status epilepticus/unknown-not stated
- Headache no/yes/unknown-not stated
- Drowsiness no/yes/unknown-not stated
- Irritability/inconsolable crying no/yes/unknown-not stated
- Rash no/yes/unknown-not stated
- Painful or swollen joints/extremities/not using an extremity no/yes/unknown-not stated
- Other no/yes/unknown-not stated

Vital signs (please fill in the first value that was measured).

- Heart rate [value] per minute/unknown-not stated/recorded as normal, no number available
- Respiratory rate [value] per minute/unknown-not stated/recorded as normal, no number available.
- Cutaneous saturation [value] in %/unknown-not stated/recorded as normal, no number available) in room air/with oxygen supplied
- Systolic blood pressure [value] in mm Hg/Unknown-not stated/recorded as normal, no number available
- Body temperature [value] in °C/ unknown-not stated/recorded as normal, no number available Method: oral / rectal / tympanic / axillary / temporal

Physical examination

- Ill appearance no/yes/unknown-not stated
- Color of skin, lips or tongue normal/cyanotic/pale/mottled/ashen

- Signs of dehydration no / yes (definition : dry mucous membranes /sunken eyes / reduced skin turgor , no tears)
- Increased work of breathing defined as presence of
    - chest wall retractions no/mild / severe /unknown-not stated
    - nasal flaring no/ mild/ severe /unknown-not stated
    - grunting no/ mild/ severe /unknown-not stated
    - apnoea no/yes/unknown-not stated
- Air entry normal / abnormal/unknown-not stated
- Breath sounds normal / stridor / crackles / wheeze /unknown-not stated
- Location of abnormal breath sounds RLL / RUL / LLL / LUL/generalised/ unknown-not state
- Abnormal ENT signs no/yes/unknown-not stated
- Peripheral capillary refill normal = <3 sec/prolonged>3sec/unknown-not stated
- Oedema no / yes/unknown-not stated
- Heart sounds normal / murmur / other/unknown-not stated
- Abdominal pain no / yes if yes: RUQ, LUQ, RLQ, LLQ. /unknown-not stated
- If yes, pain score ….. Used score system NIPS / FLACC / VAS/unknown-not stated
- Liver enlarged no/yes [cm below RCM] /unknown-not stated
- Spleen enlarged no/yes [cm below LCM] /unknown-not stated
- Jaundice no / yes/unknown-not stated
- Meningeal signs (defined as presence of Kernig, Brudzinski, tripod phenomenon, neck stiffness, bulging fontanelle for <1 year) no/yes/unknown-not stated
- Focal neurological signs no/yes/unknown-not stated
- Pupils PERL/abnormal, if abnormal please describe/unknown-not stated
- Fundoscopy not performed / normal/abnormal / Blurred disc margins / Papilloedema | Haemorrhage / Not visualised/unknown-not stated
- Consciousness A=alert, R=responsive to verbal stimulation, P=responsive to pain, U=unresponsive / unknown-not stated
- Activity level
    - normal/not responding to normal social clues/no response to social clues
    - smiles / no smile / appears ill to a healthcare professional
    - Stays awake or awakens quickly / wakes only with prolonged stimulation / does not wake or if roused does not stay awake
    - Strong normal cry or not crying / decreased activity / weak, high-pitched or continuous cry
- Rash none/petechiae/other/unknown-not stated
- If other: purpuric/other non-blanching/maculopapular/pustulous/desquamation/ itching/mucosal laesion/strawberry tongue/red lips/conjuncitivitis/involvement of hands or feet (target lesions, erythema palmare-plantare)/other
- Lymphadenopathy no / yes, if yes: cervical / submandibular / axillary / inguinal / epitrochlear, nuchal. Largest size of involved lymph nodes: …. Cm/unknown-not stated
- Painful joints [number] /unknown-not stated
- Swollen joints [number] /unknown-not stated

Diagnostics ***In the final form local units for all lab tests will be pre-filled in. Only when performed at this ED visit. Please fill in the results if they become available within a few days after presentation.***

- *CRP performed* no/yes [value]/unknown-not stated
- *White bloodcell count performed* no/yes [value]/unknown-not stated
- *Absolute neutrofile count: no/yes* [value]/unknown-not stated
- *PCT performed* no/yes [value]/unknown-not stated
- *Chest X-ray performed* no/yes: normal/local infiltrate/diffuse abnormalities /other: unknown-not stated
- *Urinalysis performed* no/yes: normal/no leucoyturia or nitrite/leucocyturia positive/nitrite positive/ leucocyturia and nitrite positive/unknown-not stated
- *Urine culture* performed no/yes [value]/unknown-not stated
- *Stools culture/rapid viral Ag test* performed no/yes [value]/unknown-not stated.
- Blood culture performed no/yes [value]/unknown-not stated
- Rapid Strep Test no/yes: positive/negative/unknown-not stated
- *Lumbar puncture performed* no/yes unknown-not stated: white blood cell count [value]/unknown-not stated glucose level [value]/unknown-not stated /protein level [value]/unknown-not stated culture [value]/unknown-not stated
- Nasopharyngeal resp viral tests performed no/RSV/influenza/other/negative /unknown-not stated

Treatment

- Antibiotics prescribed at this ED visit or first day of admission no/yes/unknown-not stated
  - If yes, type/generic name: .....
  - If yes, duration 1,2,3,4,5,6,7,10,14, other ……days
  - Prescription mode none /oral/ intravenous/intramuscular/topical/unknown-not stated
- Time and date first antibiotic dose given [24 hour clock]
- Oxygen therapy: no/yes/unknown-not stated
- Inhaled medication: none/ ipratropium/salbutamol or albuterol/epinefrine/budesonide/ unknown-not stated

Immediate lifesaving interventions: no/yes/unknown-not stated. If yes:

1. Airway/breathing support:
   - non rebreathing mask no/yes/unknown-not stated
   - intubation/ventilation. no/yes/unknown-not stated
2. Haemodynamic support (significant iv fluid > 20ml/kg) no/yes/unknown-not stated if yes please specify for first 6 hours: normal saline/albumin/gelofusin/blood/platelets/fresh frozen plasma
3. Other emergent procedures or medication no/yes/unknown-not stated like
   Electrical therapy (defibrillation)
   Emergency procedures (e.g. chest needle decompression, pericardiocentesis, or open thoracotomy)
   Emergency medications: (atropine, adenosine, inotropics, epinephrine, nalaxone, dextrose in case of hypoglycaemia) /other

Evolution

- Destination: discharged home with out-patient clinic follow-up/ discharged home with out-patient clinic telephone follow-up/ discharged home with follow-up by GP/ discharged home without follow-up / discharged home with follow-up unknown/ admission <24h/ admission to ward / admission to ICU/ dead in ED/unknown-not stated.
- Time spent at the ED* (total duration): < 1 hour/ 1-2 hours /2-3 hours /3-4 hours />4 hours
- Date of hospital admission [date]/unknown-not stated
- Date of hospital discharge [date] /unknown-not stated
- Duration of hospital stay in days ……/unknown-not stated
- Duration of PICU/NICU days …../unknown-not stated
- Days on oxygen …../unknown-not stated
- Days on invasivie ventilation …../unknown-not stated
- Days on non-invasive ventilation …../unknown-not stated
- Days on inotropes …../unknown-not stated
- Days on antibiotics …../unknown-not stated
- Patient died no/yes. If yes, date of death [date] /unknown-not stated

Definition: time of arrival until time of discharge or admission

Working diagnosis and final diagnosis

- Focus [**required field]** upper respiratory/lower respiratory/urinary tract/enteric/cutaneous/bones & joints/sepsis/meningitis/fever without focus/unknown-not stated
  If other, specify:……
- Final diagnosis* definite bacterial /probable bacterial / definite viral/probable viral/uncertain/unknown-not stated

***Use the same definition as in BIVA ED, a link will be provided in the final form.**
Please use information from cultures if available.

APPENDIX 1B – MOFICHE QUESTIONNAIRE: DESCRIPTION OF THE SETTING
Filled in once for every emergency department

Description of the setting
Information about hospital, ED and healthcare organisation

Date of registration (ddmmyyyy)
Code of hospital, name and country (in final web form each participating hospital will be included in a list where to choose from)
Adherence area (number of inhabitants < 16 year)
Setting (inner city/ rural/mix)
Socioeconomic status of the population in the adherence area (generally high/generally low / mixture of high and low SES / other)
Type (academic/teaching/ non-teaching)
Number of paediatric emergency care admittance annually from the ED (number)
Number of paediatric emergency visits annually (number)
Type of triage system used (none/MTS/CTCS/ESI/other), If other: please specify .....
How is the temperature measured in general: oral / rectal / ear / axillary / temporal (forehead) / other?
Total number of inpatient beds for paediatric patients ……..
Is there a paediatric ICU at this hospital?
What is approximately the percentage of hospitalisation of paediatric ED patients (<5%, 5-10%, 10-20%, 20-50%, >50%).
What is the age-range at your ED defined as paediatric
Is the emergency department in your hospital mixed adult and paediatric ED or an ED with paediatric patients only?
What is approximately the percentage of self-referrals visiting the ED:
(<5 %, 5-10%, 10-20%, > 20%)
Who is the first person in charge of paediatric patients visiting the ED (multiple answers possible):
Resident …. %
Paediatrician …. %
Surgeon …. %
Emergency physician …. %
Paediatric emergency physician …. %
Other: paediatric specialist (e.g. neurologist, cardiologist) …. %
During day shifts a supervising specialist is available for *
During out of hours a supervising specialist is available for *

**Direct Supervision – the supervising physician is physically present onsite with the resident and patient.*
**Indirect Supervision: (i) with direct supervision immediately available – the supervising physician is physically within the hospital and is immediately available to provide Direct Supervision.*
**Indirect Supervision: (ii) with direct supervision available – the supervising physician is not physically present within the hospital or other sites of patient care, but is immediately available by means of telephonic and/or electronic modalities, and is available to provide Direct Supervision in person within 20-30 minutes at all times.*
**Oversight – the supervising physician is available to provide review of procedures/encounters with feedback provided after care is delivered.*

This document is part of a project that has received funding from the European Union's Horizon 2020 research and innovation programme, under grant agreement nr. 668303

The majority of children visiting the ED has a primary care physician (yes / no)
Primary care physicians deliver acute primary care during office hours in the adherence area of your ED (yes/no)
Primary care physicians deliver acute primary care outside office hours (yes/no)
Patients can (officially) attend the ED without a referral from their GP
Availability of specific guidelines:
a) Guideline for children with fever (NICE/local/other/none)
if other, please specify:.....
b) Guideline for children with urinary tract infections (NICE/local/other/none)
if other, please specify:.....
c) Guideline for children with respiratory infections (British thoracic society/NICE/local/other/none).
If other, please specify:.....
d) Guideline for children suspected for meningitis/sepsis (NICE/local/other/none)
If other, please specify:.....
e) Other guidelines (yes/no). If other, please specify:.....
The national immunization schedule included

a) National immunization coverage: ...%
b) DKTP availability yes/no; administration schedule....;
c) HIB availability yes/no; administration schedule....;:...
d) S. Pneumoniae availability yes/no; administration schedule....; name vaccine:...
e) N. Meningococcus C availability yes/no; administration schedule....; name vaccine:...
f) Hepatitis B availability yes/no; administration schedule....; name vaccine:...

g) N. meningococcus B availabiltity yes/no; administration schedule….: name vaccine:….. introduced….

Is there a paediatric haem/onc department at this hospital?

How many patients with haematological malignancies are:

Diagnosed on a yearly bases

In treatment on a yearly basis

In follow up on a yearly basis

How many episodes of febrile neutropenia are seen on an annual basis

Is there a paediatric stem cell transplant unit at this hospital?

How many patients are being transplanted on a yearly basis?

How many are haem/onc

How many are primary immunodeficiencies

How many are other (e.g. autoimmune)

How many episodes of suspected infection are seen on annual basis?

Is there a paediatric rheumatology department at this hospital?

How many patients are seen with autoinflammatory diseases on an annual basis?

How many patients are on biologicals on an annual basis

Checklist quality control variables

General characteristics

- Are all patients at your ED registered

Patients signs and symptoms

- Is working diagnosis assessed at the end of the ED visit
- Is working diagnosis made by the treating doctor
  - If not, is it made by the treating nurse
- Are vital signs truly measured yes/no

Can vital signs be estimated? Yes / no

Diagnostics and interventions

- Are diagnostic tests recorded in the patient ED file?
- In approximately what percent of the cases? (evt keuze-categorieen toevoegen) are they recorded in the patient file?
- In approximately what percent of the cases is this information complete?
- Who records this information? Physician / nurse / student / other.
- Are diagnostic tests recorded in the patient file during the ED visit or is this done retrospectively?
- In what percent of the cases is this done during the ED visit
- If recorded retrospectively, what could be a reason for this? E.g.: urgency of the patient, ED crowding, other reason.
- If recorded retrospectively, is there a risk of bias?

- Are interventions recorded during the ED visit or is this done retrospectively?
  - same questions as for diagnostic tests
- Are immediate life-saving interventions reported during the ED visit or is this done retrospectively.
  - same questions as for diagnostic tests

Follow-up

- same questions as for diagnostic tests

Is hospitalization > 24 uur mainly (> 90..%) for medical reasons ?

.

Are children admitted to the ward if the stay at the ED takes longer than advised by local guidelines?

- Is ICU admission mainly (> 90..%) for lifethreatening diseases or abnormal vital signs including conscientiousness ?

If ICU admission occurs for other reasons, please specify: e.g. monitoring of vital signs, not enough beds at the regular department, other.

- Is mortality at ED reported in the patient ED file?

Time points

- Is the duration of ED visit measured electronically or noted? Please specify.
- Are time points measured accurately? yes/no

Is the time between arrival and triage recorded?

In what percent of the cases is this recorded accurately?

Is the time between arrival and discharge recorded?

In what percent of the cases is this recorded accurately?

Are there guidelines on the timeframe within triage has to be performed? Please specify

APPENDIX 2- BIVA-PIC CRF Subject num: _____

Episode num._____

If Neonate: Gestational age: _____ weeks Weight: _____kg

INVESTIGATIONS

Classification (tick all that apply)

☐ **Child with Fever**
☐ **Suspected Sepsis**
☐ **Healthcare-associated Infection (HAI)**
☐ **Surgical Patient**

If HAI:

- **Surgical site infections** **Y** **N**
- **Catheter-associated Urinary Tract Infections** **Y** **N**
- **Ventilator-associated Pneumonia** **Y** **N**
- **Central Line-Associated Bloodstream Infections** **Y** **N**
- **Laboratory-Confirmed Bloodstream Infection** **Y** **N**
- **Others _____ (specify)** **Y** **N**

If Surgical Patient:

- **Specify kind of Operation:** _____
- **Date of Operation** ___/___/___

HAEMATOLOGY	Units	Units (alt.)	1st research bloods BASELINE	2nd research bloods 48h after 1st	Follow Up 28d after 1st
DATE:					
Hb	**g/dL**				
WCC	**10^9/L**				
Plt	**10^9/L**				
Hct	**%**				
Neutrophils	**10^9/L**				
Lymphocytes	**10^9/L**				
Monocytes	**10^9/L**				
Eosinophils	**10^9/L**				
Basophils	**10^9/L**				
PT	**%**				
INR	**-**				
APTT	**sec**				
Fibrinogen	**mg/dL**				
D-Dimer	**µg/ml**	**µg/l**			
BIOCHEMISTRY					
Crea	**mg/dL**				
Urea	**mg/dL**				
Na	**mmol/L**				
K	**mmol/L**				
Cl	**mmol/L**				
Calcium	**mmol/L**				

Gluc	**mg/dL**				
Alb	**g/dL**				
CRP	**mg/L**	**mg/dL**			
CRP max.	**mg/L**	**mg/dL**			
ProcalcitoninPCT	**ng/mL**				
IL-6	**ng/L**				
LDH	**U/L**				
Alanine Transaminase	**U/L**				
Bilirubine	**mg/dL**				
CK	**U/L**				
Ferritin	**µg/l**				
BLOOD GAS ANALYSIS					
Arterial PaO2	**mmHg**				
CO2	**mmHg**				
HCO3	**mmol/L**				
BE	**mmol/L**				
Lactate	**mmol/L**				

GMSPS (MENINGOCOCCAL ONLY)

	Y	N
Hypotension (SBP < 75 if less than 4 years, < 85 if older)	☐	☐
Core - peripheral temperature gap > 3 degrees	☐	☐
Base deficit < -8	☐	☐
Coma score < 8 at any time or deteriorated > = 3 in last hour	☐	☐
Lack of meningism	☐	☐
Parental opinion that child is worse over last hour	☐	☐
Widespread ecchymoses	☐	☐

PRISM SCORE

PRISM data recorded should be worst in first 24hours of PICU admission

Respiratory rate:		**PaO_2 (kPa):**	
Apnea:	**Y N**	**$PaCO_2$ (kPa):**	
GCS (total)		**HCO_3 (mEq/L):**	
- Eyes			
- Verbal			
- Motor			
Pupils fixed and dilated:	**Y N**	**Glucose**	
Systolic BP (mm Hg):		**PT/PTT>1.5 x contro (mmol/dL):**	
Diastolic BP (mmHg):		**Bilirubin > 60 µmol/L if > 1 month:**	**Y N**
Heart rate (bpm):		**Potassium (mEq/L):**	
FiO_2:		**Calcium (mmol/L):**	

SEPSIS-3-CRITERIA	**Value**	**Units**	**Units (alternative)**	**Comments**
PaO_2		**kPa**	**mmHg**	
Platelets		**$\times 10^3/\mu L$**		
Bilirubin		**mg/dL**	**µmol/L**	
MAP		**mmHg**		
Dobutamine	**Y N**			
Dopamine		**µg/kg/min**		**for at least one hour**
GCS				
Creatinine		**mg/dL**	**µmol/L**	
Urine output		**mL/d**		

Microbiology:		**Test methods: Culture | PCR | CF (Complement fixation) | EIA (Rag/IF)**			
Material/Analysis/	***Date:***	***Test method (C,P,CF,E)***	***Results***		
Urinanalysis			**Leuc Y / N**		**Nit Y / N**
Urine micro					
Urine culture			**Pos / Neg / ND**	**Organism:**	
Blood culture			**Pos / Neg / ND**	**Organism:**	
Throat swab			**Pos / Neg / ND**	**Organism:**	
CSF micro					
CSF			**Pos / Neg / ND**	**Organism:**	
NPA			**Pos / Neg / ND**	**Organism:**	
BAL			**Pos / Neg / ND**	**Organism:**	
Pernasal Swap			**Pos / Neg / ND**	**Organism:**	
Endotracheal Aspirate			**Pos / Neg / ND**	**Organism:**	
Stool			**Pos / Neg / ND**	**Organism:**	
PCR material			**Pos / Neg / ND**	**Organism:**	
Other pathogen identified:					
Screening for MRE | MRSA | MRGN/ESBL					

Imaging / Others – if conclusive for diagnosis		
Method	**Performing Date**	**Results**
X-Ray		
MRT		
CT		
Sonography		
EEG		
others		

THERAPIES / TREATMENT

Urinary Catherisation	**Y**	**N**
Central Line Catheter Implantation	**Y**	**N**
Renal Replacement Therapy	**Y**	**N**
Chest Drains	**Y**	**N**
ECMO	**Y**	**N**

Antibiotics	**Route**	**Start date (dd/mm/yy)**	**Stop date (dd/mm/yy)**
Virostatics	**Route**	**Start date (dd/mm/yy)**	**Stop date (dd/mm/yy)**
Antifungals	**Route**	**Start date (dd/mm/yy)**	**Stop date (dd/mm/yy)**
Steroids	**Route**	**Start date (dd/mm/yy)**	**Stop date (dd/mm/yy)**
IVIG	**Route**	**Start date (dd/mm/yy)**	**Stop date (dd/mm/yy)**
Other Fluids/Treatments	**Route**	**Start date (dd/mm/yy)**	**Stop date (dd/mm/yy)**

Route: IV=intravenous, O=oral, Neb=Nebulised, IM=intramuscular, T=topical, IT=intrathecal, SDD= selective decontamination of digestive tract

OUTCOME

Full recovery	**Y**	**N**	**UNKNOWN**
Skin graft	**Y**	**N**	**UNKNOWN**
Amputation	**Y**	**N**	**UNKNOWN**
Hearing loss >40dB	**Y**	**N**	**UNKNOWN**

Disability (non/mild/moderate/severe/coma or vegative state/brain death/unknown)

Other: _____

Severity of Illness:

	First results	**1st research bloods**	**2nd RB**	**further timepoint**
DATE:				
	yes no	**yes no**	**yes no**	**yes no**
In oxygen	☐ ☐	☐ ☐	☐ ☐	☐ ☐
Invasive ventilation	☐ ☐	☐ ☐	☐ ☐	☐ ☐
Non-invasive ventilation (CPAP, CPAP/PS, BiPAP, IPPV/similar, HFOV, face mask, nasal prongs)	☐ ☐ …….	☐ ☐ …….	☐ ☐ …….	☐ ☐ …….
Inotropes	**yes no** ☐ ☐	**yes no** ☐ ☐	**yes no** ☐ ☐	**yes no** ☐ ☐

Sepsis: (***see definitions***)

Focal infection	**Y**	**N**
Suspected Sepsis	**Y**	**N**
Proven Sepsis	**Y**	**N**
Severe Sepsis	**Y**	**N**
Shock	**Y**	**N**

Organ dysfunctions/failure: (***see definitions***)

Renal failure	**Y**	**N**
Liver failure	**Y**	**N**
Respiratory failure	**Y**	**N**
DIC	**Y**	**N (pTT > 1,5 x normal, platelets < 100 000/µl and elevated D-dimeres)**
Seizures	**Y**	**N**

APPENDIX 3– BIVA-HR CRF Subject num: _____
Episode num. _____

Data to be collected in addition to BIVA-ED

Lymphocyte count ……. mm^3/ml
Neutrophil count If neutropenic: since when
Underlying diagnosis
Treatment protocol
Transplant yes/no
If yes: pretransplant/conditioning for transplant/post transplant. If post transplant:
Day … post transplanttransplant
Neutrophil engraftment no/yes; if yes: day …. Post transplant
Lymphocyte engraftment no/yes; if yes: day …. Post transplant

GVHD: grade…..

treatment
Immunoglobulins schedule: no/yes : weekly/three weekly
Immunoglobulins route: iv/sc
Immunoglobulinsdose: …..g/kg

Current medication
PCP prohylaxis (yes/no); if yes what: septrin/pentamidine/atovaquone/otherAntiviral prophylaxis (): acyclovir/valaciclovir/other ie.....
Antifungal prophylaxis (yes/no) ambisome/voriconazol/caspofungin/itraconazole/other
Antimycobacterial prophylaxis: rifampicin yes/no, isoniazid yes/no, other yes…/no

Antibiotics: ()
Antibiotic 1 dose, frequency since
Antibiotic 2 dose, frequency since
Antibiotic 3 dose, frequency since

Antifungal 1 type, dose, frequency since
Antifungal 2 type, dose, frequency since
Antifungal 3 type, dose, frequency since

Antiviral 1 type, dose, frequency since
Antiviral 2 type, dose, frequency since
Antiviral 3 type, dose, frequency since

Immunesuppression yes/no: if yes:
Steroids : (yes/no)
Type:
Systemic: dose mg/kg/day equivalent
Topical:
Type: hydrocorticone/emuvate

Tacrolimus (yes/no)
Cyclosporine (yes/no)
Others yes/no, if yes…

Antacids (yes/no)

Defibrotide (yes/no)

G-CSF (yes/no)

Central line (yes/no) If yes: type: hickmann/portacath Number of lumen: 1/2/3
Note for these patients culture form central line is allowed please indicate if bloodculture was taken from: peripheral stab / CVL

APPENDIX 4– BIVA-INF CRF

Subject num: _____
Episode num. _____

Data to be collected in addition to BIVA-ED

Underlying diagnosis (Kawasaki, Systemic JIA, treatment-naïve JIA)
If included for another rheumatic disease, mention which: (SLE, PFAPA, FMF, MVD, TRAPS, CAPS, MWS, CINCA/NOMID, FCAS, Blau syndrome)
Genetic confirmation no/yes; if yes: gene mutation
Treatment protocol (local center ---)

Year/month/Day of diagnosis:
Assumed day of onset:

Treatment with antibiotics (last 10-14 days) yes/no
If yes, mention which antibiotics:

Current medication: no/yes, if yes:
IVIG: no/yes dose: …..g/kg schedule weekly/three weekly route: iv/sc
Day of administration:

NSAIDs: yes/no	If yes, since when: mm/yyyy
Colchicine yes/no	If yes, since when: mm/yyyy
Azathioprin yes/no	If yes, since when: mm/yyyy
DMARDs (MTX): yes/no	If yes, since when: mm/yyyy
Cyclophosphamide yes/no	If yes, since when: mm/yyyy
Infliximab yes/no	If yes, since when: mm/yyyy
Rituximab yes/no	If yes, since when: mm/yyyy
Tacrolimus yes/no	If yes, since when: mm/yyyy
Cyclopsorin yes/no	If yes, since when: mm/yyyy

Biological (anti-TNF blockade): yes/no If yes, since when: mm/yyyy
Started during current admission: yes/no

Anti-IL-1 drug (anakinra): yes/no If yes, since when: mm/yyyy
Started during current admission: yes/no

Anti IL6yes/no If yes, since when: mm/yyyy

Steroids: yes/no
Methylprednisolone 3 day course (15-30 mg/kg): yes/no
Started during current admission: yes/no
Oral prednisone: yes/no
If yes, since when: mm/yyyy

Disease considered to be controlled: yes/no

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Organ systems involved:
Skin
Joints
Muscle
Cardiac
Lungs
CNS
Vasculitis
Serositis
Eyes
Hearing loss
GI Liver
GI other