BRIEF REPORT

Prospective cohort study of the predictive value of inflammatory biomarkers over clinical variables in children and young people with cancer presenting with fever and neutropenia [version 1; peer review: 1 approved, 1 approved with reservations]

Bob Phillips [1,2]

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v1 **First published:** 22 Oct 2021, **10**:1070
https://doi.org/10.12688/f1000research.73075.1
Latest published: 03 Feb 2022, **10**:1070
https://doi.org/10.12688/f1000research.73075.2

Abstract

Introduction

Fever during chemotherapy induced neutropenia is a common and potentially life-threatening complication of the treatment of childhood cancer. Predictions of poor outcome could be enhanced by incorporating serum biomarkers of inflammation at presentation and reassessment.

Methods

A prospective cohort study was conducted of children under 18 years old, being treated for cancer or a cancer-like condition, who presented with fever ($\geq$ 38.0°C) and neutropenia (neutrophil count < $0.5*10^9$/L). Clinical features were recorded, along with three experimental inflammatory biomarkers: procalcitonin (PCT), interleukin-6 (IL-6) and interleukin-8 (IL-8). Outcomes included serious medical complications (SMC): any infection related mortality, critical care and organ support, severe sepsis, septic shock, significant microbiologically defined infection, or radiologically confirmed pneumonia.

Results

Biomarker assessments were undertaken in 43 episodes of fever and neutropenia, from 31 patients aged between four months and 17 years old (median six years): 20 were female and 22 had acute leukaemia. Five episodes of SMC were noted. PCT, IL-6 and IL-8 had poor individual discriminatory ability (C-statistic 0.48 to 0.60) and did not add to the value of clinical risk stratification tools. Insufficient data were collected to formally assess the value of repeated assessments.

Conclusions

Incorporating serum biomarkers of inflammation at presentation of

Open Peer Review

Approval Status ✓ ✓

	1	2
version 2 (revision) 03 Feb 2022	✓ view	✓ view
version 1 22 Oct 2021	? view	✓ view

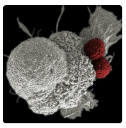
1. **Paul J. Gibson**, McMaster University, Hamilton, Canada

2. **Rejin Kebudi**, Oncology Institute, Istanbul University, Istanbul, Turkey

Any reports and responses or comments on the article can be found at the end of the article.

episodes of fever with neutropenia in childhood does not clearly improve risk stratification. Repeated assessments over time may be of value.

Keywords
febrile neutropenia, neutropenic sepsis, childhood cancer, inflammatory biomarkers, IL6, IL8, procalcitonin



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This article is included in the Multicancer early detection and screening collection.

Corresponding author: Bob Phillips (bob.phillips@york.ac.uk)

Author roles: Phillips B: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Dr Phillips (Post-Doctoral Research Fellow, PDF10872) was funded by the National Institute for Health Research (NIHR) for this research project. The views expressed in this paper are those of the author and not necessarily those of the NIHR, NHS or the UK Department of Health and Social Care.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Phillips B. **Prospective cohort study of the predictive value of inflammatory biomarkers over clinical variables in children and young people with cancer presenting with fever and neutropenia [version 1; peer review: 1 approved, 1 approved with reservations]** F1000Research 2021, **10**:1070 https://doi.org/10.12688/f1000research.73075.1

First published: 22 Oct 2021, **10**:1070 https://doi.org/10.12688/f1000research.73075.1

Introduction

Infection, frequently presenting as fever during chemotherapy induced neutropenia, is a common and potentially life-threatening complication of the treatment of childhood cancer. Modern management approaches have promoted the use of clinical decision rules to stratify patients at low risk of serious medical complications during febrile neutropenia (FN), enabling them to be safely managed with less intensive therapy as an outpatient.[1]

In addition to the role of clinical risk stratification, there is an increased desire to use modern biochemical markers of inflammation in predicting the risk of severe sepsis. There is increasing interest in inflammatory biomarkers such as procalcitonin (PCT), an inflammatory marker that has been shown to rise in response to bacterial infection in non-immunosuppressed children,[2] and various cytokines including interleukin (IL)-6 and IL-8 to improve the diagnostic accuracy of a prediction rule in children with FN.[3]

A systematic review and meta-analysis of the discriminatory ability of biomarkers in children with FN[4,5] concluded that while several small studies suggest PCT, IL-6 and IL-8 may be valuable for predicting severe infection in children with FN, the true impact remains unknown. A smaller number of studies explored the role of serial (i.e., 0 h, 12–24 h, 48 h) biomarkers to detect documented infection or sepsis. In one study, the difference between mean C-reactive protein (CRP), PCT and IL-8 at 24 hours in children with and without sepsis was more pronounced than at presentation.[6] These data suggest the optimal value for prediction may be made by incorporating biomarkers at early reassessment (i.e., within 12–24 h or 24–48 h), rather than at presentation.

This study aimed to undertake a focused analysis of three promising inflammatory biomarkers: PCT, IL-6 and IL-8 in both initial and value of serial testing and their additional discriminatory value above routine clinical features using the PICNICC model[7] and AUS-score.[8]

Methods

This study took place in Leeds Children's Hospital between March 2016 and March 2018. It recruited patients on presentation of FN or pre-enrolled them during routine appointments where they, or their parents or guardians, affirmed their consent in written form. In addition to formal information sheets, a link to an animated video summary of the research was provided https://www.youtube.com/watch?v=Z1AXzJqatds.[9] Ethical approval was given by the Leeds West NHS Research Ethics Committee [15/YH/0357].

Children could be included who were: younger than 18 years old, who had cancer, or received a stem cell transplant, or who had Langerhans cell histiocytosis in need of cytotoxic chemotherapy, or haemophagocytic lymphohistiocytosis undergoing active treatment, or severe aplastic anaemia, and attended with febrile neutropenia. Fever was defined as temperature $\geq 38.0°C$ and neutropenia, an absolute neutrophil count ≤ 0.5 g/L.

All patients were admitted to hospital and managed according to institutional FN pathways; these consisted of full evaluation and empiric piperacillin/tazobactam (or suitable alternatives) until afebrile 48 hours and with no other reason for antimicrobials, regardless of neutrophil count. Inflammatory biomarkers, with the exception of CRP, were analysed in batches and their results masked from the treating team. Using this unselective approach, with treatment unaffected by the results of the biomarker analyses, we minimized the biases which can arise through cherry-picking of patients and altering medical treatment on the basis of the test under evaluation (selection and incorporation biases).

Demographic data, variables and outcomes included core items as devised by the International Paediatric Fever in Neutropenia Working Group (see Extended Data, Table 1)[9]. Clinical assessments and blood samples for biomarkers were taken at presentation (within 12 h of fever or admission) and daily until the end of the FN episode or discharge as part of usual clinical care.

Table 1. Distribution of diagnosis in 31 patients with biomarker data.

Diagnosis	Number of patients
Acute leukaemias	21
Other haematological malignancies	2
Solid tumours	5
Brain tumours	3

The primary outcome measure was 'serious medical complication (SMC)'[10] (defined by any of (i) infection related mortality (ii) admission to ICU/HDU/other ward/unit for organ support (iii) severe sepsis or (iv) septic shock) or significant microbiologically defined infection, or radiologically confirmed pneumonia. Secondary outcomes measures were the component parts of the primary outcome, death within 30 days, infection related mortality, clinically defined infection, infection with antibiotic-resistant bacteria and relapse of primary infection. Initial power calculations estimated ~400 episodes would detect an additional benefit C-statistic discriminatory of +0.10.

Results were analysed descriptively, and assessment made of the individual discriminatory value of the inflammatory biomarkers using the C-statistic for SMC, and their additional value to clinical prediction rules (PICNICC prediction and AUS-score). Analyses were undertaken using base R version 3.6.0 (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) (RRID:SCR_001905) and the package pROC (pROC: an open-source package for R and S+ to analyze and compare ROC curves) Further analysis of the results was planned to be by hierarchical logistic regression modelling of episodes within patients, assessing the predictive value of clinical and then biomarkers of inflammation at admission, day one and subsequent timepoints. Additional assessments of the sensitivity and specificity of the markers, alone and in combination, were also proposed.

Results

Data was collected from 65 patients with an episode of fever and neutropenia. Of those, 43 episodes in 31 patients had biomarker samples taken, with 31 episodes with 'day one' data. The distribution of diagnoses is shown in Table 1. The patients ranged from four months to 17 years old (median six years), and 20 were female.

Of these 31 episodes, five were assigned an SMC: two significant viral infections with oxygen requirement, and one each of: *Fusobacterium* blood stream infection, *Escherichia coli* urinary tract infection, and culture-negative severe sepsis requiring fluid boluses. No patient received intensive care support or died. There were eight non-serious viral infections and one non-serious central line colonization.

All biomarkers performed poorly in distinguishing those who developed an SMC (see Figure 1), with C-statistic estimates ranging from 0.48 to 0.60 (see Table 2). The biomarkers were ineffective in improving the discriminatory value of the PICNICC risk prediction (change in C-statistic: −0.10 to +0.04), and only marginally useful in improving the simpler

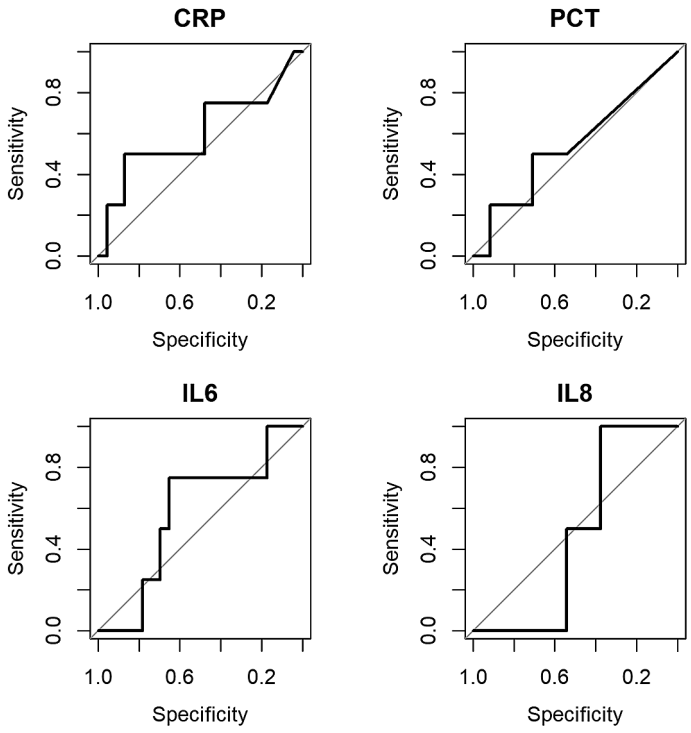

Figure 1. Plots of discriminatory values of admission biomarkers in distinguishing patients with serious medical complication (SMC). CRP = C-reactive protein; PCT = procalcitonin; IL-6 = interleukin-6; IL-8 = interleukin-8.

Table 2. C-statistic values for biomarkers to distinguish patients with serious medical complication (SMC). CRP = C-reactive protein; PCT = procalcitonin; IL-6 = interleukin-6; IL-8 = interleukin-8.

Biomarker		With PICNICC prediction	With AUS-score
CRP	0.60	0.69	0.71
PCT	0.54	0.55	0.59
IL-6	0.57	0.55	0.57
IL-8	0.48	0.64	0.58
Rule alone		0.65	0.53

Table 3. Mean biomarker levels in patients who did, and did not, appear clinically unwell at presentation. CRP = C-reactive protein; PCT = procalcitonin; IL-6 = interleukin-6; IL-8 = interleukin-8.

Biomarker	Mean 'severe' clinical appearance (n = 5)	Mean 'non-severe' clinical appearance (n = 26)
CRP	111 mg/dL	42 mg/dL
PCT	5.8 ng/ml	0.03 ng/ml
IL-6	105 pg/ml	67 pg/ml
IL-8	210 pg/ml	176 pg/ml

AUS-score (+0.04 to +0.18) (See Extended Data, Table 2)[9] Given the paucity of data and lack of diagnostic value, no complex analyses were undertaken.

The biomarker levels concurred better with the physician assessment of "severe" clinical illness, though in no case was this statistically significantly different (see Table 3, $p > 0.10$).

Repeated measures of the biomarkers were available in 25 episodes. Development of SMC did not occur in patients who presented with non-severe symptoms and consistently low inflammatory biomarkers despite ongoing fevers. Of those with SMC, 4/5 had reductions in biomarker levels as their infection resolved; they stayed high in the one case of culture negative severe sepsis.

Discussion

This prospective study of inflammatory biomarkers in paediatric febrile neutropenia found little support for their use as indicators of covert infection. Technical and administrative challenges limited the number of samples collected during the study, despite enthusiasm from the patients and their families. This adds to the body of evidence describing a relatively limited role in initial stratification.

Serial use of these markers, where they can be used to suggest an infection is under control, or controlled, is an area of active testing. PCT has been studied in several patient groups to diagnose sepsis and monitor response to treatment. A systematic review[11] and subsequent large (>1500 patients) RCT with pragmatic study design examining PCT-guided decision-making in neonates (NeoPIns) with suspected infection and critically ill adults on ICU (SAPS) found significant reductions in antibiotic duration in the PCT arm.[12,13] Two large UK studies looking at the same question in adults and children with suspected or proven serious bacterial infections are ongoing.[14,15] These studies specifically exclude immunocompromised patients such as children with cancer, and work exploring this is required to enhance patient experience and reduce antimicrobial resistance.[16–18]

The results of this study should dissuade clinicians from routinely using inflammatory biomarkers in making initial stratification of children with febrile neutropenia. The onward investigation of their serial use in antimicrobial stewardship should be pursued within carefully monitored studies.

Data availability

Underlying data

The data for this study contains the following elements, which when combined would make the patients identifiable given the rarity of childhood cancer in the identified geographical area and time of this study:

Demographic data: (ii) episode date; (iii) age; (iv) cancer diagnosis & treatment.

FN episode data: (i) inpatient or outpatient onset; (ii) time of start of temperature; (iii) time of presentation.

A reduced dataset taking out all items to produce sufficient anonymity (for sensitive data; childhood cancer) would severely limit their utility for researchers.

The patients and families who took part in this study, and the Research Ethics Committee who granted permission for it, agreed the data should be available for sharing in ethically approved secondary use projects. Such studies are typically individual participant data meta-analysis collaboratives. Anyone who has such an approved project, investigating aspects of paediatric febrile neutropenia and biomarker profiles, is encouraged to approach the author at bob.phillips@york.ac.uk for access to the dataset.

Extended data

Open Science Framework (OSF): Extended data for 'Prospective cohort study of the predictive value of inflammatory biomarkers over clinical variables in children and young people with cancer presenting with fever and neutropenia', https://www.doi.org/10.17605/OSF.IO/CVFZB.[9]

This project contains the following extended data:

- Extended Data Tables.docx (Extended Table 1: Data items collected and Extended Table 2: AUC-ROC (C-statistic) values for biomarkers to distinguish patients with SMC)

- Consent Video (mp4 format)

Consent

Written informed consent for publication of the patients' details was obtained from the patients/parents/guardian of the patient.

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Open Peer Review

Current Peer Review Status: ? ✓

Version 1

Reviewer Report 21 January 2022

https://doi.org/10.5256/f1000research.76698.r100188

✓ **Rejin Kebudi**
Division of Pediatric Hematology-Oncology, Oncology Institute, Istanbul University, Istanbul, Turkey

The manuscript *Prospective cohort study of the predictive value of inflammatory biomarkers over clinical variables in children and young people with cancer presenting with fever and neutropenia* investigates the role of PCT, IL6, IL8 at first day and serial monitoring in paediatric febrile neutropenic episodes.

The unselective approach for the biomarkers (the inflammatory biomarker results except for CRP were masked from the treating team) minimized the biases for medical treatment. Did the results of CRP (which the medical team could see) effect treatment decisions?

There were 43 episodes and 31 patients, only 31 episodes had first day data. Despite that, the biomarker levels concurred better with the physician assessment of severe clinical illness, however, this was not statistically significant. The conclusion was that at presentation of febrile neutropenic episodes, serum biomarkers of inflammation do not improve the risk stratification, repeated assessment over time may be of value.

Were there any differences in patients who had acute leukemia vs solid tumors? Were all of the febrile neutropenic episodes, in which biomarkers were investigated, high risk? If there were both high risk and low risk episodes, did the biomarkers differ within the risk groups?

In table 3 the mean inflammatory biomarker values in severe clinical appearance are numerically much higher than the non-severe ones; what was the range of each biomarker in the severe and non-severe group. Do the authors define the severe clinical appearance (table 3) the same as SMC?

Although the use of these biomarkers were not statistically significant in initial stratification, the evaluation of a larger cohort with higher number of SMC, may give more information on the value of the initial inflammatory biomarkers for predicting a SMC or sepsis. A larger cohort may also

give more information for the value of the use of serial biomarker monitoring and which one/ones to use cost-effectively. The discussion may include some of these concerns.

Do see this for further reference. [1]

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pediatric oncology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response ( ) 01 Feb 2022

Bob Phillips, University of York, UK, York, UK

There are a number of interesting questions raised in the review:

1. Were there any differences in patients who had acute leukemia vs solid tumors?

This is a very fair question, and no, there were no qualitative differences in patient type. Given the small numbers, multiple analyses for variation were felt to be unwise and only the highest level (risk-grouping and biomarkers) were undertaken.

1. Were all of the febrile neutropenic episodes, in which biomarkers were investigated, high risk? If there were both high risk and low risk episodes, did the biomarkers differ within the risk groups?

The risk groups are shown in Extended Data Table 2; they included all levels of risk.

1. In table 3 the mean inflammatory biomarker values in severe clinical appearance are numerically much higher than the non-severe ones; what was the range of each biomarker in the severe and non-severe group.

This is a good question and Table 3 has been modified to add the (range) as well as the mean values.

1. Do the authors define the severe clinical appearance (table 3) the same as SMC?

No – severe clinical appearance and significant medical complication are defined differently. SMC is defined in the fifth methods paragraph, "severe clinical appearance" is the gestalt impression of the examining physician.

Competing Interests: Author of the manuscript

Reviewer Report 10 November 2021

https://doi.org/10.5256/f1000research.76698.r97615

?

Paul J. Gibson
Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada

General Comments:

This is a nice small study that tackles an interesting and still unanswered question with regards to the utility of biomarkers in the risk stratification of Pediatric Febrile Neutropenia. However, it appears there were challenges in recruitment and sample collection that are not specifically outlined. It may be helpful to describe those challenges to guide future efforts.

Abstract:

Conclusions: "Repeated assessments over time may be of value". I'm not convinced that the body of the paper nor the discussion justify this statement based on the results of this study. While other studies are referenced that may point to the utility of serial assessments, the concept is not substantially discussed in this paper.

Introduction:

The second paragraph mentions interest in newer biomarkers but fails to address CRP until it used as a single example in the 3rd paragraph. I suggest the authors make earlier reference to this 'older' biomarker and its role (or lack thereof) in risk stratification and highlight why PCT IL-6 and IL-8 may be preferable.

Methods:

"Using this unselective approach, with treatment unaffected by the results of the biomarker analyses, we minimized the biases which can arise through cherry-picking of patients and altering medical treatment on the basis of the test under evaluation (selection and incorporation biases)". This sentence may be more succinctly stated. such as "PCT, IL-6 and IL-8 values were not provided to clinicians to avoid bias"

As Table 1 states results, it is more appropriate in the results section.

The methods reference power calculations suggesting ~400 episodes were required. but only 43 were captured, yet there is no explanation for planned duration or reason for termination of the study.

Results

Data was collected on 65 patients, yet only 31 have had biomarkers taken? Why? Did they refuse collection? Did they present outside of times feasible for biomarker collection? The Discussion references technical and administrative challenges, but maybe expand what you mean?

You may consider showing graphically the changes in biomarkers in the 5 SMC patients.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am a clinical pediatric oncologist with research experience in supportive care. I cannot however, confidently suggest all statistical methods quoted in the paper are appropriate.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response ( ) 01 Feb 2022

Bob Phillips, University of York, UK, York, UK

"General Comments:

1. This is a nice small study that tackles an interesting and still unanswered question with regards to the utility of biomarkers in the risk stratification of Pediatric Febrile Neutropenia. However, it appears there were challenges in recruitment and sample collection that are not specifically outlined. It may be helpful to describe those challenges to guide future efforts."

This is an extremely good point, and paragraph 3 has been added to the Discussion.

"Abstract:

1. Conclusions: "Repeated assessments over time may be of value". I'm not convinced that the body of the paper nor the discussion justify this statement based on the results of this study. While other studies are referenced that may point to the utility of serial assessments, the concept is not substantially discussed in this paper."

This is also reasonable – the few data there are congruent but an overstatement from this report. Modified.

"Introduction:

1. The second paragraph mentions interest in newer biomarkers but fails to address CRP until it used as a single example in the 3rd paragraph. I suggest the authors make earlier reference to this 'older' biomarker and its role (or lack thereof) in risk stratification and highlight why PCT IL-6 and IL-8 may be preferable. "

A worthwhile negative to explicitly state. Added to into paragraph 3 to explain why the newer ones are possibly better.

"Methods:

1. "Using this unselective approach, with treatment unaffected by the results of the biomarker analyses, we minimized the biases which can arise through cherry-picking of patients and altering medical treatment on the basis of the test under evaluation (selection and incorporation biases)". This sentence may be more succinctly stated. such as "PCT, IL-6 and IL-8 values were not provided to clinicians to avoid bias"

This is definitely true, but I'd really like to keep the longer explanation. As a tutor of critical appraisal, I have found the word "bias" is often thrown about as a correct answer, but more knowing it's the code to use than the meaning of it. Additionally, it conforms more strongly to the STROBE checkpoint 9.

1. As Table 1 states results, it is more appropriate in the results section.

Fair. This is now a supplementary Table, and Table 1 is within the results.

The methods reference power calculations suggesting ~400 episodes were required. but only 43 were captured, yet there is no explanation for planned duration or reason for termination of the study.

Again, reasonable request for the missing information. Added in Methods and Discussion.

"Results:

1. Data was collected on 65 patients, yet only 31 have had biomarkers taken? Why? Did they refuse collection? Did they present outside of times feasible for biomarker collection? The Discussion references technical and administrative challenges, but maybe expand what you mean? "

Agreed – discussion paragraph added.

Final Comment:

- You may consider showing graphically the changes in biomarkers in the 5 SMC patients.

It is definitely worth considering. Given the very small numbers and the lack of real value of the work in serial measurements arising from this report, as correctly emphasised earlier, adding a graph might well over-egg this part of the pudding again and is probably better left text-based.

Competing Interests: Author of the manuscript