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Short communication

Natural variability of TRAIL, IP-10, and CRP in healthy adults – The "HERACLES" study

Annefleur C. Langedijk [a,*], Katrien Oude Rengerink [b], Eline Harding [a], Annemarie Wensing [c], Rianne van Slooten [a], Yael Israeli [d], Michal Rosenberg [d], Tanya Gottlieb [d], Eran Eden [d], Louis J. Bont [a]

[a] Department of Paediatric Infectious Diseases and Immunology, Wilhelmina Children's Hospital, UMC Utrecht, Utrecht, the Netherlands
[b] Julius Center, UMC Utrecht, Utrecht, the Netherlands
[c] Department of Virology, UMC Utrecht, Utrecht, the Netherlands
[d] MeMed Diagnostics, Israel

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ABSTRACT

A novel host-protein score (called MMBV) helps to distinguish bacterial from viral infection by combining the blood concentrations of three biomarkers: tumour necrosis factor related apoptosis inducing ligand (TRAIL), interferon gamma induced protein 10 (IP-10), and C-reactive protein (CRP). These host biomarkers are differentially expressed in response to bacterial versus viral acute infection. We conducted a prospective study, with a time series design, in healthy adult volunteers in the Netherlands. The aim was to determine the variability of TRAIL, IP-10, and CRP and the MMBV score in healthy adults across time. Up to six blood samples were taken from each healthy volunteer over a period of up to four weeks. In 77 healthy participants without recent or current symptoms, MMBV scores (maximal) were bacterial in 1.3 % and viral (or other non-infectious etiology) in 93.5 % of participants. There was little variation in the mean concentrations of TRAIL (74.5 pg/ml), IP-10 (113.6 pg/ml), and CRP (1.90 mg/L) as well as the MMBV score. The variability of biomarker measurement was comparable to the precision of the measurement platform for TRAIL, IP-10, and CRP. Our findings establish the mean values of these biomarkers and MMBV in healthy individuals and indicate little variability between and within individuals over time, supporting the potential utility of this novel diagnostic to detect infection-induced changes.

1. Introduction

A novel host-response based score was previously shown to differentiate between bacterial and viral infections in children with respiratory tract infections (RTI) and fever without source [1,2] and in adults with lower respiratory tract infections [3]. This blood-based score (MeMed BV®, MMBV) integrates concentrations of three host biomarkers: tumour necrosis factor related apoptosis inducing ligand (TRAIL), interferon gamma induced protein 10 (IP-10), and c-reactive protein (CRP). While CRP has been extensively studied as a biomarker that is induced in bacterial infections in febrile patients, the evidence relating to TRAIL expression and infection is relatively new [4]. Its concentration increases in viral infection and decreases in bacterial infection [2]. New host-proteins that are up-regulated in viral infections may be an innovative complement to bacterially induced proteins in current clinical use [1]. The expression dynamics of individual biomarkers in healthy subjects have not been reported. In the present study, we examined the natural variability of TRAIL, IP-10, and CRP expressed in the healthy individual across time.

The Hospital Employees Response Ante COVID-19 Listed Early Symptoms (HERACLES) study aimed at detecting viral infections including COVID-19 during the pre-symptomatic phase. However, none of the study participants were detectably infected with any respiratory viruses during the study period. The lack of infections enabled the present study, where we post-hoc decided to use the data to determine the natural variability of TRAIL, IP-10, and CRP in healthy adults across time.

* Corresponding author.
E-mail address: a.c.langedijk@umcutrecht.nl (A.C. Langedijk).

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2. Methods

2.1. Study design and participants

All adult hospital staff employed at the Wilhelmina Children's Hospital with high exposure to COVID-19 were invited to participate in the HERACLES study. Employees who considered themselves to have any contact with patients in relation to their work were defined as having high exposure. Invitations for participation in the study were posted in newsletters and sent out individually to all employees.

Participants were excluded in case of a previous episode of acute RTI in the past two weeks or at time of enrolment. Other exclusion criteria were: previously proven COVID-19 infection, a proven or suspected HIV, HBV, or HCV infection, active malignancy, current treatment with immune-suppressive or immune-modulation therapies, and severe illnesses that affect life expectancy and quality of life (other than suspected COVID-19 infection).

2.2. Sample collection

Multiple blood samples were collected prospectively from subjects over a period of up to four weeks during the first COVID-19 wave (Fig. S1). Blood was collected by trained study personal up to six times within the study period and/or when respiratory symptoms occurred (Fig. S2). When respiratory symptoms were reported by the participant to the study team, additional blood samples and respiratory samples were collected on three subsequent days after onset of symptoms. During every blood draw, a serum sample and an RNA sample were collected which were stored at −80C and −20C, respectively. Respiratory samples (nasopharyngeal and oropharyngeal) were collected and placed in Universal Transport Medium (UTM) when participants showed symptoms. Respiratory samples were taken using flocked swabs by qualified staff members and were stored at −80C until analysis. PCR analysis for 18 respiratory viruses (adenovirus, bocavirus, coronavirus (229E, HKU1, NL63, OC43 and SARS-CoV2), human metapneumovirus (hMPV), influenza virus type A, influenza virus A(H1N1)pdm09, influenza virus type B (influenza virus), parainfluenza virus types 1 through 4 (PIV1-4), RSV types A and B (RSV), rhinovirus and enterovirus) was performed for all symptomatic participants [5].

2.3. Study procedure

Study visits took place six times for a period up to four weeks with a minimum of 48 hours and a maximum of 7 days between blood draws (Fig. S3). At 21 days after the sixth sample, a seventh sample was taken for serologic assessment. Nasal congestion, defined as the blockage of nasal passages, was examined by asking the participants to close one nostril and breath through the other, and vice versa.

At enrolment, data on demographics, medical history, medication, and possible COVID-19 exposure were collected through a questionnaire. When symptoms were notified during one of the visits, physical examination took place including measuring temperature, heart rate, respiratory rate, and saturation. In addition, a respiratory sample was collected. Symptomatic subjects were followed for three subsequent days taking blood samples and respiratory samples. After the third visit, participants were excluded from further participation. Symptomatic participants could be sampled at home according to the hospital policy at that moment.

Blood samples of all participants were also tested for SARS-CoV-2 IgG antibodies by the Afinity IgG ELISA. Samples with IgG ratios >1.4 were considered to be positive. Two samples from two individuals after a proven COVID-19 infection served as controls in the serology analysis.

For this study of biomarker variability in healthy subjects, subjects were excluded if there was a suspicion of infection, as indicated by one or more of the following: positive serology results, clinical symptoms, or a change in MMBV score across the time course.

MeMed BV® (MMBV, MeMed, Israel) tests were conducted using blood samples of healthy participants. The tests were run on MeMed Key® (MeMed, Israel) a multi-purpose immunoassay analyser for quantitative diagnostic immunoassays that provides MMBV results in 15 minutes, and is therefore designed for on-site diagnostics.

2.4. Statistical analysis

Two to three MMBV measurements were performed per time point and the average per time point used for each subject across their time course. For values below the instrument's limit of quantitation, LoQ, (15 pg/ml for TRAIL, 100 pg/ml for IP-10 and 1 mg/L for CRP [6]) the LoQ was used in all calculations.

2.5. Ethical considerations

This study was approved by the Medical Research Ethics Committee of the UMC Utrecht (IRB number 20–206/D). Informed consent procedures followed in compliance with UMC Utrecht guidelines. Virologic and serologic results were shared with participants after complete analysis.

3. Results

3.1. Clinical results

Between April 14 and May 22, 2020, 294 healthcare workers (HCWs) were enrolled in the study of which 291 (99 %) participants provided a first blood sample (Figs. S2 and S3). A total of 286 (98 %) participants completed the final study visit. The median age was 44 years (range 18–65) and almost all (92.4 %) participants were female (Table S1). Out of 286 participants, 9 had possible serology findings and 17 had clinical symptoms; the remaining 260 were considered as healthy subjects.

3.2. Healthy subjects

Of the 260 healthy subjects, here we present the natural variability of infection biomarkers in 77 healthy subjects. As most participants were females, to explore relevant differences between males and females, samples from every enrolled male (n = 22) and a randomly selected subset of females (n = 55) were measured across all time points (3–6). In healthy individuals without current or recent symptoms 93.5 % of maximal MMBV results and 98.7 % of mean MMBV results were within the "viral (or other non-infectious)" range (Fig. S4).

In these 77 healthy participants without any symptoms during sample collection, mean biomarker concentrations for TRAIL, IP-10, and CRP were 74.5 pg/ml (standard deviation (SD) 15.6), 113.6 pg/ml (SD 23.6), and 1.90 mg/L (SD 2.1), respectively (Fig. 1). Healthy biomarker variability across time was comparable to the precision of the measurement platform [6] (Fig. S5.).

3.3. Symptomatic subjects

Of the 286 HCWs who completed the study, 17 (5.9 %) developed RTI symptoms during the study period. RTI episodes in all participants were characterised by mild symptoms, including a runny/blocked nose, minimal coughing, sore throat, headache, muscle pain, or fatigue. Temperature was marginally increased in two participants (38.0 °C and 38.1 °C). All symptomatic participants were negative for 18 respiratory viruses tested for by PCR, including SARS-CoV-2. Of 17 symptomatic participants, 8 had been diagnosed with hay fever or allergies previously. All seven blood samples from two of the HCWs showed SARS-CoV-2 antibodies, indicating that they entered the study with antibodies.

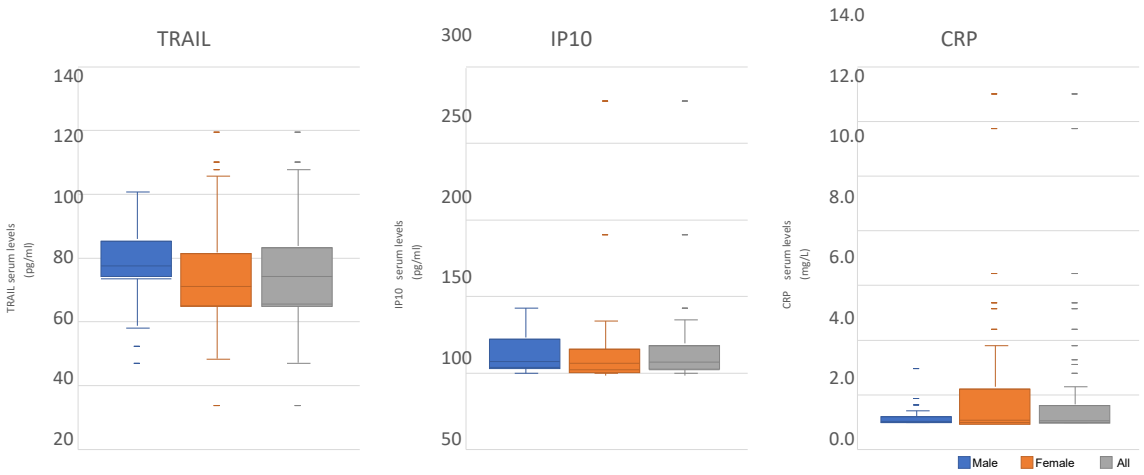

Fig. 1. Healthy biomarker concentrations in male (n = 22), female (n = 55) and all participants with sequential MMBV measurements (n = 77). Each data point is the mean of a subject's time course.

4. Discussion

We conducted a prospective study over a two-month period in HCWs in a children's hospital in the Netherlands. With the HERACLES study, we present a baseline of host biomarker dynamics in 77 healthy adults, selected from 260 healthy subjects . Our findings provide mean values of TRAIL, IP-10, and CRP and the MMBV score in healthy individuals and indicate little variability between and within individuals over time. This finding supports the potential utility of this novel diagnostic in detecting acute infection-induced changes.

To the best of our knowledge, this is the first study examining inflammatory host biomarkers in healthy adults. There are some limitations to our study. Although we received ethical approval within a short time (few days), the study started after the peak of the first COVID-19 wave in the Netherlands (Fig. S2). This could explain why we did not detect any PCR-confirmed SARS-CoV-2 infection in the HCWs. Although this clinical study did not achieve its objective of capturing the dynamic expression of the biomarkers during natural SARS-CoV-2 infection, we consider the dynamics of the biomarkers in healthy individuals to be a valuable baseline finding. A strength of the HERACLES study is that 291 HCWs were successfully recruited at a challenging time for global healthcare. The low SARS-CoV-2 incidence among the HCWs at a paediatric facility could reflect its low incidence among children. Other studies have reported similar infection rates [7].

In conclusion, the dynamics of novel and traditional host proteins in a large sample size of healthy subjects contributes to our understanding of the healthy baseline of these host biomarkers. A translational benefit of this finding is that these biomarkers may serve to detect early infection with viruses such as RSV and SARS-CoV-2 [8]. Future challenge studies are warranted to explore this further.

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CRediT authorship contribution statement

Annefleur C. Langedijk: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **Katrien Oude Rengerink:** Conceptualization, Writing – review & editing, Methodology. **Eline Harding:** Investigation, Project administration, Writing – review & editing, Data curation. **Annemarie Wensing:** Investigation, Writing – review & editing. **Rianne van Slooten:** Investigation, Writing – review & editing, Data curation. **Yael Israeli:** Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **Michal Rosenberg:** Conceptualization, Formal analysis, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **Tanya Gottlieb:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Eran Eden:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – review & editing. **Louis J. Bont:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2024.156530.

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