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**Conclusions:** Identification of a cytokine signature specific for the pre-seroconversion stage of PHI may help to understand the earliest HIV pathogenic events and identify new potential targets for immunotherapy aimed at modulating the cytokine response to HIV infection.
Title: A cytokine pattern that differentiates pre- from post-seroconversion phases of primary HIV infection

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Introduction

Acute HIV infection (AHI) is commonly defined as the period between the HIV transmission event and the development of an antibody response to HIV antigens defined as seroconversion\(^1\). Recent infection generally is considered the phase up to 6 months after infection during which anti-HIV-1 antibodies are detectable. Throughout this article, the term primary HIV infection (PHI) is used to refer to both acute and recent HIV-1 infection\(^2\).

AHI is characterised by high HIV viral replication, massive gut-associated lymphoid tissue (GALT) CD4+ T cell destruction, immune activation and the establishment of viral reservoirs\(^3\). As a result of high viraemia in bodily fluids and high levels of genital viral shedding, individuals are considered hyper-infectious at this stage\(^4,5\). Current HIV antibody tests typically become positive within 3–6 weeks of infection and 1–3 weeks after the onset of acute HIV symptoms\(^6\). This seronegative window is thus of crucial importance for early treatment outcome, vaccine development and public health\(^7,8\).

Despite the importance of AHI, there are immense difficulties identifying AHI patients for treatment initiation. Patients are often asymptomatic, or experience a non-specific febrile illness; a diagnostic dilemma compounded in the African setting by a significant symptom overlap with malaria\(^9,10\). To diagnose AHI, the presence of the virus itself must be detected by nucleic acids or proteins in the absence of HIV-specific
antibodies. Fourth-generation HIV assays have demonstrated good sensitivity and specificity for detecting HIV infection and AHI but remain less sensitive than nucleic acid amplification technology\textsuperscript{11}. Thus, current gold-standard test for confirming viraemia is RT-PCR for plasma HIV RNA\textsuperscript{12}, while the most widely accepted assay for confirmation of HIV antibody testing is Western Blot (WB) or indirect immunofluorescence assay (IFA)\textsuperscript{13}.

PHI is a rapidly evolving phase characterized by the stepwise gain in positivity for the detection of HIV RNA and HIV-specific antibodies. Different HIV-1 antigen specificities appear in sequence after HIV-1 transmission as do immunoglobulin G (IgG) subclass responses\textsuperscript{3}. As such, using different diagnostic tools, PHI has been categorised into ‘Fiebig stages’\textsuperscript{14} that are useful in approximating infection date with relative accuracy\textsuperscript{15}.

As HIV viraemia increases, there is a striking cascade response of acute phase reactants and inflammatory cytokines which has been referred to as the ‘cytokine storm’\textsuperscript{3}. Although many of the cytokines present are common inflammatory pathway effectors, their study can shed light on key pathogenic pathways occurring prior to the generation of the humoral response\textsuperscript{16–20}. We hypothesize that the expression patterns of these early response cytokines may be different between pre and post-seroconversion stages of the PHI phase. These effectors could provide clues for vaccine or immunomodulator development aimed at reducing the irreversible immune damage inflicted during AHI.
Methods

Study population

The study population was enrolled between 2013 and 2014 at the Manhiça District Hospital (MDH) in the district of Manhiça, Southern Mozambique. The present analysis is a sub-study of a prospective cohort of primary HIV infected adults enrolled and followed up for 12 months in the study of gastrointestinal inflammation (GAMA cohort). This study was approved by local institutional review boards at Barcelona Clinic Hospital (2011/6264) and by the Ministry of Health of Mozambique (461/CNBS/12). Written informed consent was obtained from patients prior to participation.

Adults over 18 years of age, who were residents of the established District Surveillance System (DSS) study area, and who presented to the outpatient clinic of the MDH for non-specific febrile symptoms or voluntary HIV counselling and testing (VCT) were invited to participate in the study.

HIV diagnosis

Blood was collected via finger-prick for HIV rapid antibody testing with Determine HIV 1/2 (Abbott Laboratories, Chicago, IL). Positive results were then confirmed with a more specific Unigold rapid test (Trinity Biotech Co., Wicklow, Ireland) following the national guidelines. Individuals with a positive HIV serology in both rapid tests were referred for clinical management and were not eligible for enrolment into the study. Primary HIV infection was diagnosed in patients with a negative or indeterminate rapid test serology (first test negative or first test positive and second test negative) and positive HIV viremia by RT-PCR testing on frozen plasma using Abbott Real Time HIV-1 assay (Illinois, U.S.) according to manufacturer’s instructions, with a sensitivity of
detection of 150 copies/ml. HIV-RNA was quantified by applying a multi-level pooling scheme of 10 samples/pool as described and validated in previous work. A subgroup of HIV-negative time-matched controls was selected via computer randomisation.

**Clinical follow-up**

Individuals identified with PHI and the control subgroup of HIV-negative individuals were invited to attend a study visit 1 month after the screening date to start the follow up in the context of the GAMA cohort. In this paper, the 1 month visit sample was only employed to establish the reference level for the biomarkers in a non-febrile non-HIV infected group and the coinfections and CD4 and CD8 T-cell counts in both study groups. Demographic and clinical data was collected in a specific questionnaire. Medical consultation and HIV counselling was provided. CD4 and CD8 T cell counts were determined using CD3, CD8, CD4 and CD45 fluorochrome-labelled antibodies on fresh whole blood in a single platform system using *Trucount tubes* (BD Biosciences, New Jersey, U.S.). Clinical and microbiological evaluation was performed, testing for the most prevalent infections in the area.

**Quantification of immunoglobulins and definition of primary HIV infection phases** (see *Detailed Methodology* section in the *Supplementary data*).

HIV specific antibodies present in plasma screening samples were assessed by Western Blot assay (WB) for sg-p120, gp41, p31, p24, p17 antigens and sgp105 and gp36 antigens for HIV type 2 infection using INNO-LIA™ HIV I/II Score (Innogenetics N.V. Gent, Belgium). Patients with a negative or indeterminate WB result were considered to be in the pre-seroconversion acute stage and patients with a positive WB result were considered to be in the post-seroconversion recent stage of HIV infection.
Thus, in our study cohort seropositive status was defined as positive WB result at screening. Viral Load (VL) and WB results at screening visit were employed to categorize individuals into Fiebig stages I-III (VL positive, WB negative), IV (VL positive, WB indeterminate), V (VL positive, WB positive with p31 band negative), VI (VL positive, WB positive with p31 band positive) according to previous work.

Seven plasma antibody isotypes (IgA, IgM, IgG1, IgG2, IgG3, IgG4 and total IgG) were quantified in the screening samples by an in house ELISA. HIV specific antibodies of different subtypes (IgG1, IgG2, IgG3, IgG4) for HIV antigens (gp41-Env, p24-Gag and p31-integrase) were performed by multi-parametric flow cytometry.

Quantification of plasma cytokines (see Detailed Methodology section in the Supplementary data). Multiplex cytokine profiling was performed for a total of 54 cytokines in screening plasma samples with particular interest in biomarkers of intestinal damage (10 biomarkers), T, B and monocyte cells function (23 biomarkers) and general biomarkers of inflammation and cell death (21 biomarkers) (Supplementary table 1). Determinations were performed by ELISA assay or Luminex multi-analyte profiling technology.

Statistical analysis

Group comparisons by Fiebig stage were performed using the non-parametric Kruskal-Wallis test and Chi-squared test for categorical variables. Multiple tests were adjusted using the Bonferroni correction. Post hoc pairwise comparisons between Fiebig stages were performed by Dunn’s test with Bonferroni adjustment. Comparisons between PHI and non-HIV infected controls were based on the non-parametric Mann-Whitney U-test. Identification of cytokines with the best performance in distinguishing
between pre- and post-seroconversion stages was performed using Random Forest analysis. Random forest is a supervised learning method based on the ensemble of multiple classification trees \(^{22}\). Relation between early and late Fiebig stages with respect the selected cytokines were explored by Principal Component Analysis (PCA). Patterns of the selected cytokines by PHI phases was assessed by multivariate logistic regression. Outcome was a binary variable where 1 represents pre-seroconversion AHI group (Fiebig I-IV) and 0 represents recent HIV infected group (Fiebig V-VI). In both PCA and regression model, MIG, MCP-1 and BAFF values were log transformed for a better adjustment of the data. Clinical variables (age, gender, VL and other co-infection) were tested for inclusion in the model by backward step-wise elimination approach (inclusion criteria was p-value<0.05 in multivariate analysis). Statistical analyses were performed using R-3.2.2 and Stata14 software.

**Results**

**Identification of primary HIV infected individuals**

Among the 4011 outpatients screened at the MDH, 3000 (74.8\%) presented with fever-like symptoms and 1011 (25.2\%) for voluntary HIV testing. Seven hundred and ninety-nine (20\%) were seropositive on rapid testing, and a further 92 (2.3\%) were excluded prior to serological testing, mainly due to residence outside of the study area (Figure 1). A majority (64.4\%) of screened patients were under the age of 30, and most (62.8\%) were female. These age and gender tendencies were similar between seropositive patients and those subjects who met criteria for inclusion in the study (data not shown).
Of the 3116 rapid test seronegative or indeterminate patients who entered pyramid pooling for HIV RNA testing, 87.1% were individuals presented for non-specific febrile symptoms. Ninety-three had a detectable level of HIV-RNA and 4 patients were excluded for presenting an inconclusive HIV-RNA result. Eight of these rapid test-seronegative HIV-RNA-positive cases were found to be positive for antibodies against all the HIV antigens by subsequent Western Blot testing and excluded (Figure 1). Eighty-five individuals thus fulfilled criteria for PHI; a prevalence of 2.73% (95% confidence interval 2.18-3.36) among all enrolled patients, and 3.02% (95% confidence interval 2.41-3.74) among those who were symptomatic at presentation.

**Characteristics of the primary HIV infected population**

At the screening visit, the median age of PHI cases was 24 years (IQR 20-30), 58% were female and most (96%) presented with a non-specific febrile syndrome to the outpatient ward including reported fever, flu or headache (Table 1). There was no significant difference in age, gender balance or malaria co-infection between the 85 PHI cases and the 3019 HIV-negative subjects. PHI cases reported a significantly greater total number of symptoms than did HIV-negative patients, and were significantly more likely to report fever in the last 24 hours (p-value=0.024), and intestinal complaint (diarrhoea or abdominal pain) in the last week (p-value<0.001). Fifty-six PHI cases (70%) returned to commence follow-up and n=58 HIV-negative participants were randomly selected to attend the 1 month follow-up visit as a reference group. There was no significant difference in age, gender or HIV-RNA between PHI patients who returned and those who were lost to follow-up (Supplementary table 2). After one month of follow-up, median CD4 T-cell count in PHI-individuals was significantly lower
than in the non-HIV infected group, 585 (IQR 460-682) vs. 955 (IQR 773-1149) cells/uL respectively (p-value<0.0001); while median CD8 T-cell count was significantly higher, 1119 (IQR 741-1650) vs. 591 cells/uL (IQR 417-746) respectively (p-value<0.0001).

**Fiebig staging of PHI patients**

The majority of PHI cases (74.1%) was seronegative at presentation (*Table 1*), with the remainder indeterminate on rapid tests. Patient HIV-RNA levels at presentation were high, with a median of Log_{10} 6.3 copies/mL (IQR 5.2-7.3) (*Table 1*). Classification according to Fiebig\textsuperscript{14} revealed that the majority of the 85 PHI cases were in Fiebig I-III, 45 (52.9%) whereas 8 (9.4%), 12 (14.1%) and 20 (23.5%) were in Fiebig IV, V, and VI respectively. There was no significant difference in age, gender balance or initial complaint between Fiebig groups (*Table 1*). The later Fiebig stages did, however, have a higher prevalence of malaria infection (p-value=0.049), and higher rate of intestinal complaints in the previous week (p-value=0.010), when compared with the earlier stages. As expected, the proportion of PHI cases with an indeterminate result for HIV rapid test was significantly higher in Fiebig VI (85%, p-value <0.001) and the median HIV-RNA level lower (p-value=0.0001) compared with the earlier Fiebig stages.

**Kinetics of subclass HIV-specific humoral responses during PHI**

In order to quantify the nascent HIV-specific IgG response by Fiebig stage in our cohort, we assessed levels of IgG1, IgG2, IgG3, and IgG4 specific for p24, gp41 and p31 antigens. The levels of all antibodies against gp41 and p24 detected for subjects in Fiebig I-III were similar to those in Fiebig IV. HIV-specific antibody levels significantly
increased in Fiebig V and Fiebig VI as compared to Fiebig I-III and IV (Figure 2). As expected from WB results, in the case of antibodies against p31 antigen, only the Fiebig VI group demonstrated significantly higher median level of IgG1 compared with the rest of the Fiebig groups (p-value <0.05).

**Kinetics of biomarker expression patterns during PHI**

From total of 61 biomarkers assessed, 49 were quantifiable in more than 75% of the samples and were included for analysis (Supplementary table 1). Besides VL, nine biomarkers were differentially expressed across Fiebig groups including total plasma IgG, IgG1, sCD163, MCP-1, sCD23, BAFF, MIG, TRAIL and TNFα (p-value<0.1, Figure 3). Total plasma IgG and IgG1 were highly correlated (Spearman rho=0.78, p-value<0.0001); thus, only total IgG1 was maintained in subsequent analyses and 8 biomarkers were then considered differentially expressed. Patients in Fiebig I-III showed a significantly different biomarker profile compared with those in Fiebig VI (p-value<0.01). Levels of total IgG, IgG1, sCD163, sCD23 and MIG significantly increased with Fiebig stages, and showed a weak negative correlation with viral load (rho<-0.4 ). In contrast, MCP-1, BAFF, TRAIL and INFα biomarkers were expressed at high levels in Fiebig I-III and IV stages and decreased in Fiebig V and VI stages, significantly correlating with VL levels (p<0.0001, MCP-1 Spearman rho=0.60, BAFF rho=0.60, TRAIL rho=0.48 and INFα rho=0.47). There was no significant difference in the plasma level of these biomarkers between Fiebig I-III and Fiebig IV or between Fiebig V and Fiebig VI (p-value>0.05).

**Cytokine expression profile can classify primary HIV infection into phases**
In order to determine whether the subgroup of eight biomarkers was associated with the acute pre-seroconversion stage of the PHI, cases were grouped into two categories: 1) the pre-seroconversion acute group roughly covering the 1st month of PHI (Fiebig I-IV) and 2) the post-seroconversion group referring to recent PHI (Fiebig V-VI) when HIV-specific antibodies are detectable by Western Blot.

Random Forest analysis models consistently selected BAFF, MCP-1, sCD163 and MIG as having the best power to discriminate between the pre and post-seroconversion phases. Additionally, a biplot representation generated from principal component analysis (PCA) showed differential expression patterns for the selected biomarkers according to the grouping (Figure 4A). Multivariate regression was performed in order to assess the power of the four cytokines to distinguish between the two categories (Figure 4B). Based on regression diagnostics measures, five outlier observations were excluded from model fitting. Clinical variables (age, gender, VL or other co-infection) showed no significant association or interaction with the selected biomarkers (data not shown) and were thus eliminated from the model.

BAFF and MCP-1 were positively associated with the pre-seroconversion group while sCD163 and MIG were negatively associated (Fig. 4B). The multivariate model was capable of correctly identifying 49 out of the 53 individuals in the pre-seroconversion phase, and 26 out of the 32 in the post-seroconversion phase. This goodness of fit allowed the model to identify patients in the pre-seroconversion phase with a sensitivity of 92.5% and a specificity of 81.2%.
Discussion

This study has identified a signature of four cytokines composed of BAFF, MCP-1, sCD163 and MIG that is highly associated with PHI prior to development of the HIV-specific humoral response as determined by WB. BAFF and MCP-1 are highly expressed in the first month of PHI whereas sCD163 and MIG rise several weeks later.

Despite roll out of ART and HIV prevention activities, the 3.0% (95% CI 2.4-3.7) prevalence of PHI among people presenting with fever-like symptoms at the outpatient ward of this rural area of Mozambique has remained unchanged as compared to a study performed 5 years earlier which showed an PHI prevalence of 3.3% (95% CI 1.3–6.7). Given the high risk of transmission during this phase, these data represent an important threat to public health. Periodic cross-sectional PHI screening of people presenting with fever like symptoms in malaria-endemic high HIV incidence settings could act as an indirect surrogate to monitor evolution of HIV epidemic and compare tendencies between different communities or assessing the impact of prevention campaigns.

In our cohort of primary HIV infected individuals, the nascent HIV-specific antibody response showed a distinct pattern of expression by Fiebig stages. The kinetics of antibody generation during PHI may vary according to individual factors, subtype and dose of infecting virus. Previous studies have shown that anti-Env HIV-1 plasma antibodies are predominantly IgG1 subclass, followed by anti-Env IgG3 subclass during PHI. Results from our cohort are largely similar. However, very few individuals in the earliest Fiebig stages (I-IV) showed positive IgG3 responses to gp41. These
results slightly differ from Yates et al.\textsuperscript{24,25} where they found anti-gp41 IgG3 to be positive for all PHI individuals. The timing of detection of antibodies could be influenced by the extent of immune complex formation preceding detection of free antibody. This could differ by viral load, geographic location or HIV clade. In contrast to our Mozambican clade C cohort, their PHI cohort included 37% African individuals infected with HIV clade C, which could explain discrepancies.

In parallel to the stepwise HIV-specific antibody generation, AHI is a period when many other immune processes are occurring, specifically the innate immune response. A general cytokine storm occurring early after HIV infection has described peak levels of many inflammatory cytokines prior to seroconversion.\textsuperscript{18–20,26,27} In order to fine-tune the immune response pattern, we have assessed those previously described cytokines as well as other relevant innate immunity effector molecules. From a total of 49 analytes that were quantifiable in this cohort, 9 biomarkers demonstrated significant difference in the expression level across Fiebig groupings. Furthermore, four of these biomarkers, BAFF, MCP-1, sCD163 and MIG, exhibited the best predictive power to distinguish between individuals in pre and post-seroconversion stages of PHI. Modelling the expression profile of these four cytokines identified with high sensitivity and specificity those individuals in which the antibody generation was still not developed.

Most of the participants (87.1%) were screened for PHI through sampling of individuals presenting with febrile syndrome. While this is the most practical way of finding PHI, the cytokine and antibody expression profile in symptomatic individuals may not be generalizable to asymptomatic individuals. Although Hepatitis B, syphilis
and intestinal pathogen co-infections were not found to be associated with stage of HIV infection or levels of cytokines, we cannot exclude that other infections commonly present in HIV-infected individual, could affect plasma levels of these biomarkers. Another limitation is the lack of resolution of early Fiebig stages due to sample volume restrictions. We cannot exclude that cytokine expression may differ between Fiebig I-III. A detectable gradient of cytokine expression could become visible particularly in the transition through Fiebig III, similar to Fiebig IV. However this would likely maintain or even strengthen the signature association.

To our knowledge, no cytokine signature has been previously described to be associated with the pre-seroconversion phase of PHI. In our cohort, increased pre-seroconversion expression of MCP-1 was accompanied by elevated BAFF. In contrast, sCD163 and MIG showed a delayed increase during the early infection process which paralleled the decrease in BAFF and MCP-1. Monocyte chemotactic protein (MCP-1) and B-cell activating factor (BAFF) are both produced by monocytes, macrophages and dendritic cells after infection or tissue inflammation in order to assure a proper immune response and maintenance of normal immunity\textsuperscript{28–30}. In contrast, macrophage low-grade inflammation marker (sCD163) and monokine induced by IFN-γ (MIG) are cytokines involved in T-cell trafficking to inflammatory sites and control of T-cell proliferation\textsuperscript{31–33}. Since BAFF and MCP-1 expression is associated with early innate responses, they may be the very first effectors in the HIV immune cascade followed closely by sCD163 and MIG, associated with the initiation of adaptive immune responses. Further studies including gut biopsies in humans or non-human primates may show whether BAFF and MCP-1 expression are associated with viral shedding or
early HIV-induced GALT destruction.

There is an interest in approaching AHI as a phase of infection in which early ART coupled with adjunct targeted immune therapy could limit the destruction of the mucosal lymphoid tissue and seeding of the HIV reservoir. Approaches include boosting HIV-specific immunity, reducing inflammation, activating latency and targeting HIV apoptosis regulatory pathways. Recent studies also point out that cART initiated during early AHI either prevents loss of (at Fiebig stage I/II) or restores (at Fiebig stage III) mucosal Th17 cells. Consequently, very early ART is associated with normalization of local and systemic immune activation, reversing a hallmark of HIV pathogenesis.

Although further analysis will be required to define whether there is a causal relationship between BAFF and MCP-1 expression and antibody generation, these cytokines might be potential targets to boost the humoral response in vaccination strategies or to accelerate the HIV-specific antibody response during PHI. Modulation of these cytokines might promote an earlier development of autologous neutralizing antibodies that may contribute to faster and long lasting control of HIV.
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**Author’s contributions:**

DN and JB study design. LP, EP, LF and DR recruited subjects and collected clinical data. LP, EP LF, LL and CJ performed laboratory analysis at the field. LP and JC performed biomarker quantification at the laboratory and validation of the data. LP, EP and VU performed statistical analyses. LP, EP, JC, JB and DN interpreted the data. LP and EP drafted the paper. DN, JB and IM coordinated the work and supervise manuscript writing. All authors read and approved the final version of the manuscript.

**Competing Financial Interests:**

All authors do not have any commercial or other associations that might pose a conflict of interest with the data presented in this paper.
References


25. Yates NL, Stacey AR, Nolen TL, et al. HIV-1 gp41 envelope IgA is frequently


FIGURE LEGENDS

**Figure 1: Screening profile:** VL=viral load, WB=Western Blot test, Sympt.=symptomatic patients, VCT=patients presented for voluntary counselling and testing. *Study protocol was changed to include rapid test indeterminate patients, however four individuals were excluded prior to this change.

**Figure 2. HIV-specific antibody expression according to Fiebig stage.** The median MFI (mean fluorescence intensity) is shown for antibodies specific for A) gp41, B) p24 and C) p31. Box as IQR, middle line as median, whiskers as Tukey values (1.5 IQR). Values are Log_{10} scale for a better adjustment of the data. Nonparametric pairwise multiple comparisons were performed by using Dunn’s test. Bonferroni-adjusted significance is indicated as *** if p<0.001, ** if p<0.01 and * if p<0.05. Median values for the non-febrile non-HIV infected reference group are indicated as dotted lines.

**Figure 3. Expression of selected parameters varies by Fiebig stage.** Nine biomarkers were differentially expressed across Fiebig groups including total plasma IgG, IgG1, sCD163, MCP1, sCD23, BAFF, MIG, TRAIL and TNFα (p-value<0.1), as was VL (p-value<0.001) by Kruskall-Wallis range comparison (Bonferroni adjusted p-value <0.01). Median levels of selected parameters are represented as middle line, box as IQR and whiskers as Tukey values (1.5 IQR). Comparison of individual difference in expression level by Fiebig group was performed by Dunn’s comparison test adjusted by Bonferroni for multiple comparisons as shown above. Significance is indicated as *** if p<0.001, ** if p<0.01 and * if p<0.05. Median values for the non-febrile non-HIV infected reference
group are indicated as dotted lines.

**Figure 4. Cytokines with best discriminatory power to differentiate between early and late Fiebig stages.** A. Component analysis of cytokine levels. Principal component analysis of BAFF, MCP-1, MIG and sCD163 levels in patients in pre- or post-seroconversion stages (Fiebig I-IV, Fiebig V-VI respectively). B. Multivariate logistic-regression to identify cytokines associated with pre-seroconversion stage of HIV infection (Fiebig I-IV). Significance of the variable in the model is indicated as ** if p<0.01 and * if p<0.05. In both PCA and regression model, MIG, MCP1 and BAFF values were log transformed for a better adjustment of the data.
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Table 1. Clinic and demographic characteristics of study population according to HIV-status and Fiebig stage. Comparisons for proportions were performed by chi2 test* and continuous variables by Mann and Whitney U-test* for the two group comparison and global comparison by Kruskal Wallis test**. p-value obtained from (1) comparison by study group or (2) comparison by Fiebig group.

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<th>HIV-neg at Screening (n=3019)</th>
<th>PHI at Screening (n=85)</th>
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<th>Fiebig I-III (n=45)</th>
<th>Fiebig IV (n=8)</th>
<th>Fiebig V (n=12)</th>
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<td>Age (years) [Median (IQR)]</td>
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<td>24 (20-30)</td>
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<td>Gender [F (%)]</td>
<td>1900 (63)</td>
<td>50 (58)</td>
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<td>Initial complaint [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>80 (3)</td>
<td>1 (1)</td>
<td>0.401*</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.395*</td>
</tr>
<tr>
<td>Febrile syndrome</td>
<td>2385 (79)</td>
<td>75 (88)</td>
<td>0.039*</td>
<td>41 (91)</td>
<td>8 (100)</td>
<td>11 (92)</td>
<td>15 (75)</td>
<td></td>
</tr>
<tr>
<td>Flu symptoms</td>
<td>153 (5)</td>
<td>6 (7)</td>
<td>0.412*</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (20)</td>
<td></td>
</tr>
<tr>
<td>Voluntary testing</td>
<td>395 (13)</td>
<td>3 (4)</td>
<td>0.010*</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>1 (5)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>6 (0)</td>
<td>0 (0)</td>
<td>1*</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Fever last 24h [n (%)]</td>
<td>2503 (83)</td>
<td>80 (94)</td>
<td>0.024*</td>
<td>43 (96)</td>
<td>8 (100)</td>
<td>11 (91)</td>
<td>18 (90)</td>
<td>0.703*</td>
</tr>
<tr>
<td>Malaria [n (%)]</td>
<td>337 (17)</td>
<td>7 (11)</td>
<td>0.234*</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>3 (33)</td>
<td>3 (17)</td>
<td>0.049*</td>
</tr>
<tr>
<td>Intestinal complaint last week [n (%)]</td>
<td>84 (3)</td>
<td>9 (11)</td>
<td>&lt; 0.001*</td>
<td>1 (2)</td>
<td>1 (12)</td>
<td>1 (8)</td>
<td>6 (30)</td>
<td>0.010*</td>
</tr>
<tr>
<td>RT Serostatus [n (%)]</td>
<td>-</td>
<td>63 (74)</td>
<td>44 (98)</td>
<td>8 (100)</td>
<td>8 (67)</td>
<td>4 (33)</td>
<td>3 (15)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Negative Indeterminate</td>
<td>-</td>
<td>22 (26)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>17 (85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral Load (RNA Log 10 copies/mL) [Median (IQR)]</td>
<td>-</td>
<td>6.3 (5.2-7.3)</td>
<td>-</td>
<td>7.1 (6.2-7.5)</td>
<td>7.0 (6.3-7.4)</td>
<td>5.6 (4.9-6.3)</td>
<td>4.8 (4.3-5.5)</td>
<td>0.0001**</td>
</tr>
</tbody>
</table>
Europe PMC plus has received the file 'Supplementary data_LuciaPastor.docx' as supplementary data. The file will not appear in this PDF Receipt, but it will be linked to the web version of your manuscript.