Interferon-γ–Inducible Protein 10 (IP-10) as a Screening Tool to Optimize Human Immunodeficiency Virus RNA Monitoring in Resource-Limited Settings

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Background. Achieving effective antiretroviral treatment (ART) monitoring is a key determinant to ensure viral suppression and reach the UNAIDS 90-90-90 targets. The gold standard for detecting virological failure is plasma human immunodeficiency virus (HIV) RNA (viral load [VL]) testing; however, its availability is very limited in low-income countries due to cost and operational constraints.

Methods. HIV-1–infected adults on first-line ART attending routine visits at the Manhiça District Hospital, Mozambique, were previously evaluated for virologic failure. Plasma levels of interferon-γ–inducible protein 10 (IP-10) were quantified by enzyme-linked immunosorbent assay. Logistic regression was used to build an IP-10–based model able to identify individuals with VL >150 copies/mL. From the 316 individuals analyzed, 253 (80%) were used for model training and 63 (20%) for validation. Receiver operating characteristic curves were employed to evaluate model prediction.

Results. From the individuals included in the training set, 34% had detectable VL. Mean age was 41 years, 70% were females, and median time on ART was 3.4 years. IP-10 levels were significantly higher in subjects with detectable VL (108.2 pg/mL) as compared to those with undetectable VL (38.0 pg/mL) (P < .0001, U test). IP-10 univariate model demonstrated high classification performance (area under the curve = 0.85 [95% confidence interval [CI], .80–.90]). Using a cutoff value of IP-10 ≥44.2 pg/mL, the model identified detectable VL with 91.9% sensitivity (95% CI, 83.9%–96.7%) and 59.9% specificity (95% CI, 52.0%–67.4%), values confirmed in the validation set.

Conclusions. IP-10 is an accurate biomarker to screen individuals on ART for detectable viremia. Further studies should evaluate the benefits of IP-10 as a triage approach to monitor ART in resource-limited settings.

Keywords. global health; cytokines; implementation research; scale-up viral load; sub-Saharan Africa.

Received 16 April 2017; editorial decision 13 June 2017; accepted 4 July 2017.
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Clinical Infectious Diseases® 2017;XX(00):1–7
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DOI: 10.1093/cid/cix600

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Expression of several inflammatory and immune response cytokines is increased during HIV replication [10]. Previous studies have shown that plasma levels of interferon-γ-inducible protein 10 (IP-10) correlated with VL [11, 12] and VL set-point [13] during untreated primary HIV infection and decline after ART initiation both in early [14] and chronically HIV-infected individuals [15]. Similarly, we have seen that from a total of 42 inflammatory biomarkers, IP-10 shows the strongest association with VL and the best predictive power to identify acute HIV infection among febrile seronegative patients (Pastor et al, manuscript in preparation). We hypothesized that, because of its strong association with VL, plasma IP-10 level could be a surrogate marker of detectable viremia in ART-treated individuals, providing a simple and affordable screening tool to detect individuals with VF in LMICs.

METHODS

Study Population

The present analysis is a substudy of a cross-sectional cohort for detecting drug resistance in ART-treated adults enrolled between February and March 2013 at the Manhiça District Hospital (MDH), Maputo, southern Mozambique [16]. At the time of the study, current HIV national guidelines recommended ART initiation in patients with a CD4 T-cell count ≤350 cells/μL, and no routine VL monitoring was provided after ART initiation. The study protocol was approved by the institutional review boards and ethics committees of the Barcelona Clinic Hospital, Badalona Germans Trias i Pujol Hospital, Spain, and the National Committee on Health Bioethics, Mozambique. All study participants provided signed informed consent.

In brief, adults >18 years of age attending routine scheduled outpatient visits for clinical management of HIV/AIDS at the MDH were enrolled in the study. All patients had documented HIV infection, documented ART initiation ≥12 months earlier, and provided written informed consent. The sample and data collection procedures have been previously described [16] and include a single blood sample and sociodemographic and clinical data collected in a specific questionnaire.

Laboratory Procedures

HIV RNA levels were determined in plasma samples by reverse-transcription polymerase chain reaction (Abbott m2000 RealTime System with a detection limit of 150 copies/mL), and CD4+ T-cell counts were determined in whole blood by flow cytometry using FACSCalibur (BD Biosciences) as previously described [16]. IP-10 level was measured in plasma samples by enzyme-linked immunoabsorbent assay (Human Duo-Set ELISA, R&D Systems, Minneapolis, Minnesota) according to the manufacturer’s instructions; 0.05% Tween-20 (Sigma-Aldrich, St Louis, Missouri) 1% bovine serum albumin (Sigma-Aldrich) in phosphate-buffered saline was used as blocking solution, 3, 3′, 5, 5′-tetramethylbenzidine (Sigma-Aldrich) as substrate, and 4N sulfuric acid as stop solution. Optical density was measured at 492 and 620 nm. Values assigned to data falling outside quantification limits were the double and the half of the upper and lower quantification limits, respectively.

Statistical Analysis

Data were double-entered using Fox Pro version 2.6 (Microsoft Corporation, Redmond, Washington) and analyzed using R-3.2.2 software and Stata version 14 software (StatCorp, College Station, Texas).

Proportions and continuous variables were compared using χ² and nonparametric Mann-Whitney U test, respectively. IP-10 values were log transformed for a better adjustment of skewed data. Spearman test was used to assess correlation coefficients for continuous variables.

To assess the capacity of IP-10 levels and clinical variables to correctly identify the cases, logistic regression with penalized likelihood was performed [17]. According to random selection of the 316 individuals included in this analysis, 80% were used for data analysis and model construction (n = 253) and 20% for model validation (n = 63). A multivariate logistic regression model was built applying a stepwise selection to the set of variables: IP-10, sex, age, CD4 T-cell count, body mass index (BMI), days on ART, and presence of symptoms. In the selection, variables with P values <.05 could enter into the model whereas a P value <.10 was required to be retained. Outcomes tested were detectable VL (defined as HIV RNA >150 copies/mL) and VF (defined as HIV RNA >1000 copies/mL). Diagnostic capacity was determined using receiver operating characteristic (ROC) analyses. ROC curves from univariate and multivariate models were compared for the best prediction.

RESULTS

Population Characteristics

Of the 332 individuals included in the cross-sectional analysis for drug resistance [16], IP-10 was determined in 316 (95.2%). We thus trained our model on 253 (80%) of the individuals with available IP-10 data. The mean age of the 253 individuals included in the training set was 41 years (standard deviation [SD], 10 years) and 70% were females. Median time on ART was 3.4 years (interquartile range [IQR], 2.1–5.3 years) and 89% were receiving zidovudine/lamivudine/nevirapine at the time of the survey. Mean BMI was 23.2 kg/m² (SD, 3.9 kg/m²), median CD4 T-cell count was 439 cells/μL (IQR, 273–593 cells/µL), and 38% presented any type of symptoms at the time of the survey. Thirty-four percent had detectable VL (86/253) and 25% (64/253) met criteria for the standard definition of VF (VL >1000 copies/mL) [4]. In contrast, when clinical and immunological criteria were used, only 12% (29/245) of subjects were...
suspected to have ART failure. Population characteristics did not significantly differ from those of individuals included in the cross-sectional analysis of resistance [16] nor of those included in the validation set (P > .1).

Evaluation of an Interferon-γ–Inducible Protein 10–Based Model to Identify Detectable Viremia

Median IP-10 levels were significantly higher among individuals with detectable VL compared to those with undetectable VL (108.2 pg/mL vs 38.0 pg/mL, respectively; U test P < .0001; Figure 1). IP-10 levels did not significantly differ from those of individuals included in the validation set (P > .1). Among those individuals with detectable VL, IP-10 levels were significantly correlated with VL (ρ = 0.33, P = .002).

Univariate analysis showed that IP-10 was significantly and positively associated with detectable VL (odds ratio, 1.47 per 10% IP-10 pg/mL increase; P < .0001). The ROC curve demonstrated high predictive power for classification of individuals with detectable VL with an area under the curve (AUC) = 0.85 (95% confidence interval [CI], .80–.90). Sex, age, BMI, CD4 T-cell count, days on ART, and presence of any symptoms at the visit day were considered for inclusion in a multivariate analysis together with IP-10. However, only IP-10 and CD4 T-cell count were retained in the model and the resulting multivariate model did not increase the classification performance (AUC = 0.85 [95% CI, .80–.90]; Figure 2).

Then, ROC curve for the univariate IP-10 model was used to evaluate several cutoff values prioritizing the highest sensitivity. A cutoff of IP-10 ≥44.2 pg/mL was selected, providing a sensitivity of 91.9% (95% CI, 83.9%–96.7%) and a specificity of 59.9% (95% CI, 52.0%–67.4%) for predicting detectable viremia (Figure 2B).

The IP-10 model with a cutoff of ≥44.2 pg/mL was assessed for predictive accuracy to identify individuals with detectable VL. We calculated positive and negative predictive values (PPV and NPV, respectively) using model sensitivity and specificity and their respective 95% CIs (Figure 3). Applying the prevalence of detectable VL of 36% observed in the cross-sectional resistance study [16] and the IP-10 model sensitivity and specificity, the PPV would be 56.3% (Figure 3A) and the NPV would be 92.9% (Figure 3B).

Validation of the Model Accuracy

When we applied the IP-10 model to the validation panel of samples, 80% of the 20 individuals with detectable VL and 58% of the 43 individuals with undetectable VL were correctly classified. The sensitivity and specificity derived from the validation panel were not statistically different from those obtained with the training set (equality of proportions test P = .115 and .953, respectively), thus confirming the predictive power of the univariate IP-10 model for identification of ART-treated individuals with detectable VL.

The prevalence of standard VF (VL >1000 copies/mL) observed in the study population was 25% (64/253). Both univariate and multivariate models were also tested for their ability to detect individuals with standard VF, showing lower specificity for a given sensitivity than the model designed to identify individuals with detectable VL (VL >150 copies/mL; data not shown).

Comparison of Plasma Interferon-γ–Inducible Protein 10 Versus Human Immunodeficiency Virus-RNA Quantification to Predict Detectable Viral Load

We then combined the training and validation panels to estimate the number of VL assays required for ART monitoring in this population using either the IP-10–based algorithm or standard VL testing (Table 1). Whereas using VL monitoring alone would require 316 VL determinations to detect 106 individuals harboring detectable VL, the use of an IP-10 screening test followed by VL confirmation would only require 180 VL determinations. The IP-10 screening test would thus require 43% fewer VL determinations and identify 89.6% of those with detectable VL in this sample population.

An analysis from the Global Fund’s Price and Quality Reporting Tool found that VL reagent costs alone varied from US $13.13 to US $43.34 between countries [3, 18]. Considering US$28 as the average reagent cost for HIV-VL testing, translated to dollar values, the VL testing of these 316 treated individuals would imply around US$88850 per year to the health system. On the other hand, considering a unit cost of US$1.50
based on costs for other similar cytokines [19], the IP-10 screening, together with the 180 VL determinations required in this cohort, would imply a cost of around US$5510 per year. This means that introducing an IP-10–based screening for ART monitoring would save US$3340 a year for this cross-sectional study cohort, resulting in savings of 38% in VL-associated cost.

**DISCUSSION**

We have demonstrated that a cutoff value for IP-10 of ≥44.2 pg/mL gave a sensitivity of 91.9% (95% CI, 83.9%–96.7%) and a specificity of 59.9% (95% CI, 52.0%–67.4%) for predicting detectable viremia in individuals on ART for more than a year. Thus, we have shown that IP-10 is a simple biomarker that can be used to screen individuals on ART for VF, reducing the number of costly VL determinations required to monitor ART in LMICs.

The most recent WHO guidelines recommend routine tracking of ART effectiveness using VL testing at 6 and 12 months after treatment initiation and every 12 months in stable patients to minimize treatment failure [5]. However, the high cost and technical complexity of VL testing has hampered scale-up in resource-limited settings. In absence of routine VL testing, the use of clinical and CD4 monitoring in many sub-Saharan African countries has been shown to favor the emergence of...
VF and drug resistances [8, 16]. In Mozambique, recent cross-sectional surveys reported that 23% [7] and 36% [16] of individuals on ART, in Maputo and Manhiça District, respectively, had detectable HIV viremia.

IP-10 is an inflammatory cytokine produced as part of the innate immune response to different pathogens [20]. IP-10 has been explored for its use as both a diagnostic and prognostic marker for several infectious diseases, such as malaria, hepatitis C, or tuberculosis [20–22]. In the case of HIV infection, previous data suggested that IP-10 levels were predictive of disease progression [11, 23] and significantly decreased between 6 months [14] and 2 years [15] after ART initiation; however, to our knowledge, the use of IP-10 as a biomarker for VL levels has not been assessed for its accuracy to detect VF in individuals on ART.

Our results show that IP-10 can indeed be used as a surrogate marker of VL with high accuracy to screen ART-treated individuals and identify patients most likely to have VL levels >150 copies/mL. This is particularly relevant in countries with scarce resources where the scale-up of ART often leads to delays and even failure to return VL results due to congested and/or centralized health facilities, which compromises both quality health services and patient retention in care [3]. Health and laboratory system strengthening together with reductions in costs and decentralization of services are required to implement the effective VL monitoring necessary to reach UNAIDS 90-90-90 targets in low-income settings [3]. Health and laboratory system strengthening together with reductions in costs and decentralization of services are required to implement the effective VL monitoring necessary to reach UNAIDS 90-90-90 targets in low-income settings [3].

As IP-10 plasma level is influenced by immune recovery [24], we assessed a multivariate model including clinical variables; however, predictive power was not significantly increased. Further studies should evaluate whether a combination of IP-10 with another inflammatory cytokine involved in virological responses or any other surrogate of immune activation could improve the accuracy for predicting detectable viremia and reduce misclassification. Moreover, optimization of model parameters across HIV subtypes and comorbidities present in different populations as well as sample types including dried blood spots (DBSs) could render this approach generalizable. Indeed, recent studies have shown accurate VL measurements by using DBS to monitor patients on ART in low-income settings [25]. IP-10 levels have also been demonstrated to be easily and precisely quantifiable in DBSs [26], and this approach has been recently validated for the diagnosis of tuberculosis.

<table>
<thead>
<tr>
<th>VL Result (Gold Standard)</th>
<th>IP-10 Classification</th>
<th>Undetectable VL</th>
<th>Detectable VL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No case</td>
<td>125 (59.5)</td>
<td>11 (10.4)</td>
<td>136 (43.0)</td>
<td></td>
</tr>
<tr>
<td>Potential VF</td>
<td>85 (40.5)</td>
<td>95 (89.6)</td>
<td>180 (57.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>210 (100)</td>
<td>106 (100)</td>
<td>316 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as No. (%). IP-10 model with a cutoff of ≥44.2 pg/mL was compared to gold standard VL for classification performance of individuals with detectable VL (>150 copies/mL). Note that both training and validation sets were included to simulate a hypothetical classification in a cross-sectional cohort in Mozambique.

Abbreviations: IP-10, interferon-γ-inducible protein 10; VF, virologic failure; VL, viral load.
and monitoring antituberculosis treatment response [28]. Further studies should evaluate the predictive power of IP-10 levels quantified in DBSs to identified cases with detectable viremia among ART-treated individuals. This strategy would offer decentralized VF diagnosis, facilitate sample storage and transportation, and reduce test unit cost [29].

We thus propose a novel IP-10 screening algorithm for detection of VF. At a time when UNAIDS estimated that 18.2 million people were receiving ART in 2016 and nearly 12.7 million people will initiate ART in the next few years [30], further studies are warranted to assess the impact of implementing this simple triage approach to reduce the volume of VL determinations required for monitoring viral suppression.

Notes

Author contributions. L. P., J. C., R. P., J. B., and D. N. were responsible for conceptualization and study design. M. R. and S. M. recruited subjects and collected and validated clinical data. M. R. and C. J. coordinated sample collection and processing at the field. L. P. performed biomarker quantification at the laboratory and validation of the data. L. P. and A. C. performed statistical analyses. L. P., A. C., J. B., and D. N. interpreted the data. L. P. drafted the paper. J. B. and D. N. performed critical data review and revision of manuscript writing. All authors read and approved the final version of the manuscript.

Acknowledgments. The authors are grateful for the continued support of the clinical staff at the Manhiça District Hospital, as well as the study staff working exhaustively at the field and laboratory at the Centro de Investigação de Saúde de Manhiça (CISM). The authors thank Víctor Urrea and Helder Bulo for their contribution to study and laboratory coordination. The authors thank Victor Urrea and Helder Bulo for their contribution to study and laboratory coordination at CISM. The authors thank Víctor Urrea and Helder Bulo for their contribution to study and laboratory coordination at Manhiça District Hospital. The study which provided samples was supported by Gilead Sciences.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


