Low Lopinavir Plasma or Hair Concentrations Explain Second-Line Protease Inhibitor Failures in a Resource-Limited Setting

Gert Uves van Zyl, MBChB, FCPath(SA) Virol, Thijs E. van Mens,† Helen McIlleron, MBChB, PhD,‡ Michele Zeier, MBChB,§ Jean B. Nachega, MD, PhD,§‖ Eric Decloedt, MBChB,‡ Carolina Malavazzi, MD,¶ Peter Smith, PhD,‡ Yong Huang, PhD,# Lize van der Merwe, PhD,**†† Monica Gandhi, MD, MPH,‡‡ and Gary Maartens, MBChB, FCP, DTM&H‡*

Background: In resource-limited settings, many patients, with no prior protease inhibitor (PI) treatment on a second-line, high genetic barrier, ritonavir-boosted PI-containing regimen have virologic failure.

Methods: We conducted a cross-sectional survey to investigate the aetiology of virologic failure in 2 public health antiretroviral clinics in South Africa documenting the prevalence of virologic failure (HIV RNA load >500 copies/mL) and genotypic antiretroviral resistance; and lopinavir hair and plasma concentrations in a nested case–control study.

Results: Ninety-three patients treated with a second-line regimen including lopinavir boosted with ritonavir were included, of whom 50 (25 cases, with virologic failure and 25 controls) were included in a nested case control study. Of 93 patients, 37 (40%) had virological failure, only 2 of them had had major PI mutations. The negative predictive values: probability of failure with lopinavir plasma concentration >1 μg/mL or hair concentrations >3.63 ng/mg for virologic failure were 86% and 89%, and positive predictive values of low concentrations 73% and 79%, respectively, whereas all virologic failures with HIV RNA loads above 1000 copies per milliliter, of patients without PI resistance, could be explained by either having a low lopinavir concentration in plasma or hair.

Conclusions: Most patients who fail a lopinavir/ritonavir regimen, in our setting, have poor lopinavir exposure. A threshold plasma lopinavir concentration (indicating recent lopinavir/ritonavir use) and/or hair concentration (indicating longer term lopinavir exposure) are valuable in determining the aetiology of virologic failure and identifying patients in need of adherence counselling or resistance testing.

Key Words: hair concentration, lopinavir, medication adherence, plasma concentration, protease inhibitor resistance mutations, resource-limited settings

(*J Acquir Immune Defic Syndr* 2011;56:333–339)

Received for publication October 5, 2010; accepted December 29, 2010.

From the *Division of Medical Virology, Department of Pathology, NHLS Tygerberg and Stellenbosch University, Tygerberg, South Africa; †Amsterdam Medical Centre, University of Amsterdam, The Netherlands; ‡Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa; §Centre for Infectious Diseases and Division of Infectious Diseases, Department of Medicine, Tygerberg Academic Hospital and Stellenbosch University, Tygerberg, South Africa; ‖Departments of International Health and Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; ¶Medicins Sans Frontieres, Cape Town, South Africa; #Drug Studies Unit, Department of Biopharmaceutical Sciences, University of California, San Francisco, CA; **Biostatistics Unit, Medical Research Council of South Africa, Cape Town, South Africa; ††Department of Biostatistics, University of Western Cape, Cape Town, South Africa; and ‡‡Division of Infectious Disease, Department of Medicine, University of California, San Francisco, CA

Supported by South African Department of Health, Comprehensive Care, Management and Treatment Grant (CCMT). J.B.N. is supported by The United States National Institutes for Allergy and Infectious Disease (NIAID/NIH), Division of AIDS (DAIDS): K23 AI 068582-01; The European Developing Countries Clinical Trial Partnership Senior Fellowship Award: TA-08-40200-021 and the Wellcome Trust Southern Africa Consortium for Research Excellence: WT087537MA and the NIH/FIC/HRSA/PEPFAR Medical Education Partnership Initiative (MEPI):T84H21126-01-01. G.M. is supported by the National Research Foundation, South Africa.

Authors contributions: G.V.Z. initiated the investigation. T.V.M. conducted patient recruitment and performed data collection; G.M., H.M., J.B.N. and M.Z. provided advice on study design, analytical methods, and interpretation of the data; E.D. provided methods and practical advise on hair collection; M.Z. and C.M. organized patient recruitment at the Tygerberg and Khayelitsha sites, respectively; and C.M. provided data on the Khayelitsha patients; G.V.Z. wrote the article draft with the help of G.M., J.B.N., H.M., and M.G.; LvdM provided statistical help and advice; M.G. and Y.H. conducted and developed the hair LPV and ritonavir analysis and described of the method and P.S. performed and validated the plasma LPV analysis. All authors approved the article.

The authors have no conflicts of interest to disclose.

Correspondence to: Gert U. van Zyl, MBChB, FCPath(SA) Virol, Division of Medical Virology, NHLS Tygerberg and Stellenbosch University, PO Box 19063, Tygerberg 7505, South Africa (e-mail: guvz@sun.ac.za).

INTRODUCTION

Adult HIV-infected patients in resource-limited settings who qualify for antiretroviral therapy (ART) are started on a first-line nonnucleoside reverse transcriptase inhibitor (NNRTI) –based regimen.[1,2] Lamivudine, a nucleoside reverse transcriptase inhibitor (NRTI) commonly used in first-line regimens, and NNRTIs both have low genetic barriers to

resistance—hence a single mutation confers a high level of resistance.[3–5] Therefore, if patients do not have adequate adherence to this first-line regimen, antiretroviral (ARV) resistance can arise rapidly resulting in virologic failure[6] and necessitating a switch to second-line therapy agents. The recommended second-line ART regimen in resource-limited settings is one composed of dual NRTIs and a ritonavir (RTV) -boosted protease inhibitor (PI), typically lopinavir/ritonavir (LPV/r).[2,7]

LPV/r has a high genetic barrier to resistance.[8] However, both LPV/r and didanosine, which until recently was one of the NRTIs recommended in combination second-line regimens, are commonly associated with gastrointestinal side effects,[9,10] which can limit this regimen's tolerability.

The prevalence of failure in patients on a second-line regimen has been reported to be as high as 33% in South African patients on LPV/r-based regimens.[11] Unlike virological failure on an NNRTI regimen, very few patients who fail a LPV/r-based regimen have resistance to LPV/r, provided they have not had prior exposure to PIs.[11–13] This suggests that most second-line virologic failures are due to inadequate adherence rather than viral resistance.[13] Because the appropriate management of patients, failing second-line therapy depends on the underlying aetiology of failure, it is important to distinguish inadequate drug exposure (likely due to adherence lapses) from ARV resistance. The identification of patients with poor drug exposure can limit unnecessary genotypic ARV resistance testing (GART), which is costly, enabling GART to be reserved for those who fail despite adequate drug exposure. This selective use of GART could aid in the choice of the next optimal regimen, either through using currently available drugs, or by guiding the choice of third-line regimen agents, once newer ARVs become accessible in resource-limited settings.

Self-report or pill counts are the adherence measurements used most often by ART programs in resource-limited settings, but both measures tend to overestimate adherence.[14] Measuring drug concentrations or therapeutic drug monitoring has the potential to provide an objective estimate of adherence. Trough or random lopinavir (LPV) plasma concentrations reflect recent adherence as the plasma half-life of LPV used in a LPV/r formulation is 5–6 hours, whereas LPV concentrations in hair specimens estimates longer term exposure. Recently, hair specimens have been used to measure ARV drug exposure, using techniques of liquid chromatography coupled with tandem mass spectrometry,[15] and PI concentrations in hair have been shown to strongly correlate with ARV success.[16]

METHODS

We conducted a cross-sectional study of virologic failure in adult patients on a second-line LPV/r-containing regimen. Patients were consecutively enrolled at Tygerberg Hospital Family Clinic and Ubunthu Clinic in Khayelitsha, Cape Town. Adult patients on a second-line LPV/r-containing regimen, for at least 1 month, who gave informed consent, were eligible for inclusion into the study. Basic demographic information, blood specimens for HIV-1 RNA load and LPV plasma concentrations, and hair specimens for LPV and ritonavir measurement were collected. Genotypic resistance testing was done in patients with HIV-1 RNA loads >500 copies per milliliter. Genotypic resistance testing was funded as part of a larger descriptive study and was performed at the Tygerberg Laboratory. However, as we had limited funding available and hair analysis was only available at a remote laboratory in San Francisco, California, we chose a nested case–control design as the most efficient strategy to evaluate the value of hair and plasma LPV concentrations. The recruitment target was 25 cases and 25 controls. Patients were included in the nested case–control study, based on having an appropriate matched control, matched by age and gender in the clinic. Patients were individually matched for gender and paired with the closest available age match; with a window of not more than 5 years, we could include 19 patients only; another 6 patients were included on the basis of a age difference no more than 8 years. Patients were only excluded from the case–control study on the basis of not having a suitable match or having insufficient specimens for testing.

Patients whose hair was too short to cut using 2 fingertips to hold it were excluded from hair analysis. The study was approved by the Stellenbosch University Committee for Human Research.

Hair was collected from the occipital area, as it has the lowest variability in hair growth rate.[17,18] At least 25 strands (3–4 mm tuft) of hair was cut as close to the scalp as possible. The distal end was clearly marked with tape because the proximal part of the specimen is expected to yield concentrations most reflective of recent exposure. The hair specimens were wrapped in aluminum foil to avoid excessive light exposure and stored at room temperature in a plastic bag with desiccant until analysis.

For any particular patient, all specimens (HIV viral load, genotypic resistance test, hair LPV concentration, and plasma LPV concentration) were collected concurrently at a single clinic visit.

Laboratory Methods

For viral load testing, we used the NucliSENS EasyQ HIV-1 version 2.0 (bioMérieux Boxtel, Netherlands). For the purpose of this study, we defined virologic failure as having a viral load >500 HIV-1 RNA copies per milliliter plasma because this is the sensitivity threshold for GART to be performed using the in-house assay. ARV resistance testing was done by an in-house validated genotypic polymerase chain reaction and sequencing method, which has been described in prior publications.[19,20]

LPV plasma concentration was measured by tandem liquid chromatography–mass spectrometry (University of Cape Town, Clinical Pharmacology laboratory). As it was logistically not possible to collect trough specimens for LPV concentration measurement, a random specimen was collected, along with the time of the last dose.

Hair LPV and ritonavir concentrations were measured by liquid chromatography and tandem mass spectroscopy at the University of California, San Francisco, using a modified method.[15,16] The LPV and RTV in each hair specimen was extracted with MeOH/FTA(9/1), then analyzed by the

API-5000 liquid chromatography and tandem mass spectroscopy machine. Approximately 2 mg of proximal hair was analyzed. The other conditions were identical to the published methods article by the same group.[15]

Statistics

The statistical analysis was performed using STATA version 10.0. For comparison of the medians of matched data, the Wilcoxon signed rank test was used and for categorical data Fisher exact test was used. To describe the association between LPV plasma or hair concentrations and failure, we used conditional logistic regression to calculate odds ratios and 95% confidence intervals (CIs). We selected LPV hair and plasma cut-off concentrations with receiver operating characteristics, choosing cut-offs that maximized the sum of sensitivity and specificity. When investigating the number of cases and controls categorized according to a cut-off LPV plasma or hair concentration, we defined a LPV concentration above the cut-off, which indicates adequate therapy exposure, as "negative" and a concentration below this cut-off, which would be expected to predict virologic failure, as "positive".

RESULTS

From the first study site, Tygerberg Hospital Family Clinic, we identified 99 patients on a LPV/r second-line regimen in the clinic database, of whom we were able to ask 77 patients to join the study; of them, 70 were eligible and agreed to participate. From the second study site, Ubunthu Clinic, Khayelitsha, we recruited patients attending 2 sequential clinic visits dedicated to patients failing a second-line LPV/r regimen; 25 patients were asked to join, and all 24 were included as they were eligible and agreed to participate. In total, 94 patients were thus included and specimens, demographic information, and clinical information were collected. In 1 patient, viral load testing failed and was thus excluded. Thirty-seven of the 93 patients (40%; 95% CI: 30% to 50%) had virologic failure, defined as having an HIV-1 RNA load of >500 copies per milliliter. Seven patients were taking rifampicin-containing antituberculosis therapy, 5 of these patients had virologic failure, 2 of whom were not using appropriately adjusted doses of LPV/r. Although more patients on concurrent treatment for tuberculosis (TB) failed than those not on TB therapy, this difference was not statistically significant (Fisher exact test: $P = 0.11$). Resistance testing was unsuccessful in 4 of the 37 patients with virologic failure. Of the 33 patients who had resistance tests, 28 patients had no resistance to any of the ARV drugs in their current regimen and 5 patients had resistance mutations to the current regimen. Only 2 patients had major PI resistance mutations. Both patients also had NRTI mutations (Table 1).

Of the 93 patients studied, 50 were included in the nested case–control study: 25 cases (with virologic failure) and 25 controls. For 19 matched pairs, an age match with an age difference between 1 and 5 years were available, another 6 pairs were included who had an age difference of 6–8 years. The group ages were well matched as the mean age difference between the case and control groups was 0.3 years. The results of the cases and control patients are summarized in Table 2. LPV plasma concentrations, LPV hair concentrations, and RTV hair concentrations were lower in patients with virological failure compared with controls. Patients with virologic failure also had a shorter duration of therapy and significantly lower concurrent CD4 counts than controls (Table 2).

Although we recruited 25 cases and controls, hair specimens were available in 19 cases and 19 controls only, as many patients had shaven their hair too short (Table 2). LPV plasma concentrations and LPV hair and RTV hair concentrations were compared in patients with or without virological failure. There was a clear separation of LPV hair and plasma concentrations between patients with or without virologic failure. However, RTV hair concentrations (Table 2) were not clearly separated between those with and without virologic failure. The odds ratio and 95% CI of virological failure for each 5 μg/mL decrease in LPV plasma concentration was 2.1 (95% CI: 1.2 to 3.6) and 71.9 (95% CI: 0.6 to 8518.2) for each 5 ng/mg decrease in LPV hair concentration. In post hoc analysis the inclusion of hair LPV concentrations, in addition to plasma LPV concentrations, provided a better model—as evident from a lower score according to Akaike information criterion of 11.5 versus 26.4. Receiver operating characteristic optimal cut-offs (that maximised the sum of sensitivity and specificity for predicting failure) were a LPV plasma concentration below 1 μg/mL and hair LPV concentration below 3.63 ng/mg (Table 3). These LPV plasma and LPV hair concentrations had respective calculated negative predictive values (NPVs) of 86% and 89%, and calculated positive predictive values of 73% and 79% when the prevalence of virologic failure is 40%. A scatterplot of LPV hair and plasma concentrations is shown in Figure 1. Thirty-one percent patients had viral loads >1000 copies; at this prevalence and viral load threshold, the calculated NPV for LPV plasma and

TABLE 1. Patients With Clinically Significant Resistance Mutations to Current Regimen

Patient Number	PI Mutations	NRTI Mutations	Plasma HIV-RNA Load	LPV-Plasma	LPV-Hair
99	I54V, V82A	M184V	13 000	10.3	*NAV*
32	L10F, L23F, M46I, I54V, T74S, V82A	D67N, T69N, K70R, M184V, T215F, K219Q	5 500	*NAV*	*NAV*
12	T74S	D67N	940	BLD	0.97
74	T74S	D67N, T69N	3700	*NAV*	*NAV*
109	None	M41L, M184V T215FIST	35 000	BLD	*NAV*

BLD, below limit of detection (<0.2 μg/mL); LPV-plasma, LPV-plasma concentration in μg/mL; LPV-hair, LPV hair concentration in ng/mg hair; NAV, Not available; Plasma HIV RNA load: copies per milliliter.

TABLE 2. Descriptive Statistics of Demographic and Clinical Characteristics of Patients Included in the Case–Control Study

	Descriptive Statistics:Median (IQR) (Except for Gender)		
Factor	**Nonfailure (n = 25) (VL < 500 copies/mL)**	**Failure (n = 25) (VL > 500 copies/mL)**	***P***
Age (yrs)	38 (34–3)	36 (30–46)	NA (matching criterion)
Gender	Female = 16	Female = 16	NA (matching criterion)
CD4 count (cells/μL)	297 (236–544)	168 (105–227)	0.0069
Log10VL (Log_{10} copies/mL)	<1	4.1 (3.0–5.5)	NA (matching criterion)
BMI	24.5 (22.1–26.1)	24.1 (21.3–29.6)	0.322
Duration of LPV therapy (months)	28 (13–44)	11.5 (9–21.5)	0.0217
LPV-plasma (μg/mL)	10.2 (6.31–12.70)	0 (0–0.81)	0.0037
LPV-hair (ng/mg)	8.36 (5.63–12.13), (n = 19)	0.97 (0.27–3.15), (n = 19)	0.0009
RTV-hair (ng/mg)	0.81 (0.46–1.22), (n = 19)	0.13 (0.04–0.54), (n = 19)	0.0084

The *P* values are from Signed rank test.
BMI, body mass index; IQR, interquartile range; NA, not applicable; RTV, ritonavir; VL, HIV-1 RNA load.

hair concentrations were 92% and 96%, respectively. LPV plasma concentrations were not affected by timing of the blood specimens as there was no association with taking the last LPV/r dose the previous day or the same morning and having a concentration below or above the cut-off (Fisher exact test: $P = 1.0$). Amongst patients with virological failure, 6 had LPV plasma concentrations above the cut-off (that is false negative LPV concentrations—Table 3). Three of the 6 patients with high LPV plasma concentrations had low LPV hair concentrations, suggesting a mechanism for failure (good short-term exposure, but poor long-term exposure). Two others had viral loads below 1000 copies per milliliter and could be excluded from the false negatives as classified by LPV plasma or hair concentrations by changing the viral load threshold to 1000 copies (Table 3). The only remaining patient with failure despite high LPV plasma concentrations, number 99, had significant PI and NRTI resistance (Tables 1 and 3). Also at a viral load threshold of 1000 copies per milliliter, the only remaining patient with failure despite a high LPV-hair concentration (Table 3) had a LPV plasma concentration below the limit of detection—suggesting omission of recent doses as an explanation of failure. Of the case–control group, the only patients with concurrent rifampicin therapy for TB were cases whose LPV plasma and hair concentrations were both below the respective cut-offs.

TABLE 3. LPV Plasma and Hair Concentrations as Predictors of Virologic Failure

LPV Concentration	**HIV RNA Load below cut-off**	**HIV RNA Load above cut-off**	**Total**
LPV plasma concentration <1 μg/mL as predictive of virological failure using an HIV RNA cut-off of 500 copies/mL			
<1 μg/mL	1	19	20
>1 μg/mL	24	6	30
Total	25	25	50
LPV plasma concentration <1 μg/mL as predictive of virologic failure using an HIV RNA cut-off of 1000 copies/mL			
<1 μg/mL	4	16	20
>1 μg/mL	27	3	30
Total	31	19	50
LPV hair concentration <3.63 ng/mg as predictive of virologic failure using an HIV RNA cut-off of 500 copies/mL			
<3.63 ng/mg	2	16	18
>3.63 ng/mg	17	3	20
Total	19	19	38
LPV hair concentration <3.63 ng/mg as predictive of virologic failure using an HIV RNA cut-off of 1000 copies/mL			
<3.63 ng/mg	5	13	18
>3.63 ng/mg	19	1	20
Total	24	14	38

DISCUSSION

We observed a very high rate of virologic failure (40%) in patients on a second-line LPV/r-based regimen in our setting, which was even higher than another study from South Africa that reported a failure a prevalence of 33%.[11] Virologic failure increases the risk of disease progression and death.[21] Poor drug exposure, most likely due to inadequate adherence, was the major cause of ARV failure in our study. However, 2 of the 33 patients who had resistance tests had major PI resistance mutations, despite the fact that this was their first PI exposure. The 2 patients with major PI mutations also had NRTI mutations. As expected, the CD4 count was lower in patients with virologic failure compared with controls. Patients with virologic failure had a shorter history of LPV/r therapy than controls, this could, however, not be explained by insufficient time to allow suppression as the shortest second-line therapy duration was 2 months and the interquartile range was 9–21.5 months. As there are currently no third-line options available in most resource-limited settings, patients are often retained on the second-line regimen irrespective of having virological failure. PI resistance mutations in patients on a boosted PI regimen usually occur at a low rate with moderate levels of adherence.[22] Therefore, although the rate of acquisition of PI resistance mutations is low, it is likely that the number of patients with significant PI resistance will accumulate over time in resource-limited settings, resulting in a growing

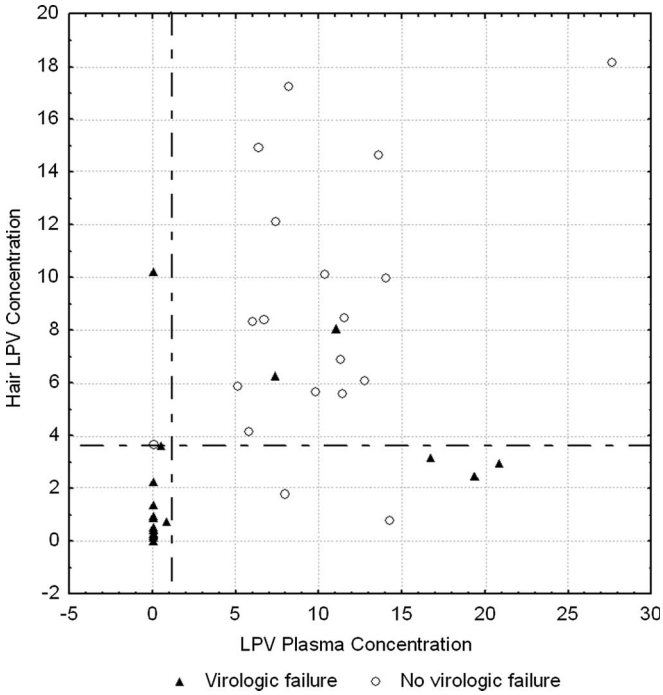

FIGURE 1. Scatterplot of Lopinavir hair (ng/mg) and plasma concentrations [(μg/mL) in patients with virologic failure (triangles) and nonfailure patients (open circles)]. The dashed lines indicate the respective concentration cut-offs: LPV plasma concentration of 1 μg/mL and LPV hair concentration of 3.63 ng/mg.

number of patients needing a third-line regimen. The use of better-tolerated ARVs, as is recommended in the current updated World Health Organization's guidelines,[7] may improve adherence and therefore decrease the proportion of failures.

LPV plasma and hair concentrations and RTV hair concentrations were all significantly lower in patients with virological failure relative to controls. We found cut-off LPV drug concentrations for plasma and hair that could discriminate between failure and nonfailure. Using a viral load failure criterion of 1000 copies per milliliter, a LPV plasma concentration <1 μg/mL and LPV hair concentration of <3.63 ng/mg had NPVs for virologic failure of 92% and 96%, respectively. In a further exploration of the false-negative cases (patients with either a high LPV plasma concentration or hair concentration, whilst having virologic failure), all cases except one could be explained by having poor drug exposure, as was evident from either having a low LPV plasma or hair concentration, the remaining case of failure had major PI and NRTI resistance.

We therefore propose a diagnostic algorithm (Fig. 2) that includes viral load measurement, random LPV plasma, and hair concentration measurement to identify patients who are likely to have virologic failure due to genotypic resistance and not poor adherence. At the outset of the study, we defined virologic failure as >500 copies per milliliter as this allowed us to do resistance testing. However, a criterion of sustained viral load above 1000 copies per milliliter is used to define failure in the South African public sector, and although a viral load between 50 and 1000 copies per milliliter would warrant clinical attention, such as adherence counseling, it would not indicate the need for a therapy change in patients on a high genetic barrier second-line regimen. At a viral load threshold of 1000 copies per milliliter, a random LPV plasma concentration <1 mg/mL or hair concentration <3.63 ng/mg due to their excellent NPV could exclude patients with poor drug exposure from unnecessary costly ARV resistance testing. Indeed, patients with a LPV or hair concentration below the threshold can be targeted for adherence interventions with repeat viral load testing at a follow-up visit. Although GART could be performed at HIV-1 RNA loads of below 1000 copies, commercial assays are only licensed to detect resistance above this threshold and the intensive investigation of patients treated with such a high genetic barrier regimen with low-level viremia may not be cost effective. In the algorithm we suggest only analyzing LPV concentrations in hair and plasma in patients with viral loads above 1000 copies per milliliter. The proposed algorithm will be less costly than GART for patients failing second-line regimens as the cost of a plasma LPV concentration measurement is about US $27 and hair measurement US $40, whereas GART typically cost from US $250 to US $530 in the South African setting. The benefit of using a random plasma concentration and a hair concentration, in combination, is that a low plasma concentration would indicate "recent" poor adherence and a low hair concentration, measured in a 1-cm hair specimen, "average" poor adherence over a period of about 1 month, as hair grows at about 1–1.5 cm per month, even when the recent adherence as indicated by a high plasma LPV concentration may be adequate (but too short to suppress viral replication). These tests therefore could also provide some insight in the adherence patterns of patients. A low *random* plasma concentration, in the absence of trough measurements, proved to be informative as most patients with virologic failure had very low random plasma LPV concentrations.

Our study was explorative with a few limitations as follows: (1) the cross-sectional design did not enable us to longitudinally monitor the effect of LPV exposure on virologic failure; (2) due to logistical reasons we measured random LPV concentrations rather than trough concentrations, which could have been more accurate; (3) our study was limited by small sample size; (4) a final limitation is that stable patients are referred out to primary clinics which could result in a selection bias with a higher LPV failure rate in the study population compared with other settings, as problematic patients are often retained in care.

CONCLUSIONS

We found a very high prevalence of virologic failure amongst adult patients in our South African setting on a LPV/ based regimen. The majority failed due to poor drug exposure, most likely related to poor adherence, as was evident from either a low LPV plasma or hair concentration. Therefore, the use of plasma and hair LPV concentrations could be valuable in diagnosing the cause of virologic failure and allow targeted GART only in those patients where failure is not explained by poor drug exposure.

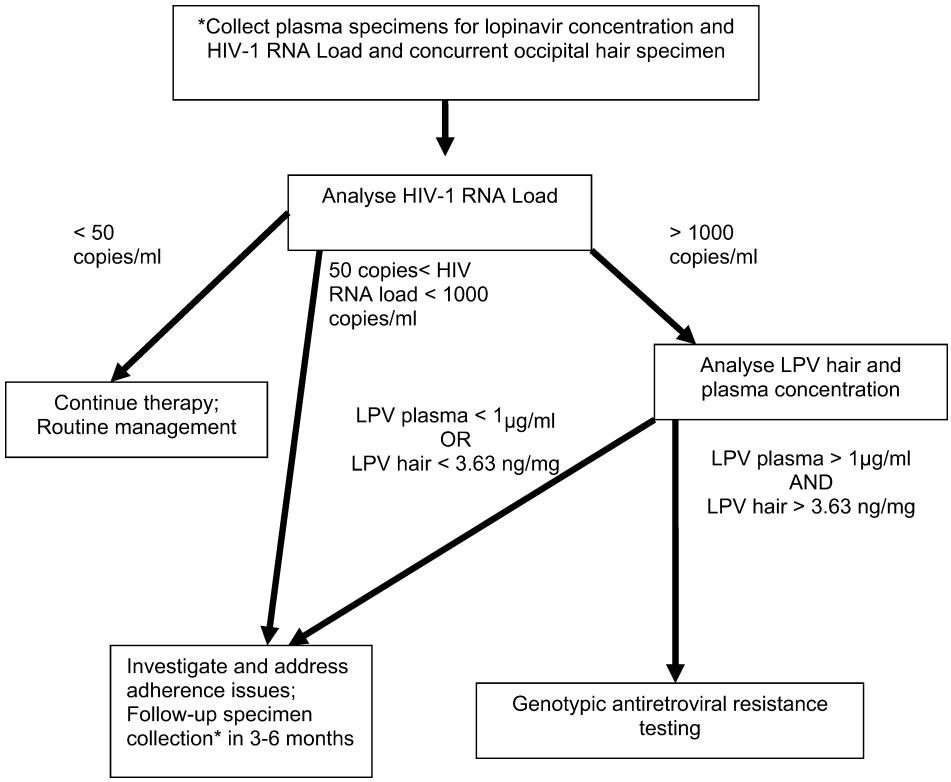

FIGURE 2. Proposed algorithm to investigate the cause of virologic failure of a Lopinavir boosted with low-dose ritonavir (LPV/r) containing second-line regimen in resource-limited settings. LPV concentrations in plasma and hair will only be analyzed when the viral load is >1000. This would allow the differentiation of patients who are failing due to poor adherence versus probable genotypic resistance. *Indicating the tests that are done at the ''viral load'' visit.

ACKNOWLEDGMENTS

The authors thank Dr. Hans Prozesky, Department Medicine, Stellenbosch University and Tygerberg Academic Hospital who manages the Tygerberg Infectious Diseases Clinic Database as part of a PEPFAR-funded project; Dr. Jantjie Taljaard, Department Medicine, Stellenbosch University and Tygerberg Academic Hospital for supporting the study at the Infectious Diseases Clinic; Dr. Shaheed Matthee, Ubunthu Clinic Khayelitsha and Department of Health, Western Cape Province, and Dr. Gilles van Cutsem, Ubunthu Clinic Khayelitsha and Medicins Sans Frontieres, South Africa for supporting the study.

Dr. Ruth Greenblatt, MD, Division of Infectious Disease, University of California, San Francisco, CA, for involvement in development of the hair drug analysis method. Prof. Wolfgang Preiser, MD, PhD, for advice on article preparation.

REFERENCES

1. World Health Organization. *Scaling Up Antiretroviral Therapy in Resource-Limited Settings: Guidelines for a Public Health Approach*. Geneva, Switzerland: WHO; 2002.
2. World Health Organization. *Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach, 2006 Revision*. Geneva, Switzerland: WHO; 2006.
3. Quiros-Roldan E, Airoldi M, Moretti F, et al. Genotype resistance profiles in patients failing an NNRTI-containing regimen, and modifications after stopping NNRTI therapy. *J Clin Lab Anal*. 2002;16:76–78.
4. Delaugerre C, Rohban R, Simon A, et al. Resistance profile and cross-resistance of HIV-1 among patients failing a non-nucleoside reverse transcriptase inhibitor-containing regimen. *J Med Virol*. 2001;65: 445–448.
5. Frost SD, Nijhuis M, Schuurman R, et al. Evolution of lamivudine resistance in human immunodeficiency virus type 1-infected individuals: the relative roles of drift and selection. *J Virol*. 2000;74:6262–6268.
6. Gardner EM, Sharma S, Peng G, et al. Differential adherence to combination antiretroviral therapy is associated with virological failure with resistance. *AIDS*. 2008;22:75–82.
7. World Health Organization. *Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach, 2010 Revision*. Geneva, Switzerland: WHO; 2010.
8. Mo H, King MS, King K, et al. Selection of resistance in protease inhibitor-experienced, human immunodeficiency virus type 1-infected subjects failing lopinavir- and ritonavir-based therapy: mutation patterns and baseline correlates. *J Virol*. 2005;79:3329–3338.
9. Gathe J, da Silva BA, Cohen DE, et al. A once-daily lopinavir/ritonavir-based regimen is noninferior to twice-daily dosing and results in similar safety and tolerability in antiretroviral-naive subjects through 48 weeks. *J Acquir Immune Defic Syndr*. 2009;50:474–481.
10. Barreiro P, Corbaton A, Nunez M, et al. Tolerance of didanosine as enteric-coated capsules versus buffered tablets. *AIDS Patient Care STDS*. 2004;18:329–331.
11. El-Khatib Z, Ekstrom AM, Ledwaba J, et al. Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. *AIDS*. 2010;24:1679–1687.
12. Arribas JR, Delgado R, Arranz A, et al. Lopinavir-ritonavir monotherapy versus lopinavir-ritonavir and 2 nucleosides for maintenance therapy of HIV: 96-week analysis. *J Acquir Immune Defic Syndr*. 2009; 51:147–152.
13. Riddler SA, Haubrich R, DiRienzo AG, et al. Class-sparing regimens for initial treatment of HIV-1 infection. *N Engl J Med*. 2008;358:2095–2106.
14. Berg KM, Arnsten JH. Practical and conceptual challenges in measuring antiretroviral adherence. *J Acquir Immune Defic Syndr*. 2006;43(Suppl 1): S79–S87.
15. Huang Y, Gandhi M, Greenblatt RM, et al. Sensitive analysis of anti-HIV drugs, efavirenz, lopinavir and ritonavir, in human hair by liquid chromatography coupled with tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2008;22:3401–3409.

16. Gandhi M, Ameli N, Bacchetti P, et al. Protease inhibitor levels in hair strongly predict virologic response to treatment. *AIDS*. 2009;23: 471–478.
17. Cassani M, Da Re N, Giuliani L, et al. Experience with hair testing in the clinical biochemistry laboratory of Ca' Granda Niguarda Hospital, Milan, Italy. *Forensic Sci Int*. 1997;84:17–24.
18. Kosuge K, Uematsu T, Araki SI, et al. Comparative dispositions of ofloxacin in human head, axillary, and pubic hairs. *Antimicrob Agents Chemother*. 1998;42:1298–1302.
19. van Zyl GU, Claassen M, Engelbrecht S, et al. Zidovudine with nevirapine for the prevention of HIV mother-to-child transmission reduces nevirapine resistance in mothers from the Western Cape, South Africa. *J Med Virol*. 2008;80:942–946.
20. van Zyl GU, van der Merwe L, Claassen M, et al. Protease inhibitor resistance in South African children with virologic failure. *Pediatr Infect Dis J*. 2009;28:1125–1127.
21. Pujades-Rodriguez M, Balkan S, Arnould L, et al. Treatment failure and mortality factors in patients receiving second-line HIV therapy in resource-limited countries. *JAMA*. 2010;304:303–312.
22. Bangsberg DR, Moss AR, Deeks SG. Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. *J Antimicrob Chemother*. 2004;53: 696–699.