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Differences of Nine Drug Resistance Interpretation Systems in Predicting Short-term Therapy Outcomes of Treatment-experienced HIV-1 Infected Patients: A Retrospective Observational Cohort Study

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Abstract

Objective: Drug resistance interpretation systems are used to select the optimal antiretroviral therapy in HIV-infected patients. It is unclear how the systems perform in predicting therapy success and failure and in how far the interpretations are affected by insufficient drug levels.

Methods: The accuracy of nine different interpretation systems in predicting therapy outcomes was evaluated using virological, immunological, pharmacological, and clinical data of 130 patients treated at 13 outpatient centers. Individual susceptibility scores of the interpretation systems were converted into active drug scores (ADS) and correlated with therapy success and failure, defined as viral load reduction of equal to or more (n=66) and less than 1 log10 copies/ml (n=64) at three months after drug resistance testing.

Results: Three interpretation systems considered the respective therapies as more active compared to the other interpretation systems ($p<0.01$). These systems predicted therapy success better than the other systems, while the others performed better in predicting therapy failure. Thus, the overall rate of correctly predicted treatment outcomes was comparable between the different systems (73.1-80.0 %). Univariate and multivariate regression analysis revealed significant correlations between the ADS of all interpretation systems and virological therapy outcomes ($p<0.0001$). In contrast, only three interpretation systems were significantly correlated with immunological therapy outcomes in univariate and just one in multivariate models ($p<0.05$). Among 128 determinations of drug levels in 64 patient samples, 19.4 % revealed no detectable drug levels. The consideration of insufficient drug levels significantly improved the prediction accuracy of all interpretation systems ($p<0.005$).

Conclusion: Differences between interpretation systems in predicting therapy failures and success need to be considered for future consensus algorithms. The prediction accuracy of interpretation systems can be improved by consideration of plasma drug levels.

Key words: HIV-1, drug resistance, interpretation systems, therapy failure, drug levels.

Background

Drug resistance testing is increasingly being recognized as a valuable tool in the management of antiretroviral treatment in HIV-infected patients, now being recommended for a variety of clinical situations [1,2]. The technical quality of detecting drug resistance-associated mutations has improved considerably, although problems with resistant minorities remain to be solved [3]. The interpretation of complex mutational patterns, however, is still a major challenge. More than 25 interpretation systems are available, which vary greatly in scientific basis, output, and clinical validation [4]. Several of these systems can be accessed online by submitting sequences of the viral protease and reverse transcriptase (RT) and receiving instantaneous interpretations for most antiretroviral drugs. Amongst these are rule-based systems which incorporate knowledge about correlations between genotype and phenotype as well as correlations with treatment history and clinical response (e.g. Stanford database, RetroGram, TruGene, REGA algorithm, CHL algorithm, ANRS AC11), and database-driven systems which use database matching search or bioinformatic approaches to extract information from a large set of geno-/phenotype pairs (e.g. Virtual Phenotype, geno2pheno) [5]. Comparisons between outputs of widely used algorithms revealed different degrees of concordance, with the greatest variability in the interpretation of resistance against nucleoside RT inhibitors (NRTI) [6-10]. These variations were similarly detected for the prediction of treatment outcomes in a retrospective analysis of 11 interpretation systems [11].

Comparisons of interpretation systems, in particular with retrospective databases, are often confounded by adherence issues. Adherence to the antiretroviral

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regimen is significantly associated with suppression of viral replication [12] and can be assessed by manual or electronic pill count, pharmacy refill data, questionnaires, diaries, and by the physician or the clinical nurse specialist [13]. Complex pharmacokinetic interactions are most reliably revealed by therapeutic drug monitoring [14, 15]. Retrospective studies correlated suboptimal drug levels with therapy failure [16-18]. In contrast, prospective studies have revealed conflicting results; three studies confirmed lower viral loads in patients with therapeutic drug monitoring and high trough levels [19-21], whereas two other studies failed to establish this correlation at least for short-term virological response [22, 23]. Finally, prediction accuracy improved significantly when trough levels of drugs were combined with drug resistance data, as has recently been suggested by the concept of the phenotypic or genotypic inhibitory quotient [24, 25].

For the comparison of drug resistance interpretation systems, we retrospectively analyzed virological, immunological, and clinical data of 130 patients from 13 German outpatient centers. Our study focused on three aspects: (i) to evaluate the prediction accuracy for short-term virological responses and failures, (ii) to reveal the influence of insufficient drug levels on the interpretations, and (iii) to find out how resistance data and drug levels as marker for non-adherence can be combined to facilitate the comparison of interpretation systems within retrospective databases.

Material and Methods

Patient Population

A total of 130 patients were included in this study. They were treated in 13 outpatient centers specialized in the care for HIV-infected individuals in Nürnberg (n = 54) or in and around Aachen (n = 76) from January 1997 until June 2002. The criteria for patient selection were availability of viral load data and genotypic drug resistance testing at baseline, detailed information about previous and study drug regimens, and viral load data at 3 months after drug resistance testing. These informations were contributed by the treating physicians as part of the regular diagnostic monitoring.

Drug resistance Testing

Genotypic drug resistance testing was either performed at the National Reference Centre for Retroviruses, Erlangen, using an in-house method for amplification and sequencing [26] or at the PZB in Aachen using the TruGene™ HIV-1 Genotyping Kit (Bayer Diagnostics, Fernwald, Germany). The detection limit for minority species was about 30% with both methods.

Drug Resistance Interpretation Systems

The drug resistance interpretation systems and the respective versions as well as abbreviations used throughout the text are listed in Table 1. For each interpretation system, individual susceptibility scores for antiretroviral drugs were converted into active drug scores (ADS) ranging from 0-1 for each drug in the study regimen. Ritonavir was not counted as antiretroviral drug if used as low-dose booster for other protease inhibitors. If mixtures of resistant and wild-type variants at a relevant position for the respective interpretation system were present, the resistance mutation was counted.

Determination of Viral Load

Viral loads were either determined with the b-DNA 3.0 test (Bayer Diagnostics, Fernwald, Germany) or the COBAS AMPLICOR HIV-1 MONITOR test, v. 5.1 (Roche Diagnostics, Mannheim, Germany). Viral loads below the detection levels of the respective system (50 and 40 copies/ml) were replaced by 49 copies/ml for the analysis of viral load changes.

Determination of Drug Levels

Drug levels for non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI) were retrospectively determined in the samples at 3 months after drug resistance testing, using a validated method of liquid chromatography-tandem mass spectrometry [27]. For further analysis, drug levels were normalized using doubled percentile rankings for each drug. To adjust for the fact that all drug levels were untimed and may thus include peak as well as trough levels, all values above the median (= 1) were counted as equally active, as recently suggested by Baxter and colleagues [28].

Statistical Evaluation

All statistical analyses were performed using the SPSS statistical software package, version 12. Chi square and Student's t-test were used for discrete and continuous variables, respectively; two-tailed p values < 0.05 were considered statistically significant. The correlation between the viral load change at 3 months after resistance testing and the respective ADS was analyzed using linear and logistic regression models. The correlation between the change in CD4+ cell count and the respective ADS was analyzed using univariate and multivariate linear regression models. Parameters included in the multivariate analysis were gender and age of the patients, baseline levels of CD4+ cell count and viral load, the number of drugs which were recycled, used previously or in the study regimen, the use of NNRTI in previous and study regimen and recycling of these drugs, and the active drug score of the antiretroviral study regimen.

Results

Patient Characteristics

The mean age of the patients was 40 years (range, 26-61 years) and a total of 22 patients (17%) were female. Mean viral load at baseline was 4.3 log (range, 2.2-5.9 log), and the mean CD4+ cell count was 293/µl

Table 1. Attribution of active drug scores (ADS) to the individual susceptibility scores of nine drug resistance interpretation systems used for further evaluation. The versions of the interpretation systems used were those of June 2002

interpretation system	abbreviation[1]	access	attribution of ADS to individual susceptibility scores			
				0.5		
			0	0.33	0.66	1
geno2pheno, v. 2.0[2] (decision trees)	g2p	http://www.genafor.org/	R (cf 0.7-1.0)	R (cf 0.5-0.7)	S (cf 0.5-0.7)	S (cf 0.7-1.0)
STDB (ß version)	STDB	http://hiv-4.stanford. edu/cgi-bin/hivtestweb.pl	HL	I	LL	S, PL
RetroGram, v. 1.4	RG	http://www.retrogram. com	D	C	B	A
TruGene, v. 5.0	VGI	http://www.trugene.com	R	I		S
Centre Hospitalier de Luxembourg, v. 3.2	CHL	http://www.ablsa.com	R	I		S
Agence Nationale de Recherche sur le SIDA AC11, v. 2000	ANRS	http://www.hivfrench-resistance.org/index.html	R	I		S
Detroit Medical Center, v. 2000	MED	http://www.ablsa.com	R	I		S
Grupo de Aconselhamento Virológico	DAP	http://www.ablsa.com	R	I		S
REGA algorithm, v. 5.5	REGA	http://www.ablsa.com	R	I		S

cf confidence factor, S susceptible, PL potential low-level resistance, LL low-level resistance, I possible or intermediate resistance, R resistant, HL high-level resistance, v. version.

[1] used throughout the text
[2] the confidence factor is a measure for the expected accuracy of the respective prediction [30]

(range, 7-1019). The patients had been treated with a mean of 5.5 antiretroviral drugs (range, 0-14) prior to the study regimen, which consisted of a mean of 3.8 antiretroviral drugs (range, 2-8). Drugs included in the previous or study regimens, respectively, were zidovudine (72% and 24%), zalcitabine (30% and 1%), didanosine (48% and 42%), stavudine (58% and 52%), lamivudine (78% and 55%), abacavir (23% and 38%), tenofovir (0% and 4%), nevirapine (31% and 17%), delavirdine (6% and 3%), efavirenz (22% and 23%), indinavir (44% and 15%), saquinavir (50% and 16%), ritonavir (39% and 22%), nelfinavir (41% and 15%), amprenavir (5% and 22%), and lopinavir (5% and 31%). Antiretroviral therapy was changed in 101 patients at a mean of 27 days (± 23 days, standard deviation) after drug resistance testing, whereas medication remained unchanged in a total of 29 patients. It remained unclear if the treating physicians selected the antiretroviral drugs in the study regimen according to the results of drug resistance testing.

Virological and Immunological Therapy Outcomes

The patients were divided into two groups with respect to the reduction of viral load observed at 3 months after drug resistance testing: 66 patients had a reduction of at least 1 log10 copies/ml, which was defined as therapy success, whereas the remaining 64 patients had a reduction of less than 1 log10 copies/ml, which was considered as treatment failure. In the first group, viral load decreased from 4.2 ± 0.8 to 2.2 ± 0.6 log10 copies/ml, whereas viral load remained at 4.2 ± 0.8 log10 copies/ml in the group of patients experiencing therapy failure. Both groups were not significantly different for age, gender, viral load and CD4+ T cell count at baseline (Table 2). There were also no significant differences detected for the total number of antiretroviral drugs or NNRTI which were used previ-

Table 2. Comparison of patients with therapy success (n = 66) vs. therapy failure (n = 64) at 3 months after drug resistance testing.

Parameter	Therapy success (n = 66)	Therapy failure (n = 64)	p-value
age (mean/range)	38.9 (26-61)	40.7 (28-59)	0.20 (n.s.)[1]
gender (f/m)	10/56	12/52	0.75 (n.s.)[2]
No. of drugs in previous regimen (mean ± standard deviation)	5.2 (± 3.0)	5.8 (± 3.5)	0.33 (n.s.)[1]
No. of patients with NNRTI in previous regimen (%)	32 (48.5%)	29 (45.3%)	0.86 (n.s.)[2]
No. of drugs in study regimen (mean ± standard deviation)	3.8 (± 0.9)	3.7 (± 0.8)	0.45 (n.s.)[1]
No. of patients with NNRTI in study regimen (%)	28 (42.4%)	25 (39.1%)	0.84 (n.s.)[2]
No. of recycled drugs in study regimen (mean ± standard deviation)	1.4 (± 1.3)	1.9 (± 1.5)	0.06 (n.s.)[1]
No. of patients with recycled NNRTI in study regimen (%)	6 (9.1%)	10 (15.6%)	0.39 (n.s.)[2]
Log baseline viral load (mean ± standard deviation)	4.2 (± 0.8)	4.2 (± 0.8)	0.78 (n.s.)[1]
Baseline CD4+ cell count[3] (mean ± standard deviation)	305 (± 219)	283 (± 217)	0.58 (n.s.)[1]
CD4+ cell count 3 months after drug resistance testing[4] (mean ± standard deviation)	358 (± 215)	277 (± 222)	0.05[1]

n.s. not significant; f female; m male, NNRTI non-nucleoside inhibitors of the reverse transcriptase
[1] Student's t-Test
[2] Chi-Square Test
[3] data available for 117 patients
[4] data available for 109 patients

ously or in the study regimen. However, a statistical trend was observed with respect to the number of recycled drugs in the study regimen: patients experiencing therapy failure had 1.9 ± 1.5 recycled drugs (mean ± SD) in their study regimen, compared to 1.4 ± 1.3 recycled drugs in the group of patients with therapy success (p = 0.06; Student's t-test). At three months after resistance testing, the CD4+ cell count was higher in the group of patients with therapy success vs. treatment failure (mean increase of 53 CD4+ cells/µl vs. mean decline of 6 CD4+ cells/µl; p = 0.05; Student's t-test).

Comparison of ADS between the Different Interpretation Systems

To uncover differences between the interpretation systems with respect to their individual assessment of drug resistance, the mean ADS of the antiretroviral therapies of all study patients (n = 130) were compared with each other. The mean ADS of the three interpretation systems CHL, MED, and ANRS was between 2.3 and 2.4, whereas the mean ADS of the other

Table 3. Comparison of active drug scores (ADS) of the antiretroviral therapies of all patients included in the study (n = 130), determined by the different interpretation systems.

No.	Interpretation system	ADS (Mean ± standard deviation)
1	G2p	2.0 ± 1.4
2	STDB	2.0 ± 1.3
3	VGI	1.9 ± 1.4
4	RG	2.0 ± 1.3
5	CHL	2.4 ± 1.3[a]
6	ANRS	2.3 ± 1.4[b]
7	MED	2.3 ± 1.2[c]
8	DAP	2.0 ± 1.3
9	REGA	2.0 ± 1.3

Student's t-test for paired samples
[a] p <0.01 for the comparison of system 5 with 1, 2, 3, 4, 8 und 9
[b] p <0.01 for the comparison of system 6 with 1, 2, 3, 4, 8 und 9
[c] p <0.01 for the comparison of system 7 with 1, 2, 3, 4, 8 und 9
The ADS of the other interpretation systems were not signifi-

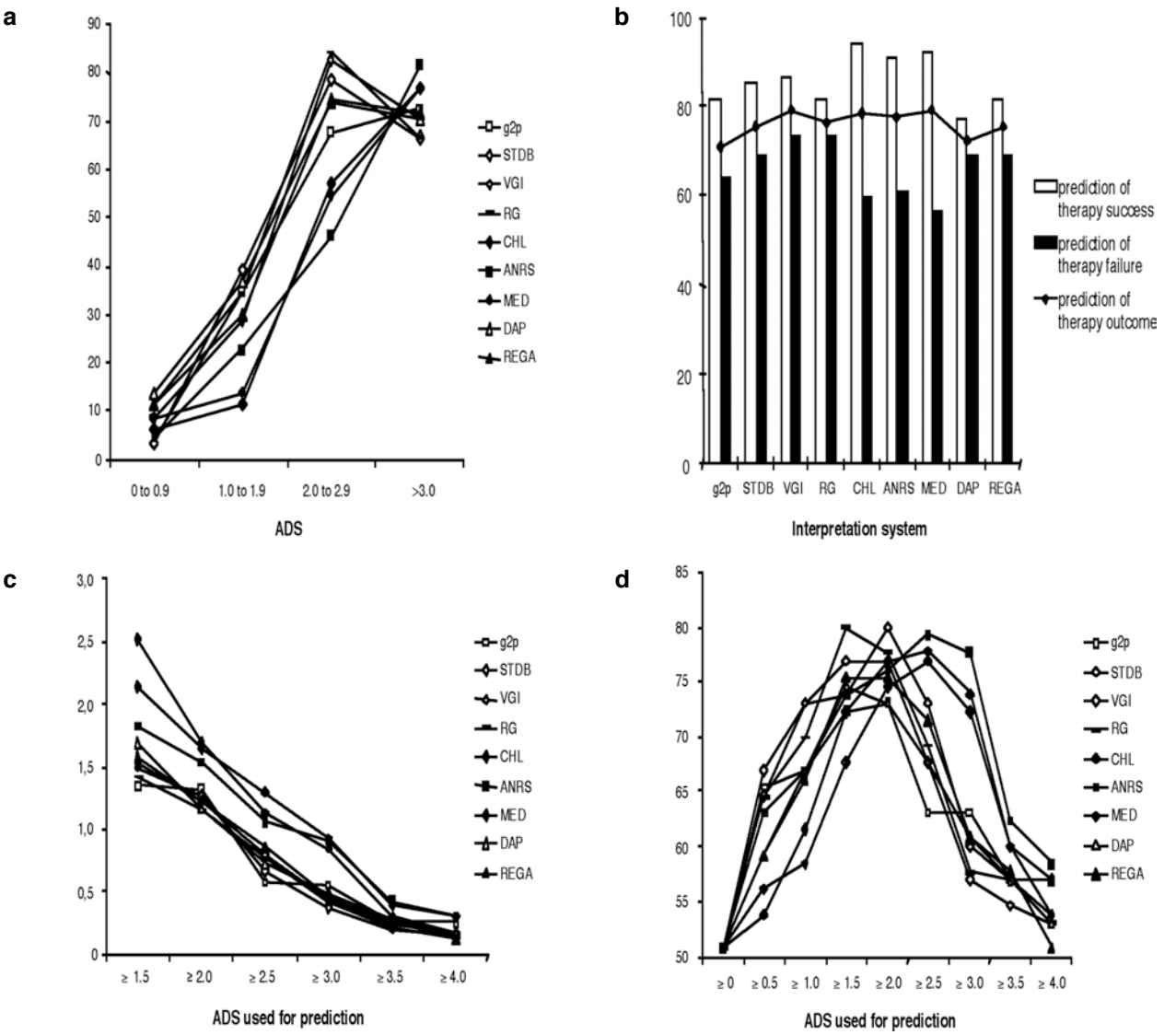

Fig. 1. Prediction of therapy outcomes by nine different drug resistance interpretation systems in 130 HIV-infected patients. Therapy success and failure were defined as viral load change of equal to or more and less than 1 log10 copies/ml at 3 months after drug resistance testing, respectively. For abbreviation of interpretation systems see Table 1. (a) Overall distribution of active drug scores (ADS) of the different drug resistance interpretation systems with respect to the proportion of patients with therapy success. (b) Proportion of correctly predicted therapy outcomes, separated for therapy success and failure, expecting "success" if the antiretroviral regimen contained two or more active drugs ("ADS ≥ 2") according to the respective interpretation system. (c) Proportion of correctly predicted therapy success divided by the proportion of correctly predicted therapy failure for the respective drug resistance interpretation systems, expecting "success" with different numbers of active drugs ("ADS ≥ 1.5" to "ADS ≥ 4.0") for the prediction. (d) Proportion of correctly predicted therapy outcomes for the respective interpretation systems, using different numbers of active drugs ("ADS ≥ 0" to "ADS ≥ 4.0") for the prediction.

6 interpretation systems ranged from 1.9 to 2.0 (Table 3). This difference was statistically significant at the 1% level, considering a total of 36 permutations. These data indicated that the interpretation systems ANRS, CHL, and MED considered the respective therapies as more active compared to the other interpretation systems.

Correlation of ADS with Therapy Outcomes

We compared the distribution of the ADS with respect to the proportion of patients with therapy success. Therapy success increased concomitantly with increasing ADS for all interpretation systems (Fig. 1a). A continuous increase in the proportion of patients with therapy success was observed for CHL, MED, and ANRS, whereas the other interpretation systems showed the highest proportion of therapy success at ADS between 2.0 and 2.9 and a decrease for ADS of 3.0 and higher.

Prediction of Therapy Outcomes

Next we compared the predictions by the different drug resistance interpretation systems with the actual therapy outcomes. In a first analysis, we evaluated the

Table 4. Comparison of predictions of therapy outcomes by four different interpretation systems using their versions of June 2002 and August 2006.

	Interpretation system							
	G2p[a]		STDB		ANRS		REGA	
Proportion of correct predictions for ADS ≥2	2002	2006[b]	2002	2006[b]	2002	2006[b]	2002	2006[b]
Prediction of therapy success	81.8 %	83.3 %	84.8 %	69.7 %	90.9 %	93.9 %	81.8 %	89.4 %
Prediction of therapy failure	64.1 %	67.2 %	68.8 %	73.4 %	60.9 %	62.5 %	68.8 %	59.4 %
Prediction of therapy outcome	73.1 %	75.4 %	76.9 %	71.5 %	76.2 %	78.5 %	75.4 %	74.6 %

[a] decision trees (version 2002), support vector machines (version 2006).
[b] as provided by the HIV GRADE algorithm homepage (http://www.hiv-grade.de/cms/grade/).
ADS active drug score.

proportion of correctly predicted therapy outcomes expecting "success" if the antiretroviral regimen contained two or more active drugs ("ADS ≥ 2") according to the respective interpretation system. Under these conditions, the overall rate of correct predictions of therapy outcomes was comparable between the different interpretation systems (73.1% - 80.0%, Fig. 1b). However, when the predictions of therapy success and therapy failure were analyzed separately, three interpretation systems (CHL, MED, and ANRS) showed a better prediction of therapy success than the other interpretation systems, whereas the prediction of therapy failure was performed more successfully by the other interpretation systems (Fig. 1b). This trend was not statistically significant, when median values of both groups were compared for the prediction of therapy success or failure ($p = 0.12$ and $p = 0.46$, respectively; Chi square test). However, CHL, MED, and ANRS always performed better than the other interpretation systems in the prediction of therapy success compared to therapy failure, irrespective of the number of active drugs ("ADS ≥ 0.5" to "ADS ≥ 3.0") at which "success" was expected (Fig. 1c). When the proportion of correctly predicted therapy outcomes was evaluated for increasing ADS according to the respective interpretation systems, CHL, MED, and ANRS showed an optimum with ≥ 2.5 active drugs, whereas the other interpretation systems peaked at ≥ 2 active drugs (Fig. 1d).

To evaluate if a more stringent definition of treatment success would change the results, the data were re-analyzed defining a viral load reduction of 2 log10 copies/ml or more as evidence of successful therapy. Using this definition, the percentage of correctly predicted therapy outcomes was lower for all interpretation systems (53.2-64.6 %), with the greatest effect on CHL, MED, and ANRS (data not shown). However, these three systems still predicted therapy success better than the other interpretation systems. All these data indicate that a less stringent interpretation of drug resistance was associated with an improved prediction of therapy success, but a worsening in the prediction of therapy failure.

Interpretation systems are continuously improved and updated to reflect current therapeutic strategies. Reanalysis of our data using up-to-date versions of four different interpretation systems showed that ANRS still predicted therapy response better than the other algorithms (Table 4). The results of the other interpretation systems (STDB, REGA, g2p) were comparable to the data obtained with the 2002 versions, with an improved prediction of therapy success seen in three of the four interpretation systems (Table 4).

Adherence and Variability of Drug Levels

A total of 128 drug levels were obtained from 64 patient samples. Excluding low-dose ritonavir, 20 of 103 determinations (19.4%) revealed no detectable drug levels, indicating that about one fifth of the patients had not sufficiently adhered to the prescribed medication (Fig. 2a). We next compared the inter-individual variability of eight antiretroviral drugs (2 NNRTI, 6 PI). Among detectable drug levels, a variability of more than 1 log was observed for indinavir, saquinavir, ritonavir, nelfinavir, and amprenavir, and of less than 1 log for nevirapine, efavirenz, ritonavir, and lopinavir (Fig. 2a). Because of the limited number of drug levels, no meaningful conclusions could be drawn for the effect of ritonavir boosting on PI drug levels.

Effect of Combined Drug Levels and ADS on the Prediction of Therapy Outcomes

The ADS determined by the different interpretation systems was correlated with the viral load change at 3 months after drug resistance testing using linear regression analysis. To compare correlations with and without incorporating drug levels, only patients for which drug levels were available (n = 64) were analyzed. Without drug levels, the best correlation was found for ANRS ($r = -0.60$), followed by CHL ($r = -0.50$) (Fig. 2b), MED and VGI ($r = -0.49$), RG ($r = -0.48$), STDB ($r = -0.47$), REGA ($r = -0.46$), g2p ($r = -0.45$), and DAP ($r = -0.43$). Drug levels were incorporated into the analysis by multiplying the ADS for a certain drug with the respective normalized drug level.

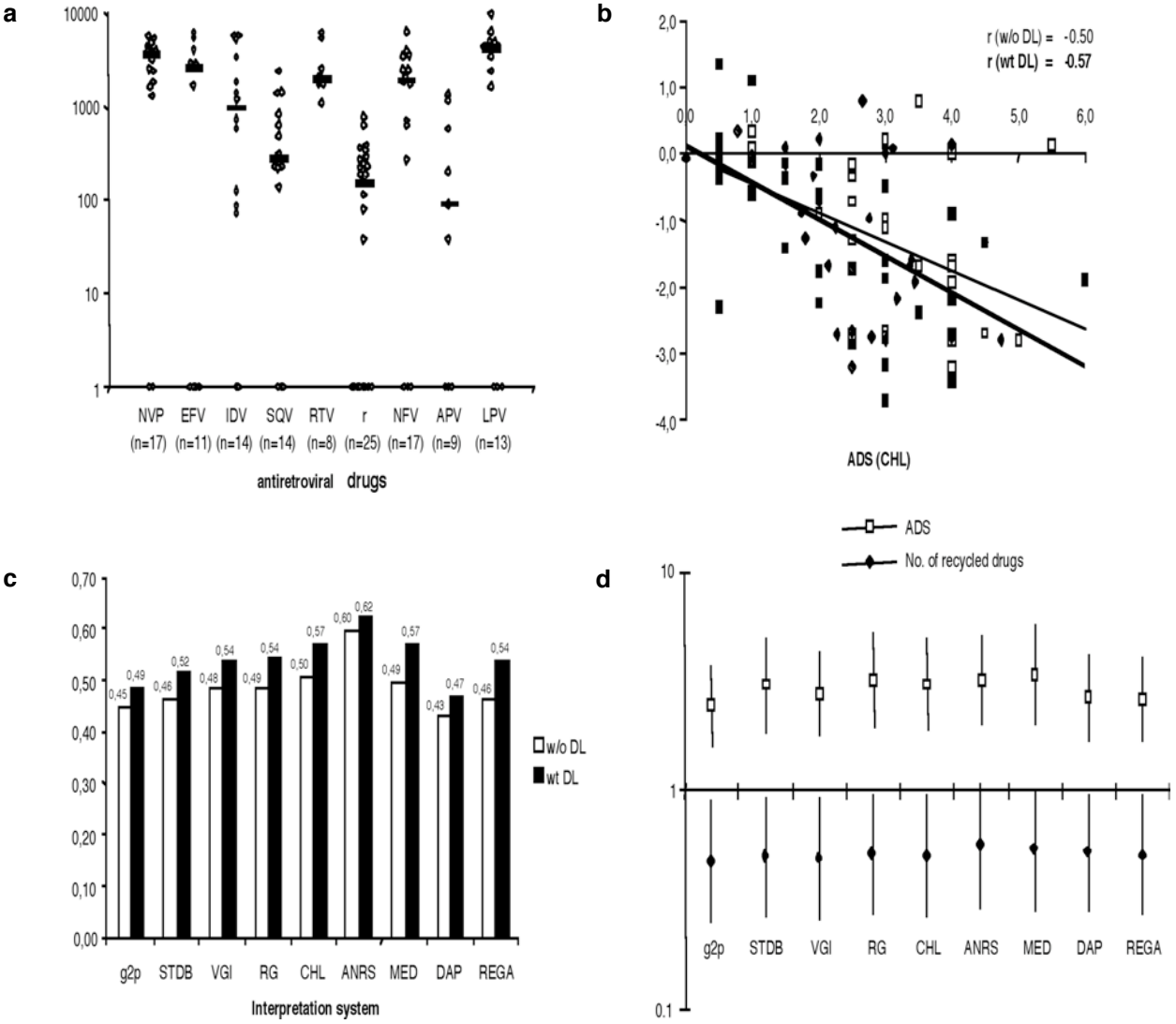

Fig. 2. Effect of drug levels (DL) combined with active drug scores (ADS) on therapy outcomes. (a) Distribution of plasma levels for nevirapine (NVP), efavirenz (EFV), indinavir (IDV), saquinavir (SQV), full-dose (RTV) and low-dose ritonavir (r), nelfinavir (NFV), amprenavir (APV), and lopinavir (LPV) in 64 HIV-infected patients at 3 months after drug resistance testing. The respective median drug levels were 3660, 2870, 989, 275, 2000, 150, 1939, 90, and 4220 ng/ml. (b) Linear regression analysis of the ADS of the interpretation system CHL with respect to the viral load change at 3 months after drug resistance testing before and after incorporation of drug levels (w/o and wt DL, respectively); r correlation coefficient. (c) Correlation coefficients of all nine interpretation systems (for abbreviation of interpretation systems see Table 1) before and after incorporation of drug levels (w/o and wt DL, respectively) in 64 HIV-infected patients. (d) Multivariate logistic regression analysis of active drug scores (ADS) and the number (no.) of recycled drugs in a total of 130 patients with respect to the prediction of therapy success, defined as viral load increase of equal to or more than 1 log10 copies/ml at 3 months after drug resistance testing. For abbreviation of interpretation systems see Table 1. OR odds ratio, CI confidence interval.

Although drug levels were obtained for eight antiretroviral drugs only, correlation coefficients significantly improved for all interpretation systems ($p < 0.005$, Wilcoxon signed rank test) (Fig. 2c), indicating that viral load decreases were predicted more accurately. The smallest improvement ($r = -0.62$) was observed for ANRS, which already showed the best correlation before drug levels were incorporated ($r = -0.60$), whereas the correlation coefficients of the other interpretation systems improved to a larger extent. The incorporation of drug levels also resulted in steeper slopes of the regression lines of all interpretation systems (Fig. 2b, and data not shown), suggesting a greater reduction of viral load per active drug in patients with good adherence.

Univariate and Multivariate Regression Analysis

For the prediction of virological therapy outcomes, a total of 117 patients were analyzed with complete data for age, gender, baseline viral load, and CD4+ cell count as well as the number of drugs and NNRTI, which were used previously, in the study regimen or recycled. Linear regression analysis revealed a highly significant correlation between the ADS of all interpretation systems and the viral load decrease at 3

Table 5. Association of baseline active drug scores (ADS) with the viral load change at 3 months after drug resistance testing (n = 117), using univariate and multivariate linear regression models.

	Change of viral load at 3 months after drug resistance testing per unit increase in ADS, log10 copies/ml			
	Univariate model		Multivariate model	
Interpretation System	mean (95% confidence interval)	p-value	mean (95% confidence interval)	p-value
G2p	-0.389 (-0.521 to 0.257)	< 0.0001	-0.378 (-0.524 to -0.232)	< 0.0001
STDB	-0.454 (-0.594 to -0.315)	< 0.0001	-0.448 (-0.604 to 0.291)	< 0.0001
VGI	-0.423 (-0.554 to -0.291)	< 0.0001	-0.382 (-0.527 to -0.238)	< 0.0001
RG	-0.448 (-0.594 to -0.302)	< 0.0001	-0.420 (0.583 to -0.257)	< 0.0001
CHL	-0.462 (-0.601 to -0.324)	< 0.0001	-0.440 (-0.599 to -0.280)	< 0.0001
ANRS	-0.478 (-0.598 to -0.358)	< 0.0001	-0.456 (-0.594 to -0.318)	< 0.0001
MED	-0.499 (-0.651 to -0.347)	< 0.0001	-0.481 (-0.650 to -0.312)	< 0.0001
DAP	-0.410 (-550 to -0.269)	< 0.0001	-0.405 (-0.565 to -0.244)	< 0.0001
REGA	-0.432 (-0.576 to 0.288)	< 0.0001	-0.397 (-0.555 to -0.239)	< 0.0001

Table 6. Association of baseline active drug scores (ADS) with the CD4+ cell count change at 3 months after drug resistance testing (n = 109), using univariate and multivariate linear regression models.

	Change of CD4+ cell counts at 3 months after drug resistance testing per unit increase in ADS, cells/µl			
	Univariate model		Multivariate model	
Interpretation system	mean (95% confidence interval)	p-value	mean (95% confidence interval)	p-value
G2p	+13 (-3 to +28)	0.11 (n.s.)	+12 (-6 to +30)	0.20 (n.s.)
STDB	+14 (-2 to +30)	0.10 (n.s.)	+15 (-4 to +34)	0.11 (n.s.)
VGI	+12 (-3 to +28)	0.12 (n.s.)	+11 (-6 to +28)	0.22 (n.s.)
RG	+14 (-3 to +31)	0.11 (n.s.)	+13 (-6 to +33)	0.18 (n.s.)
CHL	+18 (+2 to +35)	0.03	+18 (-1 to +37)	0.06 (n.s.)
ANRS	+17 (+2 to +32)	0.03	+17 (0 to +36)	0.05
MED	+16 (-2 to +34)	0.09 (n.s.)	+15 (-5 to +35)	0.15 (n.s.)
DAP	+16 (0 to +32)	0.05	+17 (-1 to +36)	0.07 (n.s.)
REGA	+14 (-2 to +31)	0.09 (n.s.)	+14 (-5 to +32)	0.15 (n.s.)

months after drug resistance testing ($p < 0.0001$, Table 5). The mean decrease of viral load was -0.389 to -0.499 log10 copies/ml and -0.378 to -0.481 log10 copies/ml per unit increase in ADS in univariate and multivariate regression models, respectively.

The prediction of immunological therapy outcomes was analyzed for all patients with CD4+ cell counts at 3 months after resistance testing (n = 109) (Table 6). Univariate linear regression analysis revealed a significant correlation for CHL, ANRS, and DAP with mean increase of 16–18 CD4+ cells/µl per unit increase in ADS ($p < 0.05$). Multivariate models were adjusted for all parameters described in Material and Methods. In this analysis, only the baseline ADS of ANRS was significantly associated with an increase in the CD4+ cell count ($p = 0.05$). The p values of the other interpretation systems ranged between 0.06 and 0.22.

Logistic Regression Analysis for the Prediction of Therapy Success

Using multivariate models, ADS of all interpretation systems were significantly associated with the prediction of therapy success with odds ratios ranging between 2.46 and 3.41 ($p < 0.0001$, Fig. 2d). All other parameters were not significantly correlated with the

prediction of therapy success except for the number of recycled drugs ($p < 0.05$ for all interpretation systems except for ANRS and MED, with odds ratios ranging between 0.47 and 0.56).

DISCUSSION

Our study shows that three interpretation systems (CHL, MED, and ANRS), which were mainly developed in a clinical setting, performed better in predicting therapy success, but worse in predicting therapy failure than the other systems (Fig. 1b, 1c). This finding was confirmed with up-to-date versions of four frequently used interpretation systems (Table 4), although the re-analyses were limited in several respects: (i) most of the current algorithms do not provide an interpretation for full-dose ritonavir, which was administered to a number of patients in our study population, (ii) some current algorithms predict responses to boosted PI only, and (iii) prediction of response to DDC is no longer available. CHL, MED, and ANRS were less stringent in attributing resistance to a certain drug than the other interpretation systems (Table 2). This effect went hand in hand with a prediction optimum at 2.5 active drugs for these three interpretation systems, whereas for the other systems the optimum was closer to 2.0 active drugs (Fig. 1d). Thus, an optimum closer to the clinical experience of a working triple drug regimen may improve the prediction of therapy success, which should further be evaluated in prospective analyses.

All predictions of therapy outcomes are dependent on predefined variables, in particular the definition of therapy success or failure as a certain degree of viral load reduction after a certain time. We considered a viral load reduction of equal to or more than 1 log10 copies/ml as sufficient for therapy success, because the patients in our study were pretreated with a mean of 5.5 antiretroviral drugs. However, this criterion may not be adequate for different patient populations, since the ultimate goal should be suppression of viral load below the limit of detection. When therapy success was defined as viral load reduction of equal to or more than 2 log10 copies/ml for our study population, the total number of patients experiencing therapy failures increased, which favored the interpretation systems predicting therapy failure more reliably than treatment success. As important it is to compare interpretation systems with each other, it will be equally pivotal to compare algorithms between the different systems to find out which combination of mutations drives the algorithm into which direction.

Multivariate linear and logistic regression analysis showed that the ADS derived from all interpretation systems correlated well with virological therapy success ($p < 0.0001$, Table 5). Compared to data published by de Luca et al. [29], our study revealed a higher viral load decrease per active drug with -0.38 to -0.48 log10 copies/ml vs. -0.01 to -0.23 log10 copies/ml. One reason may be the more extensive pretreatment of the Italian patients who were included in the study after a mean of two failing antiretroviral regimen. Besides the ADS, only the number of recycled drugs proved to be an independent predictor of virological treatment outcome for most of the interpretation systems ($p < 0.05$, Fig. 2d). Resistance mutations that were induced after previous therapies may no longer be detectable in the plasma at the time of resistance testing, but nevertheless show up rapidly again and contribute to treatment failure if the respective drugs are being recycled [30]. In contrast, the correlation of the ADS with the immunological therapy outcome was modest: only three (CHL, ANRS, DAP) and one interpretation system (ANRS) showed a significant correlation with the CD4 increase in univariate and multivariate models, respectively ($p < 0.05$, Table 6). Two of these systems (CHL, ANRS) performed better in the prediction of therapy success, suggesting that this discrimination is important for the assessment of an interpretation system.

For our study, we faced the challenge to incorporate retrospectively obtained drug levels into the analysis. External reference values show considerable variation [31]. Retrospective studies used trough levels with reference to the concentration required to reduce viral replication by 95% [32], concentration ratios by dividing the concentration of the samples by the time-adjusted population value in the standardized pharmacokinetic curve [33], and the median in the study population for untimed plasma levels [34]. Prospective studies used protease inhibitor peak and trough levels to adjust the drug dosage [35], average steady-state concentrations for zidovudine/lamivudine and trough concentrations for indinavir in therapy-naïve patients [36], and drug concentration ratios for indinavir and nelfinavir [37]. Furthermore, consensus values for trough levels are available within published [38] or internet-based guidelines (http://www.hivpharmacology.com/). It was unclear whether the drug levels in our study represented peak or trough values. However, these samples mainly came from one treating physician who advised his patients to take their pills after the blood draw. Thus, the drug levels reported in our study most likely represent trough levels, as most of them range within published data. Indinavir levels were higher than in other studies with 989 ng/ml vs. 50 ng/ml [39], 100 ng/ml (www.hivpharmacology.com), 150 ng/ml [40], 152 ng/ml [41], 130 ng/ml [42], and 335 ng/ml [43], possibly caused by the additional use of low-dose ritonavir or another protease inhibitor in 8 of 14 patients receiving indinavir. In contrast, amprenavir levels in our study were lower than in other studies with 90 ng/ml vs. 280 ng/ml [44], 326 ng/ml [45], and 400/1200 ng/ml (www.hivpharmacology.com), which may be due to the relatively small number of samples with amprenavir drug levels ($n = 9$). In addition, amprenavir was frequently administered without boosting at that time.

The importance of adequate drug levels for treatment success becomes obvious from several results of our study. Almost one in five patients did not have measurable drug levels, which shows the frequency of adherence problems, an important reason for therapy failure [46]. In this respect, our retrospective study may reflect the daily clinical practice more accurately than prospective studies. In addition, the incorporation of drug levels into the ADS significantly improved the prediction accuracy of all interpretation

systems ($p<0.005$) (Fig. 2b, 2c). This concept was similarly realized within the phenotypic or genotypic inhibitory quotient [47,48]. Notably, our results were obtained although drug levels were only available for selected drugs and not in a standardized time frame after drug administration. It would be interesting to see how much the correlations would have profited from incorporating NRTI drug levels [49]. The data provided another reason why MED, CHL, and ANRS predicted therapy success better than the other interpretation systems: ANRS already showed the best correlation before the drug levels were incorporated into the prediction (Fig. 2c). Most other systems came close after drug levels had been incorporated, suggesting that these systems were affected to a larger extent by adherence issues. Our data encourage the determination of drug levels in retrospective databases to minimize the confounding effect of insufficient plasma levels on the prediction accuracy of interpretation systems.

The challenge of the future will be to generate a consensus interpretation system from all currently available interpretation systems, combining the best algorithms for the prediction of therapy success and failure. Notably, improved prediction of therapy success and failure went at the expense of one another, indicating that interpretation systems need to find the right balance. Large retrospective databases with quality-controlled virological, clinical, pharmacological, and immunological data will be an important tool to achieve this goal. Machine-learning techniques will be of great value, if not only applied to geno-/ phenotypic databases [50], but to complex clinical databases as well. These systems will allow to modify all parameters for predictions systematically, facilitating comparisons between existing interpretation systems. Furthermore, they will allow the calculation of clinically relevant cut-offs and then retrospectively extracting the rules behind the algorims. Considerable efforts to establish, maintain and update this consensus interpretation system will be rewarded by improved and individualized guidance to choose the optimal antiretroviral treatment for each patient.

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