AIDS Rev 2000; 2: 111-118

Second Spanish Consensus on the Use of Drug Resistance Testing in Clinical Practice (Madrid, March 2000)

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Abstract

A workshop was organized in Madrid on March 2000 to update recommendations for the use of drug resistance testing in HIV infection in Spain, based on new information and tests currently available. A panel of 30 physicians with wide experience in the field of antiretroviral therapy and/or resistance testing convened in a full-day session. Available clinical and laboratory data reported in the medical literature, conferences, and panel expert opinion were presented and discussed in an open fashion. The panel agreed to identify situations in which resistance testing should be recommended, others in which it might be considered, and others in which it should not be used. In summary, drug resistance testing should be recommended in HIV-positive pregnant women, in children (infected) born to treated mothers, in primary HIV infection or recent seroconversion, in early virological treatment failures, and before introducing a salvage regimen in heavily pre-treated subjects. Two situations were recognized in which resistance testing might be considered: in chronic naive infected subjects before beginning therapy, and in post-exposure prophylaxis. Lastly, testing should not be recommended when no treatment options exist for a given patient, or when plasma viremia is below the limit of detection. In summary, specific situations have been identified in which drug resistance testing might be of value for choosing antiretroviral therapy either in naive or pre-treated subjects. The advantages of this new tool remain controversial in any other circumstances.

Key words

Drug Resistance. Antiretroviral Therapy. Genotype. Phenotype.

Introduction

In March 18th 2000 a group of experts in the field of antiretroviral therapy and/or drug resistance met in Madrid in order to reach a consensus on the use of drug resistance testing in clinical practice. This workshop mainly tried to update the guidelines adopted the previous year in a similar meeting[1]. Advances in molecular biology techniques have allowed to consider the information about drug resistance as valuable for the best care of HIV-infected subjects. At least 3 prospective studies[2-4] have demonstrated that resistance testing provides advantage over empirical management in patients failing a previous antiretroviral regimen. On the other hand, recent studies have underlined that trans-

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Table 1. *Recommendations for drug resistance testing according to different guidelines.*

Conditions	1999 Spanish Consensus[1]	1998 IAS[17]	1999 BHIV[18]	2000 DHHS[19]	1999 French Recommendations[20]	2000 IAS[21]	Euroguidelines[22]	2000 Plan Nacional del SIDA, Spain[23]
Pregnant women	R	C	NR	?	C	C	R	R
Infected children from mothers on ART	C	C	NR	?	R	C	R	R
Seroconversion or recent infection (< 6-12 months)	R	R	R	C	R	?	C	R
After first failure (early detection)	R	NR	R	R	NR	C	R	C
After further failures	R	C	R	R	R	R	R	R
Naive, chronically infected	R	C	R	NR	NR	NR	C	NR
Post-exposure prophylaxis	R	NR	NR	?	NR	?	R	C
Without further therapeutic options	R	C	R	R	C	C	R	C
Viral load < 1000 cop/mL	NR	NR	NR	NR	NR	?	NR	?

R: Recommended
C: Consider
NR: Not recommended

mission of drug-resistant strains might be increasing in recent years, causing a raise in primary resistance to antiretroviral drugs in a growing proportion of newly infected persons[5-16]. During the last two years, several panels of experts have released guidelines for the use and implementation of drug resistance testing in clinical practice[17-22], including two reports from Spain[1,23]. Table 1 summarizes their main recommendations.

A panel of 30 physicians with wide experience in the field of antiretroviral therapy and/or resistance testing convened in a workshop in Madrid in March 2000. Available clinical and laboratory data reported in the medical literature, conferences, and panel expert opinion were presented and discussed in an open way during a full one-day workshop. The panel agreed to identify situations in which resistance testing should be recommended, others in which it might be considered, and others in which it should not be used.

Table 2 summarizes the main situations in which drug resistance testing was examined. The panel agreed that it should be recommended in five situations: In HIV-positive pregnant women, in children (infected) born to treated mothers, in primary HIV infection or recent seroconversion, in early virological treatment failure, and before introducing a salvage regimen in heavily pre-treated subjects. Two situations were recognized in which resistance testing might be of value in a limited number of cases: In chronic naive infected subjects before beginning therapy, and in post-exposure prophylaxis. Lastly, testing should not be recommended when no treatment options exist for a given patient, or when plasma viremia is below the limit of detection. We will discuss separately in detail each of these situations.

Table 2. *Guidelines for the use of drug resistance testing in clinical practice.*

Recommended
- HIV+ pregnant women
- Children (infected) born to treated mothers
- Primary HIV infection or recent seroconversion
- After first or second treatment failures
- Before beginning salvage therapy in heavily pre-treated subjects

Consider
- Chronic naive infected subjects before beginning therapy
- PEP

Not recommended
- No treatment options
- Undetectable plasma viremia

HIV-infected pregnant women

Mother-to-child transmission of HIV infection has declined dramatically since the widely prescription of antiretroviral drugs during pregnancy and at the time of delivery. Among other factors, suppression

of plasma viral load to undetectable levels at birth seems to be one if not the most important variable accounting for this protective effect. Although prospective, randomized trials proving this benefit are limited to zidovudine[24] and nevirapine[25], it is generally believed that the success in preventing vertical HIV transmission is mainly dependent of the antiviral effect of drugs during pregnancy and labor. Therefore, if resistance to drug(s) in use develops during pregnancy, it should be presumed that viral rebound could prone the newborn at risk for HIV infection. Likewise, if pre-existing resistant viruses exist in the woman, treatment failure might be more common, and the risk for perinatal transmission higher[26,27]. Taking into account these considerations, the panel agreed to recommend drug resistance testing to all HIV-infected pregnant women, including those without previous exposure to antiretroviral drugs, i.e. those firstly known to be infected during a prenatal exam. As will be addressed later, a rate of primary resistance ranging from 5 to 25% in many developed countries supports this intervention.

Children (infected) born to treated mothers

Despite the number of new HIV-infected newborns is currently very low in developed countries, children who acquire HIV infection from mothers exposed to antiretroviral drugs during pregnancy are more likely to be infected with resistant viruses[26,27]. Treatment is often introduced early in HIV-infected children and, therefore, the first combination is really crucial for them. Therefore, the panel agreed to recommend drug resistance testing to all children born to HIV-infected mothers, and extended the recommendation to those born from mothers who never had been exposed to antiretroviral drugs, since the incidence of primary resistance is growing in many areas[12,15].

Primary HIV infection or recent seroconversion

The transmission of drug-resistant HIV was first described in 1993 with documentation of reduced susceptibility to ZDV in a subject with primary HIV infection[28]. Since this first report, sexual transmission of resistant viruses has been well documented, and extended to other drugs, including lamivudine, nevirapine, and protease inhibitors[6-16,29]. More recently, the transmission of multidrug-resistant HIV strains has been reported[5], and is a major concern in terms of public health[30].

Resistance to ZDV and, in a lesser extent, to 3TC are the most frequently recognized among naive individuals. In subjects presenting with primary HIV infection or in recent seroconverters, primary resistance reach rates between 10-20% for ZDV and 5-10% for 3TC in the US and Western Europe (Table 3)[6-14]. These drugs have been the most widely used, and shown either a low (3TC) or intermediate (ZDV) genetic barrier.

Response to first antiretroviral regimens has shown to be compromised in subjects carrying primary drug resistance[6,8,31], although the use of drug combinations can preclude its recognition, at least in the short-term[32]. In the ICONA trial[31], a multicenter Italian study, a poor virological response to first-line therapy was correlated with the presence of baseline genotypic drug resistance. In the ACTG 343, an induction maintenance trial, subjects harbouring ZDV resistance at baseline tended to suffer viral rebound more frequently at the maintenance phase[33].

Resistant mutations tend to disappear from plasma HIV-RNA if drug pressure is not maintained, as it occurs in viruses transmitted from treated subjects to newly infected persons who remain naive. More fit wild type strains tend to overgrowth mutant viruses in a few months[34], although mutations remain hidden in some extend as proviral DNA. So, the recognition of drug resistant HIV will become more difficult as the time from seroconversion increases.

Early virological failure

Both baseline genotyping and phenotyping have shown to predict the response to salvage therapy in patients experiencing antiretroviral failure[35-39]. Three prospective studies have provided evidence favouring the use of resistance testing for choosing the best combination of drugs as rescue intervention in persons failing therapy[2-4]. Although the virological benefit was modest on average (Table 4), it might have great clinical impact, as was shown in old trials using mono or bitherapy[40]. Preliminary data from a more recent trial conducted in France[41], however, have not confirmed the advantage of adding either genotyping nor phenotyping to the standard of care, although most criticisms to the study have been related to the fact that most patients enrolled in the trial were heavily pre-treated, for whom no new drugs were available.

However, if resistance testing is expected to provide a clear advantage in rescue interventions for patients experiencing failure with their current therapy is mainly because it allows to substitute only one or two drugs in the failing regimen instead of replacing all drugs for new ones. Compounds with low genetic barrier, i.e. lamivudine or nevirapine, are those mainly compromised in early viral rebounds after being on good response to therapy for a while[33,42]. It is at this time when a repeatedly confirmed viral rebound can permit to intensify if resistance is not recognized[43,44] or to make a selective substitution if just one or two drugs are compromised by resistance.

Before introducing salvage regimens in multi-treated patients

The chances of reaching undetectable viremia in persons with broadly exposure to all current available antiretroviral compounds are very limited[45,46].

Table 3. *Prevalence of primary resistance to antiretroviral drugs in naive subjects with primary HIV infection or recent seroconversion.*

	Yerly *et al.*[6]	Boden *et al.*[7]	Little *et al.*[8]	Brodine *et al.*[9]	Salomon *et al.*[10]	Wegner *et al.*[11]	Briones *et al.*[13]	Miró *et al.*[14]
Country	Switzerland	USA	USA	USA	Canada	USA	Spain	Spain
Population	Seroconverters	Seroconverters	< 1 year infection	Seroconverters	Seroconverters	< 3 years infection (< 1 year in 56%)	< 6 months infection	< 3 months infection
Period	1996-1998	1995-1999	1989-1998	1997-1998	1997-1999	1997-1998	1997-1999	1997-1999
No.	82	80	141	31	81	114	30	25
Risk group (homosexuals)	40%	94%	80%	45%	64%	> 90%	70%	60%
Genotypic resistance								
* **Any**	10%	16%	1.4%	26%	-	22.1%	26.7%	16%
* **NRTI**	10%	12.5%		10%	18%	4.3%	23.3%	4%
* **AZT**	9%	-		6.7%	8%	-	20%	4%
* **3TC**	2.4%	-		3.3%	5%	-	3.3%	0
* **NNRTI**	2.4%	7.5%		13%	4%	15.8%	3.3%	8%
* **PI**	4%	2.5%		16%	7%	9.5%	6.7%	4%
* **MDR**	1.2%	4%		6.7%	10%	4%	6.7%	0
Phenotypic resistance	PI > 4 fold: 4% > 10 fold: 2.4%	85% concordance with genotype	Any: > 2.5 fold: 26% > 10 fold: 7% NRTI: 1.4% NNRTI: 0.7% PI: 1.4%	Not done	NRTI: 36% IP: 14%	Any: 29.7% NRTI: 7.7% NNRTI: 26.4% PI: 1.1% MDR: 3%	Any: > 4 fold: 23% > 10 fold: 7% NRTI: 6.7%	Not done

Table 4. *Virological response to salvage therapy guided by either resistance testing or standard of care in 3 prospective studies[2-4].*

Study	Design	1st PI failure	↓ log HIV-RNA (w16)	< 400 HIV-RNA cop/mL (w16)
VIRADAPT	Gen vs SOC	40%	-1.04 vs –0.46	29% vs 14%
GART	Gen + Exp Adv vs SOC	50%	-1.19 vs -0.61*	34% vs 22%*
VIRA 3001	Phen vs SOC	100%	-1.27 vs –0.75	38% vs 23%

* week 12
Gen: genotype
Phen: phenotype
SOC: standard of care
Exp Adv: expert advise

As long as these subjects remain under therapy, the virological failure is rarely associated to the appearance of clinical opportunistic events. Therefore, antiretroviral therapy often provides protection against AIDS even when no complete virus suppression is affordable[47-50]. A low replicative capacity of escape mutants carrying multiple drug-resistant mutations could explain this observation[51]. In this context, only drug-related toxicity issues can justify the recommendation of drug holidays[52]. Alternatively, the recycling and concurrent administration of multiple drugs (mega-HAART) is often explored[53]. Resistance testing allows to exclude from this strategy those drugs for which no antiviral activity is expected and that will contribute to accumulate toxicities, either causing side effects directly or through interaction with other compounds.

Chronic naive infected persons

Persons infected with HIV for many years or for an unknown length of time are less likely to harbour mutant viruses than those infected recently. The current armament of drugs was only introduced in the market after 1995, and the proportion of patients beginning therapy expanded dramatically only after that time. Persons infected previously were less likely to be exposed to other individual with antiretroviral drug experience. Moreover, resistance could have been selected only against a few drugs, mainly for ZDV. All these circumstances explain why the rate of primary resistance to antiretroviral drugs is much lower among naive individuals with long-lasting HIV infection than in recent seroconverters. In the Spanish ERASE-1 and -2 studies, two multicenter trials in which samples collected since 1993 were examined, the prevalence of primary resistance was below 10% in naive subjects with prolonged HIV infection (Table 5)[54,55]. Therefore, the panel agreed to consider that resistance testing should not be mandatory in chronic HIV-infected persons in the absence of particular circumstances which could justify it, i.e., in the sexual partner during several years of a person known to be on antiretroviral therapy, who decided to be tested for HIV antibodies for the first time and yielded positive results. Table 6 records the priority for drug resistance testing in naive individuals according to the estimated duration of their HIV infection.

Table 5. *Prevalence of genotypic resistance to nucleoside analogues in naive individuals (mostly with chronic HIV infection) in Spain[53,54].*

	1993 n = 75	1997 n = 75	1998 n = 52
ZDV (codons 41,70,215)	8	9	6
ddI (codon 74)	1	0	0
ddC (codon 69)	0	0	2
3TC (codon 184)	1	0	4
Total number of patients	10 (13.3%)	9 (12%)	9 (17%)

Table 6. *Recommendation for drug resistance testing in naive subjects as a function of the time since HIV exposure and the knowledge of treatment in the patient's source at that moment.*

Souce Of infection \ Naive Person	Primary HIV infections or recent seroconversion	Chronic HIV infection
On treatment	Yes	Yes
Unknown	Yes	No
No treatment	Yes	No

Post-exposure prophylaxis

The risk of acquiring HIV infection after accidental exposure to HIV is on average of 0.3%[56]. Health care providers (i.e., nurses) who suffer an accidental inoculation with needles recently used to drawn blood from HIV-infected patients can acquire resistant HIV strains from patients having been exposed to antiretroviral drugs[57]. In this circumstance, hopefully the early administration of antiretroviral compounds (within the first few hours) could reduce the rate of establishment of HIV infection[58]. The use of non-nucleosides or protease inhibitors, which act directly on viral enzymes, seems to be the best option for preventing cells to become infected with HIV. Drugs to be administered need to be selected empirically, taking into account and excluding those used for the patient acting as potential source of the infection[59]. Moreover, the tolerability of the regimen should be promoted avoiding the use of

badly tolerated drugs as much as possible[60]. If resistance testing may play a role in this context it is for providing information from the patient's blood[61]. As soon as the results are available, the preventive regimen administered to the health care provider can be adapted appropriately.

When no treatment options exist

The management of subjects on virological failure who have been exposed to all drugs available and/or have developed toxicity related to their use face limited therapeutic options. In this context, the best care should be focused on the prevention of opportunistic infections. The benefit of knowing the resistance profile in these individuals is mainly academic, since the opportunity for choosing new drugs does not exist. One of the criticism to the French NARVAL trial[41], in which the information provided by genotyping or phenotyping was compared to the standard of care was that most subjects recruited in the trial were heavily pre-treated, leaving few options for any rescue intervention. As expected, no differences in the virological response were seen when all three arms were compared.

Subjects with undetectable plasma viremia

Current methods for genotyping or phenotyping require a minimal level of plasma viremia to provide results, in the range of 1000 HIV-RNA copies/mL[62]. The detection as early as possible of emerging drug resistant mutant viruses might provide the opportunity for switching therapy before multiple mutations accumulate. It is well known that further mutations will increase the level of resistance and/or produce cross-resistance to other compounds of the same family[63]. Since the current viral load tests permit the quantification of samples with viral load levels in the range of 20-50 HIV-RNA copies/mL, most experts agreed to consider that drug resistance tests should improve their performance in specimens with low levels of viremia. One limitation, however, concerns the reliability of the results obtained in these circumstances since the reproducibility of PCR testing is greatly dependent of the nucleic acid concentration. Therefore, ideally drug resistance testing of specimens with very low viremia should be confirmed repeatedly.

Additional considerations for an appropriate use of resistance testing

The technology available for measuring drug resistance is still complex, and reproducibility is far to be consistent in different labs, as it was shown in the ENVA-2 study[64]. The performance of the current assays when testing non-B subtypes should be explored in detail, since the proportion of new infections caused by these viral variants is increasing in Europe and the United States[65]. Cost-effectiveness analyses need to be performed in order to prove the benefit of adding the resistance information to the parameters guiding antiretroviral treatment. Drug levels and IC_{90} values must be integrated in new algorithms designed to yield a more reliable interpretation of genotypes and phenotypes[66]. Search for new genotypes accounting for resistant phenotypes must be pursued and identified[67], and specific situations in which resistance testing provides a precise support for rescue interventions need to be defined, i.e. when early failure occurs on nelfinavir[68] or nevirapine[69].

Drug Resistance Panel

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