

GESIDA consensus statement on the use of resistance studies in clinical practice

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Introduction

The current use of highly active antiretroviral therapy (HAART) against the human immunodeficiency virus (HIV), has led to excellent control of viral replication in a large number of patients. This is associated with an immunological improvement and an important reduction in mortality and morbidity¹.

Although the first studies with protease inhibitors (PI) in monotherapy^{2,3} suggested that the selection of resistance was the only cause of virological failure, at present it is well known^{4,5} that several factors can contribute to the failure of antiretroviral therapy. These include poor adherence, insufficient potency of antiretroviral regimens, and pharmacological and pharmacokinetic interactions among drugs.

HIV has developed different mechanisms to escape from pressure of the immunological system, and from drugs. The poor accuracy of reverse transcriptase (RT) in replication of viral RNA, together with the large population of existing virions (around 10^{12}),

and the extraordinary kinetics of HIV replication (with a half-life in plasma below 6 hours)⁶, favor the spontaneous appearance of different genomic variants known as *quasispecies*⁷. Thus, when selective pressure is put on the different *quasispecies* in a person infected, the smaller pre-existing resistant populations are selected. They then acquire a replicative advantage which makes them, over time, the predominant viral population.

Therefore, resistance to drugs, while not the only factor, is very important in explaining virological failure of a given antiretroviral treatment. At present, several types of tests enable the resistance of the majority viral population to one or more drugs to be determined. Although these tests are very helpful, there are still technical problems involving cost and interpretation.

The AIDS Study Group (GESIDA) of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC), in a panel of experts held in March 2000, and in a monographic symposium held in May during the SEIMC Congress in May 2000, examined several studies on this topic and evaluated their own existing recommendations (see below). Resistance testing, unless otherwise specified,

Translated from *Enferm Infecc Microbiol Clin* 2001;19(Monográfico): 53-60.

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refers here exclusively to genotypic tests, that is, to sequencing RT and protease (PRT) genes.

Tables 1 and 2 show a comparison of the recommendations of several groups of experts for the indication of genotypic resistance studies in different situations.

Potential indications

Patients with no previous antiretroviral therapy

Today, there are reports of HIV mutants which are resistant to all the drugs used in antiretroviral treatment^{8,9}. The prevalence of primary resistance (those detected in patients who have never received antiretroviral therapy) is, according to some studies, between 1% and 26%. The width of this range can be explained by the methodological differences in the studies: analysis of different pharmacological groups, different geographical areas and different types of resistance tests, as well as different cut-off points used to define resistance⁹⁻¹¹. However, it is logical to think that the current generalization of antiretroviral therapy will result in an increase in the transmission of resistant viruses and, therefore, an increase in the prevalence of primary resistance¹².

Another controversial area is the cut-off point at which the prevalence of primary resistance detected in a specific region would justify the routine performance of resistance studies in patients before beginning antiretroviral treatment. In the latest consensus guidelines of the Panel of the *International AIDS Society-USA* published recently in *JAMA*¹³, resistance testing was proposed at the beginning of antiretroviral treatment, where

TABLE 1. Comparison of recommendations in patients with no previous antiretroviral treatment

	Naives			
	< 6 months	> 6 months	PEP	Pregnant women
GESIDA	R	C*	C*	R
SPNS	R	NC	C*	R
IAS	C	C	NC	R

R: recommended; C: consider; NC: not commented; C*: according to context; GESIDA: Grupo de estudio de Sida de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica; SPNS: Secretaria Plan Nacional sobre el Sida; IAS: *International AIDS Society-USA*; PEP: post-exposure prophylaxis.

TABLE 2. Comparison of recommendations in pre-treated patients

	Pre-treated			
	1 st failure	2 nd -3 rd failures	> 3 failures	Pregnant women
GESIDA	R	R	C*	R
SPNS	C	R	NC	R
IAS	R	R	R(j)	R

R: recommended; C: consider; NC: not commented; C*: according to context; GESIDA: Grupo de estudio de SIDA de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica; SPNS: Secretaria Plan Nacional sobre el Sida; IAS: *International AIDS Society-USA*.

the prevalence of primary resistance was between 5% and 10%.

Finally, another decision required in this group of patients would be, depending on the probable date of infection by HIV, whether or not to evaluate the need for resistance testing, which would not be the same in recently infected patients as in those who were infected before the appearance of PI, non-nucleoside reverse transcriptase inhibitors (NNRTI) or in those in whom the duration of HIV infection is unknown.

Primary infection and recent infection

Primary infection is defined as the period between the moment of infection and complete seroconversion. Recent infection refers to the first three months after infection^{8,14}. The arguments in favor of starting treatment during this phase are exclusively biological and are not based on clinical studies. They include: a) the drop in the number of infected cells; b) achieve almost complete restoration of the immunological system¹⁵, and c) drop in viral load with potential improvement in prognosis of the disease¹⁴. At present, starting treatment at this point of infection is controversial. In those cases where it is decided to start antiretroviral therapy, it is recommendable to do so in controlled studies which can provide useful information on unresolved issues as a possible long-term clinical benefit¹⁴.

One problem found in evaluating prevalence studies of primary resistance in patients with primo-infection or recent infection is that some of these studies do not distinguish patients who fulfill the primary infection criteria as previously defined and include patients in whom the infection has already evolved for up to one year.

The widest study in the literature which exclusively evaluates patients in the first three months of HIV infection was carried out in Switzerland by Dr. Perrin's group. In this study, the incidence of genotypic resistance observed in 82 patients recruited between 1996 and 1998 was 10% for nucleoside analog reverse transcriptase inhibitors (NRTI), 1% for NNRTI and 4% for PI. This study associated the existence of baseline phenotypic or genotypic resistance with a poor virologic response and therefore concluded that it is necessary to test for resistance in this group of patients⁸.

Dr. Markowitz's group found a 16.3% prevalence of primary mutations with NRTI, 7.5% with NNRTI, 2.5% with PI and 3.8% with multiple drugs (MDR) in a study of 80 patients with primary infection, mainly in the area of New York. Agreement between the genotypic and phenotypic studies was 85%, with discordances (except in one patient with phenotypic resistance to 3TC and without the M184V mutation, which was later detected in a limit dilution of PCR) in patients with a phenotypic intermediate resistance pattern¹¹.

Dr. Richman's group included 141 patients in a primary resistance prevalence study, of whom only 62 were recruited in the primary infection phase. The final results were not stratified by group according to the time of infection. The rate of phenotypic resistance (understood as a > 10-times reduction in susceptibility to the drug) was 2% for NRTI, 1% for NNRTI and also 1% for PI¹⁰. The usefulness of resistance studies in selecting antiretroviral therapy was confirmed in two patients.

In June last year in San Diego, Dr. Miller's group presented data on the prevalence of primary resistance in 46 patients with acute infection included in the QUEST study. Primary mutations against NRTI were observed in 8%, 4.3% against NNRTI and none against PI, with 43% secondary mutations¹⁶. Also in San Diego, Tamalet et al presented data on the prevalence of primary and secondary mutations in 53 patients with primary HIV-infection in France. Prevalence of resistance to AZT was 10%, to 3TC 2%, to NNRTI 0% and to PI 4%, with 75% secondary mutations. No differences in response to treatment were found between patients with primary mutations and those without, with the result that

they suggested that the existence of genotypic resistance in patients with primary infection did not necessarily precede therapeutic failure¹⁷.

In Spain, two recent studies evaluated the prevalence of primary resistance in patients with primary HIV infection. Briones et al analyzed data on the resistance in 30 patients (70% homosexuals) from Madrid with documented infection of less than 6 months selected between 1997 and 1999; in 26.7% they detected primary mutations which confer resistance to some antiretrovirals; 23.3% had resistance mutations to some NRTI (85% to AZT, 15% to 3TC), 6.7% to PI and 3.3% presented a pattern of multidrug resistance. No resistance to NNRTI was detected. In the phenotypic study, 23% of patients presented some level of resistance¹⁸. In data presented in Durban at the XIII World AIDS Conference, Miró et al reported the prevalence of primary mutations in 25 patients with primary infection (< 3 months) (60% homosexuals) from the Barcelona area. They detected primary mutations in 4 of the patients (16%); one presented mutation K70R for AZT (4%), two presented mutations (V106A and V108I) for NNRTI (8%) and a last one (4%) presented a mutation in position 82 which confers resistance to IDV and RTV¹⁹.

In the recommendations of the *International AIDS-Society USA*¹³, recently published in JAMA, resistance testing in this group of patients is an option to be considered.

In the GESIDA Consensus Meeting on Resistance, the decision to consider this an indication for genotypic resistance testing before starting treatment, if treatment is indicated, was unanimous. The possibility of extending the period to patients who were going to start treatment within the first six months of infection was considered. It is important to insist, however, that resistance testing in these cases should not delay the start of treatment, as appropriate changes could be made once the results are received and depending on the response to therapy. Similarly, in cases where this study might not be available, it was considered advisable to save a sample of plasma before starting treatment for possible future studies.

Resistance testing in patients with primary infection has also been recommended by the National AIDS Plan Secretariat (SPNS), according to its report «Resistance to antiretro-

viral drugs: use of studies in clinical practice» dated March 2000 (<http://www.msc.es/sida/asistencia/home.html>).

Lastly, in another meeting of a panel of experts held in Madrid in March 2000, resistance testing before beginning HAART was also recommended for patients with recent HIV infections²⁰.

Chronic infection (over 6 months)

After considering the possibility of stratifying the indications for resistance testing in naïve patients according to time of infection (6-12 months; > 12 months), it was finally decided not to distinguish time of infection, with regard to indication of a resistance study to guide the initial treatment regimen, after the first 6 months of infection.

The predominant viral population is similar to that of the source case during the first weeks after infection, and presents the same pattern of resistance. In the following weeks, in the absence of pharmacological pressure which selects and maintains the transmitted mutant strain, the wild type virus, being fitter, will gradually replace the original *quasi-species* and become the predominant population²¹⁻²³, diluting the original pattern of mutations. The «loss» of mutation over time without pharmacological pressure varies according to the drug. A recent study showed the disappearance of the majority of mutations after 13 weeks²³. In a French study of naïve patients, Descamps et al concluded that most patients who had accumulated genotypic mutations had been infected less than one year before²⁴. The margin of time considered necessary before indicating a resistance study in naïve patients might thus be extended to one year from the date of infection.

The 1998 ERASE-2 study carried out in Spain evaluated the prevalence of primary mutations in 52 naïve patients; 17% presented primary mutations for NRTI, 6% for PI. Mutations for NNRTI were not evaluated. The main mutations found for NRTI were K70R isolated in 66%, M184V in 44%, T215Y in 33% and M41L in 11%. The most frequently detected mutation in protease gene was in position 82 in 16% of the patients²⁵.

In a study presented in San Diego²⁶, the prevalence of phenotypic and genotypic mutations was evaluated in 230 naïve patients recruited in 9 U.S centers. In the genotypic

study, primary mutations were isolated for NRTI in 2% and secondary in 5%. For NNRTI, the prevalence of primary mutations was 6% and for secondary mutations it was 42.2%. Lastly, primary mutations for PI were detected in 2% and secondary mutations were detected in 38.2%. In the phenotypic study, the prevalence of high-level resistance was 1.6% for NRTI and PI and 7.3% for NNRTI, whereas the prevalence of intermediate resistance was 4% for NRTI, 21.9% for NNRTI and 3.1% for PI. At the same meeting, a British prevalence study of genotypic resistance carried out on 54 naïve patients was presented²⁷. No primary mutations were detected for NRTI or PI, whereas in 37% secondary mutations were detected.

The options put forward at the consensus meeting were:

1. Resistance testing for all patients.
2. Resistance testing only for those patients who were infected after 1996, since in prevalence studies before this date the rates were very low for NRTI and zero for PI and NNRTI.
3. No resistance testing for any patient infected for more than 6 months.

At present, in our environment, the prevalence of mutations which confer resistance to NRTI having clinical impact is approximately 8%, and 6% for PI²⁵. According to the recently published consensus guidelines of the *International AIDS Society-USA*, these studies are recommended where prevalence of resistance is above 5%-10%. Therefore, we do not believe resistance testing should be considered routine in this group of patients at this time. Nevertheless, we are convinced of the importance of epidemiological surveillance studies which can give us more detailed information on the current situation of the prevalence of primary resistance in our population of seropositive patients. A significant increase in prevalence could force us to recommend resistance testing before therapy in all patients, regardless of the duration of HIV infection. Both GESIDA and the *International AIDS-Society USA*¹³, believe that resistance testing in these patients should be considered, but not recommended, in all cases. If it is not carried out, a plasma sample should be kept for retrospective analysis in the case of therapeutic failure.

Pregnant women

In the latest «Guidelines for the use of anti-retroviral therapy in HIV+ adults and adolescents» drawn up by the United States Department of Health and Human Services published in January this year, it is believed that in general, the recommendations on resistance studies during pregnancy are identical to those in non-pregnant people; that is, the suitable indications for carrying out these tests are acute HIV infection, virological failure with an antiretroviral regimen or sub-optimal suppression of viral load after starting antiretroviral therapy²⁸.

Another factor which favors the importance of virological control –undetectable viral load (VL)– in this group of patients, is prophylaxis of mother-to-child transmission. Many studies have shown that the existence of an undetectable VL in the mother represents one of the main predictive factors for the absence of vertical transmission²⁹. For this reason, it was considered advisable to recommend the routine practice of resistance studies in naïve pregnant women, regardless of the date of infection. It must be pointed out that this is also an indication accepted by the SPNS.

Post-exposure prophylaxis

This indication is also controversial. There are few arguments for carrying out a test which can take between 2 to 3 weeks when designing urgent treatment. Particularly, because the efficacy lies in the speed of initiation of therapy, and in the mechanism of action of drugs used, more than in determining a resistance test of the source case.

At the 7th *Conference on Retroviruses and Opportunistic Infections*, Cheingsong et al³⁰ presented a prevalence study of genotypic resistance in 42 source cases of accidents in the workplace. The resistance prevalence for NRTI was 14% (88% for AZT and/or 3TC), 5% for NNRTI (mutation 103N), 5% for PI and 21% MDR, with 85% genotypic/phenotypic concordance (6/7).

We believe that, in this case, speedy start-up of treatment and use of drugs not requiring intracellular metabolization are vital. The pharmacological history of the source case would also have to be considered. Thus, if virological control is good (VL undetectable), treatment should be the same as for the source case. Otherwise, a resistance

study should be carried out on the source case and the initial regimen modified according to the results. This recommendation has also been accepted by the SPNS.

Children

Resistance studies are indicated in children born of seropositive mothers who have positive PCR for HIV regardless of the mother's viral load.

Resistance studies of the child were also considered in cases of late diagnosis. In a prevalence study of primary resistance in children presented by Ruiz at Lago Maggiore in 1998 (ERASIN), primary resistance prevalence detected was <5%-10%³¹.

Pretreated patients

Good virological response (undetectable viral load)

The «classical» concept of impossible development of mutations conferring resistance to treatment in patients who have achieved good virological control reported in two studies published in *Science* in 1997^{32,33}, was recently rejected in studies showing the appearance of new mutations in spite of an apparently good virological control. Martínez-Picado et al in San Francisco presented the results of a study wherein 4/5 patients with VL of < 50 copies/ml suffered transitory rebounds of VL (*blips*) and new mutations were identified which had not existed previously³⁴.

The clinical significance of the new mutations which appear in this situation and their possible translation into therapeutic failure is unknown. Determination of mutations in patients with such low viral loads is also technically very complex. Therefore, at present, resistance testing is not indicated in this situation.

First therapeutic failure

Resistance testing for the design of rescue therapy may be indicated in patients with a first virological failure. Several studies have evaluated the usefulness of these studies in patients for whom treatment has failed; however, it must be pointed out that in most of the published studies the number of previous treatments is not specified and patients with one or more failures are all included in the same study.

Of the retrospective studies, that by Deeks et al in which 18 patients who failed on a

regimen with indinavir (IDV) were evaluated may be highlighted. The existence of phenotypic sensitivity to two or more drugs in rescue treatment was associated with a better virological response than when it was sensitive to one or no drugs³⁵. Harrigan et al also observed retrospectively that in 84 patients pretreated with NRTI, a rescue regimen with sensitive drugs had a 12-fold greater possibility of responding to treatment than a regimen with resistant drugs³⁶.

Few prospective studies evaluate the usefulness of resistance studies in rescue therapies. The VIRADAPT study carried out on 108 patients, which compared the use of genotypic resistance testing (65 patients) to SOC (*standard of care*) (43 patients) in the design of rescue therapy, showed the greater effectiveness of the first with an average decrease of VL at 24 weeks of 1.15 logs compared to only 0.67 logs in the SOC group; and 32% of patients with VL < 200 copies/ml in the genotype group compared with 14% in the SOC arm³⁷. Only in 46.2% of the patients included in the genotyping group and in 32.1% of those in the clinical group did this involve the first therapeutic regimen, without evaluating the final response to this parameter. In the other study, GART³⁸, with a design similar to the one above, results were similar, with 29% of patients with VL < 200 copies/ml in the genotyping group against 17% in the clinical group. Finally, the preliminary results of the first study, which compared the use of phenotypic resistance testing to SOC in the design of rescue therapy in patients who failed their first treatment regimen with PI (VIRA 3001), showed that at 16 weeks, VL rose to < 400 copies/ml in 62% of patients in the phenotypic group compared to only 33% in the clinical arm³⁹.

Results of the Havannah study have recently been presented. This study was designed in part to determine whether the advice of experts improves the clinical interpretation of genotypic data. This prospective study included 315 patients who had previously been in stable antiretroviral treatment for at least 6 months and who presented virological failure (VL > 1,000 copies/ml). Patients were randomized to change treatment according to four strategies: a) no genotype, no expert advice; b) no genotype, with expert advice; c) genotype, no expert advice and d) genotype, with expert advice. The advantage of genotype

over no genotype was observed using intention to treat at 3 and 6 months. The role of expert advice followed the same pattern. At 3 and 6 months, the group of patients with expert advice (with or without genotype) had a better virological outcome than the group which did not receive this advice (regardless of whether or not they had had genotype). Similarly, in the group with virological failure on the third treatment, genotype with expert advice was greater than the genotype alone. This study confirms the findings of previous studies such as VIRADAPT and agrees with the GART study in which, despite less patient follow-up, also showed that both the genotype and expert advice contributed to the differences observed⁴⁰.

Two recently published studies which aimed at evaluating the effectiveness of induction-maintenance regimens in antiretroviral therapy (ACTG 343 and TRILEGE)^{4,41}, have indirectly shown that virological failure is secondary on many occasions to problems of adherence or pharmacokinetic problems and not to resistance. Therefore, resistance studies early after detection of treatment failure would enable design of new therapies by rescuing drugs which had already been used, as well as detecting and correcting existing problems both of adherence and of pharmacokinetic interactions or absorbance. We therefore feel that it is advisable to consider this situation as an indication for genotypic resistance testing, although it is essential that other evident reasons for failure (poor adherence, pharmacologic interactions, subtherapeutic levels of antiretroviral drugs) be given particular attention and, if detected, must be corrected.

The *International AIDS-Society USA Panel*¹³, recommends resistance testing in these patients and the SPNS agrees that this possibility should be considered.

More than one therapeutic failure

The studies to date do not specify results according to the number of previous therapeutic failures.

Patrick et al evaluated the usefulness of knowing the mutations in 28 patients of whom 80% had failed with two PI regimens, and concluded that there was a significant relationship between the number of mutations and the virological response⁴². Zolopa et al evaluated the predictive factors for res-

ponse in 54 patients who had failed with PI and were in rescue therapy with a regimen of SQV-RTV. The main predictor of a good response was the existence of mutations previous to rescue therapy, and it was concluded that the use of resistance studies would improve the predictive power of a good response by 70%⁴³. Of the prospective studies carried out, the most noteworthy are VIRADAPT³⁷ and GART³⁸, mentioned above, which include patients with more than one failure.

At the recent 4th International Workshop on HIV Drug Resistance and Treatment Strategies in Sitges, Girard et al presented the preliminary results of the NARVAL prospective study, which compares the efficacy of rescue therapy according to whether it is designed by SOC, genotypic or phenotypic study. The study includes 541 patients who had just failed with a regimen containing PI (average previously used drugs = 7) and who presented a median CD4 lymphocyte count of 280 and VL of 4.3 logs. In the 12th week, the percentage of patients with VL < 200 copies/ml was 34% in the clinical decision group, 41% in the group in which the change was made based on the results of the genotypic resistance study, and 33% in the group in which the change was made according to the phenotypic study. A fall in VL greater than 1 log was observed in 51%, 59% and 55%, respectively. The results in 427 patients evaluated in the 24th week were similar, with a percentage of patients with VL < 200 copies/ml of 27%, 36% and 26% in the clinical, genotypic and phenotypic arms, respectively⁴⁴.

A relevant question in the group of patients who failed more than one treatment regimen is after how many failures may it not make sense to carry out new genotypic studies as long as no new drugs are available. The conclusion is that it would not make sense to carry out new resistance studies, as long as at least one or two previous studies had been carried out in patients who had already failed 2-3 HAART regimens, and there were no usable drugs, according to the mutations which appeared in the genotypic study carried out. For example, if a patient started antiretroviral treatment with three NRTI, after a first failure, he was rescued with two NRTI and one NNRTI, and after a new failure he went on to a third regimen

with two NRTI and a PI, resistance testing should not be ruled out because three HAART regimens had already been tried. Resistance testing would be indicated in the design of a new rescue treatment, if efficacious drugs exist, especially NNRTI and PI. Now, if the patient has received more than three regimens of HAART and the resistance studies carried out (at least one) do not show the existence of other potentially efficacious medication, it would not make sense to continue carrying out new genotypic resistance studies. There was therefore consensus that at least one resistance study would be indicated in the case of two or more failures, and more than one study in the case that there might exist potential alternative medication without cross-resistance.

In the recommendation for the year 2000 of the *International AIDS-Society USA Panel*⁴³, resistance testing is recommended in these patients without specifying the number of previous failures. This indication is also accepted by the SPNS. It would not make sense to carry out more than one or two genotypic resistance studies if the circumstances have not changed.

Pregnant women

In spite of the fact that the latest «Guidelines for the use of antiretroviral therapy in HIV+ adults and adolescents» drawn up by the United States Department of Health and Social Services²⁸ consider that the indications for resistance testing in pregnant women must not differ from those which apply to the non-pregnant women, we feel that, regardless of the number of previous treatments carried out on the woman, in the case of a pregnancy, all the tools available must be sought to obtain the best virological control possible and that resistance testing can contribute to this end. It must therefore be recommended. This opinion is shared by the SPNS.

Methodology

At present, two different methods are available for determining resistance to antiretroviral drugs, genotypic and phenotypic.

Genotypic methods

These determine the existence of mutations in the viral genome (protease and RT

genes) which have proven to be associated with phenotypic resistance. Phenotypic changes associated with specific mutations may be consulted in the *International Medical Press 2000*⁴⁵ and different databases accessible on the Internet⁴⁶⁻⁴⁸.

All the genotypic techniques use PCR, although there are three ways of identifying the mutant population:

1. Hybridization of the amplified product with specific probes which identify mutations in specific positions (LiPA[®]). This allows greater speed in the technique and greater sensitivity in detecting lower populations⁴⁹, although it has the disadvantage that not all mutations can be determined.
2. Sequencing using multiple hybridization chips (e.g., Affimetrix[®]), not available in Spain.
3. Automatic sequencing (using sequencer systems such as Perkin-Elmer or Visible Genetics), which are the most widely used and enable sequencing of all or almost all the RT and protease, both from plasma RNA and from proviral DNA.

The main problem arising from these studies is the difficulty in interpreting the results due to the large number of possible combinations of mutations, the appearance and difficulty in interpreting new mutations and possible interactions among these mutations (cross-resistance, phenotypic resensitization phenomena or hypersensitization phenomena). At present, computer programs are available to help interpret results (e.g., Retrogram[®]); however, these have not yet been validated. Therefore, in the consensus meeting, it was stressed that assistance from a specialist is required to interpret the results. There is also a need for fluid communication between the clinician and virologist in order to maximize the benefits of the results.

Other disadvantages of genotypic methods are:

1. The impossibility of evaluating mutations in viral subpopulations which are not very prevalent (several dilutions of the initial sample would be necessary, which poses important technical problems and would increase the cost of the technique).

2. The absence of mutations for a specific drug does not guarantee its effectiveness.
3. A VL above 1,000 copies/ml is necessary to ensure correct amplification and later sequencing of the genome.
4. The need for the patient to be in treatment at the time the test is carried out, since if this is not the case, some mutations can revert rapidly²³. Likewise, except in the case of significant toxicity problems, treatment should not be withdrawn while waiting for the results of the resistance test.

One aspect which caused great concern in the consensus meeting was the need to validate the sequencing technique (e. g. the ENVA-2/3 panel). In a study presented by Schurman et al at the ICAAC last year, sequencing by the ENVA-2 panel was evaluated in 33 laboratories. In the 100% wild-type strains, agreement was 100% for RT and 94% for PRT. For 100% mutant strains, agreement was 66% for RT and 71% for PRT and in the 1:1 mixed strain (5 mutations RT/5 mut PRT) it was only 37% for RT and 49% for PRT, which stresses the incorrect performance of the technique in several cases.

Total blood samples can be delivered to the reference laboratory at environmental temperature and in a period of 6-8 hours. The plasma extracted must later be kept at -70° C. If plasma is delivered, it should ideally be done at a minimum temperature of -20° C. The time necessary to carry out the technique and obtain the laboratory results should not be more than 2-3 weeks. Finally, we feel that it is important to insist on the need to confirm the existence of a virological failure before carrying out a resistance test.

Phenotypic methods

These techniques determine the degree of sensitivity to a drug in a cell culture, in terms of the concentration of the drug necessary to inhibit 50%, 90% or 95% viral growth, in terms of increased concentration necessary to inhibit the problem strain with respect to a wild-type reference strain without giving absolute values.

Real phenotype

This provides an objective evaluation of the degree of resistance of the virus to the drug. Analysis can be of the isolated patient virus in a co-culture with donor lympho-

cytes or in a laboratory lymphoid line. A technique which uses recombinant viruses, obtained after integrating fragments of RT or PRT in a defective viral genome of these regions of the pol gene⁵⁰ that will allow the variability of results with the cell line used to be avoided, has recently been developed. At present, two commercially available tests, Virco[®] and ViroLogic[®] use recombinant viruses to determine real phenotype.

An advantage of phenotypic studies is that they assess the sensitivity of the virus to each drug with greater accuracy. In view of this sensitivity, in the future, the possibility of overcoming resistance by increasing the plasma levels of the drug might be evaluated. The main disadvantages are an occasional lack of correlation between the level of sensitivity reflected by the study and the real efficacy of the drug in clinical practice and, above all, the complexity and laborious nature of the technique.

Therefore, for the moment, phenotypic resistance studies should be considered as not very feasible in the routine clinical context.

Virtual phenotype

In this approach, the sequence obtained from the patient is compared with those in a very large database in which all the real phenotypes are known for most combinations or individual genotypic alterations. Last year in San Diego, Larder et al from Virco[®] presented a database made up from a total of 7,000 samples⁵².

At the 4th *International Workshop on HIV Drug Resistance and Treatment Strategies* in Sitges, the results of obtaining a virtual phenotype from the interpretation, using a data analysis system (DAP; «*data analysis plan*»), of the mutations selected in the genotypic study, with a database based on 27,000 genotypes and 40,000 phenotypes, were presented. The results obtained⁵³ show very good concordance between the virtual phenotype obtained by interpreting the genotype using a DAP data analysis system and the real phenotype.

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