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Guidelines for laboratory monitoring of treatment of persistent virus infections

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1. Introduction

The last decade has witnessed an exponential rise in the availability of antiviral compounds licensed for clinical use. In the main, these drugs are active against persistent infections, which are associated with significant morbidity and mortality. Their availability has been a key driver to the corresponding development of sophisticated laboratory tests for guiding the use of these drugs. Since many of these drugs have associated toxic-

ities, as well as being expensive, it is important that their use is limited to those most likely to obtain clinical benefit, and that inappropriate use is avoided. The emergence of antiviral drug resistance is now well documented, and it is important to ensure that this is limited in clinical practice by appropriate drug use.

From a laboratory prospective, qualitative and quantitative molecular assays, and sophisticated phenotypic or gene sequence based analyses of drug susceptibility are routine. These techniques have transformed the role of clinical virologists, who are in the vanguard of molecular diagnoses within laboratory medicine. However, the financial stringencies now placed upon such laboratories means that full justification for introduction

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of expensive assays is required, as well as co-ordination between laboratories with regard to the validation and use of such assays.

For these reasons, the UK Public Health Laboratory Service initiated the development of consensus guidelines for the use of laboratory tests in monitoring antiviral therapy for persistent infections. What follows is the result of these discussions.

We have attempted to keep the format of these protocols similar between viruses whilst allowing flexibility to expand on areas pertinent to specific infections. An attempt has also been made to identify those areas of research activity which have yet to generate the basis for specific guidelines, as well as those issues which we feel should become 'standard of care' for laboratory support of these relevant patients.

2. Herpes simplex virus infection

2.1. Background to antiviral therapy

Aciclovir is the most frequently prescribed therapy. Aciclovir resistance is associated with high levels of virus replication almost exclusively in seriously immunocompromised hosts (Reusser, 1996; Gadreau et al., 1998). Foscarnet is the main therapy and is licensed for treatment of aciclovir-resistant HSV infection (Safrin et al., 1991; Balfour et al., 1994); cidofovir is a potential alternative (Lalezari et al., 1998; Safrin et al., 1999).

2.2. Recommended specialist techniques for monitoring therapy

1. PCR has become the detection system of choice for identifying herpes simplex infections in those anatomical sites usually free of this virus, e.g. CNS.
2. HSV drug resistance assays should be available for testing isolates obtained from immunocompromised patients failing therapy (see below).

2.3. Baseline screening

The overwhelming majority of HSV infections in immunocompromised patients represent reactivation of existing virus, rather than primary infection. Thus, donor/recipient virology is rarely of use in diagnosis. By contrast, recipient serostatus is often used to allocate HSV prophylaxis to such individuals following transplantation.

2.3.1. Diagnostic criteria for initiating therapy

- [i] appropriate sample taken for virus detection
- vesicle/ulcer: swab from base (or biopsy) in virus transport medium (VTM)
 - disseminated systemic disease/suspected neonatal herpes: EDTA/citrated blood for PCR (Diamond et al., 1999).
 - meningitis/encephalitis: CSF for PCR for HSV (Cinque et al., 1996; Tang et al., 1999) (1 ml; minimum 200 μ l)
 - intra-ocular disease: ocular fluid or vitreous biopsy for HSV PCR
 - pneumonitis: bronchoalveolar lavage (BAL)/induced sputum

It is recognised that in many cases therapy will be commenced on clinical grounds prior to result of virus detection, however, samples are important for confirmation of clinical diagnosis and HSV typing (Ashley, 1997); atypical lesions may be confused with Varicella-zoster virus (VZV) infection (or vice versa).

In suspected cases of HSV encephalitis or disseminated internal HSV disease therapy should not be delayed pending PCR or other results, and should be given intravenously.

- [ii] direct detection method for HSV
- direct immunofluorescence with monoclonal antibodies
 - nucleic acid amplification by PCR (Espy et al., 2000)
 - electron microscopy can be helpful when readily available
- N.B. direct detection methods should use reagents that will identify and type HSV.
- [iii] virus isolation in cell culture
- identification to include typing by type-specific monoclonal antibodies

- consider rapid systems such as early detection of antigens by fluorescence in centrifuge-enhanced cultures and the commercial enzyme-linked virus inducible system (ELVIS) (Patel et al., 1999).
- EIAs should not be used for sole initial diagnosis due to the inability to type and therefore, provide prognostic information.
- any sample tested by rapid systems should also be inoculated into cell culture in parallel.

[iv] in transplant recipients, prophylaxis with low dose oral aciclovir is commonly given, based on recipient serostatus pre-transplant. Nevertheless, HSV breakthrough can still occur, and detection of virus from symptomatic lesions should be undertaken, as above.

2.3.2. Monitoring treatment

Immunocompetent patients: monitoring not required.

Immunocompromised: no routine sampling is advocated, however regular clinical monitoring of response is advised in the most compromised. Early re-sampling (as in A[i]) from any site of persisting infection is recommended.

2.3.3. Monitoring of antiviral failure

- In those with HSV encephalitis who have not responded to or have relapsed after the standard course of IV aciclovir a repeat CSF for HSV DNA PCR and other agents is advised. HSV PCR-positive result suggests a need for further IV aciclovir, but quantitative results could be more useful. (This is not an issue of antiviral resistance but of control of replication in CNS).
- Persisting mucocutaneous ulcerative lesions should be swabbed (in VTM) to provide a sample for virus detection and for isolation.
- Frequent breakthrough lesions of genital herpes patients on long-term suppressive therapy should also be swabbed for culture. Dose increase is indicated before considering drug resistance as a cause (Fife et al., 1994).
- Patients with HSV neurological disease, and immunocompromised patients with symptomatic HSV infection should be treated with

at least 14 days of IV therapy. Failure of such therapy in the immunocompromised may be due to drug resistance and isolates should be submitted for susceptibility testing.

- Isolates of HSV from [b] and [c] should be submitted for antiviral susceptibility assay. Susceptibility to cidofovir and foscarnet should be determined.
- An algorithm for the precise role of susceptibility testing is given in Fig 1.

3. Cytomegalovirus

3.1. Background to antiviral therapy

The mainstay of anti-CMV therapy is ganciclovir (GCV), a nucleoside analogue with proven efficacy as treatment, pre-emptive therapy and prophylaxis against CMV infection and disease. Other drugs include foscarnet, a pyrophosphate analogue, and cidofovir, a nucleotide analogue, both of which are usually utilised as second-line therapies. GCV is now available as an oral as well

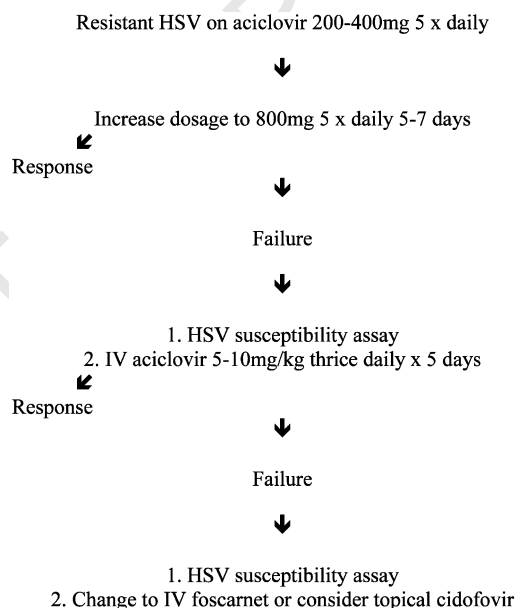


Fig. 1. Algorithm for the management of possible aciclovir resistant herpes simplex virus infections in immunocompromised patients.

as intravenous preparation, although there is a suggestion that the oral form is less potent (Emery and Griffiths, 2000). However, the pro-drug of GCV, valganciclovir, is in late stage clinical trials. Oral dosing with valganciclovir achieves comparable plasma levels to intravenous GCV and therefore, might be expected to be of similar efficacy (Pescovitz et al., 2000).

Aciclovir, and its prodrug valaciclovir have anti-CMV activity when used as prophylaxis (Prentice et al., 1994; Emery et al., 1999b). By contrast, there is no evidence that these drugs have utility in the treatment of active CMV disease.

3.2. Recommended specialist techniques for monitoring treatment

In view of the variety of techniques available for detecting CMV, it is important to appreciate the advantages and disadvantages of each method. Serological (antibody) techniques are unsuitable for diagnosis of infection in the immunocompromised host. It is essential to be able to provide a sensitive test with a rapid turn-round time (within 48 h of receipt of specimen). Such tests include antigenaemia, qualitative and quantitative PCR methods and hybridisation techniques. Within this group, molecular methods are generally more sensitive, and are more suitable for specimens transported from a distant site. By contrast, the antigenaemia assay is subject to reduced sensitivity if samples are not first processed within a few hours of being taken from the patient. The more time consuming conventional cell culture system is not appropriate for diagnosis or monitoring of infection. However, other reasons can be made for maintaining some level of cell culture, e.g. providing isolates for phenotypic analysis.

1. In light of the recent development of real-time PCR techniques, and the increasing use of quantitative PCR in published studies of CMV therapy, we recommend that quantitative PCR techniques be used to monitor therapy. Although there are theoretical reasons why viral load will differ between plasma samples and whole blood samples, these differences are

probably minor in the context of routine use; it is, however, important to be consistent with specimen type in the longitudinal follow-up of patients.

2. In view of the increasing evidence for the emergence of CMV resistance to GCV, and also foscarnet and cidofovir, we recommend the availability of drug resistance assays for studying patients with a rebound of CMV viral load while receiving therapy. Since molecular assays have overtaken virus isolation techniques in the monitoring of such patients, the optimal method for rapid detection of resistance is by sequencing of the relevant area of the virus UL97 gene, which will detect the majority of GCV resistance associated mutations. At this point in time, the UL54 (DNA polymerase) resistance associated mutations remain less clear, and the routine use of such an assay is not appropriate. Where virus isolates do exist, a standard plaque reduction assay for antiviral drugs susceptibility should be performed.

3.3. Baseline screening

A number of factors determine the appropriateness of antiviral therapy to treat or prevent CMV infection/disease. The following factors should be considered:

- (a) Recipient/donor antibody status. In the case of solid organ and stem cell transplants, the recipient and donor CMV status must be considered. In solid organ recipients R negative/D positive situations confer the highest risk for CMV disease. In stem cell transplants, a CMV positive recipient can face the highest risk of post-transplant disease.
- (b) Immunosuppression. Transplant recipients receiving extra immunosuppression (such as steroids or anti-T-cell antibodies) are at an increased risk of CMV infection, and this should guide clinical management (Cope et al., 1997). Likewise HIV-infected patients with CD4 cell count < 100 per μl are at risk of CMV disease (Bowen et al., 1997).
- (c) Timing of infection. Classically, CMV disease presents between one and 3 months post-

transplant or, in the case of HIV infection, at CD4 counts < 100 per ml. This should help in the formulation of a differential diagnosis. However, late CMV post-transplant CMV disease can occur following early CMV prophylaxis, and in AIDS patients, CMV can recur following immune reconstitution consequent on HAART. Care should, therefore, be taken in excluding CMV disease out-of-hand as a cause for presenting symptoms.

3.3.1. Diagnostic criteria for initiating therapy

With regard to CMV, laboratory test results must be interpreted in the light of the particular clinical scenario in order to move towards a virological diagnosis of clinical relevance. Thus, the underlying condition, time post-transplant, donor/recipient serostatus, concurrent immunosuppression and antiviral therapy must all be considered. The following main forms of CMV disease are differentiated:

- (a) CMV syndrome. This usually manifests as a non-specific pyrexial illness often associated with neutropenia. Detection of CMV viraemia in the absence of other causes of the symptoms is sufficient to establish this diagnosis.
- (b) CMV pneumonitis. This presents with respiratory symptoms, often supported by a characteristic X-ray appearance. Diagnosis usually depends on the positive identification of CMV in a bronchoalveolar lavage specimen (BAL), however, a negative result from a BAL in the presence of viraemia and a strongly suggestive clinical picture may be sufficient to initiate therapy.
- (c) CMV gastrointestinal disease. CMV may cause disease in any part of the GI tract and the clinical symptoms are commensurate with the area of gut infected. Usually, identification of the virus from a relevant biopsy sample is required. In the absence of a gut biopsy, CMV viraemia may point to this diagnosis in the absence of any other detectable cause.
- (d) CMV retinitis. This is a clinical diagnosis, almost always in AIDS patients, made on the basis of ophthalmoscopy, although CMV vi-

raemia may also be present at time of presentation.

3.3.1.1. Early identification of patients at risk of CMV disease. In addition to diagnosis of CMV disease, initiation of therapy may be based on the detection of CMV through surveillance of high-risk patients (e.g. transplant recipients). Thus, patients who are at increased risk of serious CMV disease can be identified e.g. CMV negative recipients of CMV positive organs, bone marrow transplant recipients, patients receiving potent immunosuppressive regimens, especially those given anti-T-cell monoclonal antibodies for treatment of rejection episodes (in the case of transplantation).

The use of routine surveillance programmes for CMV in the post-transplant period allows a time-updated approach to be implemented. For example, a PCR negative result followed by a PCR positive result with a high viral load/rate of change would initiate immediate therapeutic intervention (Emery et al., 2000a). However, a low viral load in this sample would indicate a patient with a low risk of future CMV disease and so treatment would not be recommended. If this same patient was PCR positive in their subsequent surveillance sample (the usual trigger for initiating pre-emptive GCV therapy, i.e. two consecutive PCR positive results), then two options are available: if the viral load is low and the rate of change low then the patient could remain untreated (low risk of disease). However, if viral load/rate of change has increased markedly (possible due to changes in immunosuppressive drugs being administered), then the patient would move into the high-risk category, and therapeutic intervention would be triggered (Gor et al., 1998; Hassan-Walker et al., 1999).

HIV-infected patients with CD4 count < 100 per μ l are also at higher risk of CMV disease, and PCR monitoring with pre-emptive therapy should be considered. In the context of HAART, the risk of disease is again reduced with a CD4 recovery, and many centres stop pre-emptive therapy at higher CD4 counts (Jouan et al., 2001). However, atypical CMV retinitis can still occur at higher CD4 counts (Johnson et al., 2001).

The variety of qualitative and quantitative techniques used within published data on prediction of CMV disease make the generation of hard and fast rules difficult. In addition, the viral load threshold predicting subsequent disease in high-risk stem cell transplant recipients, for instance, is likely to be lower than for more mildly immunosuppressed patients, such as renal transplant recipients. Nevertheless, it can be clearly stated that:

- (a) The risk of subsequent CMV disease is significantly increased with each \log_{10} rise in CMV viral load (Spector et al., 1998; Hassan-Walker et al., 1999).
- (b) Those with a significant increase in viral load between two successive samples are at higher risk of developing CMV disease than those in whom no increase is detected.

3.3.1.2. Prophylaxis of CMV infection. The decision on whether to implement prophylaxis from time of transplant, rather than a pre-emptive or treatment strategy will be based on a number of factors, such as the risk of the patient, perceived toxicities of longer-term therapy (GCV vs. aciclovir), the availability of rapid turn-round surveillance tests, and costs. If a prophylaxis protocol is implemented, it will be based on the recipient/donor serostatus pre-transplant, rather than molecular tests for detecting viraemia.

3.3.2. Monitoring treatment

3.3.2.1. Response to therapy—predicting rate of decline. Knowledge of the kinetics of replication (either based on theoretical models or derived from the up-rate of a patient's CMV load prior to therapy (see above) can be used to predict the likely duration of IV GCV (or valganciclovir when it becomes available) necessary to reduce the viral load substantially below detectable levels (Emery et al., 1999a). This approach will allow individual optimisation of therapy rather than using a standard therapy of 2 or 3 weeks IV GCV without considering the viral load at baseline or viral replication kinetics. The success of therapy can be gauged by measuring viral load at specific time points post therapy. This approach requires clinical validation.

3.3.2.2. Frequency of monitoring. Following initiation of therapy as treatment or pre-emptive therapy, monitoring is required to guide the duration of therapy. Although many different therapeutic approaches are possible, and there will be local preferences in this respect, monitoring at least weekly is recommended. Therapy should not be stopped whilst viraemia continues.

3.3.2.3. Identification of patients at risk of late CMV disease following prophylaxis. In patients receiving oral GCV prophylaxis there is a finite risk for the development of late CMV infection and disease following the period of prophylaxis (Gane et al., 1997; Bowen et al., 1998). The risk period for late CMV infection will depend on many factors including the type and quantity of immunosuppression being administered. However, the concepts outlined above are equally valid in this setting. Indeed, as these patients will probably be attending out-patient clinics on a relatively infrequent basis, the initiation of therapy based on a high viral load or the triggering of a more intensive surveillance approach to determine whether the patient is at high risk of CMV disease could be envisaged.

3.3.3. Monitoring of antiviral failure

3.3.3.1. Viral load as a surrogate marker of drug resistance. Virological failure of anti-CMV therapy could be defined as a lack of full suppression of CMV viraemia. This comprises two scenarios: firstly, a lack of decline or inadequate decline of CMV viral load and secondly, a rebound in viral load following initial suppression. It is important to note that drug resistance appears to emerge after prolonged time on therapy (> 3 months) (Limaye et al., 2000), and therefore, is most commonly observed in patients receiving CMV prophylaxis. Conversely, detectable viral load after 1–2 weeks of therapy will not generally be associated with drug resistance. It is also important to recognise that drug dosage is very important in determining the efficacy of treatment. For instance, patients receiving IV GCV for only 5 days per week will experience viral rebound during the 2 days of therapy, leading to a possible situation

whereby a persistent viraemia is observed over a long period of such therapy. It is unlikely that such a patient would have drug resistant virus. These factors should be considered when interpreting a positive CMV viraemia result in a patient receiving therapy.

3.3.3.2. Monitoring drug resistance—therapeutic options. The rationale for undertaking resistance assays in a patient failing CMV therapy is to provide a basis for changing therapy. Foscarnet or cidofovir is usually effective against GCV resistant viruses since the mutations occur in the UL97 kinase gene. However, the less frequently observed DNA polymerase mutations can confer cross-resistance to all these drugs. The decision to change therapy should also be based on the potential toxicities of these drugs, and it is worth bearing in mind that IV GCV continues to have *some* activity against the common UL97 mutants (M460I, M460V, L595F) (Emery, 1998).

Since very little data is available on the contribution of drug resistance to rebound CMV viraemia, or continuing viraemia, in the face of antiviral therapy, drug resistance testing in such patients is recommended.

4. Management of varicella-zoster infections

4.1. Background to antiviral therapy

VZV infections can be treated with nucleoside analogues such as aciclovir and famciclovir, which are activated by the virus-coded thymidine kinase (TK) and prevent DNA replication. These drugs are relatively non-toxic and are licensed for use in both primary and reactivation disease. Valaciclovir (prodrug of aciclovir) gives a higher ACV bioavailability, achieving similar blood levels to intravenous ACV administration. This can, therefore, be considered an alternative to ACV. Prolonged treatment in immunocompromised patients leads to the selection of drug resistant VZV strains, usually lacking the virus-specific TK. Foscarnet retains activity against these viruses.

4.2. Recommended specialist techniques for monitoring therapy

1. VZV PCR is the recommended method of choice for detecting virus in CSF, ocular fluids and blood, but is also appropriate for ulcer swabs (Beards et al., 1998; Hawrami and Breuer, 1999).
2. Although VZV drug resistance is very rare, facilities should exist for referring specimens for VZV phenotypic drug resistance assays (Shiraki et al., 1992; Boivin et al., 1994; Morfin et al., 1999; Sahli et al., 2000).

4.3. Baseline screening

VZV is a herpesvirus that typically causes chickenpox after primary infection following which the virus remains latent in the CNS (sensory ganglia) and may reactivate, typically producing the unilateral rash of shingles distributed in dermatomal patterns. Morbidity and mortality are low following primary infection or reactivation in otherwise healthy immunocompetent individuals. However, severe disease and significant mortality are associated with primary infection in immunocompromised individuals, and patients with profound immunocompromise (e.g. post BMT) have significant morbidity/mortality even after reactivation, which often disseminates widely.

More than 90% of people brought up in Europe have been infected by VZV by the time they reach adulthood and are immune to chickenpox.

An attenuated vaccine is available, but is currently used in many countries only to protect children at special risk of severe, life threatening chickenpox.

4.3.1. Diagnostic criteria for initiating therapy

- (i) Immunocompetent patients in the community
 - Clinical diagnosis of chickenpox and shingles usually sufficient.
 - Laboratory confirmation may be helpful if presentation atypical, including cases of

recurrent chickenpox (very rare) or shingles, and treatment contemplated or prophylaxis of contacts indicated.

- Laboratory confirmation should be by direct immunofluorescence (or similar staining method) or electron microscopy of vesicle scrapings.
- Molecular testing is more sensitive than fluorescence and may be useful, especially if the rash is crusted.
- Serology is of less value.
 - IgG seroconversion can usually be shown following chickenpox, but requires paired sera. IgM to VZV is generally present during acute chickenpox, but beware commercial kits of low sensitivity and samples taken on days 1 and 2 of the rash.
 - Serology is even less useful in assisting the diagnosis of shingles. Absent IgG antibody excludes the diagnosis. IgM antibody to VZV may occur upon reactivation.

(ii) Patients with severe atypical VZV infection without immunocompromise

- Clinical diagnosis is not reliable, therefore, clinical suspicion should be followed by laboratory investigation
- Molecular amplification methods are most likely to give the required sensitivity and specificity for
 - eye disease (e.g. acute retinal necrosis)—vitreous humour
 - CNS disease (e.g. encephalitis, meningitis, transverse myelitis)—CSF
 - Vasculitis—EDTA whole blood
 - visceral disseminated disease—EDTA whole blood (Wolff and Schunemann, 1999), skin lesion

(iii) Immunocompromised patients

- Appropriate samples: as above depending on symptoms
- Clinical presentation is often atypical especially in patients with profound immunocompromise.
- Skin rash may not be present at all.
- Treatment is almost always indicated, for both primary and for reactivation disease.

- Laboratory diagnosis is of great importance (particularly to distinguish VZV rashes from HSV rashes).
- Direct staining of skin lesion material (e.g. by immunofluorescence) is quick and reliable so long as adequate cellular material is present in the sample.
- There is no place for serology in the diagnosis of VZV disease in immunocompromised patients.
- Molecular amplification is the recommended technique in the diagnosis of eye disease, using vitreous humour.

4.3.2. Monitoring treatment

Immunocompetent patients: monitoring not required.

Immunocompromised: no routine sampling is advocated, however, regular clinical monitoring of response is advised in the most compromised, and early re-sampling from any site of persisting infection is recommended.

4.3.3. Monitoring of antiviral failure

Prolonged therapy may be required in immunocompromised individuals, and rash with successive lesion crops for weeks or months can occur with secondary infection and severe scarring.

- (a) The therapeutic index for ACV against VZV infection is small, especially for eye and CNS disease, and drug dose may need to be increased in non-response. Equally, a change from parenteral to oral administration may lead to apparent relapse (Meszner et al., 1990).
- (b) Persistent virus shedding after 14 days of therapy may be due to drug resistant virus (Linnemann et al., 1990; Fillet et al., 1998; Ida et al., 1999). After considering point [a] above, it may be appropriate to send an isolate for VZV drug susceptibility testing. It follows that clinical isolates from patients failing therapy should be inoculated into cell culture. Foscarnet is the second line therapy for cases of AZV-resistant VZV (Breton et al., 1998).

Prolonged disease despite therapy is usually encountered in immunocompromised individuals.

Children with immunocompromise (e.g. HIV infection, malignancy, congenital immunodeficiency syndromes, prolonged immunosuppressive therapy) suffer prolonged and severe chickenpox with significant mortality due to pneumonitis, encephalitis and disseminated infection, as well as unusual verrucous lesions (Lyll et al., 1994). Prolonged rash with successive lesion crops for weeks or months and severe scarring can occur with secondary infections.

Prolonged and severe reactivation disease is more commonly seen in immunocompromised adults. Mortality is lower than for primary infections but is significant in the profoundly immunocompromised (e.g. post BMT).

Eye disease and CNS disease present the additional challenge of drug delivery to the affected organ. Intra-ocular and intrathecal infusion of ACV have been used in extremely ill patients. Vasculitis of the cerebral arteries is a rare and devastating complication, which is very difficult to manage as it leads to repeated infarction.

5. HIV

5.1. Background to antiviral drugs

At the time of writing, licensed antiretroviral drugs fall into three classes, the nucleoside analogue reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Currently, fifteen drugs are licensed, however, this number is increasing both within the existing classes and also in new classes such as nucleotide reverse transcriptase inhibitors and fusion inhibitors. Development work is also being undertaken on chemokine receptor antagonists and integrase inhibitors. Finally, immunotherapeutic approaches, such as IL-2 are currently subject to clinical trials.

5.2. Recommended specialist techniques for monitoring therapy

5.2.1. Plasma HIV RNA quantitation

The lower limit of detection should reach at least down to 50 copies(c) per ml, since the

achievement of such a low level of viraemia following initiation of therapy is predictive of longer-term suppression of viral replication (Kempf et al., 1998; Delta Co-ordinating Committee and Virology Group, 1999). The upper limit of detection is also important, since the choice of initial antiretroviral regimen depends somewhat on the steady state viral load, i.e. viral loads > 100 000 c per ml may require a more potent regimen in order to achieve an undetectable viral load (Staszewski et al., 1999), although this is controversial (Staszewski et al., 1999; Cozzi-Lepri et al., 2000). Methods for detecting virus down to 2.5 c per ml are in development, however, the role of such assays in routine practice remain to be determined.

Laboratories should choose to use one of the commercial assays which have been subject to extensive clinical trials. At the time of writing, these methods include quantitative PCR, branched chain DNA and nucleic acid sequence based amplification (NASBA).

5.2.2. HIV resistance testing

Retrospective and prospective analyses demonstrate the clinical utility of HIV drug resistance tests in improving short-term virological outcome following a change of therapy. Seven prospective randomised studies have been reported to date, all differing in design (Durant et al., 1999; Baxter et al., 2000; Cohen et al., 2000; Meynard et al., 2000; De Luca et al., 2001; Tural et al., 2001).

Overall, a viral load reduction of approximately $0.5 \log_{10}$ HIV-1 RNA copies per ml at 12–24 weeks following a change in therapy is conferred by undertaking a resistance test in treatment-experienced patients. The precise benefit for any individual patient is dependent on other factors, including adherence, drug levels and experience of the physician interpreting the resistance test. At this stage, insufficient data exists to recommend either genotypic or phenotypic assays over the other. It is likely that each has strengths in different clinical scenarios, although this remains to be clarified. We recommend that resistance tests be undertaken at each therapeutic failure to inform the choice of a subsequent regimen.

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Table 1
Recommendations for use of HIV drug resistance tests

Patient group	Recommendations	Comment
Primary HIV infection (PHI)	Recommend	If therapy is indicated, use results to guide therapy, or modify therapy if result is obtained after initiation
Drug naïve, chronic infection	Consider	Resistance result may not reflect presence of transmitted resistant virus due to ongoing virus evolution. Thus, testing may underestimate presence of resistance. Also consider testing baseline specimen if initial response to therapy is poor
Drug experienced: second line or salvage	Recommend	Interpret in light of clinical and therapeutic history
Pregnancy	Recommend	Do not delay therapy in the case of late maternal presentation. Modify therapy when result obtained
Paediatrics	Recommend	If mother had detectable viral load while receiving therapy, testing is indicated. Also recommended at times of therapy failure
Post-exposure prophylaxis (PEP)	Recommend	Undertake if sample available from source patient, but use result to modify therapy rather than delay onset of PEP

Notes: (1) Ensure that laboratories undertaking viral load assays store aliquots of plasma samples appropriately so that retrospective resistance testing can be undertaken from relevant time points (e.g. before therapy, at times of previous drug failure).

In the light of data on the increasing prevalence of transmitted drug resistance (UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance, 2001), the testing of treatment-naïve patients should be considered (Table 1). Since virus will continue to evolve following such transmission, resistance tests taken several years following infection may not be sensitive enough to detect low levels of resistant virus. This should be taken into account in the interpretation of such tests. By contrast, testing in primary infection is recommended because the test result is more likely to reflect the transmitted virus.

A health economic assessment has demonstrated the cost effectiveness of genotypic resistance testing prior to initiating therapy and at times of drug therapy failure, especially in communities with transmission rates of more than 20% (Weinstein et al., 2001).

A number of factors will enhance the utility of the information provided by resistance testing:

1. In drug experienced patients, resistance tests should be undertaken on samples taken while the patient is receiving therapy, since wild type virus emerges rapidly after treatment is stopped (Devereux et al., 1999).
2. Success of the resistance test procedure is significantly enhanced with a viral load of over 1000 HIV-1 RNA c per ml.

3. The virus subtype (clade) may determine the significance of 'drug resistance associated' mutations and advice should be sought for results on non-subtype B viruses if in doubt (Cane et al., 2001; Grossman et al., 2001). Further, the efficacy of current PCR protocols may be compromised by non-B templates.
4. Resistance results should be fully discussed in the context of other clinical information, such as prior therapy, with those experienced in such interpretation.

Further information on the laboratory and clinical aspects of resistance testing can be obtained from recent reviews (EuroGuidelines Group for HIV Resistance, 2001; Vandamme et al., 2001).

5.3. Baseline assessment

The mainstay of laboratory monitoring of HIV disease is CD4 count and plasma HIV RNA determinations. In the untreated individual, these should be undertaken at approximately six-monthly intervals.

- (a) The viral load in this steady state is informative for two reasons. Firstly, a viral load of > 55 000 c per ml predicts a faster rate of decline of CD4+ cells. Secondly, a higher viral load has been demonstrated to be an

independent risk factor for subsequent disease progression and death (Mellors et al., 1997). These data originate from the pre-HAART era.

- (b) However, CD4 count remains the major determinant for initiating therapy (see below). Since patients with a rapidly falling CD4 count (e.g. falling $> 80 \times 10^6$ cells per l per year on repeated testing) have an increased risk of CD4 cell count decline to below 200×10^6 cells per l in the next 6 months, more frequent monitoring is recommended for such individuals.
- (c) The undertaking of resistance testing at first diagnosis is currently controversial. Two scenarios are possible:
 - (i) Primary infection, pre-therapy. As the prevalence of transmitted drug resistance appears to be increasing, testing in primary infection is recommended, and used to guide appropriate therapy.
 - (ii) Chronic infection, pre-therapy. In such cases, resistance testing should be considered. The aim of such testing is to detect transmitted resistant virus infection. However, the highly dynamic nature of HIV replication may lead to the apparent loss of resistance mutations some time after the initial transmission event and therefore, archived resistant virus within latently infected cells may not be detected in plasma virus. Therefore, the testing of drug-naïve individuals infected for some years may under-estimate the presence of resistance. An alternative strategy is to intensively monitor viral load changes in response to initial therapy, and be willing to test for resistance if a sub-optimal response is observed.

5.3.1. Initiating treatment

5.3.1.1. Primary infection. There is no definitive evidence of clinical benefit from treatment of primary HIV infection in the context of HAART. However, a number of small, primarily immunological, studies have identified some patients who benefit from intervention at this time. Thus, the

specific and strong CD4 helper cell HIV response which is evident shortly after primary infection may be maintained in people treated at this time, and there is some evidence that viral load suppression is maintained after cessation of therapy at a later time point in a subset of such individuals (Rosenberg et al., 2000). However, there is no evidence that any short-term benefit may be extended to longer-term protection.

Other important reasons for active ascertainment of primary infection include the identification of potential transmission of drug resistance (see above), partner notification and contact tracing and also prevention of further spread since such individuals are highly infectious at this time.

5.3.1.2. Chronic infection. Data from cohort studies suggest that patients who delay therapy until the CD4 count is below 200×10^6 per l cells have a poorer virological and immunological response, and the most recent guidelines suggest that this become a key determinant for starting therapy (BHIVA Guidelines 2001 Kaplan et al., 2001). By contrast, little long term benefit appears to be gained by initiating therapy at higher CD4 counts. However, it is accepted that decisions on initiating treatment for any one individual must taken into account a number of factors. For instance, a rapidly falling CD4 count above 200×10^6 per l may lead to initiating therapy at an earlier timepoint. The viral load is less important in this respect, and recent cohort studies have suggested that baseline viral load does not predict subsequent mortality independently of baseline CD4 count after starting therapy (Hogg et al., 2001; Sterling et al., 2001).

If the decision not to initiate therapy in such individuals is taken, then more frequent monitoring is required (two- to three-monthly) to identify rapid disease progression.

5.3.2. Monitoring of therapy

The efficacy of antiretroviral therapy is measured by reduction in viral load, and the objective is to reach < 50 c per ml within the first 16 weeks. The time taken to reach this low level is determined by the baseline viral load (Rizzarda et al., 2000) and the potency of the regimen. In some

cases, it may take up to 24 weeks to achieve undetectable viral load. The achievement of viral load < 50 c per ml after initiating therapy is predictive of longer-term suppression of replication (Raboud et al., 1999).

It is recommended that viral load is determined within a month of initiating therapy in order to assess adherence to therapy or the pre-existence of drug resistance. Thereafter, virological monitoring should be undertaken one to three-monthly.

Viral rebound may be represented by a very low detectable viral load (less than 1000 c per ml) or a higher viral load. In either case, a repeat should be undertaken within 2–4 weeks and the patient assessed to determine problems of drug interactions, poor adherence, co-existing infections and/or vaccinations which may all lead to transient increases in viral load. Some individuals experience low level 'viral blips' with subsequent re-suppression, the significance of which remains unclear (Havlir et al., 2000; Greub et al., 2001). High-level virological failure, with successive viral loads > 1000 c per ml is more indicative of treatment failure.

Falls in CD4 count and clinical disease progression are not usually seen in patients experiencing low-level viral load rebound. This is, however, the usual eventual outcome in patients whose viral load continues to rise towards pre-treatment levels.

5.3.3. Monitoring of antiviral failure

5.3.3.1. Resistance testing. It is recommended that antiretroviral resistance testing be undertaken at time of virological failure. In general, the success of generating resistance assay results requires a viral load of 1–2000 c per ml. Therefore, there may occasionally be a wish on the part of clinicians to alter therapy with a viral rebound to less than this value, without the benefit of resistance assay results. This situation has to be accepted, until such time as the sensitivity of resistance assays is increased.

Following a change of therapy based in part on resistance assay results, monitoring should be

undertaken as above. However, in general, the success of therapy in suppressing viral load to undetectable levels is reduced with each subsequent regimen.

5.3.3.2. The role of drug level monitoring. There is an increasing interest in determination of antiretroviral drug levels in plasma. At present, this is relevant for protease inhibitors and non-nucleoside reverse transcriptase inhibitors, since plasma levels of nucleoside analogues bear no relation to the intra-cellular triphosphate levels, comprising the active components. There are two major reasons for requesting drug level monitoring:

- (a) the identification of excessive drug levels as a cause of toxicity. In such cases, drug dosage could be reduced, and,
- (b) the identification of inadequate drug levels as a cause of failure of virus suppression.

Currently, these assays are only offered by highly specialist units at a national or international level, or commercial diagnostic companies. There remains little consensus on their routine use.

More detailed discussion of the clinical and virological management of HIV-infected patients can be found in the regularly updated British HIV Association (BHIVA) guidelines (BHIVA Guidelines, 2001)(<http://www.bhiva.org>).

6. Hepatitis B virus

6.1. Background to antiviral therapy

Licensed antiviral treatments include α -interferon and lamivudine (Lau et al., 2000). They are not licensed for combination therapy, although there is a rationale for the use of this combination, and limited data suggest that the combination is safe (Mutimer et al., 2000a). Further, drugs, such as adefovir (Tsiang et al., 1999) and tenofovir (nucleotide analogues), are in clinical trials. Nevertheless, these guidelines refer to monotherapy alone since this is the current state of clinical practice.

6.2. Recommended specialist techniques for monitoring therapy

1. Sensitive quantitative molecular assays for HBV DNA. These should provide a dynamic range from $>10^6$ copies per ml down to at least 10^3 copies per ml, in order to capture the antiviral efficacy of currently available drugs (Seifer et al., 1998), and provide an early indication of therapy failure (Puchhammer-Stockl et al., 2000). The poorly sensitive hybridisation methods should be replaced. Results should be expressed as DNA copies per ml, although there is a move to standardise such reporting to IU/ml.
2. HBV Resistance Testing. In view of the difficulty in propagation of HBV, detection of drug resistance mutations by sequencing (Ling et al., 1996; Cane et al., 1999a) or point mutation analysis (Ling et al., 1996; Cane et al., 1999b) is the method of choice.

6.3. Baseline screening

Antiviral treatment for HBV infection is indicated for selected patients with chronic HBV infection. Identification of suitable candidates requires:

- (a) an assessment of likely prognosis without treatment,
- (b) an assessment of likelihood of response to treatment, and
- (c) an assessment of safety and tolerability of treatment.

Thus, all relevant patients require the following baseline assessment:

HBsAg

HBeAg and anti-HBe

HBV DNA quantitation

The HBeAg-negative patient with low levels of HBV DNA does not require antiviral treatment. Antiviral treatment is indicated for selected patients with high serum HBV DNA regardless of HBeAg status. In this regard, and with the current state of knowledge, high DNA levels refer to HBV DNA $>10^5$ copies per ml. This approximates to the limit of detection of hybridisation assays which express results as pg per ml.

Liver biopsy is required for some but not all patients before commencement of antiviral therapy. Biopsy is required to establish the degree of hepatic fibrosis and to exclude other potential contributors to liver disease, for instance, alcohol.

6.3.1. Diagnostic criteria for initiating therapy

- (a) For patients with high serum HBV DNA, HBeAg positive and anti-HBe negative, either interferon or lamivudine can be used.
- (b) For patients with high serum HBV DNA, HBeAg negative and anti-HBe positive, lamivudine is the treatment of choice.

6.3.2. Monitoring treatment

- (a) For monotherapy of HBeAg positive patients, seroconversion, (i.e. HBeAg to anti-HBeAg positivity) is the aim. Spontaneous seroconversion rate is well recognised, however, it is strongly associated with pre-treatment AST/ALT. Six months of interferon or 12 months of lamivudine each enhances the spontaneous seroconversion rate by a factor of 4 over and above this baseline rate. Since lamivudine is a relatively safe drug, it may be preferable to interferon.
- (b) During interferon therapy (proposed duration 6 months) HBeAg and anti-HBe should be checked at 3 and 6 months, since complete seroconversion at either timepoint is an indication to stop treatment, with the potential for avoiding 3 months of drug therapy. If, at 6 months, HBeAg is negative, while anti-HBe persists as negative, then treatment can continue in an attempt to achieve complete seroconversion. If HBeAg persists positive at 6 months, interferon should be stopped. Subsequently (unless alternative treatment is given), HBeAg and anti-HBe should be checked annually. HBV DNA should only be measured before antiviral therapy and following HBeAg seroconversion, to confirm that seroconversion is indeed associated with inhibition of replication rather than the emergence of a pre-core mutant with continuing virus replication (expressing itself as HBeAg negative).

- (c) During lamivudine monotherapy of HBeAg positive patients, HBeAg and anti-HBe should be checked every 3 months. Complete seroconversion is an indication to stop treatment. Partial seroconversion is an indication to continue treatment. HBV DNA should be measured pre-treatment then at three-monthly intervals after the initial 6 months. In view of the increasing risk of resistance over time, it is recommended that such monitoring is continued during therapy. Lamivudine treatment should continue until, (i) a complete HBeAg seroconversion has been achieved; or (ii) a secondary rise in DNA is observed (see later).
- (d) For patients who are anti-HBe positive with high HBV DNA levels, treated with lamivudine, monitoring for HBeAg/anti-HBe during treatment is unnecessary. As above, it is recommended that serum HBV DNA be measured at three-monthly intervals after 6 months of treatment, for the duration of therapy

6.3.3. Monitoring of antiviral failure

- (a) Interferon treatment failure is defined as a failure to achieve seroconversion after 6 months of therapy. In these patients, lamivudine therapy should be considered.
- (b) A rise of HBV DNA while receiving lamivudine (defined as a rise of $> 0.5 \log_{10}$ from nadir) may be suggestive of emergence of HBV drug resistance. The incidence of emergence of resistance on lamivudine monotherapy is estimated at 15–20% per year (Lau et al., 2000). Serum HBV DNA should be tested for the presence of key mutations in the HBV polymerase gene associated with nucleoside analogue drug resistance. There is currently little consensus on subsequent management of such patients. One option is referral to a centre engaged in clinical trials of combination therapy for patients with existing drug resistant HBV.

There appears to be variable cross-resistance between the nucleoside and nucleotide drugs. For instance, famciclovir is generally ineffective

against lamivudine resistance viruses (Mutimer et al., 2000b), whereas, adefovir appears to retain activity against these variants (Perrillo et al., 2000).

7. Hepatitis C virus

7.1. Background to antiviral therapy

At present, licensed antiviral drugs for HCV comprise α -interferon and ribavirin. With interferon monotherapy, serum ALT levels fall to normal and HCV RNA disappears from serum in up to 40% of patients during short treatment courses, but relapse is common. By contrast, combination therapy with interferon α and ribavirin leads to a higher rate of sustained virological response in previously untreated patients (McHutchison et al., 1998) or patients in whom interferon monotherapy has failed (Davis et al., 1998). The recommended dose of interferon is 3 000 000 IU twice weekly, and of ribavirin is 1.2 g per day. A new form of α -interferon, pegylated interferon, has recently undergone clinical trials. This formulation incorporates the attachment of a polyethylene glycol moiety to interferon α -2a producing a compound which has sustained absorption, a lower rate of clearance and a longer half-life than unmodified interferon α . The improved pharmacokinetics of this preparation leads to a better clinical response over interferon α alone, and this is likely to be the preparation of choice in the future (Heathcote et al., 2000; Zeuzem et al., 2000). Even further benefit is observed with peginterferon and ribavirin, with up to 50% clearance rates in genotype 1 (Manns et al., 2001).

An inosine monophosphate dehydrogenase inhibitor is also in clinical trials. Intensive research on identifying inhibitors of viral helicase, protease and polymerase are ongoing.

7.2. Recommended specialist techniques for monitoring therapy

1. Qualitative HCV PCR. Since PCR positivity is

a pre-requisite for initiating therapy, and is increasingly used as a confirmatory test for HCV antibody testing, it is recommended that this assay is available.

2. Quantitative HCV measurement. The duration of therapy required is determined somewhat by the baseline HCV viral load. In this context, definitions of the cut-off between 'high and low' have been described (Pawlotsky et al., 2000). This paper collates data from the key clinical trials of interferon and ribavirin to establish this cut-off, which is described in International Units (the cut-off is calculated at 800 000 IU/ml, approximating to 2×10^6 genome copies per ml) (Pockros et al., 1999; Castro et al., 2001). This new method of reporting (IU) is likely to replace 'copies per ml' in the context of quantitative HCV assays, to allow comparison between assay types and the production of standards for use in these assays (Lee et al., 2000). It is likely that this cut-off value will be refined as further clinical trial data become available. Nevertheless, it is recommended that a sensitive quantitative assay, such as PCR or bDNA is available for patients being assessed for treatment. Some concern was previously expressed about the efficiency of HCV quantitation being dependent on the viral genotype. Improvements of some commercial assays have overcome some of these problems (Mellor et al., 1999).
3. HCV genotype. Published data suggest that HCV genotypes 2 and 3 require 6 months of therapy to achieve benefit whereas the response of genotype 1 to therapy is poorer and partially dependent on the baseline level of viraemia. Thus, genotype 1 with low viraemia requires 6 months of therapy, whereas genotype 1 with high levels of viraemia require 12 months of treatment. Thus, the assessment of HCV genotype is an essential component of assessment. A number of methodologies have been utilised to undertake HCV genotyping, including hybridisation based assays, restriction enzyme fragment analysis and sequencing (Prescott et al., 1997; Krekulova et al., 2001). Some systems merely distinguish between genotype 1 and non-genotype 1, which may be

adequate for treatment purposes, but which may also lead to the loss of potentially important epidemiological information about the spread of HCV (Mohsen et al., 2001) and this should be considered when deciding on appropriate assays.

7.3. Baseline screening

All HCV PCR-positive patients should be considered for antiviral treatment. A selection of suitable patients requires:

- (a) an assessment of the likely natural history of the patient
- (b) an assessment of likely efficacy of treatment, and
- (c) assessment of safety of each component of the antiviral therapy.

Therapy should be offered when the assessment of prognosis suggests that the patient would eventually suffer morbidity or die as a result of HCV-associated liver disease and when treatment can be safely administered with a reasonable chance of virus clearance. The cornerstones of such an assessment are as follows:

1. Liver histology. Concerning the need for treatment, an assessment of liver fibrosis may permit an estimation of the likelihood of eventual progression to cirrhosis for a given patient. Thus, for instance, absence of fibrosis despite long duration of infection in an older patient would argue against the need for antiviral therapy and its attendant complications. Significant fibrosis, including cirrhosis, identifies patients who are likely to develop clinically significant complications of liver disease that might be prevented or delayed by antiviral therapy. Concerning duration of therapy, it is recognised that significant fibrosis is an adverse determinant of response to treatment, and identifies patients who require 12 rather than 6 months of treatment.
2. Virology markers (viral load and genotype) also determine the likelihood of success of therapy and the duration of therapy required (see above) and should be considered together with the liver biopsy results.

7.3.1. Diagnostic criteria for initiating therapy

- (a) Qualitative HCV RNA detection by PCR should be performed and must be positive before treatment is considered.
- (b) HCV genotype should be assessed before treatment.
- (c) For genotype 1 viruses, the viral load should be determined before treatment, and defined as high or low as discussed above.
- (d) The aim of therapy is to achieve HCV PCR negativity and this should be undertaken at the conclusion of treatment.

7.3.2. Monitoring treatment

1. For patients receiving 6 months of combination treatment HCV RNA should be tested at this 6-month timepoint. If PCR is negative then this should be repeated 6 months later and then annually to confirm persisting negativity. The long-term risk of relapse after initial success is thought to be low but there is little data available at this moment in time, and therefore, we recommend life-long monitoring.
2. For patients who will receive 12 months of combination therapy, PCR should be performed after 6 months. RNA positivity despite 6 months treatment is an indication to stop treatment. PCR negativity is an indication to persist to completion. PCR should be repeated at the conclusion of treatment, then annually to confirm persisting negativity. It is important to recognise that most relapse on treatment occurs early (within 12 months).
3. When ribavirin is contra-indicated, monotherapy with interferon can be considered. Under this circumstance, proposed duration of therapy should be 12 months. PCR should be checked after 3 months of treatment, and persisting HCV RNA positivity at this stage is an indication to stop therapy. Most sustained responders achieve normal serum transaminases during the first 3 months of therapy.
4. A secondary rise of serum transaminases during ongoing interferon therapy (and those who are PCR negative at 3 months) is to undertake

further HCV PCR. PCR positivity is an indication to stop therapy.

5. In the absence of antiviral therapy, sustained HCV clearance is very seldom observed. Therefore, having confirmed serum HCV RNA positivity it is unnecessary to repeat HCV RNA PCR unless there has been intervening antiviral treatment. Thus, annual PCR should only be undertaken when PCR has become negative after apparent successful antiviral treatment.

7.3.3. Monitoring of antiviral failure

Patients who fail to achieve viral clearance during interferon monotherapy should be considered for combination therapy. Those who respond to monotherapy then relapse, have a high chance of successful re-treatment. Non-responders to monotherapy should be considered for combination therapy but sustained response rates are less good.

It may be useful to read the National Institute for Clinical Excellence document on treatment of Hepatitis C (National Institute for Clinical Excellence, 2002).

References

- Ashley RL. Laboratory techniques in the diagnosis of herpes simplex infection. *Genitourin Med* 1997;69:174–83.
- Balfour HH Jr, Benson C, Braun J, et al. Management of aciclovir-resistant herpes simplex and varicella-zoster virus infections. *J AIDS* 1994;7:254–60.
- Baxter JD, Mayers DL, Wentworth DN, et al. A randomised study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. CPCRA 046 Study team for the Terry Beinr Community Programs for Clinical Research on AIDS. *AIDS* 2000;14:F83–93.
- Beards G, Graham C, Pillay D. Investigation of vesicular rashes for HSV and VZV by PCR. *J Med Virol* 1998;54:155–7.
- BHIVA Writing Committee on behalf of the BHIVA Executive Committee. BHIVA British HIV Association (BHIVA) Guidelines for the treatment of HIV-infected adults with antiretroviral therapy. *HIV Med.* 2001; 2, 276–313.
- Boivin G, Edelman CK, Pedneault L, Talarico CL, Biron KK, Balfour HH Jr. Phenotypic and genotypic characterisation of aciclovir-resistant varicella-zoster viruses isolated from persons with AIDS. *J Infect Dis* 1994;170:68–75.

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- Bowen EF, Sabin CA, Wilson P, Griffiths PD, Davey GC, Johnson MA, et al. Cytomegalovirus (CMV) viraemia detected by polymerase chain reaction identifies a group of HIV-positive patients at high risk of CMV disease. *AIDS* 1997;11:889–93.
- Bowen EF, Emery VC, Wilson P, Johnson MA, Davey CC, Sabin CA, et al. CMV PCR viraemia in patients receiving ganciclovir maintenance therapy for retinitis: correlation with disease in other organs, progression of retinitis and appearance of resistance. *AIDS* 1998;12:605–11.
- Breton G, Fillet A-M, Katlama C, Bricaire F, Caumes E. Aciclovir-resistant herpes zoster in human immunodeficiency virus-infected patients: results of foscarnet therapy. *Clin Infect Dis* 1998;27:1525–7.
- Cane PA, Fillet A-M, Katlama C, Bricaire F, Caumes E, et al. Analysis of Hepatitis B virus quasispecies changes during emergence and reversion of lamivudine resistance in liver transplantation. *Antiviral Ther* 1999a;4:7–14.
- Cane P, Mutimer DJ, Cook P, Ratcliffe DM, Pillay D. Use of real time PCR and fluorescence to detect lamivudine resistance HBV mutations. *Antimicrob Agents Chemother* 1999b;43(7):1600–8.
- Cane PA, de Ruiter A, Rice P, et al. Resistance associated mutations in subtype C HIV-1 protease from treated and untreated patients in UK. *J Clin Microbiol* 2001;39(7):2652–4.
- Castro FJ, Sauleda S, Esteban JI, et al. Evaluation of hepatitis C virus RNA RT/PCR qualitative and quantitative second generation assays. *J Virol Methods* 2001;91(1):51–8.
- Cinque P, Cleator GM, Weber T, et al. The role of laboratory investigation in the diagnosis and management of patients with suspected herpes simplex encephalitis: a consensus report. *J Neurol Neurosurg Psychiatr* 1996;61:339–45.
- Cohen C, Kessler H, Hunt S, et al. Phenotypic resistance testing significantly improves response to therapy: final analysis of a randomised trial (VIRA3001). *Antiviral Ther* 2000;5(Suppl 3):67.
- Cope AV, Sabin C, Burroughs A, Rolles K, Griffiths PD, Emery VC. Interrelationships among quantity of human cytomegalovirus (HCMV) DNA in blood, donor-recipient serostatus, and administration of methylprednisolone as risk factors for HCMV disease following liver transplantation. *J Infect Dis* 1997;176:1484–90.
- Cozzi-Lepri A, Phillips AN, d'Arminio Monforte A, et al. When to start HAART in clinically HIV-infected patients? A collection of pieces of evidence from the ICONA study. In: 5th International Congress on Drug Therapy in HIV Infection. Glasgow, UK, 2000 [Abstract PL3.5].
- Davis GL, Esteban-Mur R, Rustgi V, et al. Interferon α -2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. *New Engl J Med* 1998;339(21):1493–9.
- De Luca A, Antinor A, Cingolani A, et al. A prospective, randomised study on the usefulness of genotypic resistance testing and the assessment of patient-reported adherence in unselected patients failing potent HIV therapy (AR-GENTA): final 6-month results. In: VIII Conference on Retroviruses and Opportunistic Infections. Chicago, IL, 2001 [Abstract 433].
- Delta Co-ordinating Committee and Virology Group. An evaluation of HIV RNA and CD4 cell count as surrogates for clinical outcome. *AIDS* 1999;13:565–73.
- Devereux HL, Youle M, Johnson MA, et al. Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. *AIDS* 1999;13:F123–7.
- Diamond C, Mohan K, Hobson A, et al. Viremia in neonatal herpes simplex virus infections. *Pediatr Infect Dis J* 1999;18:487–9.
- Durant J, Clevenbergh P, Halfon P, et al. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. *Lancet* 1999;353:2195–9.
- Emery VC. Cytomegalovirus drug resistance. *Antiviral Ther* 1998;4:239–42.
- Emery VC, Griffiths PD. Prediction of cytomegalovirus load and resistance patterns after antiviral chemotherapy. *Proc Natl Acad Sci* 2000;97:8039–44.
- Emery VC, Sabin C, Feinberg JE, Gryzwacz M, Knight S, Griffiths PD, et al. Quantitative effects of valaciclovir on the replication of cytomegalovirus in patients with advanced human immunodeficiency virus disease: baseline cytomegalovirus load dictates time to disease and survival. *J Infect Dis* 1999a;180:695–701.
- Emery VC, Cope AV, Bowen EF, Gor D, Griffiths PD, et al. The dynamics of human cytomegalovirus replication in vivo. *J Exp Med* 1999b;190:177–82.
- Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD, et al. Application of viral load kinetics to identify patients who develop cytomegalovirus disease after transplantation. *Lancet* 2000a;355:2032–6.
- Espy MJ, Mitchell PS, Thorvilson JN, et al. Diagnosis of herpes simplex virus infections in the clinical laboratory by LightCycler PCR. *J Clin Microbiol* 2000;38:796–9.
- EuroGuidelines Group for HIV Resistance. Clinical and laboratory guidelines for the use of HIV-1 drug resistance testing as part of treatment management: recommendations for the European setting. *AIDS* 2001;15:309–20.
- Fife KH, Crumpacker CS, Mertz GJ, et al. Recurrence and resistance patterns of herpes simplex virus following cessation of > 6 years of chronic suppression with aciclovir. *J Infect Dis* 1994;169:1338–41.
- Fillet AM, Dumont B, Caumes E, Visse B, Agut H, Bricaire F, Huraux JM, et al. Aciclovir-resistant varicella-zoster virus: phenotypic and genetic characterisation. *J Med Virol* 1998;55:250–4.
- Gadreau A, Hill E, Balfour HH Jr, et al. Phenotypic and genotypic characterisation of aciclovir-resistant herpes simplex viruses from immunocompromised patients. *J Infect Dis* 1998;178:297–303.
- Gane EJ, Saliba F, Valdecasas GJC, O'Grady J, et al. Randomised trial of efficacy and safety of oral ganciclovir in the prevention of cytomegalovirus disease in liver-transplant recipients. *Lancet* 1997;350:1729–33.
- Gor D, Sabin C, Prentice HG, Vyas N, Man S, Griffiths PD, et al. Longitudinal fluctuations between peak virus load,

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- donor/recipient serostatus, acute GvHD and CMV disease. *Bone Marrow Transplant* 1998;21:597–605.
- Greub G, Cozzi Lepri A, Ledergerber B, et al. Lower level HIV viral rebound and blips in patients receiving potent antiretroviral therapy. In VIII Conference on Retroviruses and Opportunistic Infections. Chicago, IL, 2001 [Abstract 522].
- Grossman Z, Alkan M, Bentwich Z, et al. Patterns of drug resistance: do we need to adjust treatment to clade? In VIII Conference on Retroviruses and Opportunistic Infections. Chicago, IL, 2001 [Abstract 456].
- Hassan-Walker AF, Kidd IM, Sabin C, Sweny P, Griffiths PD, Emery VC, et al. Quantity of human cytomegalovirus (CMV) DNAemia as a risk factor for CMV disease in renal allograft recipients: relationship with donor/recipient CMV serostatus, receipt of augmented methylprednisolone and anti-thymocyte globulin (ATG). *J Med Virol* 1999;58:182–7.
- Havliř D, Levitan D, Basset R, Gilbert P, Richman D, Wong J, et al. Prevalence and predictive value of intermittent viraemia in patients with viral suppression. *Antiviral Ther* 2000;5(suppl 3):89.
- Hawrami K, Breuer J. Development of a fluorogenic polymerase chain reaction assay (TaqMan) for the detection and quantitation of varicella-zoster virus. *J Virol Methods* 1999;79:33–40.
- Heathcote EJ, Shiffman ML, Cooksley WGE, et al. Peginterferon α -2a in patients with chronic Hepatitis C and cirrhosis. *New Engl J Med* 2000;343(23):1673–80.
- Hogg RS, Yip B, Wood E, et al. Diminished effectiveness of antiretroviral therapy among patients initiating therapy with CD4+ cell counts below 200 per mm³. In: VIII Conference on Retroviruses and Opportunistic Infections. Chicago, IL, 2001 [Abstract 342].
- Ida M, Kageyama S, Sato H, Kamiyama t, Yamamura J, Kurokawa M, Morohashi M, Shiraki K, et al. Emergence of resistance to aciclovir and penciclovir in varicella-zoster virus and genetic analysis of aciclovir-resistant variants. *Antiviral Res* 1999;40:155–66.
- Johnson SC, Benson CA, Johnson DW, Weinberg A, et al. Recurrences of cytomegalovirus retinitis in a human immunodeficiency virus-infected patient, despite potent antiretroviral therapy and apparent immune reconstitution. *Clin Infect Dis* 2001;32(5):815–9.
- Jouan M, Saves M, Tubiana R, et al. Discontinuation of maintenance therapy for cytomegalovirus retinitis in HIV-infected patients receiving highly active antiretroviral therapy. *AIDS* 2001;15(1):23–31.
- Kaplan J, Hanson D, Karch J, et al. Late initiation of antiretroviral therapy (at CD4+ lymphocyte count < 200 cells per ml) is associated with increased risk of death. In VII Conference on Retroviruses and Opportunistic Infections. Chicago, IL, 2001 [Abstract 520].
- Kempf DJ, Rode RA, Xu Y, et al. The duration of viral suppression during protease inhibitor therapy for HIV-1 infection is predicted by plasma HIV-1 RNA at the nadir. *AIDS* 1998;12:1619–24.
- Krekulova L, Rehak V, Wakil AE, Harris E, Riley LW, et al. Nested restriction site-specific PCR to detect and type hepatitis C virus (HCV): a rapid method to distinguish HCV subtype 1b from other genotypes. *J Clin Microbiol* 2001;39(5):1774–80.
- Lalezari JP, Holland GN, Kramer F, et al. Randomised, controlled study of the safety and efficacy of intravenous cidofovir for the treatment of relapsing cytomegalovirus retinitis in patients with AIDS. *J AIDS* 1998;17:339–44.
- Lau DT, Khokhar MF, Doo E, et al. Long-term therapy of chronic Hepatitis B with lamivudine. *Hepatology* 2000;32:828–34.
- Lee SC, Antony A, Lee N, et al. Improved version 2.0 qualitative and quantitative AMPLICOR reverse transcription-PCR tests for Hepatitis C virus RNA: calibration to international units, enhanced genotype reactivity, and performance characteristics. *J Clin Microbiol* 2000;38(11):4171–9.
- Limaye AP, Corey L, Koelle DM, Davis CL, Boeckh M, et al. Emergence of ganciclovir-resistant cytomegalovirus disease among recipients of solid-organ transplants. *Lancet* 2000;356(9230):645–9.
- Ling R, Mutimer D, Ahmed M, et al. Selection of mutations in the Hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. *Hepatology* 1996;24:711–3.
- Linnemann CC Jr, Biron KK, Hoppenjans WG, Solinger AM, et al. Emergence of aciclovir-resistant varicella-zoster virus in an AIDS patient on prolonged aciclovir therapy. *AIDS* 1990;4:577–9.
- Lyall EG, Ogilvie MM, Smith NM, Burns S, et al. Acyclovir-resistant varicella zoster and HIV infection. *Arch Dis Child* 1994;70:133–5.
- Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon α -2b plus ribavirin compared with interferon α -2b plus ribavirin for initial treatment of chronic Hepatitis C: a randomised trial. *Lancet* 2001;358:958–65.
- McHutchison JG, Gordon SC, Schiff ER, et al. Interferon α -2b alone or in combination with ribavirin as initial treatment for chronic Hepatitis C. Hepatitis Interventional Therapy Group. *New Engl J Med* 1998;339(21):1485–92.
- Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;126:946–54.
- Mellor J, Hawkins A, Simmonds P, et al. Genotype dependence of Hepatitis C virus load measurements in commercially available quantitative assays. *J Clin Microbiol* 1999;37(8):2525–32.
- Meszner Z, Gyarmati E, Nyerges G, Simon M, Koller M, et al. Early relapses of varicella-zoster virus infection in immunocompromised children treated with aciclovir. *Acta Paediatr Hung* 1990;30:263–70.
- Meynard JL, Vray M, Morand-Joubert L, et al. Impact of treatment guided by phenotypic or genotypic resistance tests on the response to antiretroviral therapy: a randomised trial (NARVALANRS 088). *Antiviral Ther* 2000;5(Suppl 3):67–8.

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D. Pillay et al. / Journal of Clinical Virology 000 (2002) 000–000

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- Mohsen AH, Group TH. The epidemiology of hepatitis C in a UK health regional population of 5.12 million. *Gut* 2001;48(5):707–13.
- Morfin F, Thouvenot D, De Turenne-Tessier M, Lina B, Aymard M, Ooka T. Phenotypic and genetic characterisation of thymidine kinase from clinical strains of varicella-zoster virus resistant to aciclovir. *Antimicrob Agents Chemother* 1999;43:2412–6.
- Mutimer D, Dowling D, Cane P, et al. Additive antiviral effects of lamivudine and α -interferon in chronic Hepatitis B infection. *Antiviral Ther* 2000a;5:273–7.
- Mutimer D, Dowling D, Cane P, et al. Selection of multi-resistant Hepatitis B virus during sequential nucleoside-analogue therapy. *J Infect Dis* 2000b;181(2):713–6.
- Institute for Clinical Excellence. Technology Appraisal Guidance No. 14. In: Guidance on the use of ribavirin and interferon α for Hepatitis C., HMSO, 2000.
- Patel N, Kauffmann L, Baniewicz G, et al. Confirmation of low-titre, herpes simplex virus-positive specimen results by the enzyme-linked virus-inducible system (ELVIS) using PCR and repeat testing. *J Clin Microbiol* 1999;37:3986–9.
- Pawlotsky JM, Bouvier-Alias M, Hezode C, et al. Standardisation of Hepatitis C virus RNA quantification. *Hepatology* 2000;32(3):654–9.
- Perrillo R, Schiff E, Yoshida E, et al. Adefovir dipivoxil for the treatment of lamivudine-resistant Hepatitis B mutants. *Hepatology* 2000;32(1):129–34.
- Pescovitz MD, Rabkin J, Merion RM, et al. Valganciclovir results in improved oral absorption of ganciclovir in liver transplant recipients. *Antimicrob Agents Chemother* 2000;44(10):2811–5.
- Pockros PJ, Bain VG, Hunter EB, et al. A comparison of reverse transcription-polymerase chain reaction and branched-chain DNA assays for Hepatitis C virus RNA in patients receiving interferon treatment. *J Virol Hep* 1999;6:145–50.
- Prentice HG, Gluckman E, Powles RL, et al. Impact of long-term aciclovir on cytomegalovirus infection and survival after allogeneic bone marrow transplantation. European Aciclovir for CMV Prophylaxis Study Group. *Lancet* 1994;343(8900):749–53.
- Prescott LE, Berger A, Pawlotsky JM, et al. Sequence analysis of Hepatitis C virus variants producing discrepant results with two different genotyping assays. *J Med Virol* 1997;53(3):237–44.
- Puchhammer-Stockl E, Mandl CW, Kletzmayer J, et al. Monitoring the virus load can predict the emergence of drug resistant Hepatitis B virus strains in renal transplantation patients during lamivudine therapy. *J Infect Dis* 2000;181(6):2063–6.
- Raboud JM, Rae S, Hogg RS, et al. Suppression of plasma virus load below the detection limit of a human immunodeficiency virus kit is associated with longer virologic response than suppression below the limit of quantitation. *J Infect Dis* 1999;180(4):1347–50.
- Reusser P. Herpesvirus resistance to antiviral drugs; a review of the mechanisms, clinical importance and therapeutic options. *J Hosp Infect* 1996;33:235–48.
- Rizzardi GP, De Boer RJ, Hoover S, et al. Predicting the duration of antiviral treatment needed to suppress plasma HIV-1 RNA. *J Clin Invest* 2000;105(6):777–82.
- Rosenberg ES, Altfield M, Poon SH, et al. Immune control of HIV-1 after early treatment of acute infection. *Nature* 2000;407(6803):523–6.
- Safrin S, Crumpacker C, Chatis P, et al. A controlled trial of foscarnet compared with vidarabine for aciclovir-resistant mucocutaneous herpes simplex in the acquired immunodeficiency syndrome. *New Engl J Med* 1991;325:551–5.
- Safrin S, Cherrington J, Jaffe HS, et al. Cidofovir: review of current and potential clinical uses. *Adv Exper Med Biol* 1999;458:111–20.
- Sahli R, Andrei G, Estrade C, Snoeck R, Meylan PR. A rapid phenotypic assay for detection of aciclovir-resistant varicella-zoster virus with mutations in the thymidine kinase open reading frame. *Antimicrob Agents Chemother* 2000;44:873–8.
- Seifer M, Hamatake RK, Colonna RJ, Standring DN. In vitro inhibition of hepadnavirus polymerases by the triphosphates of BMS-200475 and lobucavir. *Antimicrob Agents Chemother* 1998;42(12):3200–8.
- Shiraki K, Ochiai H, Namazue J, Okuno T, Ogino S, Hayashi K, Yamanishi K, Takahashi M. Comparison of antiviral assay methods using cell-free and cell-associated varicella-zoster virus. *Antiviral Res* 1992;19:209–14.
- Spector SA, Wong R, Hsia K, Pilcher M, Stempien MJ. Plasma cytomegalovirus (CMV) DNA load predicts CMV disease and survival in AIDS patients. *J Clin Invest* 1998;101:97–502.
- Staszewski S, Keiser P, Gathe J, et al. Comparison of antiviral response with abacavir/combivir to indinavir/combivir in therapy-naïve adults at 48 weeks (CNA 3005). In: ICAAC, San Francisco, 1999 [Abstract 505].
- Sterling TR, Chaisson RE, Bartlett JG, Moore RD. CD4 + lymphocyte level is better than HIV-1 plasma viral load in determining when to initiate HAART. In: VIII Conference on Retroviruses and Opportunistic Infections. Chicago, IL, 2001 [Abstract 519].
- Tang Y-W, Mitchell PS, Epsy MJ, et al. Minireview: molecular diagnosis of herpes simplex virus infections in the central nervous system. *J Clin Microbiol* 1999;37:2127–36.
- Tsiang M, Rooney JF, Toole JJ, Gibbs CS. Biphasic clearance kinetics of Hepatitis B virus from patient during adefovir dipivoxil therapy. *Hepatology* 1999;29(6):1863–9.
- Tural C, Ruiz L, Holtzer C, et al. Utility of HIV genotyping and clinical expert advice—the Havana trial. In: VIII Conference on Retroviruses and Opportunistic Infections. Chicago, IL, 2001 [Abstract 434].
- Analysis of prevalence of HIV-1 drug resistance in primary infections with the UK. *BMJ* 2001;322:1087–8.
- Vandamme A-M, Houyez F, Bânhegyi D, et al. Laboratory guidelines for the practical use of HIV drug resistance tests in patient follow-up. *Antiviral Ther* 2001;6:21–39.
- Weinstein MC, Goldie SJ, Losina E, et al. Use of genotypic resistance to guide HIV therapy: clinical impact and cost-effectiveness. *Ann Intern Med* 2001;134:440–50.

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D. Pillay et al. / Journal of Clinical Virology 000 (2002) 000–000

Wolff MH, Schunemann S. Aciclovir treatment prevents varicella-zoster virus replication in PBMC during viremia. *New Microbiol* 1999;22:309–14.

Zeuzem S, Feinman SV, Rasenack J, et al. Peginterferon α -2a in patients with chronic Hepatitis C. *New Engl J Med* 2000;343(23):1666–72.

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