BRITISH HIV ASSOCIATION GUIDELINES

British HIV Association guidelines for the routine investigation and monitoring of adult HIV-1-infected individuals 2011

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Table of Contents

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1. Levels of evidence [1]

- Ia: systematic review or meta-analysis of randomized controlled trials
- Ib: at least one randomized controlled trial
- IIa: at least one well-designed controlled study without randomization
- IIb: at least one well-designed quasi-experimental study, such as a cohort study
- III: well-designed nonexperimental descriptive studies, such as comparative studies, correlation studies, case–control studies and case series
- IV: expert committee reports, opinions and/or clinical experience of respected authorities

1.1 Reference

1 Schunemann H, Oxman A, Brozek J *et al.* for the GRADE Working Group. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. *BMJ* 2008; **336**: 1106–1110.

2. Introduction

In the mid-1990s, the clinical care of patients with HIV infection changed fundamentally as a result of the development and introduction of effective antiretroviral therapy (ART). This led to dramatic reductions in the numbers of patients under care with advanced immunodeficiency. Over subsequent years care has continued to evolve for a number of reasons, including:

- a switch in paradigm to manage HIV infection as a long-term, treatable condition;
- a decline in the proportion of patients with uncontrolled viral replication and/or viral drug resistance;
- an increase in the number of available antiretroviral drugs and changes in the use of diagnostics to support ART, including drug resistance, viral tropism and human leucocyte antigen (HLA) B*5701 testing and therapeutic drug monitoring;
- an increased recognition of non-AIDS-defining HIV morbidities, including cardiovascular, metabolic, renal and bone diseases, and certain non-AIDS-defining malignancies;
- a change in the epidemiology, with an increase in the proportion of women and Black African patients attending for care;
- an increase in the number of older individuals with HIV infection and the broadened challenge of managing HIV infection in patients with a range of comorbidities;
- increasing cost pressures and a need to demonstrate cost-effective management;
- an increased incidence of coinfection, including sexually transmitted hepatitis C;
- changing epidemiology of other sexually transmitted infections.

This is a new guideline. The aim is to present a consensus regarding the standard assessment and investigation at diagnosis of HIV infection and to describe the appropriate monitoring of HIV-positive individuals both on and off ART.

This guideline does not address the investigation and management of specific conditions related to HIV infection and ART, which are covered in other guidelines.

Systematic literature searches were performed within PubMed. In addition, limited use was made of peer-

reviewed research abstracts from the Conference on Retroviruses and Opportunistic Infections and also from The European Drug Resistance Workshop (see individual references in sections 10, 11, 14, 16, 17 and 18).

Within this guideline, assessment and monitoring of HIV-positive individuals have been categorized into the following areas:

- initial diagnosis;
- ART-naïve individuals;
- ART initiation;
- initial assessment following commencement of ART;
- routine monitoring on ART.

Summary tables of assessment/monitoring at each of these stages can be found in Section 4 of the Guideline. Following these tables, the tests are divided into different categories (e.g. immunology, virology and biochemistry) and then use of the relevant tests is discussed in relation to different stages of assessment as above.

3. Auditable targets

The following are suggested as targets that could be audited. The committee has selected topics that they consider to be important areas of practice/patient care. The percentages represent the targets for the minimum proportion of patients meeting each specific criterion. These targets have been reviewed by the British HIV Association (BHIVA) Audit and Standards Subcommittee.

- Patients with dated documentation of HIV-1 status (discriminated from HIV-2) (90%).
- Patients with a genotypic resistance test performed within 3 months of first diagnosis (or with a stored sample available for later testing) (90%).
- Adherence documented within the first 3 months of starting ART (90%) and at least annually thereafter (70%).
- All medication taken by patients on ART should be reviewed annually (100%).
- Patients with HIV viral load assessed within 6 weeks of commencing ART (80%).
- Patients on ART with HIV viral load measured within the last 6 months (80%).
- Patients with 10-year cardiovascular disease (CVD) risk calculated within 1 year of first presentation (70%), and within the last 3 years if taking ART (70%).
- Patients with a smoking history documented in the last 2 years (90%) and blood pressure (BP) recorded in the last year (90%).

4. Table summaries

4.1 Initial diagnosis

- History
  - Symptom enquiry (physical, psychological)
  - Sexual health
- Partner, status disclosed, safer sex
- Conception issues
  - Past and current medical [including TB and TB contacts]
  - Psychiatric history
  - Vaccination history
  - Children
  - Lifetime travel history, smoking, alcohol, drug-using history
  - Animal contact
  - GP contact/disclosure
- Physical examination
  - General, skin, oropharynx, lymph nodes, heart, lungs, abdominal (hepatosplenomegaly), anogenital, musculoskeletal and neurological system including cognitive function, dilated fundoscopy (if CD4 T-cell count <50 cells/μL)
  - Weight, height, BMI, blood pressure, waist circumference
- Investigations
  - CD4 T-cell count (absolute and percentage) (repeat to confirm baseline within 1–3 months)
  - HIV-1 plasma viral load (repeat to confirm baseline within 1–3 months)
  - HIV-1 drug-resistance test and HIV-1 subtype determination
  - Biochemistry: creatinine (eGFR), LFTs, bone profile
  - Haematology: FBC
  - Urinalysis: dipstick for blood, protein and glucose
  - Urine protein/creatinine ratio
  - Metabolic assessment: lipid profile [total cholesterol, HDL cholesterol, total/HDL cholesterol, triglycerides], glucose
  - Syphilis serology
  - Hepatitis A virus IgG (or total)
  - Hepatitis B virus surface antigen (HBsAg), anti-core total antibody (anti-HBc), anti-surface antibody (anti-HBs)
  - Hepatitis C virus antibody (followed by hepatitis C virus RNA testing if antibody positive and confirmation of antibody-positive status if RNA negative)
  - Toxoplasma IgG antibody (if CD4 T-cell count <200 cells/μL)
  - Measles IgG antibody

- ○ Varicella IgG antibody (unless patient has a reliable history of chickenpox or zoster; refer to [1] if IgG negative)
- ○ Rubella IgG antibody in women of child-bearing age (refer to [1] if IgG negative)
- ○ Stool for ova/cysts/parasites (if from, or spent >1 month in, tropics)
- ○ Schistosoma serology (if >1 month spent in sub-Saharan Africa)
- ○ Sexual health screen
- ○ Cervical cytology
- ■ Assessment
  - ○ CVD risk
  - ○ Fracture risk (if aged ≥ 50 years)

BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FBC, full blood count; HDL, high-density lipoprotein; IgG, immunoglobulin G; LFT, liver function test; TB, tuberculosis.

4.2 Assessment of ART-naïve individuals

2–4 visits per year (3–6-monthly). Generally, fewer visits (2–3) are recommended for those with higher CD4 T-cell counts (> 450 cells/μL) than for individuals with CD4 T-cell counts approaching or below the treatment guidelines initiation threshold (350 cells/μL) [2].

- ■ History and examination
  - ○ History/symptom enquiry (physical, psychological)
  - ○ Sexual history (6-monthly)
  - ○ Other medical problems/interventions, including STIs
  - ○ Vaccination history
  - ○ Examination weight, blood pressure, BMI (yearly)
  - ○ Targeted physical examination (guided by symptoms)
- ■ Investigations
  - ○ FBC (yearly)
  - ○ Creatinine, eGFR, LFTs, glucose, lipid profile (yearly)
  - ○ Urinalysis (yearly)
  - ○ Urine protein/creatinine ratio (yearly)
  - ○ CD4 T-cell count (>450 cells/μL, 4–6-monthly; <450 cells/μL, 3–4-monthly)
  - ○ HIV-1 plasma viral load (6-monthly)
  - ○ Hepatitis B assessment (tests will depend on previous status; 12-monthly anti-HBs in vaccine responders, 12-monthly serology (HBsAg, anti-HBc and anti-HBs) for susceptible patients including vaccine nonresponders)
  - ○ HCV antibody if previously negative [regular screening is recommended for all patients; IDUs and MSM should be screened yearly]

- ○ HCV RNA testing (12-monthly) in those who cleared a previous infection either spontaneously or after treatment and are at ongoing recognized risk of reinfection
- ○ Serological tests for syphilis (STS) [MSM, at each routine visit (3–6-monthly); others, 12-monthly]
- ○ Sexual health screen (offer 12-monthly, or more frequently if identified risks)
- ○ Cervical cytology (12-monthly)
- ■ Assessment
  - ○ CVD risk (12-monthly)
  - ○ Fracture risk in patients aged ≥50 years [fracture risk assessment tool (FRAX) score] (3-yearly)
  - ○ BMD [eg. Dual Energy X-ray Absorptiometry (DXA)] in all men aged ≥70 years and all women aged ≥65 years

Consider BMD assessment in men and women ≥50 years old if intermediate to high FRAX score and/or additional risk factors

Anti-HBs, anti-hepatitis B virus surface antibody; anti-HBc, anti-hepatitis B virus core total antibody; BMD, bone mineral density; BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FBC, full blood count; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus; IDUs, injecting drug users; LFT, liver function test; MSM, men who have sex with men; STIs, sexually transmitted infections.

4.3 ART initiation

Within 3 months prior to commencing ART.

- ■ History
  - ○ Adherence evaluation
  - ○ Medication history
  - ○ Over-the-counter, recreational drug use
- ■ Examination
  - ○ Weight, blood pressure, BMI
  - ○ Waist circumference
- ■ Investigations
  - ○ FBC
  - ○ Creatinine, eGFR, LFTs, glucose, lipid profile, bone profile
  - ○ Urinalysis
  - ○ Urine protein/creatinine ratio
  - ○ CD4 T-cell count
  - ○ HIV-1 plasma viral load
  - ○ HLA B*5701 testing (if considering use of abacavir)
  - ○ Tropism testing [if considering use of chemokine (C-C motif) receptor 5 (CCR5) antagonist – alternatively consider storing plasma sample for future testing]
  - ○ All patients should have their HBV and HCV status reviewed and an assessment undertaken of whether repeat testing is indicated or not

- Assessment
  - CVD risk
  - Fracture risk assessment in patients aged ≥50 years

ART, antiretroviral therapy; BMI, body mass index; CCR5, chemokine (C-C motif) receptor 5; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FBC, full blood count; HBV, hepatitis B virus; HCV, hepatitis C virus; HLA, human leucocyte antigen; LFT, liver function test.

4.4 Initial assessment following commencement of ART

Patients should be assessed within 2–4 weeks of commencing ART. Time of assessment within this range will be influenced by factors including the regimen selected (see text).

- History
  - Side effects
  - Adherence
- Investigations
  - FBC
  - Creatinine, eGFR, LFTs, glucose, bone profile
  - CD4 T-cell count (4 weeks)
  - HIV-1 plasma viral load (4 weeks)

ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; FBC, full blood count; LFT, liver function test.

4.5 Routine monitoring on ART

Individuals with good adherence and full virological suppression should be assessed 3–6-monthly. More frequent assessment will be required if patients are not fully suppressed or other problems present.

- History
  - Symptom enquiry (physical, psychological)
  - Sexual history (6-monthly)
  - Other medical problems/interventions, including STIs
  - Adherence
  - Vaccination history
- Examination
  - Weight, blood pressure, BMI (12-monthly)
  - Targeted physical examination (guided by symptoms)
- Investigations
  - FBC (12-monthly)
  - Creatinine, eGFR, LFTs, glucose, bone profile (3–6-monthly)

- Lipid profile (6–12-monthly)
- Urinalysis at each routine visit if taking tenofovir (3–6-monthly); otherwise, 12-monthly
- Urine protein/creatinine ratio (12-monthly)
- CD4 T-cell count (3–6-monthly; see text)
- HIV-1 plasma viral load (3–6-monthly)
- Cervical cytology (12-monthly)
- STS [MSM, at each routine visit (3–6-monthly); others, 12-monthly]
- Hepatitis B assessment [tests will depend on previous status; 12-monthly anti-HBs in vaccine responders; 12-monthly serology (HBsAg, anti-HBc and anti-HBs) for susceptible patients including vaccine nonresponders] [3]
- HCV antibody if previously negative (regular screening is recommended for all patients; IDUs and MSM should be screened yearly)
- HCV RNA testing (12-monthly) in those who cleared a previous infection either spontaneously or after treatment and are at ongoing recognized risk of reinfection
- Sexual health screen (offer 12-monthly, or more frequently if identified risks)
- Assessment
  - CVD risk (12-monthly)
  - Fracture risk in patients aged ≥50 years (FRAX score) (3-yearly)
  - BMD in all men aged ≥70 years and all women aged ≥65 years

Consider BMD assessment in men and women ≥50 years old if intermediate-to-high FRAX score and/or additional risk factors (see text).

Anti-HBs, anti-hepatitis B virus surface antibody; anti-HBc, anti-hepatitis B virus core total antibody; ART, antiretroviral therapy; BMD, bone mineral density; BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FBC, full blood count; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus; IDUs, injecting drug users; LFT, liver function test; MSM, men who have sex with men; STIs, sexually transmitted infections.

4.6 References

1 Geretti AM, Brook G, Cameron C *et al.* for the BHIVA Immunization Writing Committee. British HIV Association guidelines for immunization of HIV-infected adults 2008. *HIV Med* 2008; **9**: 795–848.

2 Gazzard BG, Anderson J, Babiker A *et al.* for the BHIVA Treatment Guidelines Writing Group. British HIV Association Guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. *HIV Med* 2008; **9**: 563–608.

3 Brook G, Main J, Nelson M *et al.* British HIV Association guidelines for the management of coinfection with HIV-1 and hepatitis B or C virus 2010. *HIV Med* 2010; **11**: 1–30.

5. Newly diagnosed and transferring HIV-positive individuals

5.1 Initial HIV-1 diagnosis

Individuals are diagnosed HIV positive in a variety of clinical settings and, with the adoption and implementation of the BHIVA/British Association of Sexual Health and HIV (BASHH)/British Infection Society (BIS) testing recommendations [1], the range of settings is set to increase. Increasing numbers of individuals are being identified as HIV positive by point-of-care testing (POCT). It is recommended that, for newly diagnosed HIV-positive individuals entering care, a confirmed laboratory diagnosis of HIV infection (including confirmation by an assay that discriminates between HIV-1 and HIV-2) should be available.

5.2 Tests to determine whether acquisition of HIV infection is recent

Both in-house avidity assays and a commercial assay [Aware BED EIA HIV-1 Incidence enzyme immunoassay (EIA) test; Calypte Life Sciences, Portland, Oregan, USA] are available to determine whether HIV-1 infection has occurred in the previous 3–6 months using antibody-positive serum or plasma [2,3]. These assays have been validated primarily for epidemiological purposes and for HIV-1 alone. Care must be taken in their clinical use and when communicating results to patients, who should be made aware of the uncertainty of the results (level of evidence IV). Misclassifications as recent infections can occur in patients receiving ART or in those who have very low CD4 T-cell counts or AIDS-defining conditions [4]. Furthermore, the BED assay is affected by subtype-related variability. If the test suggests a recent infection, a follow-up specimen taken 1–2 months later should be tested to demonstrate rising reactivity, thereby confirming the staging (IIa).

5.3 Individuals transferring care from a different HIV healthcare setting

Referring services should aim to provide clinical information to a new centre within 2 weeks of the request as such information may be critical in the ongoing care of an individual. All patients should have written confirmation of HIV status or have HIV antibody status confirmed by repeat serological testing. Documentation and/or repeat testing should include confirmatory discrimination of HIV-1 from HIV-2.

Information supplied by the referring centre should include:

- date of HIV diagnosis;
- date of most recent negative HIV antibody test;
- nadir CD4 T-cell count with date;
- current CD4 T-cell count and plasma HIV viral load with date;
- vaccination history;
- history of HIV-related illnesses;
- staging of HIV infection;
- baseline resistance test result with date;
- subsequent resistance test results with dates;
- ART history:
  - start date and reason for starting;
  - regimen details;
  - reason for starting and reason for stopping/switching;
  - ART:
- side effects;
- toxicity;
- tropism test results with dates;
- HLA B*5701 test results.

5.4 Communication with general practitioners and shared care

Many patients historically have sought all of their medical care through their HIV centre. However, increasingly GPs are responsible for many aspects of the medical care of HIV-positive individuals. Overall, a high proportion of patients consent to disclosure of HIV status to GPs and are satisfied with their involvement. The potential benefits of increased and enhanced primary care involvement include:

- improved access to care;
- enhanced management of comorbidities and risk reduction;
- experience in managing mental health problems;
- experience in managing an ageing population;
- appropriate management of unrelated medical problems.

For appropriate and safe care, it is important that regular, effective, two-way communication between the HIV centre and primary care is established. This is important in order to:

- establish a comprehensive list of prescribed medications;
- highlight and safely manage important drug interactions;
- recommend appropriate health screening (e.g. CVD risk assessment and cervical cytology), which takes account of

differences in protocol resulting from differences in HIV status or ART;

- recommend appropriate interventions taking account of HIV status;
- ensure care is comprehensive;
- reduce duplication of effort.

5.5 Recommendations

- Newly diagnosed HIV-positive individuals should have a confirmatory, positive HIV antibody result from the laboratory on file. Date of most recent HIV-negative antibody test where known should be recorded (IIb).
- Patients transferring their care should have written confirmation of laboratory-based serology including tests discriminating HIV-1 from HIV-2. Where these are not available, these tests should be repeated (III).
- Consideration of incident HIV antibody testing should be made in line with local surveillance arrangements when a recent infection is suspected (IIa).
- When an individual transfers their care to another centre, it is recommended that the referring centre supply a patient summary within 2 weeks of this being requested (IV).
- All patients should be encouraged to register with a GP and to consent to disclosure of HIV status to their GP (IV).
- With patient consent, regular summary letters (at least 12-monthly) should be sent from the HIV centre to the GP detailing current status, CD4 T-cell count, HIV viral load and medications. Important potential drug interactions should be highlighted (III).
- Where GPs are starting new medication for a patient on ART, potential drug interactions should be checked, either through the British National Formulary (BNF), with a pharmacist or through the Liverpool Drug Interaction website (www.hiv-druginteractions.org). Ideally a treatment plan or medication list should be given to the patient or alternatively a letter detailing treatment should be sent to the HIV centre (III).

5.6 References

1 British HIV Association, British Association of Sexual Health and HIV, British Infection Society. *UK National Guidelines for HIV Testing 2008*. London, BHIVA, 2008. Available at www.bhiva.org/HIVTesting2008.aspx (accessed June 2010).

2 Murphy G, Parry JV. Assays for the detection of recent infections with human immunodeficiency virus type 1. *Euro Surveill* 2008; **13**: pii=18966. Available at www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18966 (accessed June 2010).

3 Chawla A, Murphy G, Donnelly C *et al.* Human immunodeficiency virus (HIV) antibody avidity testing to identify recent infection in newly diagnosed HIV type 1 (HIV-1)-seropositive persons infected with diverse HIV-1 subtypes. *J Clin Microbiol* 2007; **45**: 415–420.

4 Marinda ET, Hargrove J, Preiser W *et al.* Significantly diminished long-term specificity of the BED capture enzyme immunoassay among patients with HIV-1 with very low CD4 counts and those on antiretroviral therapy. *J Acquir Immune Defic Syndr* 2010; **53**: 496–499.

6. Patient history

6.1 Initial HIV-1diagnosis

The patient should be reviewed by an HIV clinician within at most 2 weeks of diagnosis, or earlier if the patient is symptomatic or has other acute needs ([1]; section 6.1.3). Taking a complete history gives the opportunity to assess the patient's level of awareness about HIV infection and treatment, evaluate educational needs and determine the form that education and other support might take [2]. A full sexual history should also be taken at baseline [3].

6.2 Monitoring of ART-naïve patients

The following elements of the baseline history should, where relevant, be reviewed at least annually:

- medication and recreational drug use;
- exercise;
- contraception, plans for conception and cervical cytology;
- family history;
- social history including support network, employment, benefits and accommodation;
- sexual history (6-monthly);
- mood and cognitive function;
- patient expectations;
- vaccination history.

Depression and anxiety are common among people living with HIV disease (see 8. Identifying the need for psychological support). Suggested screening questions for depression include: 'During the last month, have you often been bothered by feeling down, depressed or hopeless?' or 'During the last month, have you often been bothered by having little interest or pleasure in doing things?' [4]. Guidelines on the management of depression and anxiety have been published by the National Institute for Health and Clinical Excellence (NICE) [4,5]. Clear pathways should be in place for further assessment when problems are identified and psychological support should be available.

Patients should be encouraged to keep a list of all their medications, herbal and nutritional supplements, vaccination history, and any allergic or adverse medication reactions. This list should be updated and reviewed at each clinic visit [6].

6.3 Pre-ART initiation assessment

Patients should have the opportunity to be involved in making decisions about their treatment. Clinicians should establish what level of involvement the patient would like and tailor their consultation style appropriately. Clinicians should also consider how to make information accessible and understandable to patients (e.g. with pictures, symbols, large print and different languages) [6]. If there is a question about the patient's capacity to make an informed decision, this should be assessed using the principles in the Mental Capacity Act 2005 [7].

Patients' beliefs about their personal need for medicines and their concerns about treatment affect how and whether they take them [6]. The following themes have been associated with adherence to ART [8]. Does the patient:

- believe their future health will depend on taking ART?
- have concerns about having to take ART?
- have concerns about the adverse effects of ART?
- have concerns that ART will disrupt their life?
- have concerns about becoming dependent on ART?
- have concerns that ART will cause embarrassment?
- have all the information they need to allow them to make a decision?

Open questions should be used to explore patients' ideas about HIV disease and its treatment: these are more likely to uncover their concerns. Nonverbal clues may indicate undisclosed concerns; these should be explored further [6]. A tool to assess readiness to commence ART has been proposed by the European AIDS Clinical Society (EACS) [9].

When there is agreement to start ART, consider the following.

- Review the baseline assessment, including:
  - current prescribed and nonprescribed drug use;*
  - allergies;
  - last menstrual period and plans for conception;
  - social support network, current occupation and hours, responsibilities as a carer, and accommodation;
  - travel plans in next 3 months;
  - system review relevant to medication, e.g. visual impairment, swallowing difficulties, diarrhoea, mood, cognitive function, memory and dexterity.
- Daily routine (waking, bed and meal times) including days off [6].

- Dosing regimen, food and storage requirements, forgiveness and time zone adjustments.
- Goals:
  - What are the patient's goals from treatment?
  - How will the patient assess its effectiveness [6]?

*Drug–drug interactions between antiretrovirals and other medications (including over-the-counter drugs, recreational drugs and herbal remedies) are frequent and can affect the toxicity and efficacy of either treatment. Common examples of interacting drugs include statins and acid-reducing agents. When prescribing a new medication that may interact with antiretrovirals or a new antiretroviral combination, check on line at www.hiv-druginteractions.org, or for advice contact the nearest HIV clinic pharmacy, when possible.

6.4 Monitoring individuals established on ART

The issues recommended for annual review with treatment-naïve individuals should also be covered with patients on ART. The following topics should be reviewed at each prescription:

- full medication history and recreational drug use;
- understanding of dosing instructions;
- adherence [6,10];
- contraception and plans for conception;
- mood;
- adverse effects using open questions (e.g. 'Tell me about problems you have had with diarrhoea' rather than 'Any diarrhoea?', or 'What do you find most difficult about taking your medications?' [4];
- patients' concerns about medication [6].

The beliefs about medication of both patients and clinicians change over time. Therefore it is important to review the rationale for the current medication at intervals agreed with the patient [6].

6.5 Assessment of adherence

NICE have concluded that self-report is the most simple and inexpensive method of measuring adherence; no specific self-report tool was recommended. It is most likely that those reporting nonadherence are correct. However, self-report overestimates adherence and is subject to recall bias, social desirability bias and errors in self-observation. Both the wording of the question and the skills of the interviewer are important [6]. The assessment should include each element of the combination, dose timing and frequency and (where relevant) food and storage requirements [11]. The following have been shown to help patients to report nonadherence.

- Explaining why you are asking the question [6].
- Asking questions without implying blame [6].
- Assuring the patient there is no right or wrong answer [11].
- Loading the question (e.g. 'How many doses have you missed . . .?') [11].
- Using open-ended questions (e.g. 'Tell me about the last time you missed your medication.') [11].
- Using words familiar to the patient [11].
- Using cues to prompt recall (e.g. 'During the last week did you sleep away from home? Did this prevent you from taking all your pills?') [11].
- Using a specific time period such as 'in the last week' [6].

There is no evidence pointing to an optimal time period to assist recall. Recall over 1–3 days may reduce forgetfulness but may only detect very low levels of adherence and may not reflect behaviour at times when routine is disrupted, such as weekends. Consider asking for more precise information about the most recent time (e.g. number of pills missed during the last 2 days) and less specific information about the more distant past (e.g. whether or not pills have been missed during the last 30 days) [11]. Pharmacy refill records may also be used to highlight possible nonadherence [6].

6.5.1 Sample adherence questions

Simoni *et al.* [12] reviewed studies employing adherence self-report for antiretroviral drugs and recommended the following validated measures preceded by a permissive statement.

'Many patients find it difficult to take all their HIV medications exactly as prescribed.'

- Put a mark on the line below at the point that shows your best guess about how much of your prescribed HIV medication you have taken in the last month. We would be surprised if this was 100% for most people, e.g. 0% means you have taken no medication; 50% means you have taken half your medication; 100% means you have taken every single dose of medication. (Line marked at 10% intervals from 0% to 100%.) [13]
- Do you ever forget to take your HIV medication? (Yes/No) [14]
- Did you not take any of your HIV medications over the past weekend? (Yes/No) [14]

Other validated questions include asking 'How many pills did you skip taking yesterday?', '. . . the day before yesterday (2 days ago)?', '. . . 3 days ago?' and '. . . 4 days ago?' [15,16] or asking patients whether they took 'all,' 'most,' 'about half,' 'very few,' or 'none' of their pills during the preceding 7 days [17]. A range of self-report questionnaires have been validated in the HIV field [13–15,17–20]; however, there is no consensus about the optimal tool [12].

6.6 Recommendations

- The beliefs of patients about their need for ART, and specific concerns they may have about it, should be explored before initiating treatment (III).
- Adherence to ART should be documented regularly (Ib).
- It is good practice to periodically review, with patients, their current ART regimen, and its acceptability and tolerability (and alternatives if appropriate) (IV).

6.7 References

1 British HIV Association. Standards for HIV Clinical Care. 2007. Available at www.bhiva.org/HIVClinicalCare.aspx (accessed June 2010).

2 Aberg JA, Gallant JE, Anderson J *et al.* Primary care guidelines for the management of persons infected with human immunodeficiency virus: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis* 2004; **39**: 609–629.

3 Fakoya A, Lamba H, Mackie N *et al.* British HIV Association, BASHH and FSRH guidelines for the management of the sexual and reproductive health of people living with HIV infection 2008. *HIV Med* 2008; **9**: 681–720.

4 National Institute for Health and Clinical Excellence. Depression in adults (update). Depression: the treatment and management of depression in adults. National Clinical Practice Guideline 90. 2009. Available at http://guidance.nice.org.uk/CG90 (accessed June 2010).

5 National Institute for Health and Clinical Excellence. Anxiety: management of anxiety (panic disorder, with or without agoraphobia, and generalised anxiety disorder) in adults in primary, secondary and community care. National Clinical Practice Guideline Number 22. 2004. Available at http://guidance.nice.org.uk/CG22 (accessed June 2010).

6 National Collaborating Centre for Primary Care. Medicines concordance and adherence: involving adults and carers in decisions about prescribed medicines. National Clinical Practice Guideline Number 76. 2009. Available at http://guidance.nice.org.uk/CG76 (accessed June 2010).

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7. Examination

General physical examination should be performed at baseline, and targeted physical examinations guided by symptoms or biomarker abnormalities at follow-up visits. Examination should be focused on eliciting HIV-associated infectious and noninfectious complications, with particular focus on the skin, mucous membranes, lymph nodes, heart, lungs, abdomen, pelvis and nervous system. Dilated fundoscopy is recommended for early detection of cytomegalovirus (CMV) retinitis in patients with CD4 T-cell counts below 50 cells/µL.

As a result of the increased risk of cardiovascular morbidity and fat redistribution among HIV-infected patients, baseline assessment of weight, blood pressure (BP), waist* circumference and body mass index (BMI) is indicated. Repeat assessment (except for BMI) immediately prior to ART commencement should be considered.

Additionally, weight and BP should be measured annually. BMI should be calculated.

7.1 Recommendations

- Complete physical examination at baseline (IV).
- Targeted physical examination guided by symptoms or biomarker abnormalities for patients in regular follow-up (IV).
- Annual assessment of weight, blood pressure and BMI (IIa).

8. Identifying the need for psychological support

Mental health problems such as depression, anxiety, post-traumatic stress disorder and suicidal behaviours are associated with HIV infection [1–3]. There are also well-established cognitive effects of HIV [4]. In addition, studies clearly demonstrate that people with some diagnosed mental health conditions have an elevated prevalence of HIV infection [5].

Over the course of HIV disease there are many traumas and mental health challenges, and high rates of referral and treatment [6]. Particular challenges are seen to cluster around hurdles of disclosure, adherence, treatment burden and relationship/sexual health issues. Commencement of

*Waist circumference measurements should be taken without clothing, or over light clothing only, at the end of normal expiration, with the arms relaxed at the sides, under the midline of the participant's armpit, at the midpoint between the lower part of the last rib and the top of the hip. Patients should wrap the measuring tape around themselves, without applying tension to the tape, and the tape should be in a horizontal position (source: www.who.int/chp/steps/manual/en/index3.html).

life-long ART can trigger mental health crises. Ageing in the presence of HIV and HIV treatments, together with long-term exposure to the virus, may raise issues around cognitive functioning.

Taking life-long treatment with a high adherence demand may also have emotional effects. Some compounds exacerbate mental health symptoms [7], while others may be associated with side effects (e.g. lipodystrophy) with mental health sequelae [8]. Poor mental health or heavy mental health burden is associated with reduced adherence, which in turn is associated with poorer outcome [6–9].

Therefore, incorporating assessment of mental health into the routine follow-up of patients at all stages is important but is particularly critical at first presentation in order to establish a baseline. It is also important prior to commencement of ART (see 6.2 Monitoring of ART-naïve patients) and in those individuals with suboptimal adherence and/or virological failure, or signs of mental health symptoms (such as depressed mood, heightened anxiety, relationship concerns, memory or functioning concerns).

Cognitive symptoms have been noted from the early days of the epidemic, ranging from mild cognitive symptoms to more severe memory loss, executive functioning difficulties and cognitive impairment [10]. The advent of treatment has clearly reduced the prevalence of severe cognitive disorders [11,12], while milder forms have continued in a proportion of patients. There is currently much debate about the prevalence, risk factors for, and prognosis of, mild-to-moderate cognitive impairment in persons taking effective ART (full virological suppression). Joint psychological support standards are currently being consulted on and it is anticipated that these will make recommendations about screening [13], although there is not yet consensus about easy-to-administer and effective measurements. The finalized standards will be available late in 2011.

Recommendations

1. Standardized monitoring of psychological wellbeing at baseline, at annual follow-up and at change points (such as treatment initiation and treatment switching) (III).
2. Having good referral mechanisms to psychological services in place and clear criteria for referral (see BHIVA guidelines on psychological support [13]) (IV).
3. Inclusion of psychological consideration in relation to fertility, drug use, treatment change, side effects, adherence, relationships and doctor–patient interaction (IV).

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9. Assessment of immune status

9.1 CD4 T-cell counts

There is no high-grade evidence for what is the optimal frequency at which to measure CD4 T cells in well-

Table 9.1 Recommendations for the frequency of CD4 T-cell count requests in patients with HIV-1 infection

CD4 T-cell counts	CD4 T-cell count interval
In ART-naïve patients	
• >450 cells/μL (i.e. 100 cells/μL above standard treatment threshold)	Every 4–6 months
• <450 cells/μL	Every 3–4 months
In patients taking ART	
• Following initiation of ART	Counts at 1 month, 3 months and then every 3–4 months
• >200 cells/μL and viral load <50 copies/mL for 1 year	Every 4–6 months

resourced health environments. We have considered three different scenarios: initial HIV diagnosis; monitoring ART-naïve patients; and CD4 T-cell counts in patients on ART. Recommendations for how often we should be measuring CD4 T-cell counts are mainly based on expert opinion [1–3]. For ART-naïve patients, we used data from a cost-effectiveness analysis using an HIV simulation model incorporating CD4 T-cell count and plasma HIV-1 RNA load as predictors of disease progression [4]. In patients on suppressive ART regimens we have combined data from a recent EUROSIDA publication [5] and expert opinion [3] into what we hope is a simple algorithm for frequency of CD4 T-cell monitoring (Table 9.1).

9.1.1 Initial HIV-1 diagnosis

Helbert and Breuer recommended three CD4 T-cell counts within the first few weeks of diagnosis [1]. This is not standard practice in the UK but it seems prudent to have two baseline counts. Repeat CD4 T-cell counts could be performed at the initial and second HIV follow-up visits, which for most clinics would vary from 1 to 3 months following initial visit depending on how well the patient is; in patients with low CD4 T-cell numbers (< 200 cells/μL) a confirmatory result should be obtained promptly.

9.1.2 ART-naïve patients

It would be reasonable to offer testing every 4–6 months for individuals with CD4 T-cell counts more than 100 cells/μL above the treatment threshold, which would be 450 cells/μL currently, and then to increase the frequency of monitoring to every 3 months in patients where the CD4 T-cell number drops below this figure [1,2]. Data from Kimmel *et al.* suggest that it is more cost-effective in ART-naïve patients to set a CD4 threshold to help guide frequency of testing rather than apply a fixed interval for CD4 T-cell analysis to all ART-naïve individuals [4].

9.1.3 Patients on ART

CD4 T-cell counts could be performed at week 4, week 12 and then every 3 months after starting antiretroviral drugs. There is debate about whether it is necessary to check the CD4 T-cell count 1 month after starting ART. Usually CD4 T-cell counts are requested in conjunction with viral load, so, pragmatically, it may be easier to continue to do this rather than make a single exception. This is obviously a matter for debate. The 4-week count could be left to the discretion of the local service.

Extending the testing interval from 3 to 6 months in patients on successful ART (indicated by a viral load below 50 copies/mL and an increase in CD4 T-cell count of 100 cells/μL from baseline) does not lead to a significant increase in treatment failure [5]. The International AIDS Society panel suggests that the CD4 T-cell count can be measured every 6 months in patients on ART who have values above 350 cells/μL [3]. This Writing Group suggests that the frequency of CD4 T-cell measurements could be reduced to every 6 months in patients who have maintained a viral load below 50 copies/mL for more than 1 year and have a CD4 T-cell count above 200 cells/μL.

9.2 CD4 T-cell percentage

The CD4 T-cell percentage is routinely utilized in paediatric practice to monitor disease progression in children aged less than 5 years [6]; however, less emphasis is placed on this marker for monitoring HIV infection in adults. One study showed that the CD4 T-cell percentage may be an independent predictor of disease progression in patients with CD4 T-cell counts above 350 cells/μL [7]. However, the number of study events was small, the threshold for definition of low CD4 T-cell percentage differed from that usually adopted in other studies of this parameter, and finally the viral load, which influences disease progression, was not assessed [8]. At this time, the Writing Group does not recommend the use of CD4 T-cell percentage to monitor disease progression in adult patients with HIV-1 infection. There are exceptions to this rule: individuals with splenectomy and patients with Human T-lymphotropic virus Type 1 (HTLV-1) coinfection [9,10] may have a CD4 lymphocytosis and, in this instance, CD4 T-cell counts may give a misleading impression as to the true extent of immune deficiency. Patients with these conditions should be monitored using CD4 T-cell percentage and ART should be offered to individuals with values of 21% or lower. A significant discrepancy between CD4 T-cell count and percentage should alert clinicians to potentially reversible causes of immune deficiency such as steroid and/or cytotoxic therapies, and intercurrent sepsis.

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10. HIV viral load

10.1 Initial diagnosis/ART-naïve

Primary HIV infection is associated with a high plasma viral load. This declines about 4–6 months after infection to a nearly steady level, with a small but appreciable increase observed over time during the asymptomatic phase of the infection [1,2]. The viral load increases sharply again in advanced disease, coinciding with the onset of AIDS. It has been long established that the set-point viral load is a strong predictor of the rate of disease progression [3–5].

While viral load results are generally highly reproducible, at least two values are required for patients with chronic infection to establish a firm set point [6]. Subsequent measurements can be taken every 6 months in asymptomatic stable patients not receiving ART. A further measurement should be taken prior to initiation of therapy if a recent value is not available. While the CD4 T-cell count is the main driver for initiation of ART, the viral load provides additional guiding information, especially in patients with a relatively high CD4 T-cell count. In addition, the viral load may influence the choice of antiretroviral agents [7].

10.2 Post ART initiation

The goal of ART is restoration of CD4 T-cell count and suppression of viral load below the quantification limit of commercial viral load assays, until recently 50 copies/mL. Newly introduced viral load assays, typically based on real-time polymerase chain reaction (PCR) technology, have a lower limit of quantification of 40 copies/mL (e.g. Abbott RealTime, Abbott Molecular, Abbott Park, Illinois, USA) or 20 copies/mL (e.g. Roche TaqMan v.2, Roche, Basel, Switzerland) and can report qualitative RNA detection below these thresholds. The interpretation of RNA detection below 50 copies/mL remains difficult in the absence of published evidence. While lack of RNA detection during ART may be regarded as a desirable outcome, evidence indicates that HIV-1 RNA persists at a low level in the plasma of treated patients who maintain suppression <50 copies/mL for several years [8]. Data presented in abstract form indicate that in treated patients monitored by the RealTime assay a viral load value between 40 and 49 copies/mL independently predicts a small but significant risk of viral load rebound above both 50 and 400 copies/mL during 12 months of follow-up [9,10]. Other recent data presented in abstract form suggest that low-but-detectable TaqMan results do not presage traditional virological failure. A clinically relevant threshold of 250 copies/mL has been proposed [11].

It is recognized that measuring viral load 4 weeks after starting ART can strongly predict which individuals will have a sustained virological response at 6 months [12] Therapy is expected to achieve a viral load suppression greater than 1 $\log_{10}$ copies/mL relative to the pre-therapy baseline value by week 4, whereas suppression below 50 copies/mL is seen within 12–24 weeks of ART initiation. In patients monitored with the Abbott RealTime assay, suppression below 50 and below 40 copies/mL occurs after a median (95% confidence interval) of 4.1 (3.3–5.1) and 4.4

(3.7–5.4) months, respectively [9]. Patients who show a suboptimal week 4 response or fail to achieve suppression of the viral load within 4–6 months of starting therapy need to be assessed as to the reasons for this and a change of therapy needs to be considered [12]. Some centres measure viral load at 2 weeks after commencement of ART. While it is expected that an effective regimen will start to show significant viral load reduction at 2 weeks, there is at present no clinically validated evidence to support this earlier time-point.

10.3 Individuals established on ART

Historically, routine follow-up has been 3–4-monthly and in most clinical trials, 12-weekly is standard. With better-tolerated and more effective treatments, there is increasing interest in reducing the frequency of follow-up (e.g. to 6-monthly). There are no prospective studies of this strategy. Reekie *et al.* for EUROSIDA [13] concluded that the risk of failure (defined as a viral load above 500 copies/mL or clinical progression) in stable patients (after more than 1 year on therapy) is low for intervals of up to 6 months. Additionally there are cohort data demonstrating that the risk of virological rebound declines significantly over time consistently across adherence strata both in individuals on first-line therapies and in those with previous virological failure [14,15]. However, the risk of viral load rebound resulting in resistance and accumulation of mutations throughout the period between visits was not assessed in these studies. Therefore, there is insufficient evidence to determine whether it would be safe to change the current practice of monitoring the viral load every 3–4 months as routine practice. However, in selected adherent patients on well-tolerated, effective, and stable regimens, 6-monthly follow-up seems reasonable to consider (for example if less frequent follow-up is requested by the patient).

In patients who achieve virological suppression, any rebound in viral load to above 50 copies/mL should be confirmed by testing of a newly collected sample, whereas repeat testing of the same sample is not generally recommended as discrepant results cannot be interpreted easily. Confirmed rebound should be addressed promptly to prevent the negative consequences of ongoing viral replication. The urgency of recall is greater for patients receiving regimens with a low genetic barrier to resistance.

10.4 Recommendations

- Every newly diagnosed patient should have an HIV-1 plasma RNA load ('viral load') measurement taken at the time of diagnosis (Ia).

- In primary infection, the viral load should be monitored at presentation and again at between 3 and 6 months to establish the 'set point' (Ia).
- Patients not receiving ART who are clinically stable should undergo viral load measurements once every 6 months (IIa).
- The viral load should be determined within 1 month prior to initiation of therapy to confirm the pre-ART baseline value (IV).
- Viral load should be tested 4 weeks after commencement of treatment, when a decline in viral load of greater than 1 $\log_{10}$ copies/mL relative to the pre-therapy baseline value should be observed (IIa).
- Further viral load measurements at 3 and 6 months are recommended to confirm full virological suppression below 50 copies/mL (Ia), taking into account that the time to undetectability is prolonged in patients monitored using new viral load assays.
- Subsequent viral load testing should be performed routinely every 3–4 months (Ia).
- In select adherent patients on well-tolerated, effective and stable regimens, 6-monthly follow-up may be considered (IIb).
- A viral load rebound to above 50 copies/mL should be confirmed by testing a subsequent sample (IIb). Repeat testing of the same sample is not recommended (IV).
- Confirmed viraemia should be addressed promptly to assess the underlying determinants and avoid accumulation of resistance (Ia).

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11. Technical aspects of viral load testing

Despite the significant improvements introduced in recent years, HIV sequence variability continues to challenge molecular viral load assays [1–3]. Mismatches between primers and probes and RNA target sequences could result in falsely low or undetectable viral loads in some samples. Testing with a second method is recommended when the viral load results are not consistent with the patient's history (IIa).

Based on available information, viral RNA in blood samples collected into ethylenediaminetetraacetic acid (EDTA) tubes is stable for at least 2–3 days at room temperature, allowing transportation of the sample by post or collection over a weekend [4,5]. If samples cannot be sent to the laboratory immediately after collection, they should be kept at room temperature (IIb). Use of plasma preparation tubes (PPT) tubes is not recommended (IIa) as they tend to produce more low-level viral load results compared with EDTA tubes [6,7].

Current assays have similar but not identical reading levels for similar values of viraemia [8–10]. It is recommended that clinicians engage actively with local laboratory services in order to discuss the performance of the viral load assay provided and appropriately interpret its results (IV). It remains important to ensure that patients are monitored with the same assay (IIa).

For routine monitoring purposes, viral load testing should be performed on plasma. The viral load assays can be adapted to perform well in other compartments including cerebrospinal fluid (CSF) and seminal plasma. However, routine monitoring of viral load in compartments other than plasma is not currently recommended because of undemonstrated clinical utility or practicality (IV). Testing of CSF collected from patients with neurological disease should be considered, especially in patients with suppressed plasma viral load (III).

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12. Viral load kinetics during ART and viral load 'blips'

Using sensitive testing methods in research settings, HIV-1 RNA can be detected in plasma in a large proportion of patients receiving standard ART regimens and showing a viral load stably below 50 copies/mL for many years [1–10]. This residual viraemia is not generally associated with the emergence of drug resistance or low antiretroviral drug levels in plasma [8,11,12], and is not responsive to short-term intensification with efavirenz, ritonavir-boosted atazanavir, ritonavir-boosted lopinavir, enfuvirtide or raltegravir [8–10]. These findings are shedding new light on the significance of low-level viraemia detected by routine viral load assays during ART, while falling short of providing clear guidance for its management in patients receiving standard ART regimens. As a consequence of technical fluctuation around the cut-off level of quantification, routine viral load assays are more likely to report low-level viraemia above 50 copies/mL in treated patients who have a level of residual viraemia just below the assay cut-off (e.g. around 30 copies/mL), as seen in some patients

[8]. The detection of this residual viraemia is likely to be technically inconsistent, leading to the phenomenon of viral load 'blips'.

Viral load 'blips' are defined as transient rises in viral load to levels above the lower detectable limit of the assay [13]. Although currently there is no consensus definition, in practice a blip is considered to be a single viral load measurement of 50–1000 copies/mL preceded and followed by a measurement of fewer than 50 copies/mL. It is controversial whether blips are associated with an increased risk of virological failure, although most studies show that isolated blips are of little clinical significance [14–17]. The scenario is different, however, for patients with two or more consecutive measurements above 50 copies/mL [17] and possibly for patients with frequent blips, as these are more likely to experience virological rebound above 400 copies/mL. These patients may benefit from intervention to review expected drug potency, adherence and tolerability, and drug resistance, and modifications of therapy should be considered in line with treatment guidelines [18].

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13. Proviral DNA load

Proviral DNA testing is predominantly used in two scenarios: for confirmation of HIV diagnosis in certain adults with equivocal serology and undetectable plasma HIV RNA, and for the diagnosis of infants born to HIV-infected women. Testing is usually qualitative in these circumstances. Some studies have suggested that quantitative monitoring of the proviral DNA load may be informative in elite controllers (patients who show undetectable plasma HIV RNA in the absence of therapy) [1] and those patients who have undetectable plasma HIV RNA on therapy [2–4]. To date, these applications are research tools only and evidence of their clinical utility remains limited.

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14. Resistance testing

14.1 Initial HIV-1 diagnosis

The prevalence of antiretroviral drug resistance among treatment-naïve patients in the UK is around 8% [1]. Although previous estimates may have been confounded by selection bias, prevalence rates have been declining over recent years [2]; however, rates are now showing a possible slight increase. While the highest rates of resistance are seen in patients born in the UK [3], rates are increasing in countries currently expanding access to ART [4–6] and may soon start to rise among immigrant populations as a result [7]. In some cases, the presence of resistance in an apparently treatment-naïve patient may in fact reflect previous undisclosed therapy. There is increasing evidence to indicate that transmitted resistance negatively impacts on treatment responses, particularly in the context of nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimens [8–17]. Most transmitted drug resistance affects reverse transcriptase and protease inhibitors (PIs), although transmitted integrase inhibitor resistance has started to emerge.

Although transmitted resistance often remains detectable in plasma for several years, gradual reversion to low-frequency and archived mutants occurs over time [18–24]. Reversion may occur through intermediates (or 'revertants', e.g. T215D/N/S from T215Y/F). Genotypic tests should therefore be used in treatment-naïve individuals as they allow the detection of such mutations that do not confer phenotypic resistance but may signal the presence of more substantial resistance. Detection of such revertants should be interpreted as an indication that fully resistant mutants are present as either low-frequency quasispecies or archived resistance.

Both genotypic and phenotypic resistance assays provide results based on the majority population of circulating viruses at the time of sampling. The level of detection of mutant viruses is around 20–30% of the population in genotypic assays and probably less in phenotypic assays. Low-frequency mutants can impact negatively on responses to therapy in the context of NNRTI-based regimens (reviewed in [12, 15–17, 25, 26]). Assays with increased sensitivity for detection of resistance mutations are under development but can be considered primarily as research tools in most circumstances at the current time [16].

14.1.1 Recommendations (Table 14.1)

- Testing for resistance is recommended in all newly diagnosed patients. This includes patients with acute seroconversion, established infection or infection of unknown duration, regardless of demographic characteristics, ethnicity or risk group (Ia).
- Baseline resistance testing should include the polymerase and protease genes. Testing for susceptibility to integrase and entry inhibitors is not recommended routinely in naïve patients at present, although this area is kept under active review (IIb).

- The most appropriate sample is the one closest to the time of diagnosis (Ia) and this should preferably be tested at the time of initial presentation (IV).

14.2 ART-naïve

The possibility exists that the resistance profile obtained at diagnosis may change in patients who acquire a new infection. The true risk of HIV-1 superinfection remains to be determined but may be significant in persons who continue to be exposed to new sources of the virus [27], especially in early stages of the initial infection [28]. Triggers to repeat resistance testing prior to starting ART may include a sudden increase in viral load, a sudden drop in the CD4 T-cell count, and a recurrence of symptoms of acute HIV infection [29,30]. It should be noted, however, that most patients with sudden changes in viral load and CD4 T-cell counts do not have evidence of superinfection [29,30]. In a London cohort study of 47 homosexual men who showed an increase in viral load of greater than 0.5 $\log_{10}$ copies/mL during routine monitoring, two (4%) showed evidence of superinfection and a change in the initial drug susceptibility profile as determined by repeat sequencing of the reverse transcriptase and protease genes [30].

14.2.1 Recommendations (Table 14.1)

- For patients who have not undergone resistance testing at the time of diagnosis, testing is recommended before starting therapy (Ia). Whenever possible, a plasma sample collected as close as possible to the time of diagnosis should be retrieved for retrospective testing (Ia). When a stored sample is not available a current sample should be tested (IV).
- Following resistance testing at the time of diagnosis, repeat testing is not routinely recommended prior to

Table 14.1 Recommendations on when to perform resistance testing

When to test	Comments	Method	Level of evidence and grading
New diagnosis	Recommended	Genotypic	Ia
Starting ART	Recommended, if not already carried out	Genotypic	Ia
	Repeat testing not routinely recommended but can be considered if superinfection likely		IIb
After starting ART	Consider resistance testing if suboptimal response to first-line therapy (<1 $\log_{10}$ copies/mL reduction after 4 weeks of therapy)	Genotypic	IV
	Consider resistance testing if viral load >50 copies/mL at 12–16 weeks after starting therapy		III
	Recommend resistance testing if viral load >50 copies/mL at 24 weeks after starting		Ia
ART failure	Recommended to guide treatment changes	Genotypic*	Ia
	Perform while on treatment (or not more than 2 weeks after stopping)		IIb

*Consider phenotype or virtual phenotype if multiple regimen failure and/or multiple mutations on genotype where interpretation is uncertain.

starting therapy, although it should be considered in selected persons who may have experienced reinfection (IIb).

14.3 Post treatment initiation

In patients without evidence of drug resistance by routine methods, a suboptimal virological response to first-line therapy (a viral load reduction of less than 1 $\log_{10}$ copies/mL by 4 weeks) may signal the emergence of drug-resistant variants that were initially present at low frequency and therefore undetectable by routine testing.

14.3.1 Recommendations (Table 14.1)

• In patients without evidence of drug resistance at diagnosis by routine genotypic methods, a suboptimal virological response to first-line therapy (a viral load reduction of less than 1 $\log_{10}$ copies/mL by 4 weeks) should prompt resistance testing at that time (IV).

14.4 ART-experienced

The prevalence of drug resistance has declined among treatment-experienced patients in the UK as a result of improved management of ART and treatment failure. Currently, approximately half of treated patients undergoing testing show evidence of resistance and around 3% have evidence of triple-class resistance affecting the nucleoside reverse transcriptase inhibitors (NRTIs), NNRTIs and PIs [1]. There are no national data on the prevalence of resistance to integrase and entry inhibitors, but integrase inhibitor resistance in particular is expected to grow with expanded use of the class. Patients who experience virological failure while on chemokine receptor 5 (CCR5) antagonists may show a change to chemokine receptor 4 (CXCR4)-using virus upon repeat tropism testing, or maintain the R5 tropism. In approximately one-third of R5 failures, the virus exhibits phenotypic resistance to the antagonist. Although certain mutations in the glycoprotein 120 (gp120) V3-loop appear to play a key role, the genotypic predictors of the resistance profile have not been clearly elucidated. Therefore, genotypic resistance testing is not routinely recommended for patients failing CCR5 inhibitor treatment at this time [31–34].

While it is recommended that confirmation of virological rebound is obtained in patients with previously undetectable viral load prior to performing a resistance test, it should be noted that mutations conferring or increasing resistance may accumulate if a patient is left on a failing regimen [35]. Resistance testing of viral load 'blips' (defined as a single viral load measurement greater than 50 copies/mL preceded and followed by values less than 50

copies/mL) is unlikely to yield significant information [36], whereas testing of confirmed low-level viraemia is highly informative [37–39]. Whereas a viral load cut-off of 1000 copies/mL has been traditionally recommended for resistance testing, specialized testing can achieve high success rates at lower levels of viraemia [37–39].

Resistant mutants selected during therapy are rapidly outgrown by wild-type virus once therapy is discontinued [40]. To be informative, resistance testing should therefore be performed on samples taken while the patient is still on therapy. Resistance testing undertaken when a patient has discontinued therapy for more than 2 weeks should be interpreted with caution as the extent of underlying resistance is likely to be underestimated. Despite the apparent disappearance of resistance, however, resistant mutants persist at low frequency in the plasma quasispecies and as archived resistance in latently infected cells [41], and can re-emerge rapidly if selective pressure is reintroduced. Therefore, resistance should be considered long-lasting. Interpretation of resistance should take into account the results of all tests performed during the patient's treatment history ('cumulative genotype') [42]. Patients who simultaneously interrupt all drugs in an NNRTI-based regimen are likely to experience a prolonged period of NNRTI monotherapy with a resulting risk of resistance that may or may not be detectable by routine methods, but may have an effect on treatment responses once NNRTI-based therapy is resumed [43–45]. Pending further data, the potential emergence and impact of NNRTI resistance should be taken into consideration in these patients [46].

The interpretation of resistance test results is complex. Although informative interpretation systems have been developed for both genotypic and phenotypic results, none is entirely accurate, and all are subject to change as new data become available. Interpretation is especially difficult with new drugs and this problem affects both genotypic and phenotypic resistance assays. Expert advice should be sought with complex or unusual resistance profiles. Sufficient information on treatment history should be provided to optimize interpretation of resistance test results in the laboratory.

14.4.1 Recommendations (Table 14.1)

• Viraemia should be confirmed before performing a resistance test in treated patients (IV). However, further assessment should be undertaken promptly because of the risk of accumulation of mutations, particularly in patients taking regimens with a low genetic barrier (IIb).
• Resistance testing is recommended in all treated patients experiencing confirmed viraemia and changes in

therapy should be guided by the results of resistance testing in these patients (Ia).

- For patients showing viraemia while receiving integrase inhibitors or enfuvirtide (T20), resistance testing should be undertaken promptly in laboratories offering the tests (IIb).

- For patients experiencing viraemia while receiving CCR5 antagonists, repeat tropism testing should be performed (Ia). If the virus is confirmed as R5, the presence of resistance to CCR5 antagonists should be suspected (Ia), although testing for this is not routinely available at present.

- The level of viraemia at which resistance testing can be performed reliably is just above 50 copies/mL in many specialized laboratories. Resistance testing where viral load levels are less than 1000 copies/mL can provide useful information and clinicians are encouraged to discuss and agree the required viral load cut-off for testing with their service providers (IV). Laboratories should review the optimal methodology for resistance testing at low viral load levels (III).

- Resistance testing should preferably be performed on samples taken while the patient is still on therapy (IIb).

- Resistance testing by routine methods is not recommended after unstructured interruption of NNRTIs because of suboptimal sensitivity in this context (IIa), although selection of NNRTI resistance should be considered possible (IIb).

- Resistance test results should be interpreted in the context of the patient's entire treatment history and the results of all tests performed in a patient should be taken into account to guide optimal treatment selection (IIb).

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15. Subtype determination

On the basis of the viral nucleic acid sequence, HIV-1 has been subdivided into nine subtypes (A–D, F–H, J and K). It is thought that these diversified soon after HIV-1 group M was established in the human population. Subsequently, as a result of dual infection or superinfection, recombinant viruses, with genomes composed of more than one subtype, emerged. Some mosaic viruses with a stable subtype structure became established in populations and are termed circulating recombinant forms (CRFs). As HIV infections spread globally, local epidemics in different geographical areas and risk groups emerged, which were dominated by a single subtype or CRF [1,2]. As more viral mixing has occurred a plethora of untypable and unique recombinants have emerged, confusing the picture further [3].

There are a number of techniques for identifying subtype but the gold standard is viral genome sequencing. In clinical practice, the subtype is usually supplied as a by-product of a genotypic test for resistance. However, this should be interpreted with caution because the pol. gene only reflects the genetic composition of a small region of the viral genome. Furthermore, different algorithms using the same sequence data can produce discrepant results. At present the REGA HIV-1 subtyping tool [4] is generally regarded as the gold standard for web-based systems.

Unless superinfection occurs, the viral subtype will not change during the course of disease.

Epidemiologically, there is interest in viral subtypes as they provide information on the dynamics of the epidemic at national and international levels. Currently, subtype does not provide much guidance for individual patient management. There are, however, a number of issues surrounding subtype that have attracted significant attention [1,2].

15.1 Disease progression

There is limited evidence that some subtypes cause more aggressive disease than others, with faster disease progression [5–8].

15.2 Transmission

Anecdotal evidence of greater transmissibility of some subtypes has not been substantiated [9].

15.3 Performance of molecular diagnostic assays

Subtype-related sequence variability can affect the performance of viral load and genotypic and phenotypic drug resistance and tropism assays.

15.4 Response to therapy

Antiretroviral drugs were designed for, tested on and predominantly used on infections with subtype B, which has been historically the dominant virus in the USA and Europe. There was concern that some subtypes may be inherently less responsive to certain therapies [10,11]. However, there is now clear evidence that the excellent virological and immunological outcomes achieved with highly active antiretroviral therapy (HAART) do not differ among the predominant subtypes [12].

15.5 Development of drug resistance

Although certain resistance mutations are more common in some subtypes than others, major mutations conferring resistance in subtype B also confer resistance in prevalent non-B subtypes and vice versa [13]. Subtle effects cannot be excluded, however, and rarer subtypes may show novel patterns.

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16. Other tests to guide use of specific antiretroviral agents

16.1 Tropism testing

16.1.1 Background
HIV gains entry into cells that express CD4 and one of two main transmembrane co-receptors, either CCR5 or CXCR4. The preferential use of one of the co-receptors is determined by the V3-loop of the envelope protein gp120.

In the current system of nomenclature, most HIV-1 strains are categorized as:

- CCR5 tropic (R5): enters CD4 cells using only CCR5 as a co-receptor;
- CXCR4 tropic (X4): enters CD4 cells using only CXCR4 as a co-receptor;
- dual tropic (R5/X4): can use either CCR5 or CXCR4 to enter CD4 cells, although one co-receptor may be favoured.

Different mixtures of R5, X4, and R5/X4 virus strains may be present in an HIV-infected patient. In these cases, the virus population is described as being mixed tropic. Cur-

rently, phenotypic tropism assays cannot differentiate between dual-tropic and mixed-tropic (collectively referred to as D/M) virus populations [1].

Throughout infection, R5 virus is most commonly detected. CXCR4-using variants are more likely to be detected in patients with advanced disease and low CD4 T-cell counts, as either R5/X4 or mixed populations of R5 and X4 strains [2–7]. The detection of exclusively X4 virus in clinical samples is rare. There is a strong relationship between the CD4 T-cell count and the likelihood of the detection of CXCR4-using virus; levels range from around 10% in patients with CD4 T-cell counts above 350 cells/μL to up to 50% at CD4 T-cell counts less than 200 cells/μL. A higher prevalence (40–50%) of CXCR4-using viruses is also seen in treatment-experienced patients, but this is reflective of low nadir CD4 T-cell counts more than of treatment *per se*, and is almost entirely attributable to an increase in R5/X4 and mixed populations. The emergence of CXCR4-using virus is associated with disease progression, but whether the emergence is a cause or a consequence of HIV disease progression has been the subject of debate. The prevailing opinion is that CXCR4-using strains emerge as a result of immunological deterioration, CD4 T-cell depletion and disease progression. The HIV-1 subtype is a further factor influencing preferential HIV-1 co-receptor use [8,9].

Virological failure of a CCR5 antagonist is often but not universally associated with a tropism shift, that is, emergence of pre-existing CXCR4-using virus (up to 63% in clinical trials) [10]. In about one-third of patients who retain R5 virus at failure, the R5 virus shows phenotypic resistance to the antagonist [11–14].

16.1.2 Determining HIV-1 tropism in clinical practice
Clinical trials of CCR5 antagonists have confirmed the specificity of the antiviral effect for R5 virus [15–22]. As these agents only inhibit the replication of R5 variants, a tropism test is essential prior to CCR5 antagonist use in order to exclude patients harbouring X4 or R5/X4 variants in whom no significant virological response to treatment is anticipated (reviewed in [23]). HIV-1 tropism may be determined phenotypically, by assessing the ability of a recombinant virus containing patient-derived envelope sequences to infect CCR5 or CXCR4 reporter cell lines that also express CD4. It may also be inferred genotypically from the sequence of the gp120 V3-loop. Both methods have advantages and drawbacks [23].

Among phenotypic methods, the original Trofile assay (Monogram Biosciences, San Francisco, CA) was used to screen patients for inclusion in clinical trials of CCR5 antagonists [1,15–21]. The Trofile assay showed a lower limit of sensitivity of approximately 10% for consistent detection of CXCR4-using virus in a clonal mixture of R5

and X4 variants [1,24]. In 2008, a modified version of the test known as the enhanced sensitivity Trofile assay (ESTA) superseded the original Trofile as a screening tool [24]. ESTA has a nominal lower limit of sensitivity of 0.3% for detecting CXCR4-using virus within clonal mixtures, but sensitivity with clinical samples appears to vary [25]. ESTA was found to more accurately identify patients likely to show a virological response to maraviroc in a *post hoc* re-analysis of the MERIT trial of maraviroc versus efavirenz (in combination with zidovudine/lamivudine) in treatment-naïve patients, which used the original Trofile assay to screen patients for inclusion [17,23,26]. ESTA also showed a marginal benefit over Trofile in a *post hoc* re-analysis of the AIDS Clinical Trials Group (ACTG) 5211 trial of vicriviroc in treatment-experienced patients [23,27].

There are a number of factors limiting the use of ESTA in routine patient care: testing is only performed in a central laboratory in California, and is expensive and labour-intensive, with a turn-around time of about 4 weeks and a relatively high failure rate (reflecting the assay complexity and stringent sample collection, storage and transport requirements) [28]. A minimal volume of 3 mL of plasma is recommended, which often poses a problem for testing of stored samples and in children. In addition, there is a minimum viral load requirement of 1000 copies/mL for reliable amplification [1], thus excluding this approach in patients with low or undetectable viral load. To circumvent this limitation, use of proviral DNA recovered from peripheral blood mononuclear cells (PBMC) is being explored but the data remain preliminary [29]. Other phenotypic assays have been developed in some laboratories that show generally good but not complete concordance with Trofile [30].

Genotypic systems use bioinformatic tools to predict tropism from gp120 V3 sequences and offer the advantage of platform portability, low cost and rapid turn-around. Examples of the interpretative systems include position-specific scoring matrices (PSSMs) and Geno2Pheno$_{coreceptor}$. The latter can also incorporate clinical parameters (most importantly the nadir CD4 T-cell count, but also the CD8 T-cell count and viral load), to improve predictive power for CXCR4-using virus. Genotypic tropism testing (GTT) is easy to implement in laboratories routinely performing genotypic drug-resistance testing, although commercial assays are not yet widely available. GTT is performed by bulk sequencing and typically shows a lower limit of sensitivity for detection of CXCR4-using virus of approximately 10–20%. Concordance with phenotypic tests was initially low [31] but adjustments such as repeat testing of individual patient samples (triplicate testing is recommended), changing assay threshold parameters, and incorporating clinical parameters can improve performance,

resulting in good-to-excellent concordance [28,32–39]. It should be noted, however, that assay comparisons are to be interpreted with caution in the absence of a reference gold diagnostic standard. The most relevant analysis is observing how effective an assay is at predicting virological responses to CCR5 antagonist use. Evidence indicates that GTT (performed and interpreted according to defined parameters) is comparable to the original Trofile assay in predicting virological responses to maraviroc in treatment-experienced patients, and comparable to ESTA in predicting virological responses to maraviroc in treatment-naïve patients [40,41]. Thus, in the latter group, both ESTA and GTT performed better than the original Trofile in identifying patients who would respond to maraviroc within the MERIT study. An increasing number of prospective cohort studies in both treatment-naïve and treatment-experienced patients starting maraviroc also indicate that GTT is reliable in terms of positive predictive value [42–44].

One advantage of GTT is the ability to circumvent the high plasma viral load requirement of phenotypic assays, and evaluate tropism in virologically suppressed patients using proviral DNA. There is limited evidence to indicate that GTT of proviral DNA may actually provide better concordance with phenotypic tropism prediction than genotypic analysis of plasma [33,34,38,42–46]. Prospective outcome data for the use of proviral DNA, however, are currently limited to case series [23,43,44]. There is limited evidence in support of the notion that, in treated patients, a tropism test result obtained prior to virological suppression remains usually unchanged during suppression [45,46] and can be used to guide a subsequent treatment switch when viraemia is suppressed.

16.1.3 Recommendations

- HIV-1 tropism testing should be performed prior to CCR5 antagonist therapy using a validated phenotypic or genotypic method. Genotypic tropism testing offers a more easily accessible, rapid and inexpensive method for tropism diagnostics than phenotypic testing and is therefore the preferred option (Ib).
- Laboratories undertaking genotypic tropisms testing should do so under quality assurance schemes and according to the prevailing consensus about preferred methodology for sampling, testing and interpretation (IV).
- In treatment-naïve patients, tropism testing should be performed immediately prior to the start of therapy whenever CCR5 antagonist use may be considered in the first-line regimen (unlicensed indication in Europe) (Ia). Alternatively a plasma sample could be stored for future testing if required (IV).

- In treated patients experiencing virological failure, tropism testing should be performed and the results should become available at the same time as those of drug-resistance testing to ensure all available therapeutic options may be considered (Ia).
- In treated patients with suppressed viraemia for whom a switch to a CCR5 antagonist is considered (e.g. because of toxicity), tropism testing may be performed using either PBMC-derived proviral DNA from a current sample, or plasma-derived RNA from a stored sample collected immediately before viral load suppression (III). The clinical utility of either approach should be monitored closely, as supporting evidence is limited.
- Detection of CXCR4-using virus at any time should be considered long-lasting. No specific recommendations can be made about the longevity of R5 predictions in patients with ongoing viral replication, although a 90-day cut-off has been commonly applied. In patients with a high risk of emergence of CXCR4-using virus (e.g. based on CD4 T-cell count) the test should be repeated as near as possible to the start of CCR5 antagonist therapy (III).
- The recommended sample for GTT is plasma in patients with viral loads greater than 500 copies/mL (Ib) and proviral DNA in patients with low-level viraemia (III). In patients with suppressed viraemia, tropism testing can be performed using the last plasma sample showing a viral load greater than 500 copies/mL (III). The patient's virological and clinical status since the sample was obtained should be reviewed to ensure consistent suppression of viraemia without blips, and no evidence of immunological or clinical deterioration (III). Alternatively, the tropism can be determined in patients with suppressed viraemia using proviral DNA (III). Both approaches require clinical monitoring.
- In patients failing therapy with CCR5 antagonists, the GTT should be repeated to determine whether the dominant virus population retains the R5 tropism, keeping in mind that detection of R5 does not exclude resistance to the antagonists (Ia). Testing for phenotypic resistance to CCR5 antagonists is not routinely available. Resistance should be assumed in patients experiencing virological rebound and reporting good adherence, especially if resistance to other drug classes is present (IV).

16.1.4 Methodological considerations

While producing good-quality V3-loop sequences may be achieved easily in laboratories with experience of genotypic resistance testing, it is important that the methodological approach to GTT should follow the prevailing consensus. Bulk sequencing of the V3-loop is recommended, followed by interpretation with the Geno2Pheno$_{coreceptor}$ tool (Ia). The assay interpretative parameter, called the false positive rate (FPR), should be set between 5.75 and 10% in the clonal model (Ib) [47]. A value of 5.75% has been shown to provide good discrimination between R5 and X4 sequences in both treatment-experienced and treatment-naïve patients [23,40,47]. To improve sampling of the viral quasispecies and sensitivity for the detection of CXCR4-using virus, triplicate testing is recommended (Ib), whereby samples undergo three separate PCR amplifications followed by separate sequencing of the three PCR products [39,40,47]. Three separate results are therefore obtained for each sample, and if any sequence is identified as X4, the presence of CXCR4-using variants is reported. In patients showing R5 tropism, if clinical data (most importantly the nadir CD4 T-cell count) are available and reliable, additional analysis may be considered using the clinical model of the Geno2Pheno$_{coreceptor}$ tool. The proposed FPR is currently 15%, but this is currently under review and may be lowered as data emerge. In patients with R5 sequences where the clinical model predicts the presence of X4, the presence of mixed populations of CCR5- and CXCR4-using virus may be considered likely [31] (IIb).

When testing proviral DNA in patients with undetectable viral load, recovery from PBMC or buffy coats is recommended (IIb); use of whole blood is not recommended because of likely loss of sensitivity (Kate Templeton, personal communication).

16.2 HLA B*5701 testing

HLA B*5701 screening significantly reduces the risk of abacavir hypersensitivity [48,49]. The test successfully identifies patients at highest risk of abacavir hypersensitivity and should be offered to all patients in whom the use of abacavir is considered. Where abacavir is frequently used in first-line regimens it may be more practical to test HLA B*5701 status in all patients at first presentation.

Data from the UK suggest that some PCR non-sequence-based typing methods for HLA B*5701 cross-react with other HLA B*57 alleles that are more prevalent in Black sub-Saharan populations [50]. Clinicians using this assay in Black sub-Saharan individuals should seek assurances from the laboratory providing testing about the specificity of the HLA B*5701 screening test.

16.2.1 Recommendations

- HLA B*5701 testing should be performed in all patients prior to commencing treatment with abacavir (Ib).

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17. Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) measures concentrations of NNRTIs, PIs, CCR5 antagonists and integrase inhibitors. Scarce data on the utility of TDM for NRTIs or entry inhibitors are available [1]; therefore, TDM is not practical for these agents.

In a recently published Cochrane review, the routine use of TDM (in randomized clinical trials) was examined in relation to outcomes of death, HIV-related events, and the proportion of patients achieving and maintaining an undetectable viral load. Overall, no benefit for achieving a viral load of less than 500 copies/mL at 1 year was seen. Safety outcomes were also similar in study arms receiving TDM and those receiving standard of care. In two trials of treatment with unboosted PIs, a significant benefit of TDM was seen [2].

However, while there is little evidence to support its routine use, TDM may be useful in the following clinical scenarios [3–5].

1. To predict/manage drug–drug interactions, by providing information to guide dose adjustments, when drugs sharing the same metabolic pathway are prescribed [6]. It is highly advisable to perform TDM at steady state (2 weeks following drug initiation, switch or withhold).

2. In pregnant women, because of the physiological changes that can affect drug pharmacokinetics (e.g. absorption, distribution, metabolism, elimination, blood flow, protein binding and intestinal transit) This is particularly true during the third trimester, when concentrations of antiretroviral agents (i.e. nelfinavir, saquinavir, lopinavir and atazanavir) have been shown to be lower than when measured post partum or when compared with nonpregnant HIV-infected subjects [7–10].

3. In pathophysiological conditions that could significantly impair drug absorption (e.g. malabsorption) or renal or hepatic function and affect drug pharmacokinetics [4].

4. To prevent/manage ART-induced concentration-dependent toxicity (e.g. indinavir-induced nephrotoxicity, efavirenz-associated central nervous system adverse events and atazanavir-related hyperbilirubinaemia) [11–13].

5. In the case of suboptimal virological response (exclude other causes of treatment failure such as poor adherence, incorrect dosing or dosing frequency, poor adherence to food requirements and drug interactions).

6. TDM and adherence: the usefulness of TDM to investigate/test adherence to antiretroviral drugs is unclear. However, a nondetectable drug concentration in a stored sample of plasma (drawn at time of failure and reporting a detectable viral load) may confirm the absence of therapeutic agent in the blood and lead to investigations of drug interaction and malabsorption and strengthen adherence support.

7. In treatment-experienced patients with virus with reduced susceptibility to antiretroviral drugs. Ritonavir-boosted PI (PI/r) doses may be increased to overcome resistance if no new drug is available and in the case of a failing regimen. The use of TDM may theoretically improve the outcome of these regimens and help to manage toxicity, although controlled clinical trials have not demonstrated this so far. One of the limitations in this setting is the absence of well-defined relationships between drug exposure and treatment response.

8. In patients with particularly high or low body weight compared with the population average [5].

9. When genetic (e.g. ethnic differences and gender) and environmental factors (e.g. grapefruit juice) are suspected to impact drug exposure and toxicity or response [14,15].

10. For unlicensed drug dosing regimens (i.e. once-daily nevirapine, saquinavir/ritonavir and unboosted atazanavir).

17.1 Recommendations

There is insufficient evidence to recommend routine use of TDM in the management of ART (I).

- TDM may be useful in individual patients (IV):
  ○ to assess and manage drug–drug or drug–food interactions;
  ○ if there is coexistent kidney or liver disease;
  ○ to assess and manage suboptimal adherence;
  ○ to assess reasons for regimen failure and to optimize treatment if resistance is present;
  ○ to manage drug-related toxicity.

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18. Biochemistry testing

18.1 Introduction

With the increased recognition of metabolic problems occurring in individuals with HIV infection (including insulin resistance, lipid dysregulation, and renal, liver and bone diseases), regular assessment of biochemical parameters has become an important focus of follow-up over the last few years. Until recently the focus was on managing toxicities in individuals taking ART. Since the analysis of the Strategies for Management of Anti-Retroviral Therapy (SMART) study, attention has also been focused on assessing these risks in ART-naïve individuals. Factors indicative of high disease risk or presence of disease are now also appearing in guidelines as criteria to consider initiation of ART. They are, as a consequence, important parameters to monitor.

Several biomarkers such as D-dimers, highly sensitive C-reactive protein (CRP), and interleukin (IL)-6 have been used in studies such as SMART and are highly correlated with risk of CVD, progression to AIDS and death [1]. It may be that they have a role in routine follow-up, for example in determining which individuals should start ART at higher viral loads, or stratifying individuals for further risk-reduction interventions; however, their case for inclusion has yet to be firmly established.

18.2 Liver function

18.2.1 Liver disease in HIV-infected individuals

The prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection is increased in HIV-infected patients compared with the general population, and the liver or biliary tree may be affected by opportunistic infections such as tuberculosis (TB), cytomegalovirus and cryptosporidium. Initiation (and discontinuation) of ART may be associated with flares of viral hepatitis, and specific antiretroviral drugs may cause liver injury, including nevirapine (hypersensitivity) and didanosine (hepatic fibrosis). Hepatic steatosis is relatively common and may occur in the presence or absence of lipodystrophy. Lactic acidosis, resulting from mitochondrial toxicity, is relatively common in patients on stavudine, and, to a lesser extent, zidovudine. Finally, many drugs used to treat or prevent opportunistic infections, including

rifamycins, isoniazid, pyrazinamide, cotrimoxazole, fluconazole, augmentin and cephalosporins, in addition to other drugs such as statins, may cause liver injury. Patients coinfected with hepatitis virus and those with low CD4 T-cell counts are at greatest risk of liver injury during treatment with antiretroviral drugs (liver enzyme flares), particularly in the first few months after treatment initiation [2,3].

18.2.2 Assessment and monitoring of liver enzymes

Routine measures of liver injury ['liver function tests' (LFTs)] include 'transaminases' [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)], alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT), bilirubin and albumin. While relatively nonspecific in isolation, when assessed in combination they are able to identify patients with cholestatic injury pattern (raised ALP and GGT, with or without raised bilirubin), or hepatocellular injury (raised ALT and AST). Other injury patterns, such as fatty or malignant infiltration or granulomatous inflammation, may result in isolated elevations in ALP or diffusely elevated liver markers. While collectively referred to as LFTs, none of these tests is a reliable measure of liver synthetic function. Albumin and the international normalized ratio (INR) as a measure of coagulation reflect liver function, but may be affected by many other factors.

18.2.3 Recommendations

- Assessments of liver function (LFTs) should include ALT and/or AST, ALP, GGT, bilirubin and albumin, and should be performed at baseline, routine clinic visits and during illness (IIa).
- More frequent monitoring is recommended during the first 3 months of exposure to (new) antiretrovirals (except nevirapine; see below), at approximately 1 month and 3 months (III).
- More frequent monitoring of LFTs (every 2 weeks during the first 2 months of treatment, at the third month, and then regularly thereafter) is recommended in the summary of product characteristics (SPC) for nevirapine.
- Patients with persistently raised markers of liver injury or newly occurring abnormal liver tests should be investigated for viral hepatitis, opportunistic infection, malignancy, drug toxicity or fatty liver disease (IIa).
- Sporadic high ALT levels are common. Apparent elevations should be confirmed (III). Acute hepatitis C should be excluded if an appropriate exposure history is obtained.

18.3 Renal function

18.3.1 Kidney disease in HIV-infected individuals

Kidney disease may affect up to 30% of HIV-infected patients. Acute renal failure is largely restricted to hospitalized patients with infection, liver disease or malignancy [4]. Chronic kidney disease (CKD) is associated with advanced HIV infection, older age, diabetes mellitus, hypertension and use of indinavir or tenofovir [5,6]. In Black patients, HIV-associated nephropathy (HIVAN) is an important cause of CKD and typically presents with heavy proteinuria and advanced renal failure at HIV diagnosis [7]. In other ethnicities, most CKD is associated with metabolic, vascular or urological disease, and drug toxicity [6]. The prognosis of Black patients with HIV-associated chronic kidney disease has improved dramatically in the HAART era, and the number of patients requiring long-term renal replacement has risen considerably in recent years [8].

CKD may be diagnosed by the presence of haematuria, proteinuria or reduced estimated glomerular filtration rate (eGFR) for more than 3 months [9]. Use of creatine supplements as a possible explanation for raised serum creatinine levels (and reduced eGFR) should be excluded. Proteinuria is a risk factor for developing renal failure [10] and (cardiovascular) death [11]. Patients with severe renal impairment, progressive decline in renal function, persistent haematuria or significant proteinuria (above 500 mg/24 h) should be investigated to establish the aetiology. ART may slow the progression of CKD, at least in patients with HIVAN [12,13].

Although most antiretroviral drugs may cause renal injury, indinavir and tenofovir have been most frequently associated with nephrotoxicity [14]. Crystallization of indinavir in the urinary tract may result in nephrolithiasis or tubulo-interstitial nephritis. Most episodes resolve with rehydration and drug discontinuation, although gradual loss of renal function and progressive or irreversible renal failure have also been reported [14]. Tenofovir has been implicated in the development of acute renal failure, progressive decline in renal function, hypophosphataemia, renal tubular acidosis, Fanconi syndrome, nephrogenic diabetes insipidus, hypokalaemia, osteomalacia, and urinary concentration defects [6,15,16]. Discontinuation of tenofovir usually leads to improvement of the renal abnormalities. Patients who receive tenofovir together with didanosine or (ritonavir-boosted) protease inhibitors, and those with advanced HIV infection, old age, low body mass and pre-existing renal impairment appear to be at increased risk [15,17], although the incidence of renal toxicity in randomized clinical trials has generally been low (less than 1%) [18,19]. More recently, atazanavir/ritonavir and, to a

lesser extent, lopinavir/ritonavir have also been associated with CKD [20].

18.3.2 Assessment and monitoring of renal function

eGFR provides a more accurate measure of renal function than serum creatinine, and should be used routinely to assess kidney function in HIV-infected patients. In addition, urinalysis should be performed to detect haematuria, proteinuria or glycosuria. The purpose of screening is early detection of CKD or drug-induced renal injury. In patients with glomerular disease, the bulk of urinary protein is albumin and may be picked up on dipstick. We advocate quantification of urinary protein by measuring the urinary protein/creatinine ratio (uPCR). This can be measured on a spot urine sample, and allows comparison of serial measurements.

Renal function in patients on indinavir or tenofovir should be monitored more closely by assessing eGFR, serum phosphate and urinalysis at each clinic visit. A progressive decline in eGFR, or the presence of severe hypophosphataemia (phosphate less than 0.64 mmol/L) or new-onset haematuria, glycosuria (in the presence of normoglycaemia) or proteinuria may indicate ART toxicity. The presence of hypophosphataemia should be confirmed on a fasting specimen. Proteinuria of tubular origin, which predominates in drug-induced renal injury, may not be detected by dipstick testing [21]. Proteinuria on dipstick should be quantified by uPCR measurement.

18.3.3 Recommendations

- Assessments of renal function (eGFR, urinalysis and urine protein/creatinine ratio) should be performed at baseline, ART initiation and annually thereafter (IIa).
- Renal function should be closely monitored during severe illness (hospitalization) (III).
- Dipstick urinalysis should be performed at all routine clinic visits in patients on tenofovir or indinavir (IV).
- In patients receiving tenofovir, new onset or worsening proteinuria and/or glycosuria may indicate tubular injury: these patients should be monitored carefully, and if renal abnormalities persist, additional biochemical tests including fasting serum and urine phosphate should be performed, and tenofovir discontinuation and/or referral to a nephrologist considered (IV).
- All patients with persistent haematuria and/or significant proteinuria (protein/creatinine ratio greater than 50 mg/mmol) should be further evaluated to exclude glomerulonephritis or urological disease (IIb).
- More frequent monitoring of renal function (every 4 weeks during the first year, and every 3 months thereafter) is recommended in the SPC for tenofovir.

- Referral to a renal physician should be considered for patients suspected to have a glomerulonephritis (haematuria and/or uPCR >100 mg/mmol) and those with a severe or progressive decline in renal function, advanced renal failure (eGFR <30 mL/min) or severe hypertension associated with renal injury (uPCR >100 mg/mmol or eGFR <60 mL/min) (IV).

18.4 Dyslipidaemia in HIV-infected individuals

HIV infection is associated with increased levels of triglycerides and decreased levels of high-density lipoprotein (HDL) cholesterol. ART may affect lipid levels and independently increase cardiovascular risk [22–26]. CVD is an increasingly important cause of mortality and morbidity in patients with HIV infection in the UK [27], emphasizing the importance of assessing lipid profiles and managing dyslipidaemia (as part of the overall cardiovascular risk) in those with HIV infection.

Lipid levels should be assessed in the context of overall CVD risk. CVD risk assessments generally incorporate age, gender, smoking, blood pressure, diabetes, the ratio of total:HDL cholesterol, and the presence or absence of left ventricular hypertrophy on electrocardiogram [28]. The Framingham CVD risk calculator works reasonably well in HIV-positive populations, although it is worth noting that it was not developed for use in non-White groups. Other algorithms may be better suited to these populations. A CVD risk calculator has been developed for use in HIV-positive populations (www.chip.dk/TOOLS) [29], although it should be noted that this provides 5-year risk estimates rather than the usual 10-year estimates. This calculator includes abacavir exposure as a CVD risk factor; the data regarding abacavir as a CVD risk factor, however, remain inconsistent. Alternatively, the QRISK calculator (www.qrisk.org) or the QIntervention tool (http://qintervention.org), which also provide an estimate of the risk of developing type II diabetes, can be used.

CVD risk can be reduced by smoking cessation, blood-pressure management (including nonpharmacological measures) and lipid-lowering interventions. Smoking cessation should be repeatedly encouraged. Weight reduction, diet and exercise may improve blood pressure and HDL-cholesterol levels. Decisions on lipid-lowering therapy should be based on overall cardiovascular risk rather than lipid levels in isolation.

D-dimer levels, highly sensitive CRP, and IL-6 have recently been correlated with cardiovascular events and death [30]. While these biomarkers may become useful in identifying high-risk patients and contribute to the debate regarding when to start ART, they remain research tools

and are not recommended for routine evaluation at present (IV).

18.4.1 Recommendations for assessment and monitoring of lipid profile

- Lipid profiles should include total cholesterol, HDL cholesterol and triglycerides and (together with blood glucose) should be performed at baseline and at least yearly thereafter (potentially more frequently in those at high CVD risk). They are required as part of the pre-ART assessment, following ART initiation or modification, and to assess targeted interventions (IIa).
- Random measurements suffice for most patients; measurements should be repeated fasting if glucose or triglycerides are abnormal (IIa).
- Total:HDL cholesterol should be used to guide lipid treatment decisions (IIa) [31].
- Low-density lipoprotein (LDL) cholesterol may be required for monitoring response to lipid-lowering treatment, but is not generally required for routine monitoring.

18.5 Other biomarkers

Amylase, creatine kinase, lactate dehydrogenase and lactate should be measured if clinical disease is present or suspected, but are not recommended for routine monitoring of stable patients.

18.6 Bone disease in HIV-infected patients

Reduced bone mineral density (BMD), including osteopenia and osteoporosis, is more common among HIV-infected patients compared with matched uninfected individuals [32,33]. Most studies have identified the importance of traditional risk factors for low bone mass (including older age, hypogonadism or early menopause, low body mass, White ethnicity, high alcohol intake) [32]. In addition, HIV parameters including increased duration of HIV infection, low nadir CD4 T-cell count, hepatitis virus coinfection and exposure to ART may contribute to bone loss [34–36]. Initiation of ART is associated with reductions in BMD, irrespective of the drugs included in the regimen. In randomized controlled clinical trials, the use of tenofovir/emtricitabine has been associated with greater initial bone loss compared with abacavir/lamivudine [37,38]. In these studies, bone loss stabilized after the first year of therapy, and the clinical significance of these modest differences in BMD remains unclear. Biochemical parameters (calcium, phosphate and alkaline phosphatase) have very limited use as screening tools for reduced BMD. Hyperthyroidism,

primary hyperparathyroidism and vitamin D deficiency should be excluded in patients with low BMD.

Low vitamin D status [25(OH)D less than 30 µg/L] is common in HIV-infected patients in the UK, and one-third of patients may have severe vitamin D deficiency [25(OH)D less than 10 µg/L]. Risk factors for vitamin D deficiency include sampling in winter and Black ethnicity. Some studies demonstrate an association with NNRTI use, particularly efavirenz [39,40]. Raised alkaline phosphatase is uncommon, even in patients with severe vitamin D deficiency. Its presence (in the context of normal liver enzymes) may reflect increased bone turnover and should be investigated. Low vitamin D status in patients receiving tenofovir has been associated with increased parathyroid hormone levels [41,42].

The clinical significance of vitamin D deficiency remains unclear. Although preliminary data suggest that the incidence of fractures may be increased in HIV-infected patients [34,43], the benefits of vitamin D replacement and/or treatment of low-risk patients with bisphosphonates remain to be established.

As low vitamin D levels are near universal in winter in HIV-infected patients living in the UK, there is little to be gained from routine vitamin D testing.

18.6.1 Bone mineral density assessment
The best method to detect low bone mass is hip and lumbar spine DXA scanning. The usefulness of biomarkers to identify patients with (or at increased risk of) osteoporosis and fragility fractures remains to be established.

18.6.2 DXA scanning
Although bone densities are lower than expected based on age (see above), severe osteoporosis and nontraumatic (fragility) bone fractures in this population remain uncommon. The data on whether HIV-infected individuals are at increased risk of fragility fracture compared with the general population are conflicting [44,45]. Therefore, routine BMD measurement is not recommended for all patients with HIV infection.

Scoring systems that incorporate age, BMI, BMD, gender and other risk factors have been developed and allow assessment of the risk of fractures and the need for treatment [e.g. FRAX WHO Fracture Risk Assessment Tool (www.shef.ac.uk/FRAX)]. The National Osteoporosis Guidelines Group (NOGG) has devised a management flow chart for patients stratified by fracture risk [high, intermediate and low (www.shef.ac.uk/NOGG)].

It is recommended that, in addition to risk assessment, women 65 years and older and men 70 years and over should routinely have BMD assessed (usually by DXA scan). Furthermore, in view of the high prevalence of low

bone density in HIV-infected patients, BMD assessment should be considered in patients aged 50 years and over if intermediate- or high-risk stratification by FRAX or additional risk factors for low bone mass or fracture are present (HIV or related risk factors, including increased duration of HIV infection, low nadir CD4 T-cell count and hepatitis virus coinfection).

As a consequence of the lack of consistent data on fragility fracture risk and also the potential cost implication of DXA scanning, there is no recommendation for routine screening in patients below 50 years of age.

18.6.3 Recommendations

- Risk factors for reduced bone mineral density should be assessed at first HIV diagnosis and prior to ART commencement. Risk factors should be further assessed in individuals on ART and 50 years or older every 3 years (IV). Bone mineral density (BMD) assessment (usually by DXA) should be performed in all men aged 70 years and older and all women aged 65 years and older. Consider BMD assessment in men and women over 50 years old if they have an intermediate to high FRAX score and/or additional risk factors.

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19. Haematology

19.1 Haematological assessment and monitoring

Anaemia, neutropenia and thrombocytopenia are common in patients with advanced immunosuppression and severe (opportunistic) infections or malignancy. By contrast, abnormalities on full blood count (FBC) are relatively uncommon in ART-naïve individuals with CD4 T-cell counts over 350 cells/μL. Thrombocytopenia (immune-mediated, without splenomegaly) may result from enhanced antibody-mediated platelet destruction and is often asymptomatic. Severe immune-mediated thrombocytopenia may result in bleeding and is an indication to commence ART. Other haematological abnormalities, including anaemia and neutropenia, are uncommon. Deficiencies in folate, iron and/or vitamin B12 should be excluded. In patients on ART, blood count abnormalities are rare with antiretrovirals other than zidovudine. They occur more frequently with some drugs used to treat or prevent opportunistic infections such as cotrimoxazole, (val)ganciclovir and dapsone. In individuals with advanced disease, more frequent haematological monitoring is indicated because of an increased risk of drug toxicity and also an increased risk of developing opportunistic infections (for example disseminated *Mycobacterial avium* complex infection) with haematological involvement. Finally, studies have demonstrated that haemoglobin is an independent prognostic factor in both ART-naïve individuals and those commencing therapy [1–3].

19.2 Recommendations

- FBC should be performed at baseline, and prior to starting ART. In stable, asymptomatic, ART-naïve individuals or individuals established on effective ART, FBC should be performed once per year. FBC should be performed in patients who are unwell (IIa).
- More frequent monitoring (at 6 and 12 weeks, and then 3-monthly) should be performed in patients who have recently commenced zidovudine (Ib).
- Although routine screening for glucose-6-phosphate deficiency (G6PD) is not recommended, it should be considered in patients at risk of severe haemolysis (Asian/Mediterranean men) when using high-risk drugs such as dapsone (III).

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20. Serology

20.1 Overview

Baseline screening for a variety of infectious agents is commonly undertaken when an HIV-positive patient is first diagnosed. While the risk factors associated with the HIV infection and the specific indications for testing will vary in the different patient groups, from a pragmatic perspective it is easier if all new patients are tested for the same pathogens (Table 20.1). Benefits for the patient from screening include the following.

1. Establishing the presence/absence of other chronic infections that are known to occur more commonly in HIV-infected patients. This provides the opportunity to treat the infection (e.g. HBV and HCV).
2. Determination of status may influence whether prophylaxis is offered following exposure to a particular pathogen.
3. Determination of status may influence whether immunization is offered, prior to an exposure to a particular pathogen. Early identification of nonimmune individuals is important as response rates may fall as HIV **disease** progresses and some live vaccines are contraindicated when the CD4 T-cell count falls below 200 cells/μL [1].
4. Published data indicate that rates of seronegativity for common viral infections [measles, mumps, rubella, varicella zoster virus (VZV), hepatitis A virus and HBV] that may be targeted by immunization are low overall in HIV-infected adults in the UK, indicating that pre-immunization testing should be used to target susceptible individuals [2]. It should be noted that the prevalence data are limited to an adult HIV-infected

Table 20.1 Summary of recommended viral screening of HIV-positive patients

Virus	Markers	Seronegativity rates*	Recommendations about testing
Hepatitis	HAV IgG (or total)	19.5%	All patients at diagnosis. Although centres may opt to limit pre-immunization testing to all or selected vaccine candidates (Ia), it is practical to make testing part of the routine screening for all patients (IV).
	HBsAg, anti-HBc (total) and anti-HBs	22.5% for all markers	All patients at diagnosis (Ia). Subsequent routine testing depends on the initial results (see text). Repeat all markers yearly in susceptible patients, including vaccine recipients (Ia).
	HCV antibody	NA	All patients at diagnosis (Ia). A positive screening antibody test should be followed by an HCV RNA test to confirm current infection (Ia). An HCV antibody **test** should be repeated regularly in those who test initially negative (IIb). IDUs and MSM are the groups at highest risk of infection and should be screened yearly (IV).
			HCV RNA (rather than antibody) testing is recommended in those who cleared a previous infection either spontaneously or after treatment and are at ongoing recognized risk of reinfection (IIb). The screening interval should be dictated by transaminase levels and/or risk behaviour and could be yearly as a general guide (IV).
			HCV RNA testing is not routinely recommended in patients who test antibody negative unless recent infection is strongly suspected or persistent and unexplained rises in transaminases are observed (IIb).
VZV	VZV IgG	1.5% overall, and 0% and 1.5% among persons with and without a history of VZV disease, respectively	All patients or selectively in those who lack a reliable history of chickenpox or zoster according to local preference (IIb).
Measles	Measles IgG	7.0%. Higher in routine screening as this does not include neutralizing antibody testing	All patients (IIb).
Rubella	Rubella IgG	6.4% among adult women below the age of 50 years	All women of child-bearing age (IIb). It may be practical to offer testing to all patients at diagnosis (IV).

Anti-HBcAb, HBV core antibody; anti-HBs, HBV surface antibody; HAV, hepatitis A virus; HBsAg, HBV surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; IgG, immunoglobulin G; VZV, varicella zoster virus.
*The prevalence data are derived from a London adult HIV-infected cohort comprising predominantly homosexual men (60.5%), of White ethnicity (75%) and born in the UK (56.5%) [2].

cohort comprising predominantly homosexual men (60.5%), of White ethnicity (75%) and born in the UK (56.5%).

20.2 Hepatitis viruses

20.2.1 Hepatitis A and hepatitis B
The reader is referred to the BHIVA immunization guidelines [1] for a detailed description of the indications and modalities for screening and vaccination. Further information is available from the BHIVA guidelines for the management of coinfection with HIV-1 and HBV or HCV [3]. For patients eligible for hepatitis A virus (HAV) vaccination, the use of pre-vaccination HAV immunoglobulin G (IgG) (or total) antibody testing should be decided locally; evidence indicates that testing may be cost-effective in most clinical settings [4,5]. Post-vaccination testing is not routinely required [1].

For hepatitis B, testing for surface antigen (HBsAg), anti-core antibody (anti-HBc, total) and anti-surface antibody (anti-HBs) is recommended at the time of diagnosis to identify both infected patients (HBsAg positive) and patients lacking immunity (anti-HBc and anti-HBs negative) who should be offered vaccination. Vaccine recipients should be tested for anti-HBs 6–8 weeks after vaccination, and yearly thereafter* [1]. Patients who test HBsAg negative, anti-HBc antibody positive and anti-HBs antibody negative should be tested for anti-HBV envelope (HBe) antibody as a further marker of past infection. Subsequent routine testing depends on the initial results. Patients with evidence of a past infection (anti-HBc and anti-HBs or anti-HBe antibody positive) should be tested for HBsAg alone at yearly intervals to detect a possible reactivation, patients with isolated anti-HBc should be vaccinated, and vaccine nonresponders should be tested yearly for HBsAg, anti-HBc and anti-HBs to identify new infections [1].

*There is little published evidence detailing the antibody metrics of HIV-positive HBV vaccine responders over time. While the cost–benefit of yearly anti-HBs screening in patients on ART with restored CD4 cell counts who show an anti-HBs response after vaccination remains to be determined, in the absence of guiding evidence cautious screening (and boosting where required) is advised.

20.2.2 Hepatitis C

All newly diagnosed patients should be tested for HCV antibodies and the test should be repeated at yearly intervals in those who initially test negative. A positive antibody result should be followed by an HCV RNA test to confirm a current infection. As false positive reactivity is possible with antibody screening tests, positive antibody status should be confirmed in patients who test RNA negative. Detection of anti-HCV antibodies is typically delayed for up to 12 weeks and occasionally longer after a recent infection. There are also reports of immunocompromised patients failing to mount an antibody response for many months after infection. In a UK study of HIV-positive MSM with acute hepatitis C, 37% and 10% of patients showed no detectable antibody 3 and 9 months after the initial presentation, respectively, while 5% remained negative after 1 year [6].

Thus, while screening antibody-negative patients for HCV RNA is not routinely recommended, it should be considered in patients at a recognized risk of a recent infection and in those with persistent, unexplained transaminase elevations. HCV-infected patients who experience RNA clearance (either spontaneously or after antiviral therapy) will maintain detectable antibody. These patients should undergo HCV RNA screening if they show persistent unexplained transaminase elevations or have a recognized risk of reinfection.

20.3 Herpes viruses

20.3.1 Varicella zoster virus (VZV)

The reader is referred to the BHIVA immunization guidelines [1] for a detailed description of the indications and modalities for screening and vaccination. Testing for VZV IgG is recommended in either all patients or in those lacking a reliable history of chickenpox or shingles, according to local preference [2]. VZV IgG-seronegative patients should be considered for vaccination according to their immune status [1].

20.3.2 Herpes simplex 2 (HSV-2)

HSV-2 coinfection is common in HIV-positive patients and may be accompanied by recognized genital disease or be clinically unrecognized. There is a strong epidemiological association between HSV-2 and HIV infections and bidirectional interactions have been described that promote viral replication and infectivity. Testing for type-specific HSV antibodies is available commercially. The tests distinguish between HSV-1 and HSV-2 infections and typically become positive from 2 weeks to 3 months after the initial onset of symptoms of primary or initial infection. HSV-2 antibody positivity is consistent with a diagnosis of genital herpes, whereas HSV-1 antibody positivity does not differentiate between genital and nongenital infections. Guidelines on the use of HSV type-specific serological testing have recently been drafted for BASHH [7] and the International Union Against Sexually Transmitted Infections (IUSTI) [8].

- Although HSV-2 seropositivity increases the risk of HIV transmission [9] and frequent HSV recurrences augment HIV replication [10,11], there is no firm evidence to inform the management of HSV-2 coinfection in HIV-infected persons without symptoms of genital herpes. Serological HSV testing is not routinely recommended in HIV-infected persons (IV).
- Limited data suggest an increased risk of perinatal HIV transmission among HSV-2-seropositive HIV-infected women [12,13]. As evidence is not consistent [14], serological HSV testing of HIV-positive pregnant women is not routinely recommended (IV).
- Serological HSV testing of pregnant women with no history of genital herpes is indicated when there is a history of genital herpes in the partner (IIb) [15–17]. HSV-1- and/or HSV-2-seronegative women should be counselled about strategies to prevent a new infection with either virus type during pregnancy.

20.4 Measles and rubella

The reader is referred to the BHIVA immunization guidelines [1] for a detailed description of the indications and modalities for screening and vaccination. Screening for measles IgG is currently recommended in all patients at the time of diagnosis, to identify seronegative patients and offer them vaccination if appropriate [1]. Testing of rubella antibody is recommended in women of child-bearing age to guide vaccination. Depending on the local clinic arrangements, selective screening of women may not be practical and testing of all HIV-positive persons may be preferred. Pregnant women will be screened for rubella as part of their antenatal tests. Post-vaccination testing is not routinely recommended.

20.5 Cytomegalovirus (CMV)

In the pre-HAART era, CMV was one of the commonest opportunistic infections in HIV-positive patients, with the risk of disease increasing as the CD4 T-cell count fell. With seropositive rates being in excess of 90% in HIV-positive patients, baseline screening was performed to identify seronegative patients who would benefit from screened blood products if required. Now, CMV disease is much less common, and blood when required is leucodepleted. In addition, molecular techniques have improved the

diagnosis of CMV disease, and a benefit of primary anti-viral prophylaxis in reducing the risk of CMV disease has not been demonstrated in HIV-infected patients [18,19]. Thus, there is little benefit from routine screening for CMV IgG. Testing for CMV IgG is therefore not routinely recommended (IV), but can be undertaken at the time CMV disease is suspected.

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21. Other microbiological screening

21.1 Tuberculosis screening

21.1.1 Tuberculin skin testing

Recommendations regarding TB screening are taken directly from the BHIVA 2011 TB guidelines [1]. The sensitivity and utility of tuberculin skin testing (TST) in HIV infection is markedly diminished [2–4] and specificity may also be compromised by bacille Calmette–Guérin (BCG) vaccination. Sensitivity may be improved by combining TST with interferon gamma release assays; however, there are presently insufficient data to recommend this [5].

As elaborated in the BHIVA tuberculosis guidelines [1], routine TST in HIV-positive patients is not recommended for either diagnosis or screening (IIa).

21.1.2 Interferon-gamma release assay (IGRA)

Assays that detect interferon-gamma release from T cells stimulated with TB-specific antigens have been shown to be more sensitive and specific than TST in HIV-seronegative individuals with latent and active tuberculosis. There are increasing data becoming available in HIV-infected individuals [6,7]. The following are the recommendations of the BHIVA TB guidelines [1] regarding screening.

21.1.3 Recommendations for screening

In an HIV-infected individual with a positive IGRA, the risk of developing active TB is based on:

○ region of origin;
○ current blood CD4 cell count;
○ duration of time on HAART.

The recommendations state that patients should be offered screening with IGRA if (and only if) they are in one of these groups and would benefit from chemoprophylaxis [BII].

Therefore, the recommendation is to consider screening in HIV-positive patients from:

• sub-Saharan Africa, if the length of current ART is under 2 years, whatever the current blood CD4 cell count;
• medium TB incidence* countries, if the length of current ART is under 2 years and current CD4 count is less than 500 cells/μL;
• low-incidence countries, e.g. Caucasians from the UK, if not on ART, or if the length of current ART is less than 6 months and current CD4 count is less than 350 cells/μL.

21.1.4 Other methods of tuberculosis screening

Routine induced sputum analysis in asymptomatic patients with no other evidence of TB is not recommended [8]. Baseline chest radiographs in asymptomatic individuals with no prior tuberculosis history are not routinely indicated, although they may be considered in those at increased risk of TB (e.g. those from a highly endemic group or with a known contact history).

21.1.5 Chest radiograph

Routine baseline chest films should be performed in those with a history of previous chest disease (including *Pneumocystis*) and may be considered in those at increase risk of TB (e.g. those from a highly endemic group or with a known contact history) and in those who have used intravenous drugs (IV).

21.2 Toxoplasma serology

All patients with a CD4 T-cell count of less than 200 cells/μL should have *Toxoplasma* serology (IgG titres) performed. If the test is IgG positive (consistent with previous exposure), then no repeat testing is required. If the test is IgG negative, then the serology should be repeated if the CD4 T-cell count declines to below 100 cells/μL (as this result will be useful in determining the optimal prophylaxis for the patient). If the patient remains seronegative for

*Medium-incidence regions include Eastern Europe, Central Asia, North Africa and the Middle East, South Asia, East Asia, and the Caribbean.

Toxoplasma then the serology should be repeated annually while the CD4 T-cell count remains below 100 cells/μL.

21.2.1 Recommendation

• All patients with a CD4 T-cell count of less than 200 cells/μL should have *Toxoplasma* serology performed. If the test is negative, this should be repeated yearly if the CD4 T-cell count is less than 100 cells/μL (III).

21.3 Tropical screening

There is relatively little information on the interactions between HIV and helminth or other tropical infections, and very scanty data on the sensitivities and specificities of routine assays for these coinfections in the setting of HIV infection [9,10].

There is some evidence that urogenital schistosomiasis is associated with an increased risk of HIV transmission [9,11], but there is presently insufficient evidence to assess whether there are any detrimental effects of other tropical infections on HIV infection, and insufficient data on whether routinely de-worming patients has a beneficial effect on HIV viral load, CD4 cell count or clinical progression [12].

There are few studies examining tropical screening in HIV-infected individuals and recommendations are therefore partly extrapolated from studies in more general populations [13–16]. All patients who have originated or spent significant time (more than 1 month) in sub-Saharan Africa should have *Schistosoma* serology performed. Any patient with an eosinophilia (absolute eosinophil count greater than 0.4×10^9 cells/L) on FBC who has originated or spent significant time (more than 1 month) in the Tropics (areas excluding Europe/Russia, North America and Australasia) should be investigated further depending on geographical exposure [13,14]: please liaise with a physician with a specialist interest or with an infectious diseases unit. Such tests will probably include (but not be limited to) stool examinations for ova, cysts and parasites, and serologies for helminths such as *Strongyloides,* filaria and *Schistosoma* (if not already performed). Patients who spend further time in the Tropics should have these tests repeated as required. It is preferable to perform all such investigations in asymptomatic patients at least 3 months after their last tropical exposure.

21.3.1 Recommendation

• Individuals with exposure longer than 1 month in sub-Saharan Africa should have screening with *Schistosoma* serology. Those with an eosinophilia (absolute eosinophil count greater than 0.4×10^9 cells/L) who originate

from or report significant time spent in tropical areas (more than 1 month) may have a helminthic infection and should be further assessed (see text) (III).

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22. Sexual health screening including anal and cervical cytology

22.1 Sexual history taking, counselling and sexually transmitted infection (STI) screening

Contact tracing and partner notification

Thorough contact tracing and partner notification are essential; careful documentation of this, and eventual outcomes, should be performed. A patient may wish to delay disclosure to partners; some delay may be acceptable if there is no urgency (i.e. no ongoing risk behaviour). Attempts should be made to encourage and support disclosure, counselling should be provided and contacts should be tested; if the patient refuses to cooperate, then additional action may be required. Testing of children is a sensitive area and specialist input should be sought.

Interventions shown to reduce transmission risk such as ART, pre- and post-exposure prophylaxis for seronegative partners, and diagnosis and treatment of STIs may all be relevant depending on specific circumstances.

Asymptomatic individuals should be offered STI screening at least yearly with consideration of more frequent screening dependent on risk [1]. There is some evidence that adding syphilis serology to routine HIV monitoring reduces time with undiagnosed syphilis and therefore potentially contributes to a reduction in onward syphilis transmission [2]. Therefore, in individuals or groups at increased risk of syphilis (currently MSM), syphilis serology should be considered with routine HIV follow-up (2–4 times yearly).

22.2 Cervical and anal cytology

22.2.1 Screening for cervical intra-epithelial neoplasia (CIN)

The following recommendations regarding monitoring for cervical dysplasia are published within [1]:

'Annual [cervical] cytology should be performed with an initial colposcopy if resources permit. Subsequent colposcopy for cytological abnormality should follow national guidelines, although immediate referral to specialist colposcopy services following an initial abnormal smear (mild dyskaryosis) is advised based on the frequent persistence of CIN in HIV-positive women. The guidelines also suggest that the age range screened should be the same as for HIV-negative women, i.e. first invitation at 25 years and ending at 65 years. There are few data regarding the prevalence of cervical lesions in sexually active HIV-positive adolescents who may have been immunosuppressed for many years. Therefore, there may be a need for more intense surveillance on a case-by-case basis.'

For many women cervical screening will be undertaken in primary care. The recommendation that routine cytology should be performed yearly differs from the national recommendation. It may therefore be helpful to specify this recommendation in communications between HIV centres and general practice.

22.2.2 Screening for anal intra-epithelial neoplasia (AIN)

HIV-positive individuals, particularly MSM, are at significantly increased risk of anal cancer despite the introduction of ART [3]. While anal cytology has been shown to be a sensitive technique with which to detect dysplasia [4,5], in some studies it has been found to have low specificity [6]. There is debate about which of anal cytology or high-resolution anoscopy performs better and is more cost effective for screening [7].

Screening for AIN has major cost and resource implications. While Goldie *et al.* found screening MSM to offer life-expectancy benefits at a cost comparable to those of other accepted interventions [8], in more recently reported models it was concluded that anal screening was not cost effective [9,10]. It is important to note, however, that these conclusions were based on important assumptions such as the rates of AIN regression, and the response to treatment, for which there are few or no long-term data [11–14].

There is insufficient evidence currently to recommend routine screening for AIN; however, this recommendation should be regularly reviewed in light of the increased research in this area.

Where a diagnosis of anal dysplasia has been made, it is important that the disease is evaluated and monitored. High-resolution anoscopy should be performed in patients diagnosed with high-grade dysplasia to document the extent of disease and confirm the grade. Patients should be instructed to report symptoms early, and to perform self-

examination regularly. Regular follow-up (6–12-monthly) should be undertaken and include enquiry of anal symptoms and a digital rectal exam.

22.3 Recommendations

- A sexual health assessment, including a sexual history documented at first presentation and at 6-monthly intervals thereafter (IIb).
- There should be a clearly documented discussion of the following issues at first presentation and at relevant times thereafter:
  ○ disclosure of HIV status;
  ○ safer sex;
  ○ importance of STI screening;
  ○ indications for post-exposure prophylaxis (PEP) and when/how to access it;
  ○ transmission risks including impact of concurrent STI;
  ○ the issue of 'reckless transmission' and litigation (IIb).
- An annual offer of a full sexual health screen (regardless of reported history) and the outcome documented in the HIV case notes, including whether declined (IIb).
- Syphilis serology should be documented at baseline and performed yearly. In individuals or groups at increased risk of syphilis (MSM), syphilis serology should be considered with routine HIV follow-up (2–4 times yearly) (IIb).
- All women should have cervical smears performed annually (IV).
- Screening for anal dysplasia by anal cytology may be beneficial; however, there is insufficient evidence at this time to support its routine introduction (IV).

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23. Routine monitoring recommended for specific patient groups

23.1 Women

- Gender-specific aspects of HIV monitoring will be discussed fully in the BHIVA women's guidelines currently under development.

23.2 Older age

Approximately 20% of HIV-infected individuals accessing care in the UK are aged 50 years or more [1].

The prevalence of ageing HIV-infected individuals continues to increase as a result of: (i) greater survival rates among HIV-infected patients; (ii) delayed recognition of the infection in older individuals; and (iii) continued new infections in older individuals.

There is a need to adapt the management of HIV-infected individuals to ensure that the clinical needs of these individuals continue to be met as they age. However, very little is known about the likely healthcare needs of these patients. Existing reports on the clinical picture of HIV infection among older individuals are largely anecdotal; HIV may accelerate several age-related conditions, and HIV-infected individuals may experience accelerated frailty, accelerated bone mineral loss and different levels of drug absorption and metabolism compared with their younger counterparts. Impaired glomerular function, impaired tubular function and proteinuria are all more common in the elderly. While this age-related decline in renal function is unlikely to result in severe kidney failure, it may affect many homeostatic processes, which may have implications for exacerbation of bone mineral loss and/or increased cardiovascular risk. The impact on adherence and potential drug–drug interactions of treatment for age-related comorbidities in patients who may be receiving ART has not been documented. HIV infection and ageing are also both associated with changes in immunity and host defence. The potential for full immune restoration among older individuals receiving HAART for prolonged periods of time has not been fully investigated.

In older individuals, drug pharmacokinetics (absorption, distribution, metabolism, and elimination) are altered [2] as a result of: (i) changes in gastric pH; (ii) body fat increase and water decrease; (iii) reductions in liver volume, blood flow and metabolic enzyme activity; (iv) decreased renal function. Therefore, close monitoring for drug (both antiretroviral and non-antiretroviral agents)-related toxicity in older individuals is recommended.

Finally, it is important to be aware of health initiatives aimed at older individuals in the general population (undertaken in general practice).

Men and women should be offered faecal occult blood screening for bowel cancer every 2 years between the ages of 60 and 70 years.

Currently, all women aged 50–70 years in the UK are offered a routine breast-screening test every 3 years by their GP. There are plans to extend the age range for routine breast screening to include women from age 47 to 73 years. For women under the age of 50 years, screening should also be considered if there is:

- a history of breast cancer in the past;
- a first-degree relative (mother or sister) who has had breast cancer at a young age.

23.2.1 Recommendations

- Enquiries regarding other health interventions/new diagnoses and co-prescribed medications should be made at all routine visits (III).
- Consider a lower threshold for TDM (IV).
- In patients with symptoms of cognitive decline, consider and investigate HIV-related as well as alternative causes (IV).
- Routine bone density scanning in women over 65 years and in men over 70 years of age (III).

23.3 Injecting drug users

Although needle and syringe sharing has declined within the UK in recent years, around one-quarter of injecting drug users (IDUs) continue to share needles and syringes. Injection of crack cocaine is now more common and this is associated with risky injection practice. In 2006, injecting drug use was the attributed risk factor for HIV acquisition in 176 individuals newly diagnosed as HIV positive [3].

In those continuing to inject, risk reduction by evaluation of injection technique should be considered. Discussion about the use of clean needles, syringes and mixing equipment is important not only to influence the risk of acquisition of other infections but also to reduce the risk of onward transmission of HIV to injecting partners. Easy access to needle exchange programmes should also be facilitated for those actively injecting.

Knowing which drugs are being taken is important particularly in relation to interactions with ART (e.g. between opiates such as methadone and NNRTIs/PIs). IDUs as a group are more at risk of ART failure secondary to poor adherence. Specialist assessment prior to initiation of ART and additional adherence monitoring and support in IDUs, particularly those actively injecting and with chaotic lifestyles, should be considered [4–6].

Injecting site infections are common, with around one-third of IDUs reporting having had an abscess, sore or open wound at an injecting site in the last year [3]. *Staphylococcus aureus* can cause disease ranging from localized soft tissue infections to severe invasive disease including septicaemia and endocarditis. Injecting drug use accounted for 1-in-5 reports of serious Group A streptococcal infections reported to the Health Protection Agency (HPA) in 2007. Clostridial infections causing wound botulism (*Clostridium botulinum*) or tetanus

(*Clostridium tetani*) are also sporadically reported. Tetanus immunization should therefore be current [7].

IDUs are at increased risk of hepatitis A and also infection with other blood-borne viruses, such as HBV and HCV. Individuals should be screened and if necessary vaccinated against HAV and HBV. Regular monitoring of HBV surface antibody should be undertaken and booster doses of vaccine given as appropriate. For individuals without hepatitis C who are actively injecting, more frequent HCV screening than yearly is justified considering the high risk of infection and the potential benefit of early intervention in those newly acquiring HCV infection. In individuals who have previously been infected with and then cleared HCV, regular screening with HCV RNA should be performed, as re-infection is possible.

23.3.1 Recommendations

- Regularly enquire whether nonprescribed/recreational/illicit drugs are being used and how these are administered (IV).
- Undertake an evaluation of injecting practice (IIb).
- Examine injecting sites for signs of infection (IV).
- Assess immunity to hepatitis A and B and tetanus and vaccinate as per protocols (IIb).
- Reassess hepatitis B immunity on a regular basis (IIb).
- Test at least 12-monthly for hepatitis C and syphilis (IIb).

23.4 Individuals coinfected with HBV and HCV

BHIVA guidelines for the monitoring and management of HBV- and HCV-coinfected patients have recently been published [8].

23.5 Late presenters

Patients who present with CD4 T-cell counts less than 350 cells/μL and/or with an AIDS condition are considered to be late presenters [9]. Patients who present with CD4 T-cell counts below 200 cells/μL are considered to be presenting with advanced HIV disease (increased short-term mortality risk) [9].

Routine screening with dilated indirect ophthalmoscopy is recommended at 3-monthly intervals in patients with very advanced disease (CD4 T-cell counts less than 50 cells/μL) [10]. While CMV viraemia is independently predictive of mortality, there is no clear evidence that primary prophylaxis with valganciclovir is helpful [11,12]. Mycobacterial blood cultures need only be performed in symptomatic patients. Toxoplasma serology should be performed in all new patients who at presentation have advanced disease (AIDS diagnosis or CD4 T-cell count <200 cells/μL). In those with positive

toxoplasma serology, primary prophylaxis should be initiated as per the opportunistic infection guidelines. We recommend that individuals presenting with advanced disease should also be screened with cryptococcal antigen before commencing ART. If positive, investigations for end-organ disease (chest radiograph and lumbar puncture) should be undertaken.

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Appendix

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