

# Clinical and immunological assessment of HIV infection

A G Bird

## Introduction

Human immunodeficiency virus (HIV) infection results in a progressive immunodeficiency primarily affecting T cell mediated immunity. It is also associated with direct damage to other organs including the brain and gastrointestinal tract which may be the direct result of either primary viral pathogenicity or a consequence of immunopathological effects. This review will cover only aspects of the clinical presentation of HIV infection resulting from the associated immunodeficiency and its laboratory assessment.

Accurate clinical staging is essential for epidemiological monitoring of the HIV epidemic, overall, in patient selection for clinical trials, and is of increasing importance in the assessment of individual patients for therapeutic intervention. Laboratory monitoring is gaining increasing importance in the initial staging of disease in individual patients, in the identification of immunological progression of those patients who remain asymptomatic, and in the recruitment to and assessment of clinical trials of new therapeutic agents against HIV infection.

## Staging of HIV disease

In most individuals infected with HIV evidence of cellular immune deficiency appears after a latent period of good health that usually extends for a number of years after primary viral infection. Available evidence from several cohort studies suggests that 10 years after infection half of adults acquiring infection by any of the recognised routes will have developed AIDS and that most of the remainder will have either symptomatic disease which does not yet fulfill the case definition for AIDS or laboratory evidence of persistent virus infection and subclinical immune deficiency.<sup>1-4</sup> However, in all studies of such duration there remains a residue of individuals with little clinical or laboratory evidence of cellular immunodeficiency. Whether this minority population identifies a group of individuals who will only show late evidence of progression or who genuinely represent individuals who are immune to the pathogenic potential of HIV will only become apparent with longer term follow up.

Clinical presentation and natural history of disease is less predictable and more diverse in children who have acquired the infection vertically during gestation or at childbirth, and is beyond the scope of this article. Readers are referred to review articles dealing specifically

with paediatric aspects of HIV infection.<sup>5-7</sup>

Because viral infection in adults, and the associated immunodeficiency, is progressive in most individuals, several clinical staging systems have been proposed which are essentially hierarchical and describe events which once occurred do not revert to earlier stages. However, with the increasing use of specific treatment in patients it is now possible in practice to return patients temporarily to earlier levels of such classifications. Such reversion is seen particularly in laboratory markers of immunological progression in patients receiving anti-retroviral treatment.

The most widely adopted Centers for Disease Control (CDC) clinical staging system of HIV infection is reproduced in table 1 but may soon be replaced by a proposed CDC/WHO classification which will incorporate both laboratory and clinical markers (table 2). The proposed new classification recognises the importance of the long asymptomatic stage of disease in determining the eventual outcome of

Table 1 Centers for Disease Control (CDC) classification of HIV infection

Stage I	Acute HIV infection and seroconversion
Stage II	Asymptomatic HIV infection
Stage III	Persistent generalised lymphadenopathy
Stage IV	A Constitutional disease (old AIDS related complex (ARC))
	B Neurological disease
	C Severe clinical immunodeficiency
	C1 CDC AIDS definition
	C2 severe infections outwith AIDS definition
	D Secondary cancers within AIDS definition:
	Kaposi's sarcoma
	Non-Hodgkin's lymphoma
	E Other conditions

Table 2 Proposed CDC/WHO classification system

I Clinical:	
Category A	—Primary clinical HIV infection (seroconversion illness)
	—Asymptomatic HIV infection
	—Persistent generalised lymphadenopathy
Category B	—Conditions indicative of HIV associated cellular immune deficiency not listed in Category C, such as,
	—Bacterial pneumonia or meningitis
	—Pharyngeal or vaginal candidiasis
	—Cervical dysplasia or carcinoma
	—Oral hairy leucoplakia
	—Recurrent or multidermatomal herpes zoster
	—Pulmonary tuberculosis
	—Idiopathic thrombocytopenic purpura
	—Unexplained constitutional disturbance
Category C	—Old CDC stages IV B, C1, C2, D, E
II Laboratory:	
	$CD4\ cells \times 10^9/l$ $Total\ lymphocyte\ count \times 10^9/l$
(1)	> 0.5      > 2
(2)	0.2-0.9      1-1.9
(3)	< 0.2      < 1

HIV Immunology  
Unit, Department of  
Medicine, Royal  
Infirmary, Edinburgh  
EH3 9YW  
A G Bird

Correspondence to:  
Dr A G Bird

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the disease process and the need to direct therapeutic trial intervention at this group of patients. It includes clinical and laboratory staging systems assessed in parallel to improve discrimination of disease stages and recognises the importance of laboratory markers of disease in the subclassification of asymptomatic patients. The addition of lymphocyte counts in this classification identifies the reality that in many countries reliable methodologies for accurate quantitation of CD4 counts, although desirable, are not routinely available.

#### *Acute (primary) HIV infection*

Acute HIV infection is identified as a clinical event in only about 15–20% of individuals. Defined as an acute viral illness resembling infections mononucleosis and less commonly associated with acute neurological manifestations, which include aseptic meningoencephalitis or neuropathy, these symptoms are temporally associated with the first appearance of HIV antibodies in infected individuals.<sup>8</sup> This acute viral illness is self limiting in virtually all cases but if prolonged has been associated with more rapid subsequent progression to symptomatic immunodeficiency.<sup>9</sup>

#### **Asymptomatic HIV disease and persistent generalised lymphadenopathy**

The proposed new category A of the CDC/WHO staging system combines primary viral infection together with asymptomatic disease and persistent generalised lymphadenopathy (PGL) as a single staging entity. This summation is a recognition of the fact that natural history studies have indicated that asymptomatic HIV seropositive (PGL) subjects of known seroconversion date have no increased propensity to disease progression when compared with similar subjects without clinical lymphadenopathy.<sup>10</sup> Most HIV seropositive individuals remain in category A for long periods of time and yet within this stage a wide range of immunological changes of different severity can be seen. It is in this category that the inclusion of immunological staging will add most additional information.

#### **Early manifestations of HIV associated immunodeficiency**

Symptomatic HIV immunodeficiency generally appears many years after initial viral infection. Most, but not all, patients pass through a sequence of mild and progressive constitutional disturbance frequently associated with minor opportunistic infections identified in category B. Few of these conditions are restricted to HIV infection and some can be frequently encountered in non-HIV infected individuals who are well or mildly immunocompromised. Oral hairy leucoplakia or recurrent or extensive herpes zoster are most typical of the earlier stage HIV immunodeficiency. Only unusually are they encountered in association with other diseases and only exceptionally in normal individuals. Pulmonary tuberculosis can obviously present in

immunocompetent individuals but appears with increased frequency and severity at all immunological stages of HIV disease in areas of endemic infection.

Most of the conditions identified in category B have been recognised for many years as being associated with cell mediated immune deficiency of any cause, and more than one of these conditions appearing together or sequentially should raise suspicion of underlying immune deficiency. Investigation must now also include assessment for and exclusion of HIV associated immunodeficiency. In contrast, bacterial infections with encapsulated species are more typically associated with antibody deficiency states but are also seen with increased frequency and severity in HIV seropositive subjects. They are particularly prevalent amongst populations of infected drug users where they can be the major cause of morbidity in early HIV infection.<sup>11</sup>

#### **Late HIV associated immunodeficiency**

Clinical presentations in this category are associated with profound immune deficiency and comprise a range of opportunistic organisms and tumours. Their character and severity of presentation are incompatible with general or immunological good health. Most presenting infections or tumours in this category were included in the original case definitions of AIDS. However, for several years this stage of disease has also included the severe constitutional symptoms of unexplained weight loss, diarrhoea, or fever previously known as AIDS related complex, an inevitable harbinger of symptomatic immune deficiency. Infections in this category of disease are progressive without appropriate treatment. Some, notably *Pneumocystis carinii*, are now largely preventable with prophylactic treatment. This realisation is resulting in increasing enthusiasm for the earlier detection of HIV infection and the monitoring of individuals to determine those at greatest risk of short term progression, to whom new therapeutic approaches are being targeted. Together with the more effective therapeutic regimens for established opportunistic infections and the use of antiretroviral treatment, the length of survival of individuals in this category has been substantially lengthened from an initial mean of six months to two years or more. However, although longer survival is now generally achievable continued disease progression is inevitable in this category as a result of the severity and irreversibility of the underlying cellular immunodeficiency. With the increasing success in treatment of certain infections, there is a rising morbidity with opportunistic agents which are more difficult or impossible to treat (cytomegalovirus, atypical mycobacterial infections, or cryptosporidiosis) and a particular tendency for the development of invasive Kaposi's sarcoma or immunoblastic or Burkitt's type lymphomas as a terminal event. Although lymphomas are reported often (30%) in patients who have been receiving long term antiretroviral treatment for periods in excess of two years,<sup>12</sup> this

probably represents a feature of severe long term immune deficiency rather than a primary consequence of treatment with the nucleoside analogue class of drugs.

### Immunological assessment of HIV infection

A more complete description of the clinical natural history of HIV infection has emerged from the long term follow up of infected subjects, from documented episodes of sero-conversion. Such studies have indicated that HIV infection is a predictably progressive disease with a mean incubation time to severe symptomatic disease of about 10 years in young adults, irrespective of mode of acquisition of the infection.<sup>3 4</sup> This finding, combined with the results of studies and trials suggesting that prophylactic treatment can delay some of the later opportunistic infections, and that specific antiretroviral treatment can slow the rate of disease progression over intermediate time periods, have forced a re-evaluation of the need for earlier identification of HIV infection and the clinical and immunological assessment and follow up of the asymptomatic subject.

Following the first detection of HIV infection, the immune status of every individual should be assessed to guide clinical and therapeutic decision making. This is forcing reappraisal of the long asymptomatic phase of the disease and is the reason for the introduction of immunological subdivisions of this stage of disease.

Increasing experimental evidence suggests that the early stages of HIV infection are generally associated with a strong and partially effective anti-HIV immune response which is associated with the reduction of initially high levels of virus replication.<sup>13</sup> This state of relative immunity can persist for many years but is apparently eroded by the ease with which HIV mutates away from effective specific antibody and cellular responses.<sup>14</sup> However, the observations from natural history studies which suggest that immune responses can effectively suppress viral replication in the early stages of infection for many years gives hope that earlier therapeutic intervention may be associated with considerably enhanced survival.

Many immunological parameters are influ-

enced by HIV infection. In the late stages of disease virtually every available investigation is abnormal. As the priority for clinical assessment is to identify who is most likely to progress among a pool of asymptomatic individuals and how often follow up assessments are required, markers must be chosen which have the characteristics given in table 3.

Experience from the classification and assessment of primary immune deficiency states has indicated that absence of cell populations responsible for immune recognition or effector responses is usually associated with clinically overt immunodeficiency. However, severe infections can also be seen in association with conditions in which loss of specific immune responses is not necessarily associated with deletion of a total cell population. Applying these observations to HIV infection means that the deletion of the CD4 cell population, which characterises the late stages of HIV infection, is associated with severe clinical immunodeficiency and complete loss of *in vitro* T cell immune responses. However, in early HIV infection loss of specific functional T cell responses generally precede overt evidence of total CD4 population depletion. There is, therefore, increasing interest in examining specific immune responses to characterise early evidence of immune compromise in asymptomatic patients to identify those individuals at increasing risk of short term progression.<sup>15</sup>

However, such approaches have three major difficulties. Assays require tissue culture of fresh blood lymphocytes, techniques which are difficult to apply on a large scale; particularly as containment facilities are required for such work. Secondly, such assays are difficult to standardise and quality control. Finally, the choice of antigen or polyclonal activator is difficult. If opportunistic organism antigens are used (the most physiologically relevant approach) then comparison of patients is a problem because it is likely that environmental exposure varies from person to person. For these reasons functional assays have not been widely evaluated in the longitudinal follow up of individual patients.

### Lymphocyte phenotypic markers

Laboratory assessment of the degree of immunodeficiency generally includes quantitation of T cell subpopulations. Serum or cellular markers of immune activation give additional information. Because the destruction of the CD4 population is the central lesion of HIV associated immunodeficiency, severe depletion of this T cell subpopulation is an invariable counterpart of the late stage disease characterised by major opportunistic infections. Moreover, several cohort studies have identified that a decline in the CD4 population in HIV seropositive subjects usually identifies a subgroup individual at high risk of short term progression (table 4). In contrast, individuals with persistently normal or stable CD4 populations have low rates of short term disease progression.

Table 3 Characteristics of markers of potential value in assessment of asymptomatic HIV disease

	<i>B<sub>2</sub>M</i>	CD4 count	p24 antigen	Viral isolation
Markers present in most infected patients (sensitivity)	No	Yes	No	Yes
Marker absent from non-progressor patients (specificity)	Yes	Yes	Yes	No
Change in marker associated with disease progression	In some	Yes	In some	If quantitated
Marker influenced by treatment	Yes	Yes	If present	?
Marker directly related to disease pathogenesis	No	Yes	If present	Yes
Assay standardisable with quality control	Yes	Yes	Yes	?
Widely available in clinical centres	Yes	Yes	Yes	No

Table 4 Incidence of AIDS in follow-up cohort studies from presentation CD4 count

Group	Presentation CD4 count	% AIDS incidence at 3 years	Reference
Homosexual	< 200	87	3
	201-400	46	
	> 400	16	
Homosexual	< 242	68	18
	243-345	40	
	346-490	23	
	> 490	11	
Injecting drug user	< 200	100	19
	201-500	51	
	> 500	22	

These cohort observations have been largely responsible for recommendations that patients with low presentation CD4 counts or whose counts fall to low levels on sequential follow up should receive primary prophylaxis against *P. carinii* infection, the most common serious opportunistic pathogen in late stage HIV infection. Current advice is that all adults with CD4 counts consistently below  $0.2 \times 10^9/l$  should receive such prophylaxis.<sup>16</sup>

Sequential follow up of asymptomatic patients has indicated that in most HIV seropositive subjects the CD4 count falls progressively with time at an overall rate of about  $0.8-1.0 \times 10^9/l/year$  in cohorts.<sup>4</sup> However, the rate of fall varies considerably from one individual to another. Experience suggests that individuals with a rapid rate of CD4 cell loss are more at risk of early clinical progression. The relative predictability of clinical progression in individuals showing sequential CD4 cell loss has led to increasing interest in directing specific antiviral treatment at this subgroup of individuals, although formal trial evidence that early treatment of such asymptomatic subjects results in prolonged overall survival is currently lacking.

Lymphocyte subpopulation numbers in blood are notoriously labile, being profoundly influenced by factors which include diurnal rhythm, stress, and intercurrent infection.<sup>17</sup> Such considerations determine that trends of CD4 count stability or progression can only be assessed after a number of estimations have been obtained from asymptomatic individuals over a number of follow up attendances. Until a number of data points have been obtained, any assessment based on laboratory investigations alone is unreliable. This consideration provides a strong argument for the early identification and long term regular followup of the HIV infected subject. During this stage of the disease assessment of T cell markers and CD4 counts, in particular, should only be performed when patients do not have intercurrent infections when spuriously low and misleading values can be obtained.

CD8 counts are also abnormal in most patients with HIV infection. However, changes in CD8 counts are biphasic. Initial very high absolute numbers, which are associated with the immune response to primary HIV infection, gradually fall towards the normal range many years later, continuing to fall to subnormal concentrations in late stage disease. The dynamics of change in CD8 count make it

unreliable as a marker in individual patients.

The divergent trends of CD4 and CD8 populations in early HIV disease has resulted in enthusiasm for use of the CD4:8 ratio in some centres. However, as the low ratio characteristic of most stages of HIV disease is also seen in several other viral infections and chronic immunological diseases, overreliance on this marker should not be encouraged. Absolute T cell subpopulation numbers provide more reliable staging indices.

#### Immunological activation markers

Activation of lymphocytes, especially CD8 cells, is a consistent feature of many primary or persistent viral infections. In HIV infection such activation persists through all stages of the disease and affects other lymphoid populations, including B cells. Several markers of activation on CD8 cells have been examined, including expression of class II histocompatibility antigens (DR), interleukin 2 receptors (CD25), and a number of cell adhesion molecules. Several of these show promise, although they have not been fully evaluated in the staging of individual patients or in the assessment of response to treatment.

Immunological activation results in the appearance of several lymphocyte and monocyte/macrophage products in serum or other body fluids. They include cytokines, immunoglobulins,  $\beta_2$  microglobulin ( $B_2M$ ) and neopterin. Their relative ease of measurement combined with the potential for retrospective analysis on stored serum samples has resulted in considerable interest. Used alone, such markers perform only moderately well partly because of low sensitivity and because levels can also be strongly influenced by other causes of immune stimulation, of which the infections complicating the later stages of HIV infection are obvious examples. However, a number of studies have shown that when used together with CD4 counts  $\beta_2$  microglobulin or neopterin provide additional staging information in individual patients.<sup>18-20</sup> Raised concentrations provide additional power to identify the subgroup of asymptomatic patients at high risk of clinical progression. Equally significantly, low concentrations of  $\beta_2$  microglobulin, when associated with relatively normal CD4 counts, identify a subgroup of patients at very low risk of short term clinical progression. Preliminary evidence suggests that  $B_2M$  also has potential as a surrogate marker to assess early benefit from HIV specific treatment.<sup>21</sup>

Titres of total immunoglobulin classes rise differentially with different disease stages. Titres of IgA rise with progression to late stage disease but too late in most individuals to be of value in asymptomatic staging.

#### Virological markers

Methodology which permits quantitation of cell or plasma HIV viral load will be valuable in the assessment of disease and treatment.<sup>22 23</sup> However, such techniques are expensive, poorly standardised, and not yet widely available.

P24 antigen, the only widely applied viral marker, performs poorly in the asymptomatic patient where it would be of greatest value as most such individuals have no detectable antigen. Even in advanced disease, the determination of p24 has poor sensitivity for disease progression. Longer experience suggests that this marker does not justify the great enthusiasm with which it was initially associated.

### Recommendations for frequency of clinical and laboratory monitoring

All identified HIV infected subjects should be offered regular clinical and laboratory follow up, irrespective of stage of disease presentation. Progress in understanding of disease natural history and evaluation of new treatments will require close study of the early stages of HIV infection.

Initial clinical assessment of asymptomatic subjects should be accompanied by baseline immunological investigation. In view of the inherent biological fluctuations associated with the CD4 count, a further count, irrespective of the initial one, is advisable within three months to establish baseline values against which subsequent trends can be assessed. Such trends cannot be extrapolated on the basis of single values for similar reasons and thus all apparent changes in CD4 values should be confirmed before clinical decisions are taken. For this reason it is advised that the asymptomatic individual is assessed at a minimum of six monthly intervals. Evidence of change will require more frequent evaluation.  $\beta_2$  microglobulin and neopterin concentrations should be measured less frequently. As such markers are less labile (but also less sensitive to change) they should be assessed about once a year.

In later stage disease immunological markers become progressively less valuable as end-stage progression approaches. In such patients clinical assessment of symptomatology and laboratory diagnosis of infectious complications are the major priorities. Once the CD4 count has fallen consistently below  $0.5 \times 10^9/l$  in any individual then further assessment is probably only justifiable to assess therapeutic response to new antiviral agents.

### Future prospects

The new CDC/WHO classification of HIV disease will probably change perceptions as attention is shifted from late to early stage disease. Moreover, the pace of assessment of new therapeutic approaches is already slowed by the widespread introduction of partially effective treatments in later stage disease, delaying the appearance of clinical endpoints in clinical trends. Future clinical trials are likely to become increasingly reliant on the enrolment of asymptomatic patients with assessment of efficacy based on the use of surrogate immunological or virological markers. Following initial evaluation of such trials it is likely that such laboratory monitoring will

become increasingly valuable in the clinical decisions about when such agents should be used. For any such laboratory investigations to be widely acceptable internal and external quality control and standardised methodologies will be essential prerequisites.

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